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Title

Mitotane directly activates estrogen receptor α: a bioinformatics and pharmacological study

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Background

Mitotane (o,p'-DDD) is the cornerstone of medical treatment of adrenocortical carcinoma (ACC), both in adjuvant [1,2] and metastatic settings [3]. Estrogenic-like side effects frequently occur in patients [4] and previous studies explored the chemical nature of interaction between estrogen receptor-α (ER-α) and toxic compounds, included the DDD derivatives [5]. Integrating bioinformatics approach with cell biology and pharmacological methods, in this study we explored the possible interaction between mitotane and the ER-α receptor, leading to a biological effect.

Methods

Molecular docking was performed using the SwissDock platform. Molecular dynamics (MD) simulations of the protein were performed by means of package GROMACS 2016. The ER-α expressing MCF-7 cells were used as experimental cell model and cells were exposed to mitotane (3-24 μM) with/without tamoxifen (1-10 μM) for three days. The cell viability/proliferation was evaluated by MTT assay and direct count. The transient ER-α silencing was performed using two ER-α siRNA (50 nM) and verified by western blot. Si-RNA scramble (40nM) was used as control. MDA-MB-231 cells were used as negative control.

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Results

Mitotane showed a similar docking configuration as 17β-estradiol and bisphenol A (BPA) and a significant binding affinity to ER-α. MD simulations showed that mitotane preserves the active conformation of ER-α, classifying it as an agonist. Exposure of MCF-7 cells to mitotane led to the concentration-dependent increase of cell viability and proliferation reaching its maximum (50% ± 11.36%) at 6 μM of mitotane. This effect was reduced in the presence of tamoxifen. Taking advantage of the RNA interference approach, silencing ER-α in MCF-7 cells resulted in the loss of mitotane stimulatory effect. The negative internal control MDA-MB-231 cell line (ER, PgR and HER2 negative) did not modify its cell viability when exposed to mitotane.

Conclusions

Integrating bioinformatic approaches with cell biology and pharmacological methods, we demonstrated that mitotane directly binds and activates ER-α.

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Title

ADVERSE DRUG REACTIONS IN THE CLINICAL PRACTICE IN AN ITALIAN ALLERGY UNIT: ALLERG-RAF RETROSPECTIVE STUDY

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Background

Adverse Drug Reactions (ADRs) were defined in the 1972 by the World Health Organization as a response to a drug that is “noxious, unintended and occurs at doses normally used in man”. The EU Directive 84/2010 has partially modified this definition, also including in the ADRs all those reactions deriving from an inappropriate use of the drug. The Italian Medicines Agency indicates that about 5% of all hospital admissions are due to ADRs, which are in fifth place among the causes of death in hospital. The monitoring of ADRs allows a continuous updating of the risk/benefit ratio of the drug and allows a more appropriate use [1, 2].

Methods

This is a retrospective analysis of all the medical records of patients evaluated in the Allergy Unit of ASST Spedali Civili and University of Brescia from 2000 to 2016. The study (ALLERG-RAF) was notified to the competent Ethics Committee. The inclusion criteria of the retrospective analysis were age $\geq$18 years and visit requested for suspected ADRs. Not complete data in the medical record was the reason for the patient's exclusion from the analysis. The following parameters were collected: sex; age at enrollment; age at the time of the reaction; pathology for which the drug was taken; drug(s); type of reaction observed; possible use of medical therapy following the suspected ADR; presence of relics; diagnosis of comorbidity and presence of atopy. Each patient was assigned an alphanumeric code and the pseudonymized data were collected in an informatics database. The data deriving from the descriptive statistical analysis are presented in an aggregate manner.

Results

In the period from 2000 to 2016, 35817 accesses to the Allergy Unit were made, of which 58% were first visits. In the same period, the number of ADRs reported was 3008 and involved 1840 patients (545 male and 1295 female). Mean age at time of reaction (min - max) was 43.2 (1-87) for males and 43.4 (0.2-94) for females. Of all the ADRs reported, 48.1% concerned antibiotics, mainly beta-lactams (61.5%), followed by quinolones (13%). Anti-inflammatory drugs, mainly NSAIDs were at the second place as ADR incidence, taking account for the 25.3% of diagnosis. Most of all reported reactions involved cases of urticaria/angioedema (43.9%) followed by general skin reactions (18.6%). Only 14.7% of patients were classified as atopic. No clinical sequelae were reported following the ADR.

Conclusions

Results of the retrospective analysis are consistent with national data and further underline the importance of pharmacovigilance activity [3]. This study presents some limits, that are 1) the retrospective direction of the study; 2) the reactions reported in the medical records are episodes reported by patients, which also occurred many years before the interview with the physician. Interestingly, the possibility of the re-administration of drugs previously suspected of ADR was the main reason for Allergy Unit access.

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Title

ANTIBODY RESPONSE EVALUATION IN MOTHERS VACCINATED AGAINST SARS-CoV-2 DURING PREGNANCY AND RESPECTIVE NEWBORNS AT BIRTH.

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Background

The risk of severe disease and mortality in pregnant women and newborns is greater if compared to non-pregnant adults' risk in case of SARS-CoV-2 infection. Vaccination against SARS-CoV-2 has been demonstrated to be safe during gestation. Nevertheless, there are no guidelines regarding the timing of COVID-19 vaccination in pregnancy. Our purpose with this analysis is to understand the occurring relation between the timing of vaccination during pregnancy and mothers' and babies' antibody titer at birth.

Methods

Participants were recruited at Niguarda Hospital of Milan, from May to November 2021. Criteria of inclusion were: pregnant women having received an anti-SARS-CoV-2 vaccination (with Pfizer/BioNTech or Moderna vaccine) during pregnancy and their respective newborns. Serological IgG antibodies anti-S1 RBD were evaluated through a quantitative chemiluminescent-assay (Abbott) and information about vaccination timing was obtained.

Results

84 women were included in the analysis. 22 mothers out of 84 received just one dose, while 62 received two doses. The geometric mean titer (GMT) of anti-S IgG is 329.2 (7.6) AU/ml for babies (%CV 280) and 571.8 (7.4) AU/ml for mothers (%CV 247) in the group of subjects who received just one dose. In the group of those who received two doses, the GMT was 6271.7 (2.7) AU/ml for babies (%CV 101) and 5480.5 (2.7) AU/ml for mothers (%CV 109). The relation between the gestational age and the serological titer of the newborn at birth is significant for the 62 mothers with 2 doses ($p<0.001$): the higher the gestational age when mothers received vaccination the higher the serological titers of the respective newborns at birth (Tobit mixed models regression).

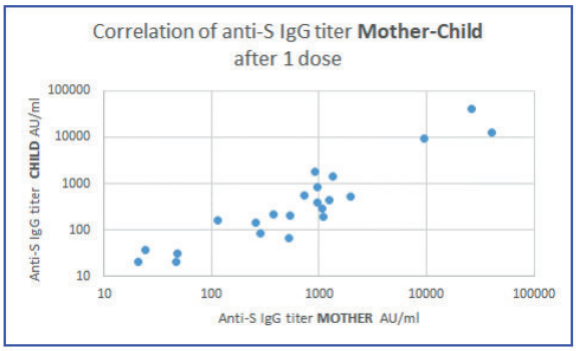
Conclusions

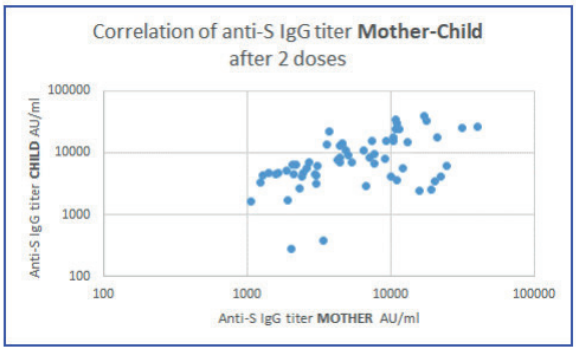
Our data suggest that:

1) The birth titer in children of mothers who received two doses of vaccine during pregnancy results equal to or higher than the mothers' titer.

2) There is a correlation between gestational age at vaccination and serological titer at birth.

References





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Title

Effectiveness and safety of monoclonal antibodies for COVID-19 in a real-life experience during the COVID-19 pandemic in an Italian hub hospital

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Background

Monoclonal antibodies (mAb) against SarsCov2 are among the most promising pharmacological strategies. From Mar-21 the European Commission has authorized the following mAb targeting the spike protein of SARS-CoV-2: casirivimab+imdevimab, regdanvimab, sotrovimab and bamlanivimab as monotherapy or in combination with etesevimab. Given their recent market entry, real word data are needed to better elucidate their impact on different clinical contexts. Here we describe their use in the hub hospital Santa Croce e Carle Hospital in Cuneo during the first year of their approval (March 21 – March 22)

Methods

A retrospective analysis was carried out on 556 high-risk patients with COVID-19 who received anti-SARS-CoV-2 mAb (bamlanivimab, bamlanivimab+etesevimab, casirivimab+imdevimab and sotrovimab) between March 2021 and March 2022. . The aim was to assess the effectiveness and safety of mAb therapies in comparison to the available Randomized Clinical Trials (RCTs) data. Number of death, hospitalizations or emergency department visits within the twenty-ninth day from the administration of the therapy were recorded.

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Results

The first available treatment was bamlanivimab (16-03-2021), used as monotherapy first and then in combination with etesevimab (261 infusions were administered), followed on 26-03-2021 by casirivimab+imdevimab, (233 infusions). Sotrovimab started to be used from 02-12-2021 (61 infusions). Among patients treated with bamlanvimab/etesevimab, sotrovimab and casirivimab+imdevimab, the highest percentage of efficacy was in the third group (1.92%,1.64%,1.43%) and was similar to the percentage of the RCTs. Interestingly our data showed that the faster was the beginning of the therapy infusion the more effective was the prognosis in terms of reduction in hospitalizations and progression to severe disease.

Analysis of the safety data showed that the most common adverse reactions for bamlanivimab+etesevimab were nausea, headache and skin rash while headache, diarrhea and nausea along with skin rash were recorded when casirivimab+imdevimab were administered.

Conclusions

Our study confirms the effectiveness and safety of the anti-SARS-CoV-2 mAb therapies, in reducing hospitalizations and deaths related to COVID-19. In this real-word experience the safety of the proposed drug treatments was excellent and confirmed the good risk/profile observed in clinical trials.

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Title

A Retrospective analysis on pharmacological approaches to COVID-19 patients in an Italian hub hospital comparing first and second pandemic wave

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Background

The first wave of COVID-19 epidemic in Italy was characterized by a very rapid spread of cases and deaths with strong territorial concentration in the north of the country (1). The lack of knowledge on the disease lead to several different attempts of drug repurposing during the first wave, with a better defined two-step approach developed during the second wave and based on the integrated use of antiviral drugs followed by immunosuppressive and immunomodulatory pharmacological strategies. Our study aims to retrospectively evaluate the different therapies administered in COVID-19 patients admitted to Santa Croce e Carle Hospital, Cuneo during the two first waves of COVID-19 epidemic.

Methods

We analyzed data from 277 patients with virological diagnosis of COVID-19 who were admitted to the hospital from Feb 20th to Apr 30th 2020 (first wave) and 322 patients admitted from Oct 20th to Dec 31st 2020 (second wave). Correlations between pharmacological treatments as well as clinical and demographic variables and clinical outcomes have been performed.

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Results

The therapies have radically changed moving from the first to the second waves of the epidemic. We documented significant changes in the consumption of glucocorticoids, mainly dexamethasone, with a massive increase from 30.7% to 89.7% (P≤ 0.0001) and heparin antithrombotics, which increased from 23.10% to 93.2%. (P≤ 0.0001). Interestingly, the use of enoxaparin increased by 13.8% (P≤ 0.0001), the use of unfractionated heparin and fondaparinux decreased by 10.9% and 1.5% respectively. The administration of antivirals decreased: from 42.6% to 3.4% (P≤ 0.0001). Specifically, the combination therapy based on lopinavir / ritonavir and darunavir / cobicistat, or drugs such as dolutegravir, etravirine and rilpivirine were almost completely replaced by remdesivir during the second wave. Hydroxychloroquine was administered to more than 7o% of patients during the first wave, while it has not been used anymore in the second wave of the epidemic.

As a consequence of the differences in the pharmacological approaches, the number of hospitalizations in intensive care units decreased from 28,9% to 22% in the second wave

Conclusions

Our study offers a picture of the pharmacological treatments proposed during the first and second wave of the COVID-19 epidemic evidencing changes in the drug treatments. Between the first and second waves of the SARS-CoV-2 pandemic, the first guidelines on the management of hospitalized patients with COVID-19 were promulgated and the clinicians have become more confident in recognizing the severity of the condition and thus managing the patient.

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Title

Gender-based evaluation of the effect of mitotane on total cholesterol, HDL, LDL and triglycerides levels in patients with adrenocortical carcinoma

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Background

In dyslipidemic patients, high plasma mitotane levels have been observed, without re-ported side effects, highlighting that plasma mitotane distribution in lipoprotein could be the major determinant of its distribution in the other tissues. In addition, mitotane bi-oavailability depends on different factors, such as gender. Sex differences in drug pharmacodynamics and pharmacokinetic, response to treatment and related toxicity have been still reported. The objective of the present project was the evaluation of the interplay between mitotane pharmacokinetics and treatment-induced dyslipidaemia. Mitotane and its metabolite o,p'-DDE, have been quantified in human plasma and the biologic implication of serum lipoprotein on drug pharmacokinetic and efficacy have been explored considering sex-related differences and menopausal period.

Methods

We performed a cohort study in ACC patients treated at the S. Luigi Hospital. All patients underwent radical surgery for ACC and then started mitotane as adjuvant treatment. Plasma o,p'-DDD and o,p'-DDE concentrations were determined from blood samples ob-tained at the end of dosing interval, before the next drug dose intake. Analytes quantification was performed by a validated High Performance Liquid Chromatography method coupled with UV detection (HPLC-UV). Substances separation, after specific liquid extraction, was achieved on a RP-C18 column.

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Results

We retrieved data of 551 ACC patients, 215 males and 336 females. Considering female population, 246 were premenopausal and 90 postmenopausal. We observed a different lipid profile between males and females and between pre- and post-menopausal women. Considering mitotane-effect on lipid levels, we observed that higher drug concentrations were correlated to higher HDL in all the considered groups ($p<0.001$), to total cholesterol both in males ($p=0.005$) and females ($p=0.036$), to triglycerides in postmenopausal females ($p=0.002$) and to LDL in male patients ($p<0.001$). An in-crease of o,p'-DDE were positively correlated with HDL levels in all the groups ($p<0.001$) and negatively with LDL in all the groups (males $p=0.008$, pre- and post-menopausal females $p<0.001$), with total cholesterol in pre- ($p=0.016$) and post-menopausal women ($p=0.01$) and with triglycerides in premenopausal females ($p=0.005$).

Conclusions

This is the first study designed to separately evaluate in male and in female and in premenopausal and postmenopausal women the effect of mitotane treatment on choles-terol, HDL, LDL and triglycerides levels in AAC patients. The obtained results suggest that a gender and personalized approach should be useful to prevent and to better control dyslipidemias. Further studies, including data about male hormonal phase, body weight and body fat distribution are indispensable to clarify the mechanism of mitotane -induced dyslipidemia, and in particular hypercholesterolemia.

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Title

Beneficial effects of MR120 in a preclinical model of chronic intestinal inflammation

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Background

CCR6, expressed on immune cells, interacts solely with its ligand CCL20, whose expression by lymphoid tissues and epithelial/endothelial cells is highly increased in inflammatory conditions, like in IBD. Our group has recently demonstrated the ability of an original small molecule targeting CCR6 (MR120) to reduce the neutrophil infiltration in two different murine protocols of acute intestinal inflammation, TNBS-induced colitis[1] and zymosan-induced peritonitis[2]. Our aim is now to expand the investigation on MR120 by assessing its anti-inflammatory effects in dextran sodium sulfate (DSS)-induced chronic colitis, a model able to more closely mimic the typically alternating phases of remission and exacerbation of human IBD[3].

Methods

Chronic colitis was induced in C57BL6/J mice through exposure to 3% DSS in tap water for 3 cycles of 5 days, interspersed with 9 days of tap water only. Sham (S) mice received only drinking water. On the 33rd day mice were euthanized by CO_2 inhalation. From day 8, MR120 1mg/Kg was subcutaneously (s.c.) applied twice daily, 8h apart, while S and control (DSS) mice received vehicle 10 mL/Kg s.c.. Disease activity index, scoring body weight, feces consistency and rectal bleeding, was registered daily. At day 33, colonic macroscopic score (MS), length and thickness and spleen/body weight were determined. Spleen and mesenteric lymph nodes (MLN) T lymphocytes were analyzed through FACS. Colon and lung myeloperoxidase (MPO) activity was determined through biochemical assays. All experiments were performed according to the guidelines for the Care and Use of Animals (DL26/2014).

Results

DSS significantly increased DAI score, colon MS and thickness, colon and lung MPO activity and induced splenomegaly, while decreased colon length and spleen and MLN T cells percentages ($P<0.05$ vs. S). MR120 significantly reduced DAI score, colonic MS and MPO activity, and antagonized splenomegaly ($P<0.05$ vs DSS), while no significant changes were produced on splenic and MLN T lymphocytes.

Conclusions

These data indicate that MR120 attenuated the severity of the systemic and local responses triggered by DSS-induced chronic colitis. In particular, the reduced recruitment of neutrophils to the colon follows closely what already evidenced in acute inflammation and presumably contributes to the protection afforded by the CCR6-targeting compound.

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Title

Functional and pharmacological characterization of ClC chloride channels in human pancreatic ductal adenocarcinoma stem cells

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Background

Pancreatic Ductal Adenocarcinoma (PDAC) is one of the deadliest cancer diseases, which malignant nature mainly relies on its high invasive capacity, and chemo- and radio-resistance.

Thus, the discovery of novel biomarkers for early diagnosis and the design of innovative therapeutic strategies for PDAC, both represent an urgent need.

Recent evidence pointed ion channels as pivotal regulators of several cancer hallmarks (e.g. cell proliferation, resistance to apoptosis, invasiveness and angiogenesis) (1).

Here, we performed a screening of ion channels expressed in PDAC and investigated the activity and function of the most upregulated channels by using specific inhibitors and activators.

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Methods

The human pancreatic adenocarcinoma cell lines (Panc-1, Mia-PACA), cancer stem cells (CSC) derived from Panc-1 and healthy pancreatic cell line (HPDE) were grown in appropriatemedium and used for gene expression experiments and functional analysis.

Comparative analysis of gene expression for ClC-2, ClC-3 and ClC-7 chloride channels and the auxiliary subunits HEPACAM and Ostm1 was performed using Taqman RT-PCR.

For functional studies, the CSC were transferred in collagen–coated dishes and used for patch-clamp experiments in whole-cell configuration. Specific protocols were applied to record chloride currents and only cells presenting currents reversibly blocked by iodide were used for analysis.

Results

Quantitative RT-PCR analysis revealed that all the cell lines showed a similar mRNA expression level for ClC-3 chloride channel. Conversely, a significant difference was observed in ClC-2 and ClC-7 channels expression: CSCs overexpressed ClC-2, ClC-7 and their auxiliary subunits (HEPACAM and Ostm1 respectively) compared to HPDE, Panc-1 and Mia-PACA cells.

Patch-clamp experiments of CSC revealed that 14 out of 47 patched-cells displayed chloride currents that were sensitive to iodide, suggesting that they were carried by ClC-2 channels. However, these currents exhibited distinct kinetics. In 3 cells, chloride currents slowly activated toward a plateau at negative voltages, suggesting that ClC-2 was expressed individually. In 11 cells, the currents were steady activated at negative voltages, suggesting an association of ClC-2 protein with the accessory subunit HEPACAM. Ongoing pharmacological experiments are performed to test ClC-2 inhibitors (e.g. meclofenamic acid) (2) and activators (e.g. lubiprostone) (3) on chloride currents and on cell proliferation, apoptosis, and migration.

Conclusions

The CSCs represent a reservoir of self-renewal cells that contribute to PDAC cancer progression and recurrence (5). We showed that pancreatic CSCs overexpress ClC-2 and ClC-7, suggesting a putative involvement of these channels in neoplastic processes. All the information will yield advances in scientific knowledge regarding ion channels involvement in PDAC and contribute to identification of promising therapeutic options for PDAC patients. Supported by grant PRIN n. 20174TB8KW_002 “Lioness” from MIUR.

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Title

Voriconazole Therapeutic Drug Monitoring: a retrospective pilot study.

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Background

Voriconazole is a widely employed triazole in invasive fungal infections, which represent a relevant clinical issue in terms of morbidity and mortality. The antifungal Therapeutic Drug Monitoring (TDM) helps clinicians to reduce the clinical impact of pharmacokinetic variability of voriconazole, in order to maximize its efficacy and to limit its toxicity[1].

The aim of this study is to explore the effectiveness and safety of voriconazole in a real-world setting.

Methods

This is a retrospective cohort study conducted at the University Hospital Friuli Centrale ASU FC in Udine between November 2020 and May 2022, evaluating patients who received voriconazole TDM as part of routine care. Voriconazole was measured by liquid chromatography–tandem mass spectrometry (LC–MS/MS) assays.

We described patients' clinical data, voriconazole serum concentration, dosage and therapeutic target attainment.

We stratified all patients according to the target range attainment (1-5.5 mg/L). We analysed the trough voriconazole plasma levels normalized for dose and weight (C/D/Kg) by Kruskal-Wallis, considering a *p*-value <0.017 as statistically significant.

Results

Voriconazole levels were analyzed from 56 patients. Of these, 75% were male, mean 64.1 years old, and 98.2% received voriconazole for treatment. The therapeutic range target was achieved by 67.9% of patients, whereas 32.1% was out of the range (25% under and 7.1% over the target). We observed a statistically significant difference of C/D/Kg among different target attainment represented by patients under, within and over the therapeutic target ranges, with a median value C/D/Kg of 0.0102, 0.054 and 0.261 mg/L/D/Kg, respectively (*p*-value <0.000001). No any other patients' characteristics were predictive of therapeutic voriconazole levels or hepatotoxicity.

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Conclusions

Real-world data from our hospital showed a relevant percentage of patients receiving a standard dose of voriconazole but not achieving therapeutic range. The C/D/Kg parameter suggests an impact of body weight on target range attainment, which could influence the voriconazole dose to administered in clinical practice, rejecting the traditional “one dose fits all” approach. This impact has to be furtherly investigated in a larger prospective cohort of patients. These data support the need for routine voriconazole TDM in critical clinical settings as invasive fungal infections.

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Title

CYTOPROTECTIVE ROLE OF A NOVEL HYDROALCOHOLIC EXTRACT OF LENS CULINARIA AGAINST CHEMOTERAPEUTIC AGENTS AND GLUCOCORTICOID DEXAMETHASONE IN MURINE AND HUMAN CELLS.

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Background

Lens culinaria showed nutritional health benefit in humans due to their composition that make theme rich in compounds like proteins, amminoacids and polyphenols. So, considering the known positive role of legumes and considering the absence of observation about a possible cytoprotective role in cancer therapy, we evaluated the effects of a novel hydroalcoholic extract (70% ethanol, 1E030521DIPFARMTDA) from Lens culinaria (Terre di Altamura SRL, Altamura BA) to prevent cytotoxic damage induced by chemotherapeutic agents such as staurosporine, a non-selective inhibitor of tyrosine kinases, the antimitotic doxorubicin, the antineoplastic cisplatin and the glucocorticoid dexamethasone targeting osteoblast.

Methods

We carried out survival crystal violet test which marked nucleus of viable cells, clonogenic test on colonies number formed, counting test on isolated fibers and a UV/VIS spectroscopy.

Results

On renal Hek293, staurosporine reduced survival cell by -20% to -60% ($p < 0.05$) and the extract was significantly effective in restoring survival ??between 70% and 100% ($p < 0.05$) with comparable activity to that of diazoxide. In clonogenic assay, it was effective to prevent the reduction of colonies numbers caused by staurosporine. Cisplatin caused a slight reduction on Hek293 and a significant reduction on SHSY5Y too. On Hek293 it caused a reduction from -10% in cell proliferation while on SHSY5H a reduction of -30% respectively after 48 and 72 hours of incubation. In both cases, the extract showed a significant cytoprotective effect. On the primary culture of murine osteoblasts, doxorubicin caused a survival cell reduction about -30%, dexamethasone reduced it by -15%. The extract was significantly effective in preventing cell count-reduction induced by dexamethasone but not by doxorubicin. On mouse isolated

fibers, the hydroalcoholic extract was not effective in preventing cell damage caused by staurosporine and doxorubicin. In the UV/VIS spectroscopy we obtained three peaks at the wavelength of 350, 260 and 190 nm which correspond to families of compounds such as flavonoids, proanthocyanins and salicylates.

Conclusions

Hence, this novel extract prevented the cytotoxic damage induced by cisplatin and staurosporine on the renal cell line, and by cisplatin also on the neuronal cell line. On murine osteoblast it was significantly effective in preventing the reduction of cell proliferation induced by dexamethasone. Despite of the UV/VIS analysis it is not yet clear which of these family's molecules composing the extract is responsible for these effects or if there is a synergistic action.

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Title

Azithromycin and COVID-19: a real-world observational study.

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Background

To assess: 1) the association between use of azithromycin and risk of hospitalization in SARS-CoV-2 tested positive individuals; 2) the association between drug exposure and disease progression in hospitalized COVID-19 patients.

Methods

This study is part of VICES-SMIRE project that assessed COVID-19 impact on healthcare system (funded by Lombardy Region). We conducted a retrospective cohort study using the healthcare administrative databases of Brescia and Bergamo. Two cohorts were created: cohort A including all individuals tested positive between 21/02/2020-10/12/2020 and cohort B including those who were also hospitalized between 21/02/2020-31/12/2020. The date of positive test and hospitalization were considered the index date (ID) for cohort A and B. Exposure to azithromycin was assessed within 7 days prior and 20 days after the positive swab for both cohorts and two treatment groups were identified: azithromycin users (AU) and antibiotic non-users (ANU). For cohort A, the risk of hospitalization was assessed, whereas for cohort B 3 outcomes were investigated: need of mechanical ventilation (MV), Intensive Care Unit (ICU) admission and all-cause

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mortality at 14 and 30 days after ID. After propensity score matching (PSM), a clustered Fine-Gray regression model was used to assess the risk of hospitalization with death as competing risk in cohort A, whereas logistic and Cox regression models were used to assess the association between exposure and risk of ICU access, need of MV or all-cause mortality. Sensitivity analyses were performed.

Results

In cohort A, 5,089 AU and 37,751 ANU were selected. AU were mainly male (51% Vs 46%; p-value<0.05), older (mean age: 54.5 Vs 48.8; p-value<0.05) and presenting higher prevalence of comorbidities compared with ANU. After PSM, 4,861 individuals were selected. The exposure to azithromycin was associated with increased risk of hospitalization during follow-up (HR: 1.59; 95%CI: 1.45-1.75). For cohort B, 1,100 AU and 6,169 ANU were selected. The mean age was 66.9 years for AU and 69.2 for ANU. In both groups, the majority of individuals were male (68% for AU and 59% for ANU). ANU had higher prevalence of comorbidities compared with AU. After PSM, 997 individuals were selected. Previous exposure to azithromycin was not associated with the risk of ICU access (Odds ratio OR: 1.22; 95%CI: 0.93-1.56) and the need of MV (OR: 1.30; 0.99-1.70) during follow-up. Similarly, azithromycin use was not associated with mortality at 14 days (Hazard Ratio HR: 0.88; 0.74-1.04) and 30 days (HR: 0.89; 0.77-1.03). Results from sensitivity analyses were consistent with those observed in the main analysis.

Conclusions

In this study, azithromycin was associated with increased risk of hospitalization in tested positive individuals. However, no effect on disease progression were observed among hospitalized COVID-19 patients. These findings raise concern on the potential consequences of inappropriate use of antibiotics against COVID-19.

References

Title

EVALUATION OF THE STANDARD NOD-LIKE RECEPTOR PROTEIN 3 (NLRP3) BLOCKER MCC950 IN A MOUSE MODEL OF MIGRAINE

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Background

Migraine is a recurrent headache disorder characterized by episodes of acute pain. It is a typically female condition. The pathophysiology of migraine is complex and more than 50% of patients are dissatisfied with their treatment. Recent findings suggest for the involvement of the inflammasome NOD-like receptor protein 3 (NLRP3) and IL-1ß in the pathogenesis of migraine. The overall goal of the present project was to test if the blockade of NLRP3 may represent a novel strategy for the management of migraine. A murine model of migraine induced by the systemic injection of nitroglycerin (NTG) was set-up and validated using standard anti-migraine drugs. Finally, the standard NLRP3 blocker MCC950 was tested under the same experimental conditions.

Methods

To induce migraine in C57BL/6 male and female mice, NTG was injected intraperitoneally (i.p.). at the dose of 10 mg/kg. As a sign of migraine, the periorbital mechanical allodynia (PMA) was assessed, using the Von Frey filaments, 30, 60, and 120 min after NTG administration. Sumatriptan (600 µg/kg), acetaminophen (300 mg/kg), olcegepant (1 mg/kg), and MCC950 (10 mg/kg) were injected i.p. 10 min after NTG.

Results

NTG caused PMA in both male and female mice, no sex-related differences were detected. The peak of the NTG effect was recorded 60 minutes after NTG administration. The standard drugs sumatriptan and acetominophen significantly reversed PMA in male and female mice. Olcegepant was able to revert NTG evoked PMA only in male but not in female mice. Under these experimental conditions the NLRP3 blocker MCC950 reverted NTG induced PMA only in female but note in male mice.

Conclusions

A mouse model of migraine induced by the systemic injection of NTG has been set-up and validated. The present study, performed using this model suggests a potential role of NLRP3 in the pathophysiology of migraine, especially in females. Thus, NLRP3 may represent an innovative target for the development of new anti-migraine drugs. Further studies are need to be corroborated this hypothesis.

References

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Title

Effect of CB2 stimulation on gene expression in B-acute lymphoblastic leukemia: new possible targets

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Background

Leukemia is the most common pediatric cancer and, despite the high survival rate showed by patients, it is the principal cause of infant mortality (1). Acute lymphoblastic leukemia (ALL) is the most diagnosed kind of leukemia, and it is characterized by clonal proliferation of lymphoid stem cells, principally B-cells (> 80%) (2). Treatment of ALL has been a great success in cancer therapy and little could be done to further improve outcomes, without increasing side effects (3). Molecular targeted therapy and immunotherapy have been suggested as solutions to overcome this limitation (4). The most used therapeutic regimen consists in administering the chemotherapy, methotrexate. Cannabinoids, enzymes involved in their metabolism and cannabinoid receptors type 1 and type 2 (CB1 and CB2) constitute the endocannabinoid system, proposed as antitumor therapeutic target (5). Endocannabinoid system is involved in inflammation, immune response, pain modulation and cancer. The selective stimulation of CB2 receptor exerts antiproliferative, pro-apoptotic and anti-invasive effects in tumors, such osteosarcoma and T-ALL (6-8).

Methods

We evaluated the effects of CB2 stimulation with JWH-133 (antagonist) on the B-ALL cell line, SUP-B15, by small RNA sequencing and Western Blotting.

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Results

We observed a lower expression of CB2 receptor in SUP-B15 than in healthy lymphocytes, thus hypothesizing the involvement of this receptor in the pathogenesis of B-ALL. After CB2 stimulation, we observed a lower expression of 4 genes (CD9, SEC61G, T-bet and Tβ4) involved in tumor growth and progression. JWH-133 also reduces the expression of proteins associated with these genes in tumor progression and maintenance.

Conclusions

We demonstrated the antitumor role of CB2 proposing it as novel anticancer target also in B-ALL. Moreover, we highlighted the functional correlation between CB2 receptor and specific genes and proteins involved in tumor, even though underlying mechanism certainly need further investigations.

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Title

Arsenic in drinking water as a potential health risk in several municipalities of the Varese province (Italy)

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Background

Arsenic (As) is a metalloid that is often found in groundwater wells constituting a common drinking water contaminant. Worldwide more than 200 million people are estimated to be exposed to unsafe levels of As. The groundwater of many countries like Bangladesh, India, China, USA (Arizona, California and Nevada), Argentina, Chile, is naturally contaminated with high levels of inorganic arsenic and most epidemiological evidences point out a correlation with numerous adverse human health impact. Even at very low concentrations, chronic exposure to As has been associated with a multiple human disorders, including dermal, renal, cardiovascular, neurological, metabolic conditions, as well as cancer. Maximum permissible limit of As in drinking water is 10 µg/L (2020/2184/Ue). Arsenic contamination of groundwater by naturally is a known public health problem occurring in some areas of Italy with volcanic origin, like Viterbo province, less known is the contamination due to a particular idrogeological conformation of soils and aquifer system in some municipalities of Varese province, Lombardy region (Italy).

Methods

Water samples from aqueducts of 17 municipatilies of Varese province were collected before and after dearsinification in a 5-years period (2007-12), and analysed with electrothermal atomic absorption spectroscopy (ETA-AAS).

Methods

Water samples from aqueducts of 17 municipatilies of Varese province were collected before and after dearsinification in a 5-years period (2007-12), and analysed with electrothermal atomic absorption spectroscopy (ETA-AAS). Golasecca and Somma Lombardo, mean concentration values of As are critical and more than 4 times the safety limit (54,5 and 47,47 µg/L respectively). In two other municipalities, Sesto Calende and Brebbia the mean values remain above the safety value (18,16 and 12,40 µg/L, respectively) (Figure 1B). Values after dearsinification are all below the safety limit (Figure 1C).

Conclusions

The daily intake of As-contaminated drinking water causes acute and chronic health effects. The occurrence of As in groundwater poses enhanced risks to human health, constant monitoring as well as the effectiveness of drinking-water treatment are effective in order not to expose the population to potential health risks.

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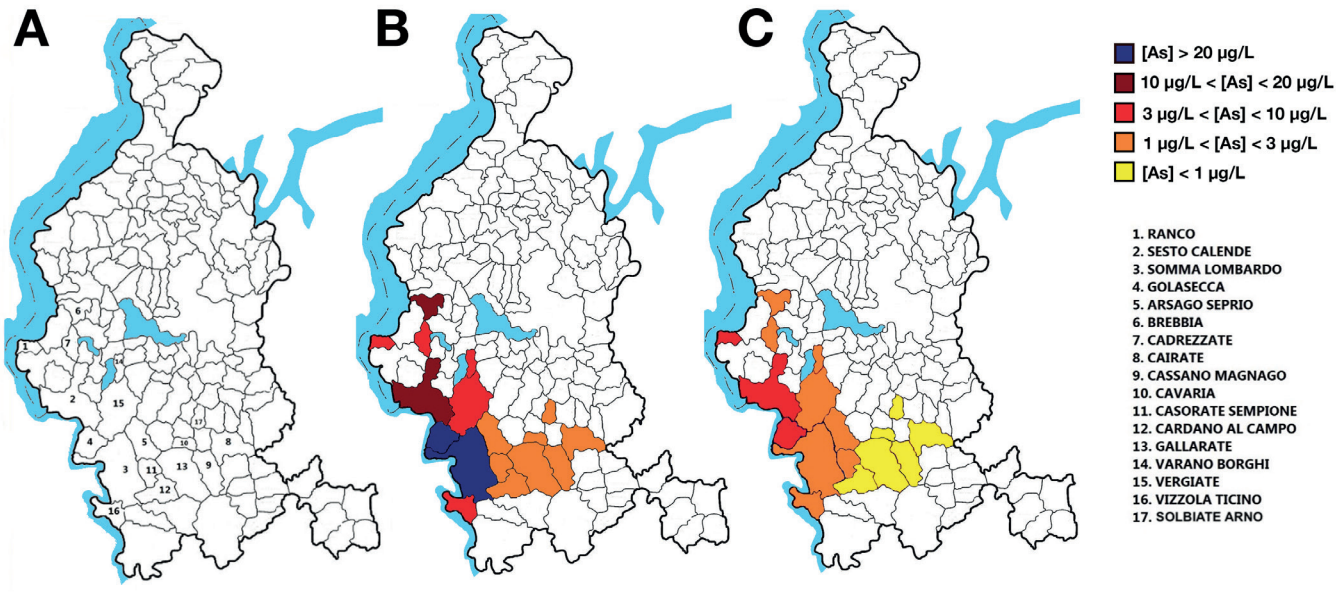

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Title

NAMPT-induced breast cancer cell reprogramming towards an invasive and metastatic phenotype

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Background

Nicotinamide phosphoribosyltransferase (NAMPT) is a pleiotropic protein that exists in two different forms: although the intracellular form (iNAMPT) is a key enzyme involved in NAD metabolism, the extracellular form (eNAMPT) has been reported to act as a cytokine and its serum concentration is increased in immunological and metabolic disorders, including cancer. For instance, in breast cancer (BC) patients eNAMPT serum levels correlate with TNM staging, tumour size, lymph node metastasis and histological grade (Assiri et al., 2016). It has been demonstrated that NAMPT overexpression promotes in mammary epithelial cells the epithelial-to-mesenchymal transition (EMT), a morphological and functional switch from mesenchymal to epithelial features that confers migratory potential (Soncini et al., 2014). The aim of our work was to investigate the modulation of EMT markers operated by eNAMPT leading to reprogram breast cancer cells toward a more invasive phenotype.

Methods

Serum samples derived from breast cancer patients were analysed with commercial ELISA anti-NAMPT kit to evaluate eNAMPT levels. The BC cell lines were cultured in their respective complete growth medium and maintained in incubator supplied with 5% CO2/ 95% air at 37°C. The analysis of eNAMPT release in a panel of different BC cell lines was performed after the concentration of supernatants with a 30 kDa molecular weight cut-off Vivaspin. The evaluation of EMT markers regulation *in vitro* operated by NAMPT was performed with Western Blot analysis and RT-PCR to investigate respectively the proteins level and the genes expression.

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Results

The ELISA analysis of serum of BC patients in a small dataset (n=52) revealed an increase of eNAMPT levels compared to healthy controls.

The *in vitro* study of eNAMPT release shown that mesenchymal-like BC cell lines expressed the highest amounts of eNAMPT, compared to epithelial-like cancer cells. These data support the correlation between eNAMPT and mesenchymal features of BC cells.

Both Western Blot analysis and RT-PCR highlighted a modulation of the principal EMT markers after the treatment with recombinant NAMPT (rNAMPT) in a dose-dependent manner. Moreover, in a non-tumorigenic epithelial cell line, rNAMPT induced EMT-like cellular changes.

Conclusions

Taken together these data support our hypothesis whereby eNAMPT has a role in the modulation of EMT markers that lead BC cells to the acquisition of a more aggressive and invasive phenotype.

For this reason, eNAMPT-targeting therapeutic approaches might be tested to prevent EMT and metastasis formation.

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Title

Plasma proteomics to improve identification of patients at high cardiovascular risk requiring early cholesterol lowering pharmacological treatment

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Background

Familial Hypercholesterolemia (FH) is the form of highest Cardiovascular Disease (CVD) risk, due to lifelong elevated LDL cholesterol content (LDL-C). Proper diagnosis and treatment are oftentimes difficult, as commonly prescribed pharmacological treatments show attenuated activity when FH is genetically determined (partial lack of LDL receptor “LDLR”; 1:500 prevalence in the population) and when high throughput genetic tests are not available. The potential of plasma proteomics is tested here, in the quest to find single markers, that we previously associated with CVD risk trajectories in the general population (1), to be considered in FH.

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Methods

We measured 274 plasma proteins (high sensitivity Proximity Extension Assay technology) in 173 clinically defined FH (according to Dutch Lipid Clinic Network Score, DLCNS) from two independent lipid clinics (Milan and St. Petersburg) and in a population-based survey of normolipidemic subjects not on lipid lowering therapies ("controls"). Next generation sequencing of *LDLR* identified 133 genetically confirmed heterozygous FH ("FH/M+") and 40 genetically negative FH ("FH/M-").

Results

Both FH/M+ (30 (13-40) y-old) and FH/M- (FH/M+ vs 39 (29-54)) presented significantly elevated levels of up to 250 proteins versus controls (55 (50-61) y-old respectively). Proteomics of FH/M+ vs FH/M- was also different (Bray-Curtis dissimilarity of principal components; PC1=66.7% vs PC2= 10%) and was explained by eight hit proteins. These proteins, increased in FH/M+ from both lipid clinics, are involved in hematopoiesis (Stem Cell Factor cell, Interleukin-7), cell proliferation (Epidermal Growth Factor Receptor; Placenta growth factor), damage (Tumor Necrosis Factor Receptor Superfamily member 10; Galectin-9), chemotaxis (Chemokine (C-C) ligand 8 (CCL8); Platelet-derived growth factor-B).

Proteomics better discriminate FH+ from FH- on top of either LDL-C or DLCN alone (AUC of the model with proteomics included= 0.972 vs AUC with LDL-C alone 0.862 vs AUC with DLCN alone= 0.795 $p<0.001$). This performance was not significant in younger FH/M+ (< 18 y-old).

Conclusions

Follow-up studies and techniques of artificial intelligence that we still applied in general population (2) are required to validate. However, these preliminary data in this cohort support the relevance of "omics" to improve the diagnosis and to start early and effective treatments against the most severe forms of CVD risk.

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Title

Novel biguanide derivatives exert anti-proliferative and anti-invasive effects in 3D models of patient-derived glioblastoma stem cells and in zebrafish xenografts

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Background

Metformin and other approved biguanides represent a new class of antiproliferative agents for glioblastoma (GBM) stem cells (GSCs) (1). High metformin concentrations are required in preclinical studies that are hardly translatable in clinical settings (2), therefore, the search for more potent derivatives is a relevant goal for new drug development. GBM organoids improve drug translational assessment of candidate therapeutics, providing patient-specific 3D models mimicking the aggressive and invasive nature of this brain tumor still invariably lethal.

Methods

Cytotoxic activity of a small library of novel biguanides was analyzed in 2D and 3D (organoids) GBM models by MTT, cell count, spherogenesis, and EdU proliferation assays. Umbilical cord-mesenchymal stem cells (ucMSCs), and rat astrocytes were used as normal reference cells. Potency and efficacy were recorded for each compound in 6 different patient-derived GSCs. In vivo antitumor activity was tested by injection of GSCs into hindbrain of zebrafish embryos, as well as effects on 2D migration (transwell assay) and 3D invasion in Matrigel®. Statistical analyses for significance of data was performed.

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Results

Five linear aryl-biguanides and 4 cyclic derivatives were synthesized and tested in GSCs. Among active compounds, Q48 (linear, IC_{50}=0.08 mM) and Q54 (cyclic, IC_{50}=0.43 mM) showed the highest potency and efficacy as compared to metformin (IC_{50}=9.78 mM, -82% at 30mM), and lower toxicity in ucMSCs and rat astrocytes (selectivity index >10). Q48 and Q54 significantly decreased viable cell number and self-renewal. Both compounds exerted higher anti-migratory effects (− 66% for Q48 and − 75% for Q54) and anti-invasive activity in 3D than metformin, representing a hallmark of GSCs in sustaining brain infiltration and GBM relapse. Q54 significantly decreased tumor mass growth in xenotransplanted zebrafish. GBM organoids, which recapitulate GBM cellular heterogeneity (containing $Sox2^+/Olig2^+$ stem and β-III-tubulin$^+$ or $GFAP^+$ differentiated cells), cell-to-cell and cell-matrix interactions occurring in vivo, revealed that the new compounds are able to significantly inhibit cell proliferation also in more complex models. Importantly, Q48 and Q54 significantly reduced $Sox2^+$-cells within organoids, indicating a selective effect on the stem population.

Conclusions

Novel biguanide-based compounds Q48 and Q54 affect GSC proliferation, self-renewal, migration, and invasion in 2D and 3D GBM models, with a 100-fold higher potency than metformin, and low toxicity in normal cells. Q54 antitumor effects were observed in vivo in the zebrafish model. Overall, our findings support a promising translational relevance of Q48 and Q54, or further analogs of these lead compounds, and show the utility for drug screening of GBM organoids.

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Title

SAFETY PROFILE OF MONOCLONAL ANTIBODIES APPROVED FOR MIGRAINE: AN ANALYSIS FROM THE EUROPEAN SPONTANEOUS ADVERSE EVENT REPORTING SYSTEM

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Background

Migraine is the second leading cause of disability, affecting about one million people worldwide. The calcitonin gene-related peptide (CGRP) plays a key role in the pathogenesis of migraine, as monoclonal antibodies (mAbs) work by blocking the CGRP pathways [1]. Given the recent approval of mAbs for migraine, the aim of the study was to investigate adverse events (AEs) related to their use through data from the EudraVigilance (EV) database.

Methods

A retrospective, observational, pharmacovigilance study was performed using EV database, a system designed for managing and analyzing information on suspected drug-related AEs. All AE individual case safety reports (ICSRs) recorded starting from the drug approval date up to 31st May 2022 with at least one of the following mAbs, reported as suspected drugs, were included: erenumab (ERE), galcanezumab (GMB), fremanezumab (FMB), and eptinezumab (EPT). Before proceeding with the analysis, all pre-marketing ICSRs with supporting literature data were excluded. Furthermore, to avoid bias, all ICSRs having other drugs as suspected have been also excluded.

Results

A total of 7,939 mAb-related ICSRs approved for migraine were collected in the EV database during the study period, of which 5,322 (67.0%) reported by healthcare professionals. More than half of ICSRs (63.4%) were detected in European Economic Area, even if all EPT-related ICSRs were identified in non European Economic Area. Of the total ICSRs, 7,899 had only one mAb reported as suspected as follows: ERE (n = 4,840; 61.3%), GMB (n = 1,631; 20.6%), FMB (n = 1,407; 17.8%), and EPT (n = 21; 0.3%). These mostly concerned females (n = 6,738; 85.3%) and adults (n = 5,384; 68.2%), with comparable data for all mAbs. Moreover, 40 ICSRs were associated with more than one mAb (n = 15 ERE plus FMB, n = 10 ERE plus GMB, n = 10 GMB plus FMB, and n = 5 ERE plus GMB plus FMB) that were mainly related to adults (n = 36; 90%) and females (n = 35; 87.5%). AEs mainly concerned gastrointestinal disorders including constipation (n = 951; 12%) and nausea (n = 468; 5.9%), followed by general disorders and administration site conditions, such as fatigue (n = 531; 6.7%), injection site pain and erythema (n = 358; 4.5% and n = 353; 4.4%, respectively), nervous system disorders including dizziness (n = 365; 4.6%) and skin disorders such as pruritus and alopecia (n = 357; 4.5% and n = 347; 4.4%, respectively). Serious ICSRs were 3,566 (44.9%), involving especially ERE (n = 2,141; 60%) and mainly related to the onset of hypersensitivity reactions (n = 171; 4.8%), cerebrovascular accident (n = 100; 2.8%) and suicidal ideation (n = 94; 2.6%).

Conclusions

This study was largely consistent with results from literature, but some serious AEs were shown. Furthermore, continuous surveillance is required and more representative database analyses and pharmacoepidemiological studies are warranted to improve the knowledge about the safety profiles of mAbs approved for migraine.

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pharmadvances

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Title

The regulatory effects of microRNAs targeting the Wnt/b-catenin signaling pathway on primary and secondary osteoporosis.

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Background

Osteoporosis is characterized by a progressive loss of bone mass determined by an alteration of the bone remodeling balance. The two most frequent forms of osteoporosis are represented by postmenopausal and glucocorticoid-induced osteoporosis (GIO). The Wnt/b-catenin signaling pathway has emerged as a critical regulatory component of the control of bone metabolism, opening new possible avenues in the management of osteoporosis, since it is also regulated by miRNAs, small non-coding RNAs that negatively regulate gene expression. The aim of this study is to identify those miRNAs involved in primary and secondary osteoporosis that interfering with the Wnt/b-catenin signaling pathway could represent therapeutic targets.

Methods

Ovariectomized mice and mice treated with prednisone were used to represent post-menopausal osteoporosis and GIO, respectively. Ovariectomized mice were sacrificed 3 months after ovariectomy, while GIO mice were sacrificed 60 days after a treatment with prednisone (5 mg/kg). Femurs were kept to establish osteoporosis by histological analysis and to isolate osteoblasts for molecular investigations. Osteoblasts were isolated from long bones, mRNA was isolated from cells, and the culture media were used to isolate miRNAs. Real-Time PCR was performed on specific targets of the Wnt signaling pathway and other targets involved in osteoporosis and miRNAs profile was carried out using a Taqman Array Card for microRNAs customized by inserting a total of 31 miRNAs that interfere with the Wnt signaling pathway in osteoporosis.

Results

The hematoxylin-eosin staining showed that both ovariectomized and GIO mice had reduced cellularity and trabecular alteration compared to Sham, confirming the induction of osteoporosis. In the postmenopausal osteoporosis model, the analysis of miRNAs and mRNA profiles revealed an up-regulation of miR-199a-5p that negatively affected the expression of CRTAP and ALPL genes, and miR-31 that inhibited WNT11. In the GIO model two up-regulated miRNAs were identified: miR-9-5p, which targeted AR and, together with miR-141-5p, also WNT5b, WNT16, ALP and PTH1R genes in GIO male mice, and let-7c-5p that negatively regulated FZD2, FZD3, FZD5, LEF1, WNT5B, WNT16, COL1A1, COL1A2, BMP7, AR, IGFBP2, MMP2 and CCND1 in GIO female mice.

Conclusions

These data suggest that the up-regulated miRNAs identified in this study negatively affect the genes involved in bone formation process and could be considered as possible therapeutic targets for the treatment of primary and secondary osteoporosis.

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Title

Chemogenetic manipulation of TMN^{HA} neurons modulates the expression of memory.

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Background

The cardinal role of brain histaminergic system in regulating several physiological processes is now a well-established concept. Histamine (HA) acting in different brain sites, modulates animals' performance in various learning paradigms [1]. However, our current knowledge of the role of neuronal histamine (HA) in memory is based on its chronic (genetic) [2, 3] and sub-chronic (pharmacological) depletion [4, 5], or acute local injections of histaminergic ligands, which present inherent technical limitations.

Here, we investigated the impact of activating or silencing endogenous histaminergic neurotransmission using chemogenetic tools in a time controlled fashion during behavioural tasks.

Methods

To interrogate the function of brain HA we used the DREADDs-driven technology injecting HDC-Cre mice bilaterally into the Tuberomamillary Nucleus (TMN) with excitatory or inhibitory viral constructs to transfect histaminergic neurons[6]. We used two different tasks, social discrimination and contextual fear conditioning to evaluate recognition and aversive memory, respectively. In the social discrimination paradigm, C57BL/6 mice received Clozapine *N*-oxide (CNO) systemic injections immediately before the acquisition session and the retention test was performed 24 hours later. In the contextual fear conditioning task mice were placed in the conditioning apparatus and received 3 mild foot-shocks (0.5mA, 2s, 30 s intervals)[1]. Immediately after, they received a systemic CNO injection. The retention test was performed in the same apparatus 24 or 48 hours later.

Results

Activation and inhibition of TMN^{HA} neurons had opposite effects on both recognition and aversive memories. When stimulated the HA neurons lead to an improvement of social and fear memories, whereas when inhibited memories were impaired.

Conclusions

We revealed that selective chemogenetic activation and inhibition of TMN^{HA} neurons facilitate and impairs memory, respectively. These results confirm and expand previous reports regarding the role of neuronal HA in the regulation of memory and pave the way for future studies using such approach for to deconstructing the specific histaminergic neural pathways involved in different memory phases (acquisition/consolidation vs retrieval) of different types of memory (recognition vs aversive).

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pharmadvances
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Title

“PEA-OXA ameliorates allodynia, neuropsychiatric and adipose tissue remodeling induced by social isolation”

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Background

Background: Chronic social isolation generates a persistent state of stress associated with obesity along with some neuro-endocrine disorders and central behavioral sequelae (eg anxiety, depression, aggression, and allodynia). In this study, we evaluated the effect of social isolation on body weight, depressive- and anxious-aggressive-like behavior, as well as on phenotypic changes of adipocytes from visceral adipose tissue of control (group-housed) or socially isolated (single-housed) male mice.

Methods

Methods: The social isolation model, exposing rodents to prolonged stress, offers a putative animal model to investigate the development of certain psychophysical and metabolic alterations 1 (Mumtaz et al., 2018). The social isolation model we used was performed by isolating mice in individual cages for 30–210 days from the 21st postnatal day (PN21). Bevahioural test like mechanical allodynia, resident intruder test, tail suspension test and hole board test was used to asses the differences between single and group housed mice. Cells cultures of mesenchymal stromal cells (MSCs) from white adipose tissue was used to asses the effect of PEA-OXA on cell proliferation, Apoptosis and Reactive Oxygen Species production. Through ELISAtest, pro-inflammatory and anti-inflammatory cytokine analyses were performed.

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Results

Results: Single or group-housed mice treated with vehicle or PEA-OXA underwent body weight, mechanical allodynia, anxious-, depressive- and aggressive-like behavior measurements. Proliferation rate, apoptosis, senescence, expression of fat lineage genes, lipid droplets and proinflammatory cytokines were measured on white adipose tissue adipocytes from group- or single-housed mice. Single housed mice developed weight gain, mechanical allodynia at the von Frey test, aggressiveness in the resident intruder test, depression- and anxiety-like behavior in the tail suspension and hole drop tests, respectively. Single housed mice receiving PEA-OXA showed a general resolution of both, physical-metabolic and behavioral alterations associated with social isolation. Furthermore, adipocytes from the adipose tissue of socially isolated mice showed an evident inflamed phenotype (i.e. a reduced rate of proliferation, apoptosis, senescence, and ROS hyper-production together with an increased expression of IL-1β, IL-10, IL-17, and TNF-α and a decrease of IL-6). The treatment with PEA-OXA on adipocytes from single housed mice produced a protective/anti-inflammatory phenotype with an increased expression of brown adipose tissue biomarker.

Conclusions

Conclusion: This study confirms that persistent stress caused by social isolation predisposes to obesity and neuropsychiatric disorders. PEA-OXA, through its multi-target activity on alpha2 adrenoceptor and histamine H3 receptors, which have recently aroused great interest in the neuropsychiatric field, reduces weight gain, systemic pro-inflammatory state, allodynia, and affective disorders associated with social isolation.

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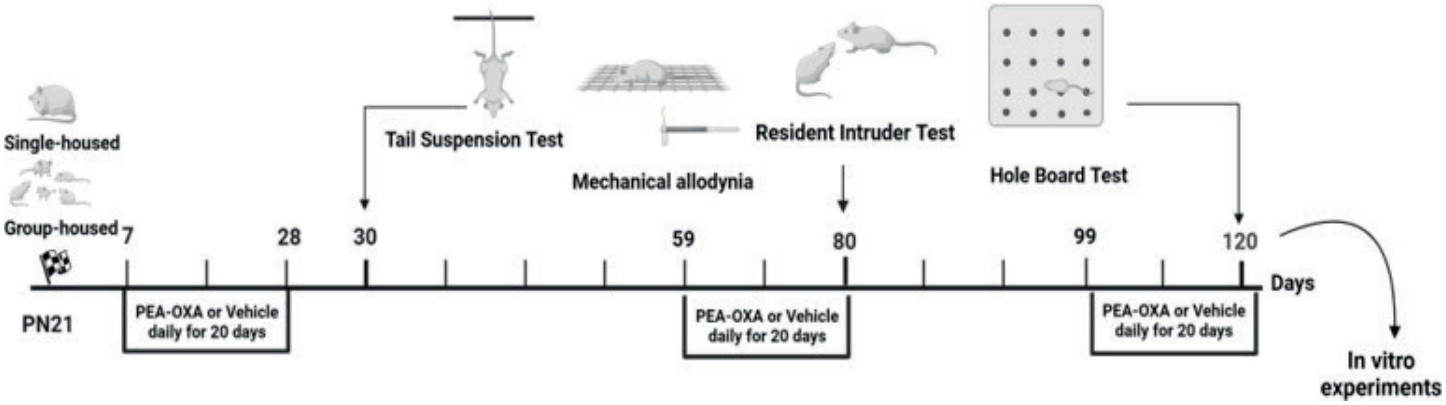

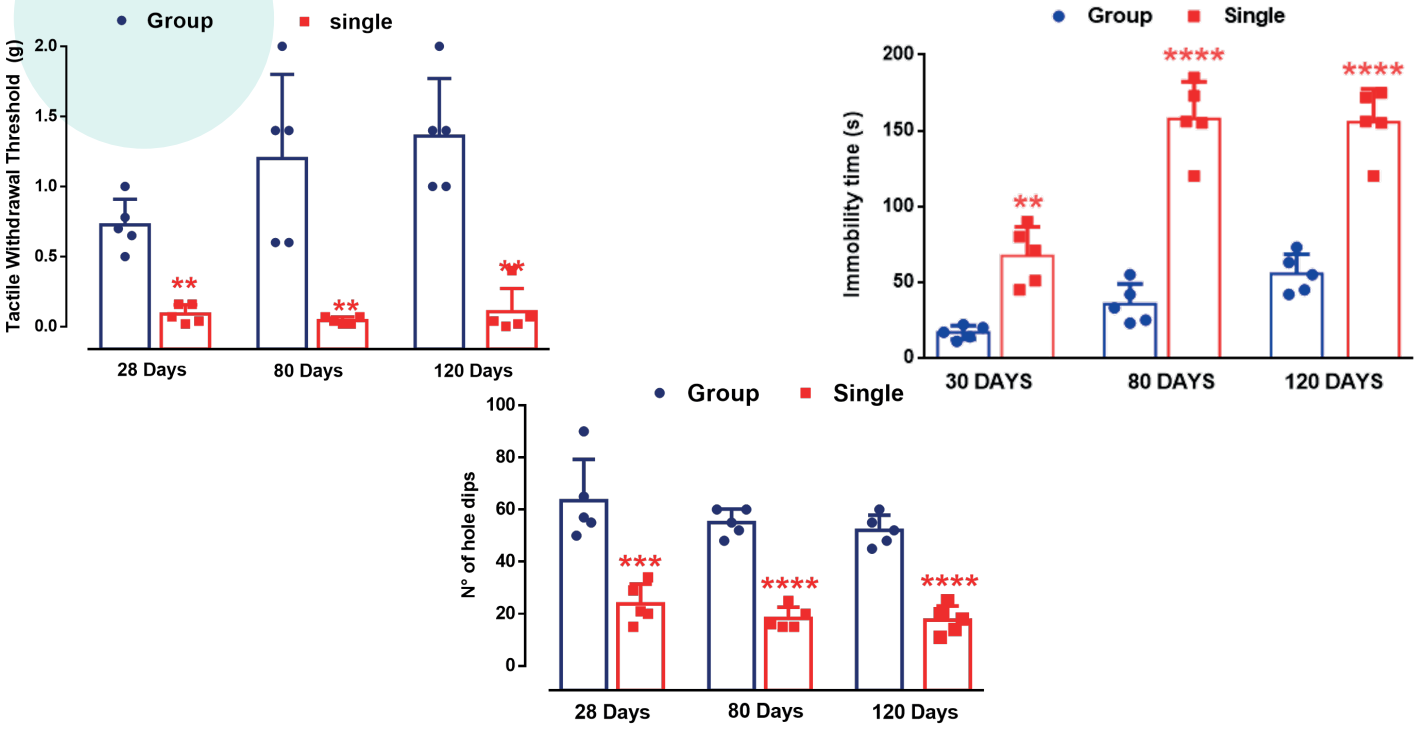
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Tactile Withdrawal Threshold (g)
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Immobility time (s)
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30 DAYS
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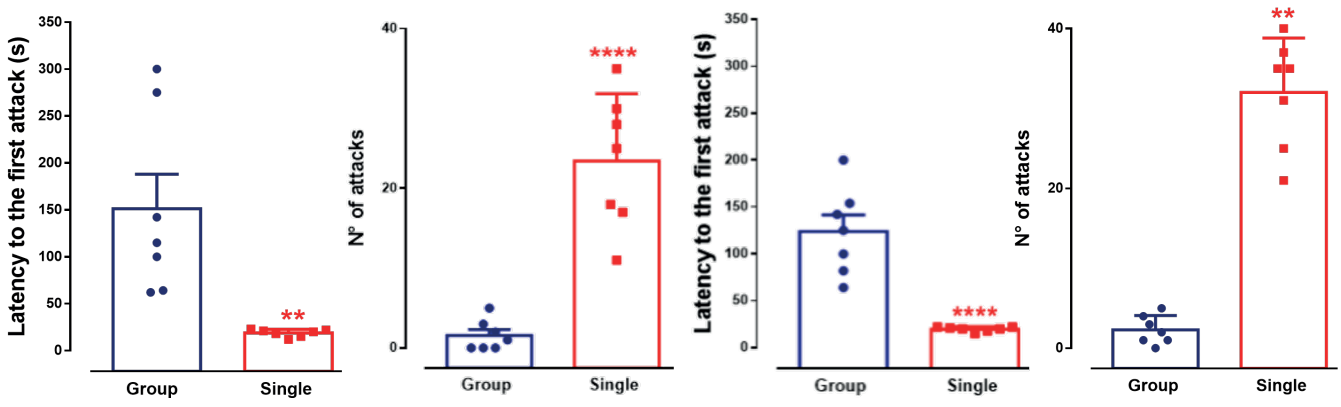
Latency to the first attack (s)
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Group
Single
N° of attacks
40
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Latency to the first attack (s)
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N° of attacks
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Group
Single

pharmadvances
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Title

Safety Profile of Antiviral Therapies for the Early Treatment/Prevention of COVID-19: Analysis of the International Pharmacovigilance Database VigiBase

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Background

After many months from the COVID-19 pandemic beginning, several anti-spike monoclonal antibodies (mAbs) and, more recently, other antiviral drugs for COVID-19 treatment in non-hospitalized patients have been marketed. Specifically, those drugs are indicated for SARS-CoV-2 infection early treatment in outpatient adults at high risk of developing severe COVID-19 [1]. The aim of this study was to evaluate the post-marketing safety profile of antivirals drugs used for early COVID-19 treatment, using the spontaneous reporting database VigiBase.

Methods

From VigiBase we identified all the individual case safety reports (ICSRs) of marketed mAbs (regdanvimab, sotrovimab, casirivimab/imdevimab, bamlanivimab/etesevimab and, specifically for COVID-19 prevention in immunocompromised patients, tixagevimab/cilgavimab) and other antiviral therapies for COVID-19 early treatment (remdesivir, nirmatrelvir/ritonavir, molnupiravir).We performed a descriptive analysis of patients' demographics (age, sex, continent of origin) type of reporter, adverse drug reactions (ADRs) and the Important Medical Events (IMEs), from their marketing date to May 4, 2022. In addition, we conducted a disproportional analysis using Reporting Odds Ratio (ROR), along with 95% confidence intervals (CIs), by comparing the frequency of ADRs for each drug of interest with distribution of all ADRs from the whole database, excluding vaccines, reported in the same period.

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Results

Overall, 15,437 ICSRs of anti-spike mAbs (casirivimab/imdevimab:27.2%; bamlanivimab/etesevimab:7.3%; sotrovimab:3.3%; tixagevimab/cilgavimab:2.7%; regdanvimab:0.2%) and other antivirals (remdesivir:54.5%; nirmatrelvir/ritonavir:4.3%; molnupiravir:0.5%) were retrieved. ICSRs mainly involved females and 45-64 years old. The percentage of ICSRs that included IMEs was 32.4%. Overall, the most frequently reported ADRs were infusion-related reaction for both casirivimab/imdevimab (20.1%) and bamlanivimab/etesevimab (19.3%), pyrexia for regdanvimab (30.0%) and sotrovimab (8.1%), increased alanine aminotransferase for remdesivir (13.3%), dysgeusia for nirmatrelvir/ritonavir (39.5%), and diarrhoea for molnupiravir (18.8%). Overall, statistically significant RORs were observed for “Investigations” with remdesivir (N=3163;ROR:5.56;95%CI:5.32-5.81), “Gastrointestinal disorders” for molnupiravir (N=178;ROR:3.43;95%CI:2.82-4.17) and “Vascular disorders” for sotrovimab (N=51;ROR:2.07;95%CI:1.55-2.76).

Conclusions

This study shows that the safety profile of anti-spike mAbs and other newly marketed antiviral therapies for the early treatment of COVID-19 is overall favourable. The most frequently reported ADRs in VigiBase are in line with those reported in the pivotal trials and Summary of Product Characteristics for all investigated antiviral drugs. The disproportional analysis identified some potential signals requiring further investigation.

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Title

GPR21 inhibition to counteract insulin resistance development

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Background

Type 2 Diabetes is a relevant pathology both from epidemiological and mortality-related aspects. Although several pharmacological treatments are available almost half of patients do not reach therapeutic goals and thus a novel therapeutic need remains.

GPR21 is an orphan and constitutively active receptor belonging to the superfamily of G-Protein Coupled Receptors (GPCRs) postulated to be involved in the pathogenesis of insulin resistance. Previous studies show that its inhibition, by gene silencing or inverse agonist, improves glucose uptake and insulin sensitivity in HepG2 cells, as well as reducing proinflammatory activity of M1 macrophages. However, significant results were achieved using the only available inverse agonist, GRA2, at the μM concentration range.

The aim of this study was to define a three-dimensional model of GPR21 to better understand the binding pocket involved in the docking of inverse agonists and then perform a virtual screen to identify higher affinity compounds than GRA2. Then, in vitro test were perfomed on Hepg2 cells to evaluate the effects of the compounds on insulin sensitivity.

Methods

In silico studies. The developed homology model of GPR21 was used in a molecular docking study to predict binding affinities and interactions occurring between the binding site's residues and designed GRA2 analogues. For *in vitro* studies, HepG2cells have been exposed to (i) high glucose (30nM)/high insulin (100nM) medium for 24 hours and (ii) to high glucose (30nM) medium for 24-72 hours, both with a final insulin stimulation (100nM, 10 min). Insulin resistance was tested by evaluating the activation of insulin signaling, in particular the phosphorylation of Akt (Ser^{473}) and Gsk-3 beta (Ser^{9}). Under the same conditions the expression of GPR21 was evaluated by Western blot.

Results

In silico studies. The detailed binding analysis from the *in silico* study identified a side pocket and two key areas of interactions in the orthosteric pocket, with higher affinity values observed in the deeper one. *In vitro* studies. Unexpectedly, no insulin resistance was established in our models. However, after 72 h of high glucose condition – a hyperglycemic condition - an increased expression of GPR21 was observed. Interestingly, all the compounds were able to increase the expression of ph-GSK-3b level over the control. One compound, 041, was able to increase the ph-GSK3β levels also in the basal condition at lower concentration in comparison to GRA2, thus showing an increased potency.

Conclusions

Our results support the utility of blocking GPR21 effects to improve insulin sensitivity. Further studies are necessary to better investigate the effects of the more promising compounds.

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pharmadvances

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Title

PHOX2B regulates neuronal excitability by modulating the expression of K^+, Na^+ and Ca^{2+} channel genes

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Background

PHOX2B encodes for a transcription factor, characterised by the presence of two polyalanine repeats of 9 and 20 residues in the C-terminus, and it is a “master gene” of the development of the autonomic nervous system (ANS). Moreover, *PHOX2B* is essential for the noradrenergic specification and neuronal differentiation by regulating cell cycle exit. Whereas the role of *PHOX2B* during neurodevelopment is well established, the exact role of *PHOX2B* in adulthood is still an open question. *PHOX2B* expression persists in important structures in the hindbrain of adult rats, among which chemoreceptors and it may also maintain the function of the noradrenergic neurons. However, very little is known about the genes regulated by PHOX2B [1]. Heterozygous mutations in the PHOX2B gene, consisting of 4 to 13 triplet expansion of the 20-alanine tract (PARM), lead to congenital central hypoventilation syndrome (CCHS), a rare life-threatening condition characterized by sleep-related hypoventilation and impaired CO_2 chemosensitivity [1]. In addition, other ANS dysfunctions are present, including cardiac and thermoregulatory abnormalities. Non-PARM mutations within exon 1, 2 or 3 that include rare missense, nonsense and frameshift mutations may occur in 5% of patients, frequently associated with the onset of neuroblastoma or Hirschprung disease. Consistent with its role as transcription regulator, transcriptional dysregulation might be an important mechanism of CCHS pathogenesis.

No pharmacological intervention is currently available, and recently progestins showed to provide partial recovery of chemoreflex impairment [1].

Here we show that ion channels are newly identified PHOX2B target genes, and the different modulation of their expression by wild-type or mutant PHOX2B proteins and by progestins, through PHOX2B expression modulation, contribute to regulate the cell excitability.

Methods

To study the effect of PHOX2B on ion channel genes expression we generated a CRISPR-CAS9 Knocked-down PHOX2B IMR32 cells model. Clones stably re-expressing PHOX2B wt or mutant proteins are obtained for rescue analyses. Current clamp recordings have measured the cell excitability.

Results

The absence of PHOX2B and the expression of PHOX2B mutant proteins increases the expression of K^+, Na^+ and Ca^{2+} channels genes, leading to impaired cell excitability that can be rescued by the re-expression of wt PHOX2B. This effect is accomplished also by progestins treatment.

Conclusions

PHOX2B modulates the expression of ion channels genes therefore contributing to regulate cell excitability. The detrimental effect of mutant proteins on their expression is indicative that cell excitability impairment is one of the pathogenetic mechanism of CCHS. The clinical effect of progestins may thus be explained by the modulation of these genes, thus prompting the idea that ion channels may be promising therapeutic targets in CCHS.

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pharmadvances

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Title

Safety Assessment of Biologics for Immune-Mediated Inflammatory Diseases Using the Italian VALORE Healthcare Database Network and the National Spontaneous Reporting System

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Background

Pre-marketing randomized clinical trials (RCTs) are designed to show efficacy but have limitations regarding safety [1]. Post-marketing surveillance plays a key role in exploring biologics safety issues, especially for long-term treatments of chronic diseases like immune-mediated inflammatory diseases (IMIDs) [2,3]. Several data sources are available to investigate the biologics safety in the post-marketing setting, such as drug registries, spontaneous reporting system (SRS) or claims databases, including a larger and heterogeneous population than RCTs and integrating evidence from pre-marketing [4].

The aim is to assess the post-marketing safety of biological drugs approved in IMIDs using the Italian VALORE database network [5] and the national SRS database.

Methods

Suspected adverse drug reactions (ADRs) related to biologics approved in IMIDs from four Italian regions (Veneto, Lazio, Emilia-Romagna, Friuli-Venezia-Giulia) were retrieved from the SRS database between 2010-2020. Safety Outcomes of Interest (SOI) related to the same biologics and person-years (py) of exposure were identified from the VALORE database in the same period and regions. Suspected ADRs were analysed at High Level Term (HLT) of MedDRA® and

stratified by drug class [TNF-α-inhibitors, interleukin (IL) inhibitors, and selective immunosuppressants]. ADR Spontaneous Reporting Rates (SRR) and SOI incidence rate (IR) were estimated using the total number of suspected ADRs and SOI as numerator and the exposure to biologics as denominator (among 100,000py).

Results

A total of 2,675 ADRs reports were identified in the SRS database: 2,042 involved TNF-α-inhibitors (76%), 478 IL-inhibitors (18%) and 155 immunosuppressants (6%). Biologics users from the VALORE database were 74,046: 176,039py for TNF-α-inhibitors (72%), 35,666py for IL-inhibitors (20%), 13,039py for immunosuppressants (8%).

The ADRs associated with the highest SRRs belonged to *Therapeutic and nontherapeutic responses* HLT category in TNF-α-inhibitors, IL-inhibitors and immunosuppressants (195, 53, and 752/100,000py, respectively); followed by skin disorders, included *Urticarias* (87/100,000py), *Erythema* (73), *Rashes*, *eruptions and exanthems (*70) and *Pruritus* (65) for TNF-α-inhibitors; *Lower respiratory tract and lung infections* (20) for IL-inhibitors; and *Neutropenia* (207) for immunosuppressants.

As regards to SOI, the highest IRs in the three cohorts were: *neoplasm* (567, 723, and 972/100,000py), *ischemic heart failure* (524, 745, and 810/100,000py), and *pneumonia* (522, 742, and 807/100,000py).

Conclusions

Among ADR reports, the highest rates were related to treatment inefficacy and immune system disorders while among healthcare data, were neoplasm, ischemic heart disease and pneumonia. These results showed the need of pooling data from healthcare databases and active surveillance as a comprehensive approach to detect different potential safety signals related to biologics.

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Title

Extinction of traumatic memory in a chronic PTSD-like model is promoted by Social Buffering phenomenon

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Background

Social Buffering is a social phenomenon by which affilliative social partners can mitigate responses to stressors[1]. Numerous studies reported that this phenomenon can occur equally among familiar and unfamiliar conspecifics in a variety of species, including laboratory rats[2,3]. Recent studies have reported that social buffering facilitates extinction of aversive memories[3,4].

Based on this evidence, the aim of the present study was to evaluate the efficacy of social vuffering in the facilitation of traumatic memory extinction in a chronic rat PTSD-like model recently developed in our laboratory[5,6].

Methods

Specifically, after one week of isolation and a footshock trauma, rats were exposed to several spaced extinction sessions to mimic the human cognitive behavioral therapy[6]. To evaluate the influence of social buffering, extinction sessions were carried out in the presence (or absence) of a social conspecific partner.

Results

Our results show that social interaction reduced fear responses (i.e., freezing behaviour) during exposure to extinction sessions as compared to rats tested in the absence of a conspefic, thus showing the efficacy of social buffering promoting extinction of traumatic memory in a rat model mimicking PTSD-like symptomatology.

edra

Conclusions

Taken together, our findings provide the basis for more mechanistics studies aimed at understanding the neural underpinnings of social buffering of fear and highlight the beneficial effects of group therapy for the treatment of trauma-related disorders, such as PTSD.

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Title

BCAAs and Di-Alanine supplementation in the treatment of sarcopenia: beneficial effects on skeletal muscle mass and function in aged mice

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Background

The association between the human aging process and sarcopenia, a progressive decline in skeletal muscle mass, strength, and function, is well established. Although the etiology of sarcopenia is still not fully understood, the major underlying mechanism is represented by a disruption in protein homeostasis, due to an imbalance between anabolic and catabolic pathways [(1)]. To date, no pharmacological remedies exist to halt or prevent age-related muscle-wasting, and a proper nutrition, often combined with physical activity, is currently considered the most effective strategy for the management of sarcopenia [(2)]. In this framework, nutritional interventions based on the dietary supplementation of amino acids (AA) with anabolic properties, such as branched chain AA (BCAAs, *i.e.* leucine, isoleucine, and valine), appear as potentially useful in the treatment of sarcopenia.

Methods

To assess the potential role of BCAAs administration in relieving sarcopenia-related muscle atrophy, we administered an oral formulation of BCAAs (2:1:1) in 60-week-old C57BL/6 male mice for 12 weeks (T12). BCAAs were administered alone or in combination with two equivalents of L-Alanine (2ALA) or the dipeptide L-Alanyl-L-Alanine (Di-ALA). In fact,

based on previous studies, the association enhances bioavailability and residence time due to fastest rate of intestinal absorption [3,4]. The outcome of the treatment was assessed on relevant *in vivo* and *ex vivo* readouts in comparison to untreated aged and adult (12-week-old) mice.

Results

At T12, *in vivo* force torque of hind limb plantar flexor muscles was improved in mice treated with BCAAs + 2ALA and BCAAs + Di-ALA compared to untreated aged mice, with a recovery score (RS) toward adult mice of +108% and 102%, respectively. *Ex vivo* recordings of isometric contraction in isolated extensor digitorum longus (EDL) and soleus (SOL) muscles, showed a recovery of muscle contractile function with a significant increase of specific isometric twitch (sPtw, kN/m2) and tetanic (sP0, kN/m2) force vs. untreated mice (RS sPtw EDL≤+22%, SOL≤+25%; sP0 EDL≤+50%, SOL≤+48%). Ultrasonography assessment at T12 highlighted a significant increase of hindlimb volume in all treated groups vs. untreated mice. This result was paralleled by a marked weight gain of hindlimb muscles, in particular gastrocnemius (GC), of all groups of treated aged mice. Quantification of GC fiber cross sectional area (CSA), performed after immunofluorescent staining of laminin, highlighted a statistically significant increase of myofibers size in all treated animals, confirmed also in SOL muscle (RS: BCAAs +69%; BCAAs +2ALA +80%; BCAAs + Di-ALA +81%).

Conclusions

Overall, these results support the potential benefits of BCAAs administration in relieving sarcopenia-related atrophy symptoms, also highlighting the importance of innovative formulation to optimize BCAAs bioavailability.

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Title

Covid-19 Vaccines-Induced Thrombosis With Thrombocytopenia Syndrome: Data From The Italian Pharmacovigilance National Network

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Background

During the COVID-19 vaccination campaign, the Italian Medicines Agency (AIFA), in collaboration with the Regional Pharmacovigilance Centres, carefully monitored Individual Safety Reports (ICSRs) on Adverse Event Following Immunization (AEFIs) associated with COVID-19 vaccines and ensured constant communication through monthly public reports[1]. During the initial months of the national immunization program, the health authorities identified a signal of sporadic thrombotic events associated with thrombocytopenia[2] related to the viral vector vaccines ChAdOx1-S and Ad26.COV2-S, particularly in young women.

To propose a thorough analysis of thrombotic and thromboembolic events linked with thrombocytopenia after COVID-19 vaccination with viral vector vaccines reported in the Italian National Pharmacovigilance Network database.

Methods

All ICSRs reported between December 27th, 2020 and December 26th, 2021 that contained Preferred Terms (PT) linked to decreased platelet counts associated with PT related to thrombotic and thromboembolic events (clinical symptoms

and/or diagnostic tests) were identified. All cases of thrombotic and thromboembolic events reporting thrombocytopenia in the report's narrative description were also reviewed. Three pharmacovigilance professionals independently assessed the ICRSs and blindly grouped them into five levels of diagnostic certainty as defined by the Brighton Collaboration Group (BCG)[3]. Plenary discussion was used to settle the conflict.

Results

During the time period evaluated, 12,166,236 doses of ChAdOx1-S and 1,500,746 doses of Ad26.COV2-S were administered in Italy, with a total of 23,358 / 117,947 ICSR related to ChAdOx1-S (19.8%) and 1,580 / 117,947 ICSR related to Ad26.COV2-S (1.3%). According to the inclusion criteria, a total of 134 reports following adenoviral vaccine inoculation were identified, with 107 cases defined as thrombotic thrombocytopenia (95 after ChAdOx1-S and 12 after Ad26.COV2-S). Based on clinical examination or investigation, or for the presence of heparin as a concomitant drug, 27 reports were classified as "non-case" (level 5, Brighton). Furthermore, 3 reports were ruled out due to hereditary thrombophilia or a history of past thrombotic events. 77 cases were classified as BCG levels 1, 2, and 3 (definite, probable, and possible cases, respectively), with a total reporting rate of about 1 case per 200,000 doses. The highest reporting rates were observed among women aged 30 to 49.

Conclusions

In Italy, rates of thrombotic thrombocytopenia after COVID-19 vaccination using viral vector vaccines are comparable to those reported in other countries.

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Title

Covid-19 Vaccines And Hypertension: Vigibase® Data And Evidence From Real-World Studies

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Background

Hypertension is a serious condition that develops when blood pressure remains consistently high over time[1]. During the vaccination campaign against COVID-19, several cases of hypertension occurred in a plausible temporal relationship with immunization have been documented.

To investigate a possible signal of risk of hypertension related with COVID-19 vaccination using VigiBase® the World Health Organization (WHO) pharmacovigilance database and to analyze the evidence available from real-world studies.

Methods

Using data from spontaneous reports recorded in VigiBase®, we conducted a disproportionality study. The data was extracted on May 8th, 2022. The reporting odds ratio (ROR) was determined as a measure of disproportionality for hypertension described by the Standardized Medical Dictionary for Regulatory Activities (MedDRA) query (SMQ) narrow. The ROR was calculated for all reports that included the MedDRA Preferred Terms (PT) "hypertension", "blood pressure increased" and "hypertensive crisis" (cases). The remaining reports have been classified as non-cases. All instances in which a COVID-19 vaccination was thought to be the causal agent were utilized as index reports, and all other reports were used as references. At least three reports of the PT of interest and ROR_{025}>1 were used to define a signal. Finally, the medical literature was reviewed via MEDLINE from January 2021 to May 2022 using "COVID-19 vaccines" AND "hypertension" as search terms to check for evidence from observational studies.

Results

As of May 8th, 2022, VigiBase® contained 3,746,090 reports of COVID-19 vaccine-related adverse events and 87,653 de-duplicated reports of hypertension as defined by the SMQ. In particular, we identified 34,955 reports of "hypertension" (ROR:1.3; ROR_{025}:1.2), 47,733 reports of "increased blood pressure" (ROR:2.6; ROR_{025}:2.6) and 3,741 reports of "hypertensive crisis" (ROR:4.0; ROR_{025}:3.8) in which a COVID-19 vaccine was indicated as a suspected causative agent. The most frequently reported symptoms (> 9%) included headache (n=16,817; 19.2%), dizziness (n=12,892; 14.7%), fatigue (n=8,406; 9.6%). Overall, 75% of cases (n=65,761) were classified as non-serious.

A meta-analysis of observational studies including 357,387 individuals reported 13,444 abnormal or increased blood pressure events[2]. These occurrences generally portrayed as shorts periods of hypertensive response and frequently observed in patients with risk factors.

Conclusions

Our findings confirmed a signal of risk of events of increased blood pressure after immunization with COVID-19 vaccines. However, there is no evidence that these episodes could lead to major complications linked with hypertension, such as stroke, aneurysms, heart failure, myocardial infarction, and chronic kidney disease.

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Title

Microglia senescence as a potential target in neuropathic pain induced by nerve injury and associated comorbidity

Authors

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Background

The management of neuropathic pain (NP) is a relevant clinical problem due to the poor responsiveness of patients to available analgesic drugs **(Cohen & Mao, 2014)**. In addition, anxiety, depression, cognitive impairment, and other mood disorders are comorbidities that characterize approximately 34% of patients with neuropathic pain. The onset of these comorbidities can drastically worsen the patients' quality of life, exacerbating painful conditions (Wrona et al., 2021). However, there are no effective and safe treatments able to manage these comorbidities (Brown et al., 2021) Microglia can adapt to any type of disturbance of the homeostasis of the central nervous system (CNS) and its lack of activity can lead to permanent and unresolvable damage (Block et al., 2007). Uncontrolled increases in microglial cell activity result in the loss of normal physiological microglial functionality, with the insurgence of microglial senescence. In this work we investigated the presence of microglial senescent cells at spinal and supraspinal level in an animal model of NP, the spared nerve injury (SNI). The timing of the onset of pain hypersensitivity as well as of various comorbidities associated with peripheral NP in mice was defined and a correlation between microglial cellular senescence and NP-associated symptoms was assessed. At the same time, we optimized an *in vitro* model of microglial senescence to deep the mechanism involved in this chronical process.

Methods

In this work we investigated the timing of the onset of the various comorbidities associated with peripheral neuropathic pain in mice, and their correlation with the presence of anxiety, depression, or memory variation of cellular senescence. Moreover, we optimized an in vitro model of microglial senescence which could help in the search for novel therapeutical approach.

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Results

28 days from surgery SNI mice developed anxiety, depression, and cognitive impairment and at the same time in the spinal cord and hippocampus of these animals an increase of microglia senescence markers (β-galactosidase and SASP) have been detected. Treating BV2 cells with LPS 500 ng/mL for 10 days (4h/day) every 72h, we observed an increase of β-galactosidase, SASP, a reduction of cell viability and an increase of SAHF

Conclusions

Therefore, present findings could represent an interesting step to better understand the pathophysiological cellular mechanisms in chronic pain states and related comorbidities. In this way, we propose that senescent microglia may be an interesting target for reducing pain and all related comorbidities in the neuropathic patient.

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Title

***Zingiber officinale* Roscoe rhizome extract as a novel nutraceutical intervention to control clinical and biological outcomes in animal model of multiple sclerosis**

Authors

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Background

Evidence suggests that, to achieve good control of multiple sclerosis (MS), treatments must be based on a multi-target approach including neuroprotective, remyelinating, or regenerative therapies combined with approved immunomodulatory drugs (Rotstein DL et al., 2018).. The side effects, therapeutic failures, toxicity, and high cost of current drugs lead to the need to consider innovative and safer therapies. Encouraging results for the management of MS have been obtained with phytoconstituents from cannabis and turmeric, and an increasing number of patients are using natural medicines in the management of MS-associated comorbidities (Ghanaatian et al., 2018). Ginger is one of the most widely used dietary supplements in the world, also used in therapy to prevent cancer and to treat emesis, bone disorders, and vascular disorders. Ginger has anti-inflammatory and antioxidant properties, effectively penetrates the central nervous system and has shown benefits in neurodegenerative disorders (Choi et al., 2017). On this basis, we decided to establish the efficacy of a standardized extract of *Zingiber officinale* Roscoe rhizome (ZOE) on clinical symptoms (i.e., motor disability, loss of body weight, hypersensitivity to pain) in the EAE mouse models of MS, and to establish its mechanism of action in the spinal cord of these animals.

Methods

Clinical score, body weights, locomotor coordination, and nociceptive threshold of all mice were assessed prior to immunization and once daily thereafter in a blinded manner, until the completion of the study. ZOE 200 mg/kg oral administration started from day 14 after immunization, corresponding to the first disease peak up to 28 days, which corresponds to the end of the model. The lumbar spinal cord dorsal horn and plasma were removed 30 days after immunization, and sample were used for biochemical analysis.

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Results

Repeated oral administration of ZOE 200 mg/kg reduces mechanical allodynia, thermal hyperalgesia, and improves motor activity in EAE mice. The repeated oral administration of ZOE reduced BBB permeability and peripheral inflammation through the reduction of plasma IL-17 cytokines. Moreover, ZOE 200 mg/kg reduced microgliosis in spinal cord tissue of EAE animals, indeed a significant IBA1 reduction was produced by ginger treatment. In the spinal cord, NF-κBp65 protein was also reduced and the most characterized microglia pro-inflammatory cytokines were down-regulated. Together with the anti-neuroinflammatory activity, ZOE reduced the demyelination produced by the EAE model, increasing both spinal levels of MBP, GAP43 and Neurofilament H.

Conclusions

Positive results from this study indicate ginger as a safe nutraceutical intervention that could be used in combination with conventional therapy to control MS symptoms and improve the patient's quality of life.

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Title

Ruxolitinib restore chemosensitivity in medullolastoma resistant cells

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Background

Medulloblastoma (MB) is the deadliest brain tumor of childhood, intrinsically characterized by fast growth, high invasiveness, and resistance to treatments. MB is associated to high frequency of relapse after treatments and aggressive chemotherapy-dependent neurocognitive and endocrine dysfunctions.[1] In the recent years only limited improvements to the therapeutic success and the clinical management of MB patients have been achieved, and the most aggressive MB tumors still have an extremely poor outcome.[2]

With the aim to deepen the molecular basis of MB aggressiveness and recurrence, we established an in vitro model of MB resistance to chemotherapy, in which, a weekly exposure to a cocktail of chemotherapeutics commonly used in MB treatment (Vincristine, Etoposide, Cisplatin, Cyclophosphamide – VECC) induces the selection of cells that progressively acquire resistance to subsequent VECC treatments.

Both gene expression and kinome profiling of naïve and resistant cells demonstrate that MB resistant (MB-R) cells are characterized by a peculiar transcriptional shift affecting cellular pathways involved in the control of cell growth, metabolism and differentiation. Among them, IL-6/JAK/STAT3 signaling resulted upregulated in MB-R cells.

Methods

In order to investigate a strategy to restore chemo-sensitivity in MB-R cells, we evaluated the effects JAK/STAT3 pathway inhibition and the possible mechanisms of action of ruxolitinib. In particular, viability and clonogenic assay, flow cytometry, western blotting, immunofluorescence staining and protein overexpression were carried out to study the effect of ruxolitinib in combination with VECC treatment in MB resistant cells.

Results

Ruxolitinib suppressed MB-R cells proliferation and synergized with standard chemotherapy, restoring VECC sensitivity in resistant cells. The inhibition of JAK/STAT3 pathway reduced MB-R cell proliferation,and induced the activation of FOXO3a transcription factor triggering the expression of pro-apoptotic proteins and repressing the expression of antiapoptotic proteins, in particular survivin, highlighting its role in sustaining chemotherapy resistance. Considering the involvement of JAK/STAT3 in the regulation of metabolic pathways[3], we evaluated the effects of ruxolitinib on cell metabolism. Interestingly, ruxolitinib induced mitochondria elongation, the reduction of mitochondrial membrane potential and the accumulation of lipid droplets.

Conclusions

Ruxolitinib restored sensitivity toward chemotherapy in resistant MB-R cells, by suppressing anti-apoptotic defense and unlocking pro-apoptotic machinery induced by FOXO3a. In addition, ruxolitinib affected cell energy metabolism and improved chemotherapy effects by striking a metabolic vulnerability of MB-R cells.

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Title

New inhibitors of NLRP3 inflammasome activation: pharmacological characterization in differentiated THP-1.

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Background

The NLR family protein NLRP3 is an intracellular signalling molecule activated by many pathogen-, environmental- and host-derived factors. NLRP3 activates caspase-1, a proteolytic enzyme that induces the cleavage and the release of proinflammatory cytokines, interleukin 1β (IL-1 β) and IL18, and causes a type of cell death known as pyropotosis. The fine regulation of inflammasome makes it a central player in the pathophysiology of numerous autoimmune and inflammatory diseases such as type 2 diabetes, gout, obesity, atherosclerosis, cryopyrinopathies, chronic inflammatory bowel diseases but also Alzheimer's and Parkinson's disease [1].

Methods

Several NLRP3 inhibitory compounds have been synthetized by SynBioMed group of the Department of Drug Science and Technology of Turin through the chemical modulation of a benzo[d]imidazol-1-one sub-moiety, which was identified as a weak inhibitor of ATPase activity. The inhibition of NLRP3 activation by these compounds was evaluated in THP-1 cell lines, differentiated with PMA and primed with LPS. NLRP3 was activated using different stimuli, such as ATP and MSU. Their protective effect on pyroptosis was evaluated by measuring LDH levels using LDH cytotox 96 non radio cytotoxicity assay (Promega). Moreover, the release of proinflammatory cytokines in supernatant and the expression of proteins involved in the signalling pathway activated by NLRP3 were evaluated. Finally, the cytotoxicity of these inhibitors was evaluated after 72h of treatment through the MTT assay.

Results

The most promising compound inhibited pyroptosis and IL-1b release in a dose-dependent manner, with inhibition of 58.7 ± 7.6% and 35 ± 1.2% respectively at the maximum concentration tested.
All the compounds were not cytotoxic at the concentration used to prevent NLRP3 activation.

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Conclusions

Future studies are required in order to perform a more accurate characterization of NLRP3 inhibitory activity and to understand the possible role of these new inhibitors in the treatment of autoimmune and inflammatory diseases.

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Title

Effect of Lactobacillus rhamnosus GG (ATCC 53103) administration on the enteric neuromuscular function of adolescent mice after antibiotic-induced dysbiosis

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Background

Early life events, including exposure to antibiotic, shape the gut microbiota and may result in dysbiosis-induced vulnerability of the enteric nervous system (ENS), favoring the onset of gut disorders, such as irritable bowel syndrome (IBS) [1]. Administration of specific probiotic strains, such as *Lactobacillus rhamnosus* GG (ATCC 53103, LGG) is proposed to positively influence the course of pediatric gut disorders [2], although its mechanisms still need to be clarified, including possible beneficial effects on the ENS. Thus, we aim to evaluate the consequences of oral LGG administration on the small intestine neuromuscular function of adolescent female and male mice undergoing antibiotic-induced dysbiosis.

Methods

Female and male C57Bl/6 mice were administered a cocktail of broad-spectrum antibiotics (1 mg/ml ampicillin, 1 mg/ml neomycin, 0.5 mg/ml vancomycin, 0.25 mg/ml ciprofloxacin and 0.015 mg/ml natamycin) from postnatal day (PND) 21 (weaning) to PND 35, in drinking water, and switched to normal tap water from PND35 to PND42. LGG was administered by oral gavage once daily ($2x10^9$ CFU) from PND21 to PND42. Small intestine neuromuscular function was evaluated by

means of in vitro and in vivo functional studies and myenteric plexus integrity by immunohistochemical approaches with confocal microscopy.

Results

LGG treatment prevented dysbiosis-induced reduction of the gastrointestinal (GI) transit in male, but not in female mice. Inhibition of carbachol- and electrical field-induced contractions after antibiotic treatment was prevented by LGG administration in male, but not in female, subjects. In dysbiotic female mice, nitrergic relaxations were significantly reduced and were not influenced by LGG, while in dysbiotic male mice, nitrergic inhibition was only slightly reduced and positively influenced by LGG. In female mice, dysbiosis induced a significant reduction of myenteric neuron number (-39% vs ctrl) which was partially restored by LGG (-19% vs ctrl). In male mice neither antibiotic nor LGG treatment affected the number of myenteric neurons (+5% and -3%, respectively vs ctrl).

Conclusions

These data indicate a long-lasting effect of antibiotic treatment on the ENS and neuromuscular function of adolescent mice, lasting one week after treatment cessation. Derangement of the neuromuscular function was more prominent in female than in male mice and involved both excitatory and inhibitory pathways. LGG prevented antibiotic-induced changes with higher efficacy in males than in females. On the whole these observations suggest that, in early life, the gut microbiota participates to the modulation of the ENS and gut neuromuscular function in a gender-dependent manner. These changes support the importance of the gut microbiota in the development of functional gut disorders characterized by sexual dimorphism, such as IBS.

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pharmadvances

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Title

Lurasidone treatment on female rats exposed to chronic mild stress: the potential role of inflammatory processes and microglia activation

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Background

Major depressive disorder (MDD) is a one of the most prevalent psychiatric conditions, with a higher incidence in females as compared to males [1,2]. Since stress is key risk factor for the development of depression, different experimental models have been used to investigate the mechanisms that may contribute to the development of depressive-like conditions. In the present study, we used the Chronic Mild Stress (CMS) paradigm, a well-established preclinical model of depression, to expand our previous observations in male rats [3,4], by investigating the effects produced by CMS in female rats and to establish the potential effects of chronic treatment with the antipsychotic drug lurasidone in normalizing such alterations.

Methods

Wistar female rats were subjected to the CMS protocol or were left undisturbed (CTRL). Sucrose consumption was monitored weekly to assess anhedonia, a potential trait marker of depression. Based on the sucrose intake following three consecutive weeks of CMS procedure, CTRL and rats that developed a vulnerable phenotype to CMS were randomized to receive a once-daily administration of vehicle or lurasidone (3 mg/kg) for five weeks (n=10 for each experimental group). Additionally, a group of CMS-Resilient animals was identified and was treated with vehicle. After five weeks of treatment, all the animals were sacrificed, and the brain regions of interest were quickly dissected. Gene Expression analyses were performed using quantitative Real-time PCR (qRT-PCR) in the Dorsal Hippocampus (DH) and in the Ventral Hippocampus (VH). Data were analyzed by using 1-way ANOVA and 2-way ANOVA, followed by Tukey's and Sidak's multiple comparison test. Significance for all tests was assumed for p value < 0.05.

Results

Three weeks of CMS exposure caused a substantial decrease in the consumption of 1% sucrose solution in 50% of female rats. On the other hand, treatment with lurasidone in CMS animals produced a gradual improvement of anhedonia, starting from the 4th week of drug administration, with a complete normalization at the end of the treatment. At the molecular level, we observed that the expression of pro-inflammatory markers (INFβ and TGFβ) and of microglia related genes (Complement C3 and C4, CX3CR1) was significantly up-regulated only in the hippocampus of vulnerable rats. Furthermore, lurasidone treatment was able to normalize the increase of the inflammatory changes, induced by the CMS exposure

Conclusions

Our data demonstrate that vulnerability to CMS exposure is associated with a significant up-regulation in the expression of immune and inflammatory players within the hippocampus, which was normalized by chronic lurasidone treatment. These results provide evidence that lurasidone may exert antidepressant effects through a modulation of immune and inflammatory system, suggesting its potential for the treatment of psychiatric disorders, particularly in patients characterized by a higher inflammatory status.

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Title

Sildenafil ameliorates MMP hyperpolarisation in MILS-NPCs lines carrying different MT-ATP6 mutations

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Background

Leigh syndrome (LS) is one of the most severe pediatric manifestations of mitochondrial disorders. LS caused by mutations in genes encoded by the mitochondrial DNA (mtDNA) affects 1/100.000 live births and is referred to as “Maternally Inherited Leigh Syndrome (MILS)”. The mitochondrially encoded ATP Synthase membrane subunit 6 gene (MT-ATP6) is frequently mutated in MILS patients[1]. Mutations in MT-ATP6 result in Complex V instability, reduced ATP production rate, abnormally high mitochondrial membrane potential (MMP), and altered calcium homeostasis[1,2].

Our previous proof-of-concept work demonstrated that neural progenitor cells (NPCs) differentiated from patient-derived iPSCs are an effective tool for phenotypic drug screening through an imaging-based MMP assay[2]. This study identified avanafil, a compound of the class of phosphodiesterase 5 inhibitors (PDE5i), as a candidate able to normalise the MMP in MILS-NPCs carrying homoplasmic m.9185T>C mutation. However, the mechanism of action by which PDE5is normalises MMP in MILS-NPCs is unknown.

Methods

We have generated additional NPCs cell lines from patient-derived iPSCs[3] carrying different MT-ATP6 mutations (8993T>C, 8993T>G). We set up an alternative method to measure MMP using the mitochondrial probe Tetramethylrhodamine Methyl Ester Perchlorate (TMRM) and Fluorescence-activated cell sorting (FACS) analysis.

Results

We found that different MT-ATP6 mutations lead to the same MMP phenotype in MILS-NPCs. Since avanafil is not approved for pediatric use, we focused on another PDE5i, Sildenafil. We validated the FACS analysis to follow the altered MMP, and we confirmed that sildenafil also normalises the MMP phenotype in MILS-NPCs.

Conclusions

Abnormally high MMP is a constant feature of MILS-NPCs. This pathological hallmark will be exploited to investigate further the mechanisms of action of PDE5is, which may modulate the intracellular levels of cGMP and/or the activity of Ca^{2+}- and ATP-dependent potassium channels located in the mitochondrial cellular membrane.

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pharmadvances

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Title

The scientific value of the aggregate data:
Real World Data in treatment of multiple sclerosis

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Background

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system characterized by demyelination and axonal loss. The prevalence of MS in Italy is approximately 115 cases per 100,000 inhabitants, with an estimate of about 126,000 affected people, for a total of about 2,000 new cases every year.

The territory of our ASL, in North-West of Italy, has about 125,000 inhabitants, of which 151 are affected by MS. We aim to describe the population of outpatients treated with oral or subcutaneous/self-injected Disease Modifying Therapies (DMT) according to the gender, age, number and the type of therapeutic shifts. The purpose of this study is to report drug use data and information derived from real life use experience.

Methods

We conducted a retrospective observational study on adult patients affected by MS. Patients included in the study received subcutaneous or oral DMT over the period from 01/01/2020 to 01/06/2022. Patients enrolled in the study were identified using a health administrative database, Erogazione e Distribuzione Farmaci-EDF, which allows access to both therapeutic plans and administrative flows.

Results

The analysis included 151 patients (76% female, median age 47 years [22,75]): 127 received only one DMT therapy, 23 patients changed 2 therapies, 1 changed three treatments and one subject received 4 different treatments.

During the follow-up period, patients discontinuing DMT therapy either for switch or interruption were 23(15%), 13(56%) changed from injection to oral therapy, 4(16%) from oral to subcutaneous therapy and 6 patients switched therapy without changing the pharmaceutical form. The period of therapy before the switch is on average 7 months.

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For patients followed by the neurologists of our ASL (56, 37%), it was possible to evaluate the Medical Possession Rate (MPR). Adherent patients (MPR>80%) were 67%: 43% treated with subcutaneous therapy and 57% with oral therapy. 7 patients changed therapy, 29% were non-adherent patients. Graph 1 shows the effective total number of packages delivered versus the expected one.

Conclusions

The results reported are in line with what emerges in literature. We have to further investigate the causes for the change from one to another biological drug. These could include adverse events, ineffectiveness, or other reasons. Considering this preliminary analysis, it is necessary to expand the tracking data, especially for patients followed by other hospitals. It would give important feedback to the physician. Moreover, investigating treatment adherence could be an important link with treatment efficacy.

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Title

Growth hormone secretagogues in Duchenne muscular dystrophy: effects on *in vitro* myogenesis

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Background

Growth hormone secretagogues (GHS) are a class of synthetic compounds analogues of ghrelin, known for their various endocrine and extra-endocrine properties, including the control of inflammation and metabolism, enhancing GH/IGF-1 mediated myogenesis, and inhibiting angiotensin-converting enzyme (ACE), all pathways of interest in Duchenne muscular dystrophy (DMD). Our study aims to investigate the potential activity of two GHS, EP80317 and JMV2894, selected for the pharmacological profile, in DMD, on the myogenic program of the dystrophic H2K-SF1 cell-line.

Methods

We analyzed: i) the myogenic potential of H2K-2B4 and H2K-SF1 cell-lines by the analysis of Pax7, Myf5, MyoD, desmin, myogenin, fast and slow myosin; ii) the effects of a treatment with GHS and methyl-prednisolone, a corticosteroid currently used to alleviate the symptoms of DMD and slow-down muscle degeneration, on myogenic markers. GHS and methylprednisolone were given alone or co-administered and the treatment lasted for 24, 48 and 96 hours or 9 days in differentiating conditions; the analysis were performed by qRT-PCR iii) the effects of GHSs and methylprednisolone on fusion index of H2K-SF1 cells, by confocal microscopy; iv) the characterization of H2K-SF1 cells in terms of GHS-R1a, IGF-1R, IGF-2R and AGTR-1 mRNA expression, by RT-PCR.

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Results

Comparing the differentiating wild-type cell line, the H2K-2B4 with the dystrophic H2K-SF1 cells, a reduced increase of MyoD, myogenin, desmin and fast and slow miosin indicated a major challenging to commit to the myogenesis. JMV2894 was the most effective in modulating the expression of these markers in differentiating H2K-SF1 cells, particularly, myogenin and slow myosin. The effects of methyl-prednisolone were similar to that of JMV2894, and could synergize with GHS as indicated by the analysis of myogenic markers expression and fusion index. The mechanism of action of GHS is not mediated by the GHS-R1a, as H2K-SF-1 cells did not show GHS-R1a expression.

Conclusions

Our results indicate that GHS can promote myogenesis in dystrophic settings and could synergize with the clinically-used methyl-prednisolone, opening new potential perspective of research in the DMD treatment.

References

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Title

Development of SARS-CoV-2 M^{pro} Peptide-Based Inhibitors Bearing Bifunctional Warheads: Design, Synthesis, and Biological Evaluation

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Background

Despite the progress of therapeutic approaches for treating COVID-19 infection, the interest in developing effective antiviral agents is still high, due to the possibility of the insurgence of viable SARS-CoV-2-resistant strains as well as novel variants, making current treatments not effective.[1,2] Accordingly, in this work we present an integrated platform, including computational, medicinal chemistry, and biological/pharmacological studies, for developing SARS-CoV-2 M^{pro} covalent inhibitors.[3]

Methods

The rational design of SARS-CoV-2 M^{pro} covalent inhibitors was performed using various computer-based techniques. Structure-based approaches, including molecular docking, molecular dynamics, free energy perturbation (FEP), and covalent docking, were used for finding suitable decorations of the peptide-based scaffold. The drug-like properties of the conceived compounds were also evaluated. The most promising compounds were synthesized using an appropriate liquid phase synthesis. The electrophilic portions (difluorostatone, aldehyde, and nitrile) were properly inserted using organometallic-based and dehydration reactions. The biological evaluation was performed employing the full-length SARS-CoV-2 M^{pro} enzyme (NC_045512) expressed in *E. coli* employing the substrate HyLite-Fluor488–ESATLQSGLRKAK–QXL520-NH2 (Eurogentec),[2] while the evaluation of the inhibitory potential of the virus replication in cells was conducted using VERO E6 cells (ATCC® CRL 1586TM) in the presence of the P-gp inhibitor CP-100356 hydrochloride to evaluate the impact of the efflux pumps. The cytotoxicity of compounds was determined by CellTiter-Glo 2.0 Luminescent Cell Viability Assay (Promega) in the cells used to subsequently test SARS-CoV-2 M^{pro} inhibitors.[4] The drug Nirmatrelvir (MCE® cat. HY-138687) was used as the reference compound.

Results

Combining several in silico techniques, we rationally designed potential peptide-based compounds with different warheads as SARS-CoV-2 M^{pro} covalent inhibitors. In silico analysis, based on molecular docking, covalent docking, molecular dynamics simulation, and FEP, indicated that the conceived compounds could act as covalent inhibitors of M^{pro} and that the investigated warheads can be used for designing covalent inhibitors against SARS-CoV-2 M^{pro}. Compounds were synthesized and biologically evaluated, and among them six compounds showed and IC_{50} against the enzyme in the submicromolar range, and they were able to inhibit the viral replication in the low micromolar range.

Conclusions

In summary, in a multidisciplinary approach enclosing computational methods, medicinal chemistry approaches, and pharmacological studies, we report the rational design and the development of potent SARS-CoV-2 M^{pro} covalent inhibitors able to inhibit the virus replication in a cell-based assay. Remarkably, our work enriches the knowledge of SARS-CoV-2 M^{pro}, providing a novel potential strategy for its inhibition, paving the way for the development of effective antivirals.

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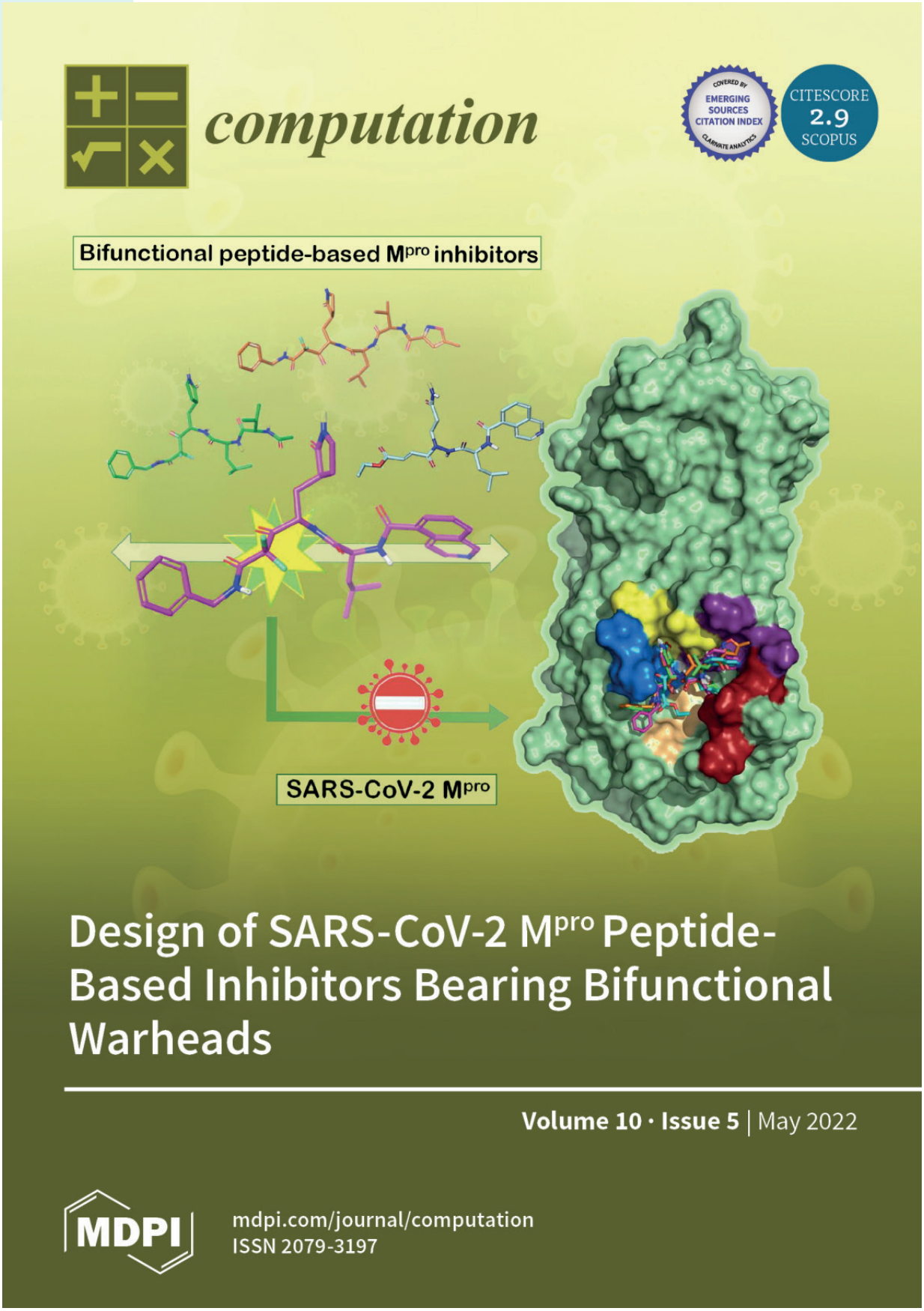
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Title

Specific deletion of cyclooxygenase-1 (COX-1) in megakaryocyte/platelets reduces intestinal polyposis in *Apc*$^{Min/+}$ mice

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Background

Several lines of evidence suggest that platelet activation represents an early event in colorectal tumorigenesis(1). It has been proposed that activated platelets induce cyclooxygenase (COX)-2-dependent prostaglandin(PG)E_2 biosynthesis, a proinflammatory and protumorigenic pathway, via the release of soluble mediators, including thromboxane(TX)A_2 (1). Thus, the antiplatelet agent low-dose-aspirin may exert antitumor effects by indirectly constraining the aberrant expression of COX-2 induced by platelets(2).

Methods

To address this hypothesis, we generated Apc$^{Min/+}$mice[developing intestinal adenomas and considered a model of human Familial Adenomatous Polyposis(FAP)(3)] with a specific deletion of *Ptgs1* (COX-1 gene) in megakaryocytes/platelets(Apc$^{Min/+}$;pPtgs1$^{-/-}$mice), thus mimicking the pharmacodynamic effects of low-dose aspirin in humans(4). In Apc$^{Min/+}$ and Apc$^{Min/+}$;pPtgs1$^{-/-}$mice, we evaluated:(i)the number and size of intestinal adenomas, their morphology, markers of macrophage infiltration and cell proliferation by immunohistochemistry and COX-1/2 gene expression by qPCR; (ii)systemic biosynthesis of TXA_2 and PGE_2, by assessing urinary levels of their primary metabolites[i.e., 2,3-dinor-TXB_2(TXM), mainly derived from platelet TXA_2, and 7-hydroxy-5,11-diketotetranorprostan-1,16-dioic acid(PGEM), mainly derived from tumor COX-2] by LC-MS/MS(4). Coculture experiments with human platelets and myofibroblasts were carried out.

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Results

Apc$^{Min/+}$;pPtgs1$^{-/-}$ mice were characterized by a significant reduction of systemic biosynthesis of TXA_2 *vs* Apc$^{Min/+}$ mice(TXM: 41±19 *vs.* 210±81 ng/mg creatinine, respectively; P<0.01), suggesting the inhibition of platelet function. The specific deletion of platelet COX-1 was associated with a significantly reduced number and size of adenomas in the small intestine *vs* Apc$^{Min/+}$mice (Fig 1A and B). The adenomas of Apc$^{Min/+}$;pPtgs1$^{-/-}$ mice were characterized by decreased F4/80+ macrophage infiltration and proliferative index and reduced expression of COX-2, as compared with Apc$^{Min/+}$mice. These effects were accompanied by diminished systemic biosynthesis of PGE_2(PGEM:0.89±14 *vs* 1.3±0.24 ng/mg creatinine, respectively; P<0.01). In cocultures of human intestinal myofibroblasts and platelets, platelet-derived TXA_2 signaling in the induction of COX-2-dependent PGE_2 in myofibroblasts was confirmed using a specific antagonist of TXA_2 receptors(SQ 29,548) or the selective exposure of platelets to aspirin.

Conclusions

Platelet TXA_2 plays a central role in developing intestinal adenomatous lesions via the induction of COX-2 in stromal cells. These results support the platelet hypothesis of intestinal tumorigenesis and provide experimental evidence that selective inhibition of platelet COX-1 is sufficient to affect early events of intestinal tumorigenesis.

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Title

Oxoeicosanoid Receptor 1 (OXER1) emerging role as a promising druggable target in Triple Negative Breast Cancer (TNBC)

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Background

OXER1 is a G-protein coupled receptor involved in inflammatory processes and in tumor development whose role and significance in TNBC are now emerging. OXER1 has been demonstrated to activate PI3K/Akt/NF-?B/RACK1 and FAK signaling pathways to promote cancer cells survival, adhesion and migration thus emerging as an important player in TNBC . GSEA, using the entire set of OXER1-correlated genes pre-ranked for their significance, identified “G2/M checkpoint” and “Mitotic spindle” as top enriched and down-regulated gene sets while and “Interferon alpha (IFN?) response” resulted significant among the top enriched and up-regulated gene sets. Down-regulated gene sets highlight the involvement of the most important members of spindle assembly and mitotic checkpoint thus indicating uncontrolled proliferation and chromosomal instability (CIN) that lead to persistent DNA damage [2]. Moreover, a study analyzing CIN in a microarray dataset of lymph node-negative primary BC patients prior to systemic therapy, found that TNBC have significantly higher CIN scores [3], which correlated with metastasis by sustaining a cell-autonomous response to cytosolic DNA [4] and INF? response [2]. Pre-clinical findings support a role of INF? contributing to metastasis formation and BC progression [5] that could be specifically attributed to triple-negative inflammatory BC (TN-IBC) [6]. Consistently with literature data, the up-regulated “IFN? response” gene set identified in our TNBC patient’s sample correlate with a non-canonical interferon stimulated genes (ISGs), which is responsible of this aggressive phenotype. Hence, the aim of the work is to corroborate our functional enrichment analysis on OXER1 role and its correlated gene sets in order to provide molecular biology and genomics data useful for the identification of TN-IBC, thus offering a personalized targeted therapy that could be tailored to OXER1.

Methods

RNA-sequence (RNA-seq) to identify TNBC cell lines displaying high and constitutive OXER1 and IFNα expression as validated *in vitro* models for our investigation context. To assess the involvement of PI3K/Akt/NF-κB pathway, luciferase reporter assay, qPCR, immunoblotting, sandwich ELISA assays, cell proliferation (MTT), migration and scratch-wound

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healing assay were performed on CAL-85-1, HCC1187 and SUM149 cells treated with OXER1 inhibitors. Analogue experiments and 3D spheroid models were also performed.

Results

Our data confirmed that OXER1 is involved in TN-IBC progression since OXER1 inhibition led to negative effects on BC cell proliferation and migration due to PI3K/Akt/NF-κB pathway antagonization which ultimately also induces RACK1 down-regulation. Our data also show that OXER1 inhibition led to non-canonical IFNα signaling decrease and reduced production of OXER1-correlated cytokines.

Conclusions

Our data support the idea that antagonizing OXER1 pathway may represent a promising strategy for the personalized treatment of TNBC.

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Title

Adolescent cocaine exposure alters recency memory and mTOR signaling in the prefrontal cortex

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Background

Adolescence is a period of vulnerability for drug abuse since the brain is extremely sensitive to external stimuli. In fact, interfering with the maturation of the brain via profound synaptic changes through early drug use might set the stage to develop memory impairment, an effect that may play a role in the continuation of drug use. We have previously demonstrated that adolescent cocaine exposure impaired recognition memory via altering the compartmentalization of the neurotrophin BDNF in the perirhinal cortex. Thus, to explore further cocaine-induced dysregulation of cognitive domains, the major aim of our work was to investigate the impact of withdrawal from cocaine exposure during brain development on the recency, temporal, memory and the involvement of the mTOR signaling.

Methods

Adolescent and adult male rats were treated subcutaneously with 5 mg/kg/day of cocaine or saline from post-natal day (PND) 28 to PND42 or from PND 65 to PND79. Following 2 weeks of drug withdrawal, rats were subjected to the temporal order object recognition (TOOR) test, a cognitively demanding test. Then, immediately after TOOR test, rats were sacrificed, and brains were collected and frozen. Infralimbic (ILc) and prelimbic (PLc) subregions of prefrontal cortex were collected through punches. Protein synthesis determinants were measured via western blot in the whole homogenate. Another cohort of adolescent rats was treated with rapamycin (15 mg/kg), inhibitor of mTORC1, and then exposed to the TOOR test.

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Results

At behavioral level, we found that withdrawal from adolescent, but not adult, cocaine exposure caused a significant impairment in the TOOR test, indicating an intact temporal memory among cocaine-exposed rats only in adult animals. At molecular level, in adolescent rats, performance in the TOOR test of saline-exposed rats activates the mTOR-S6 pathway, as shown by increased phosphorylation, in both ILc and PLc; such activation is not observed in cocaine-exposed rats. Notably, in adult rats, accordingly with the intact temporal memory observed, the activation of mTOR-S6 pathway is observed in both saline- and cocaine-exposed rats.

Interestingly, acute injection of rapamycin in naïve rats impairs the performance in the TOOR test, confirming the involvement of the mTOR pathway in the cognitive domain of temporal memory.

Conclusions

Our results point to adolescence as a crucial developmental period of vulnerability for psychostimulant-induced cognitive impairment, here emphasized by long-term cocaine withdrawal. The lack of induction of the mTOR signaling in both ILc and PLc in cocaine-withdrawn adolescent rats performing the test suggests a putative mechanism for their impaired behavioral performance.

References

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Title

Real-world evidence from administrative healthcare data of newly pomalidomide users from 2016 to 2019

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Background

Multiple myeloma (MM) is a malignant cancer with a high frequency of relapses [1]. By now and within the next few years, pomalidomide-based regimes are likely to be a substantial part of the second line treatment strategies [1]. This observational retrospective analysis aimed to describe new users of pomalidomide, their prescription pattern before its use, overall survival and integrated healthcare costs, from the perspective of the Italian National Health Service (INHS), from 2016 to 2019.

Methods

From the ReS (Ricerca e Salute) database, which collects a proportion of the Italian administrative healthcare data, patients with at least a supply of pomalidomide (ATC code L04AX06 - index date) from 2016 to 2018 (accrual period) and without any dispensation of it within 2 years before the index date were selected. Their gender, age and comorbidities were described. From the index date, other treatments for MM supplied during the preceding year, and overall survival and healthcare resource consumption costs within a variable follow-up (up to 3 years) were assessed.

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Results

From starting mean annual populations of about 7 million inhabitants from 2016 to 2018, 238 new users of pomalidomide were selected. They were mainly females (54%), with mean age 71±14 and affected by hypertension (83%), chronic lung diseases (56%) and dyslipidaemia (30%). Within one year before the index date, the majority of patients was treated with lenalidomide and/or chemotherapy. Proportions of 65%, 32% and 16% of new users survived in the first, second and third follow-up year, respectively. On average, the INHS spent €54,787, €30,560 and €36,403 per patient in the first, second and third follow-up year, respectively: pharmaceuticals accounted for >80% each follow-up year, followed by hospitalizations (>10%) and outpatient specialist care (>2%).

Conclusions

This study described the healthcare resource consumption of new users of pomalidomide and the most frequent therapeutic strategies before its use, from the perspective of the INHS, starting from shortly after the beginning of its reimbursement in Italy (2015) to recent years. Despite limitations of the use of only administrative healthcare data, this type of analyses can help to assess the real-world panorama in which new therapies and therapeutic combinations for MM are under implementation.

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Title

Real-world evidence of newly vismodegib users through administrative healthcare data from 2015 to 2019

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Background

Basal cell carcinoma (BCC) is the most common skin malignancy worldwide (1). Vismodegib is the treatment of choice for metastatic and local advanced BCC forms. This observational retrospective analysis aimed to describe new users of vismodegib and to assess their prescription patterns, surgery rate and integrated healthcare costs, from the perspective of the Italian National Health Service (INHS), from 2015 (the beginning of its reimbursement) to 2019.

Methods

From the ReS (Ricerca e Salute) database, which collects a proportion of Italian administrative healthcare data, adults with at least one supply of vismodegib (ATC code L01XX43 – index date) from 2015 to 2017 (accrual period) were selected. Patients were described in terms of sex, age and comorbidities. From the index date, this analysis assessed: length of continuous treatment (months) during two follow-up years (discontinuation was defined by the absence of supplies for >30 days), in-hospital/outpatient surgery for the removal of skin lesion/chemotherapy within one previous and two subsequent years, and two-year healthcare resource consumption costs charged to the INHS.

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Results

From a population of about 5 million inhabitants per year, from 2015 to 2017, 64 new users of vismodegib were selected. They were mainly males (70%), with mean age 78±11 and mostly affected by hypertension (69%), dyslipidaemia (25%), depression and chronic lung diseases (17%). Only 4 patients were continuously treated with vismodegib within 24 months from the index date, while 60 subjects discontinued it on average after 8 months. Among the latter, 22 were treated again with vismodegib after variable periods following discontinuation (on average, from 3 to 14 months). Within one year before the index date, 23% of patients underwent surgery and/or chemotherapy: surgery was performed in 8 subjects, chemotherapy was administered to 6 patients, and only 1 user received both therapies. Within two subsequent years, 8% of patients underwent surgery, mostly performed in an outpatient setting. On average, the INHS spent €60,311 per patient during the first follow-up year, mainly due to the cost for vismodegib (95% of the total cost), whereas €19,186 during the second year, with a slightly increase of the in-hospital expenditure (from 1% to 6%).

Conclusions

This study potentially showed the real-world burden of metastatic and local advanced BCC in Italy through the description of patients treated with its therapy of choice, in a recent and large population. Despite limitations of the use of only administrative healthcare data, these findings can show the usefulness of real-world evidence in the whole drug life cycle.

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Title

The anti-inflammatory effect immunoproteasome inhibitors in a neuro-inflammation model of Chemobrain.

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Background

Chemobrain, also called chemotherapy-induced cognitive impairment (CICI), is a side effect observed during and after chemotherapy treatment in cancer survivors. The molecular mechanisms of chemobrain involve very complicated processes including disruption of the blood-brain barrier (BBB), DNA damage, telomere shortening, oxidative stress and associated inflammatory response, gene polymorphism of neural repair, altered neurotransmission, and hormone changes. Bortezomib is a proteasome inhibitor used in multiple myeloma, ad it has been shown that may induce chemobrain in patients with a long-term life expectancy.

The proteasome is a multicatalytic complex formed by a central subunit called 20S that performs the proteolytic activity, with 4 rings of 7 subunits each. The two central catalytic rings are called β (β1-β7), while the two lateral α (α1-α7). Under the stimulation of IFN-? and TNF-α, the constitutive central particles are replaced by the newly formed immunosubunits: β5i, β1i and β2i. Bortezomib analogs have been designed with the aim of reducing neurological side effects and improve the antinflammatory activity, through the inhibition of the β5i subunit. Thus to demonstrate this effect we used an in vitro model of neuroinflammation.

Methods

Materials and methods

Human microglia cell (HMC3) were stimulated with TNF-α (10 uM) for 24 hours to induce the neuroinflammation. After induction cells were treated with 2 bortezomib analogues, KJ3 and KJ9 (0,1-10 uM each), or with Bortezomib (1 uM) for additional 24 hours. At the end of the treatment, cells were collected, RNA and protein extracted, and used for molecular analyses.

Results

Results

The blockade of the immunoproteasome by the new synthetized compounds, KJ3 and KJ9, resulted in a decrease of the activation of Nfkb and IkBα, a reduced production of oxidative products (FDA method) and in a reduced expression of proteins involved in cell cycle activation as cyclin D1 and CDK4/6.

Conclusions

Conclusion

These data support a possible therapeutic development of the compounds KJ3 and KJ9 as new antinflammatory molecules to be used in case of chemotherapy toxicity, and especially neurotoxicity induced by proteasome inhibitors.

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Title

Platelet-associated Tissue Factor: pharmacological modulation and intracellular localization

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Background

ADP mediates platelet activation and aggregation by binding to ($P2Y_1$ and $P2Y_{12}$on the platelet surface. The two P2Y receptors are differently involved in the procoagulant activity of platelets being inhibition of $P2Y_{12}$, but not of $P2Y_1$, able to prevent thrombin-induced PS exposure. The contribution of $P2Y_1$ and $P2Y_{12}$ receptors in platelet Tissue Factor (TF) modulation is still unknown. The aims of the study were to assess: 1) the involvement of $P2Y_1$ and $P2Y_{12}$ receptors in ADP-induced TF exposure on the platelet surface in healthy subjects' platelets, exploiting platelets from a $P2Y_{12}$-deficient patient; 2) TF intracellular localization through immunogold-electron microscopy and pharmacological approach, analysing platelet from two Grey Platelet Syndrome (GPS) patients

Methods

TF and P-selectin expression were evaluated by whole blood (WB) flow cytometry in platelets from 10 healthy subjects (HS; n=5 males and n=5 females; mean age 39±6y) treated with AR-C69931MX (1pM–100nM) or MRS-2500 (1pM–100nM) and stimulated with ADP (10µM), U46619 (1µM) or TRAP-6 (10µM). To assess open canalicular system (OCS) involvement in TF and P-selectin exposure, platelets were preincubated with cytochalasin D (10µM) or colchicine (10µM). Immunogold labeling and electron microscopy were also performed to define platelet TF localization. A subject

with inherited severe P2Y12 deficiency and two patients with GPS syndrome, characterized by large platelets lacking normal alpha-granules, were also studied to confirm our hypothesis

Results

$P2Y_{12}$-inhibitor AR-C69931MX, but not $P2Y_1$-inhibitor MRS-2500, concentration-dependently prevented ADP-stimulated TF exposure. To confirm the exclusive involvement of $P2Y_{12}$ in TF expression, we analyzed blood of a $P2Y_{12}$-deficient patient. Upon ADP stimulation, no increase of TF expression on the platelet surface was observed, but TF was readily detectable after stimulation with U46619 (+1.7 fold) or TRAP-6 (+1.4 fold). Unlike what was observed for TF, the membrane exposure of alpha-granule P-selectin was significantly modulated by both $P2Y_1$ and $P2Y_{12}$ thus suggesting a different cellular localization of the two proteins. To assess the presence of TF in alpha granules, we studied platelets from GPS patients: while levels of P-selectin$^+$-platelets were reduced (about - 65%), those of TF were comparable to that of HS, despite the alpha-granule defect. TF was rather associated with OCS as cytochalasin D or colchicine treatment impaired ADP-induced TF but not P-selectin expression. Immunogold labeling and electron microscopy analysis confirmed TF storage within OCS.

Conclusions

This study shows for the first time 1) the unique involvement of $P2Y_{12}$ receptor in ADP-induced exposure of TF which is associated with platelet OCS. All together our findings add new insights into the regulation of the procoagulant activity of platelets highlighting that, as for phosphatidylserine, also TF exposure is a $P2Y_{12}$ dependent mechanism

References

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Title

Functional and pharmacological characterization of a Nav1.4 sodium channel mutation in Italian kindred also affected by a ClC-1 chloride channel mutation

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Background

Non-dystrophic myotonias are rare genetic diseases characterized by disabling muscle stiffness. They are caused by mutations in the Nav1.4 sodium channel (*SCN4A* gene) or in the ClC-1 chloride channel (*CLCN1* gene) [1]. Next generation sequencing performed on five relatives with myotonic phenotype identified two novel segregated mutations: p.K1302R in *SCN4A* and p.H838P in *CLCN1*. Further genetic *CLCN1* abnormalities in the proband were excluded using MLPA method. Here we report the functional characterization of both mutations to evaluate their contribution to myotonia.

Randomized clinical trials have shown that the sodium channel blockers, mexiletine and lamotrigine, both alleviate symptoms in chloride and sodium channel myotonia [2,3]. We thus tested the effects of lamotrigine and mexiletine on WT and p.K1302R Nav1.4 mutant.

Methods

The mutations p.K1302R and p.H838P were introduced into the pRc/CMV plasmid containing the cDNA encoding wild-type (WT) hNav1.4 and hClC-1 channels, respectively. Sodium and chloride currents were recorded with whole-cell

patch-clamp technique in HEK293 cells transfected with K1302R or H838P and compared to relative WT currents. The drugs were tested in vitro on WT and K1302R sodium currents.

Results

Sodium currents generated by K1302R and WT hNav1.4 were very similar. Kinetics and voltage dependences of fast and slow inactivation were superimposed. The mutant channel showed a small negative shift (3 mV) in the voltage-dependence of activation, which increased the likelihood of the channel to open at more negative voltages. Compared to WT hClC-1, the H838P mutation caused a reduction in chloride current amplitude (-73 % at -100 mV) and a 25-mV positive shift of open probability voltage dependence, in agreement with the location of the mutation into the CBS2 domain of the C-terminus [4]. Pharmacological studies revealed that lamotrigine similarly inhibited WT and K1302R sodium currents elicited using a myotonia-like voltage-clamp protocol.

Conclusions

The results suggest that the mild functional alterations induced by p.K1302R and p.H838P may be asymptomatic when the mutations are expressed individually. In patients carrying both mutations, the combination of the functional defects is likely responsible for the expression of the myotonic phenotype. The pharmacological results suggests that lamotrigine might be useful in myotonic patients carrying the K1302R mutation. The K1302R sensitivity to mexiletine is under investigation.

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Title

Preclinical evaluation of safinamide as an antimyotonic drug in myotonic ADR mouse model

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Background

Non-dystrophic myotonias are caused by mutations in the SCN4A and CLCN1 genes encoding the Nav1.4 sodium and ClC-1 chloride channels expressed in skeletal muscle. Myotonia can be debilitating due to severe muscle stiffness and pain, which are associated with movement disorders. Mexiletine, a sodium channel blocker, is the drug of first choice in the treatment of myotonias. However, a subgroup of patients have little benefit from mexiletine, for its side effects and contraindications. Safinamide has been identified as a potential antimyotonic drug. Safinamide (XADAGO®, ZAMBON) is indicated for the treatment of mil-to-late Parkinson's disease in combination with levodopa. Beside reversible MAO-B inhibition, safinamide reduces the excessive release of glutamate through sodium channel blockade. Safinamide is also a potent inhibitor of human skeletal muscle sodium channels expressed in cell lines (1).

Methods

Safinamide effects were tested in an animal model of Myotonia Congenita, the "arrested development of righting response" (ADR) mouse. As in Myotonia Congenita, this model is characterized by genetically determined loss of function of the ClC-1 channel (2). Only the homozygous adr/adr mouse is symptomatic and shows myotonic signs, which can be appreciated by the difficulty to straighten themselves on four legs from the supine position. At the age of 4 weeks, homozygous mice were treated with safinamide to evaluate the Time of Righting Reflex (TRR). Safinamide was injected intraperitoneally at 10 mg /kg. The effects of safinamide were compared with those of saline solution and mexiletine 10 mg/kg (i.p.). The TRR was measured 20 min earlier and 20, 60, 90 and 120 minutes after drug injection.

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Results

Safinamide exerted a more potent antimyotonic effect than mexiletine. The TRR value was significantly reduced by 56 ± 3 % after 60 minutes and by 35 ± 11% after 120 minutes with 10 mg/kg safinamide. For comparison, the same dose of mexiletine was less effective, being the TRR value reduced by 47 ± 4 % after 60 minutes and by 25 ± 4 % after 120 minutes.

Conclusions

These results show that safinamide exerts a potent effect in counteracting the typical sign of myotonia in vivo (muscle stiffness assessed by TRR) and support its therapeutic use as an effective alternative to mexiletine in non-dystrophic myotonias.

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Title

NIH Heterogeneous Stock rats trained to heroin self-administration show heterogeneous response to the anti-addictive effects of the panopioid agonist Cebranopadol

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Background

Cebranopadol is a novel panoppioid agonist, binding with equi-nanomolar affinity to nociception and mu opioid receptors (NOP, MOP), while showing lower affinity to KOP and DOP receptors. In addition, cebranopadol shown low abuse liability, making it a potential candidate as pharmacological therapy for opioid use disorder (OUD). In light of the heterogeneous treatment response shown by OUD patients, we decided to test the effect of Cebranopadol on heroin self-administration (HSA) and cued relapse (CR) in genetically heterogeneous NIH heterogeneous stock (HS) rats subjected to a multisymptomatic model of OUD that we previously described (Allen et al., 2021).

We hypothesized that HS rats would show heterogeneous response to the effects of cebranopadol.

Methods

Seven behavioral measures of heroin seeking were screened in 614 (298 female) HS rats: escalation of intake, total intake, motivation, cued relapse primed relapse, cued and non-cued extinction. These behavioral measures were then used to cluster rats into OUD-vulnerable, -resilient, and -intermediate clusters using Stochastic Bayesian Model as we previously described (Allen et al., 2021).

Cebranopadol (0, 12.5, 25, 50 µg/kg) was tested on HSA on a subgroup of 44 males (11 resilient, 23 intermediate, 10 vulnerable) and 42 females (10 resilient, 15 intermediate, 17 vulnerable), and on CR on a subgroup of 59 male (14 resilient, 29 intermediate, 16 vulnerable) and 56 female (13 resilient, 30 intermediate, 23 vulnerable) rats.

Results

Cebranopadol reduced HSA in both sexes. However, analysis of individual effect size revealed that cebranopadol was not efficacious in 13/44 male and 5/42 female rats (heterogeneously distributed among clusters).

Cebranopadol also decreased CR, and also in this case, we found 7/59 males and 8/56 females (heterogeneously distributed among clusters) that did not respond to cebranopadol.

Conclusions

Globally, cebranopadol showed marked efficacy on HSA and CR in HS rats. Few rats, independently from the belonging cluster, did not respond to the drug. Sub-clustering and genetic analysis are underway to further evaluate if there are behavioral and/or genetic traits predictive of lack of sensitivity to cebranopadol.

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pharmadvances
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Title

NIH Heterogeneous Stock rats screened for multiple alcohol seeking behaviors show heterogeneous response to anti-alcohol effect of naltrexone

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Background

Alcohol use disorder (AUD) is characterized by a large phenotypic and genotypic heterogeneity [1]. Consistently, treatments efficacy varies between patient subgroups, and promising targets failed clinical expectations [2]. We hypothesized that multi-symptomatic preclinical models of individual variability in AUD would offer better translational results than group-based approaches revealing non-responder individuals.

Methods

To test our hypothesis, we subjected 40 (20/sex) NIH Heterogeneous Stock (HS) rats to a multisymptomatic screening of AUD. We initially measured alcohol consumption in a three-bottle choice (TBC) paradigm (alcohol 5%, 10%, and water). Then, we trained rats to alcohol self-administration (ASA) under a Fixed Ratio 1 (FR1) contingency (0.1 ml, alcohol 10% reward paired with a discrete house cue light). Next, motivation for alcohol was assessed by the break-point reached under progressive ratio (PR) contingency in three ASA sessions. After PR, FR1 self-administration was baselined, and cued reinstatement of alcohol seeking was tested in an extinction/reinstatement paradigm. Finally, compulsive ASA was tested in fourteen FR1 sessions in which alcohol delivery was punished with an electric foot-shock (0.25mA 0.5sec); compulsive behavior was expressed as resistance to punishment (punished reward / reward baseline) [3].

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Results

Four addiction criteria were defined [4; 5]: total alcohol intake in TBC, break point under PR contingency, cued reinstatement, and resistance to punishment. The distribution of the four criteria were z-scored and individual rats were arbitrarily defined as positive (above the 66th percentile) or negative (below the 66th percentile) for each criterion. Based on the number of positive criteria met, rats ware divided into 0crit (N=8), 1crit (N=14), 2crit (N=12), 3/4crit (N=6; 3crit/4crit matched because of low N). Finally, the effect of naltrexone (0, 0.3, 1 mg/kg intra-peritoneal) [6; 7; 8] on FR1 ASA was tested in 0-4crit rats. 1 mg/kg naltrexone decreased ASA in all groups while 0.3 mg/kg was not effective in 1crit and 2crit rats. Individual response analysis revealed that 0.3 mg/kg of naltrexone failed to reduce self-administration in 15/40 rats (37.5%). Non-responder rats accounted for 1/8 of 0crit (12.5%), 8/14 of 1crit (57.2%), 6/12 of 2crit (50%), whereas all 3/4crit rats responded to naltrexone.

Conclusions

As predicted, naltrexone showed heterogeneous efficacy when tested in a model of individual variability in AUD-like phenotype.

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pharmadvances

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Title

Characterization of in vivo, ex vivo and in vitro effects of growth hormone secretagogues in murine and cell models of Duchenne muscular dystrophy

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Background

BACKGROUND Growth hormone secretagogues (GHSs) exert multiple actions thanks to their ability to activate GHS-R1a, control inflammation, and metabolism, enhance GH/IGF-1 mediated myogenesis, and inhibit angiotensin-converting enzyme (ACE)[1]. Those mechanisms are of interest for the potential ability to target multiple steps of the cascade of pathological events in Duchenne muscular dystrophy (DMD)[2]. Our study aims to provide preclinical evidence for the potential benefits of GHSs in DMD, via a multidisciplinary *in vivo* and *ex vivo* comparison[3,4] of two *ad hoc* synthesized compounds with wide but different profiles (EP80317 and JMV2894) in *mdx* mice, paralleled by *in vitro* assays on wild type (wt, H2K-2B4) and dystrophic (H2K-SF1) cell-lines

Methods

METHODS Four-week-old *mdx* mice were treated for 8 weeks (T0-T8) with EP80317 or JMV2894 (320 µg/kg/d, s.c.). During the treatment, we performed *in vivo* measurements such as forelimb grip strength and ultrasonography. At the end of the treatment, we performed several *ex vivo* analyses from muscle physiology to histological analysis and gene expression. *In vitro* assays on wt-2B4 and dystrophic-SF1 myoblasts were performed with both drugs at different time points to exclude cytotoxic effects.

Results

RESULTS *In vivo*, both GHSs were able to increase mice forelimb force (recovery score vs wt, RS: 20% for EP80317 and 32% for JMV2894 at T8), and also reduced diaphragm (DIA) ultrasound echodensity, a fibrosis-related parameter (RS: 69% and 75%, respectively). On the other hand, only EP80317 improved DIA amplitude by *in vivo* ultrasonography (RS: 110%). *Ex vivo*, both drugs ameliorated DIA isometric contraction (*e.g.* RS: 40% for tetanic force), with EP80317 also partially preserving *mdx* DIA response to eccentric stimuli. Both drugs, and in particular JMV2894, reduced the amount of collagen (by Masson trichrome) in gastrocnemius muscle and mostly in DIA. Gene expression profiling is currently ongoing. In parallel, preliminary docking studies revealed a potential binding capability of JMV2894 on proteases involved in extracellular matrix remodeling and collagen deposition. A refinement analysis is ongoing and will drive confirmatory biological assessments.

In vitro assays on wt-2B4 and dystrophic-SF1 myoblasts excluded cytotoxicity EP80317 or JMV2894 when applied at increasing concentrations for 12, 24 and 48-hour time points. Preliminary qRT-PCR experiments showed a reduced SF1 commitment to myogenesis as revealed by a reduced time-dependent increase of Pax7, MyoD, myogenin and desmin levels. JMV2894 appeared to be more effective in modulating the expression of these markers in differentiating SF1.

Conclusions

CONCLUSIONS Our results support the interest of multi-site acting GHSs as modulators of pathology progression in mdx mice, disclosing a key anti-fibrotic action that may prove beneficial to contrast pathological remodeling and enhance regeneration [Supported by AFM-Téléthon #22199].

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Title

A signal of acquired haemophilia associated with COVID-19 vaccination: a disproportionality analysis and a systematic case review

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Background

Acquired hemophilia A (AHA) is a rare hematologic disease characterized by the development of spontaneous bleeding, with an estimated incidence of about 1.5 cases per million population/year. An autoimmune mechanism, based on the development of autoantibodies against factor VIII (FVIII) can be proposed for this event. Age over 65 years and pregnancy are in young patients are recognized as risk factors[1]. An unusual and unexpected number of AHA diagnoses temporally related to COVID-19 vaccination have been reported by some authors[2,3] (up to 17 cases per million population/year in the province of Reggio Emilia in Italy)[2]. The aim is to investigate a possible signal of risk of AHA associated with COVID-19 immunization.

Methods

To investigate the presence of an AHA risk signal associated with COVID-19 vaccines, we performed a disproportionality analysis on the World Health Organization (WHO) database (VigiBase®) by calculating the information component (IC) for all COVID-19 vaccines and for a single COVID-19 vaccine product using the entire database as a reference. AHA reports associated with any COVID-19 vaccine identified in Vigibase®, integrated with those available on the Food and Drug Administration Vaccine Adverse Events Reporting System (VAERS) and those published in the medical literature, formed a unique dataset. We systematically reviewed all selected cases for clinical plausibility.

Results

We identified 150 cases of suspected AHA associated with COVID-19 vaccines (146 included the PT "acquired hemophilia") in Vigibase in which only three vaccine products were reported as suspected causative agents of AHA. A disproportionality analysis revealed a significant IC for the preferred term "acquired hemophilia" associated with all COVID-19 vaccines (IC: 1.3; IC025: 1.1) and the BNT162b2 vaccine product (IC: 1.9; IC025: 1.6). Ninety-six unique cases of AHA following COVID-19 vaccines were examined as a result of the integration of data from the Vigibase, VAERS, and literature and the elimination of duplicates. Of these, at least one preexisting condition that can be considered a risk factor for AHA (history of AHA, cancer, autoimmune disorder) was present in 20 cases (21%), while it was excluded in 57 (59%) and not reported in 19 (20%). No cases associated with pregnancy were reported. Approximately 22% of cases occurred in patients ≤65 years old. The median time to onset was 18 days. A single case showed spontaneous resolution without specific AHA treatment, 39 (41%) cases had complete resolution after treatment, and for 10 (11%) patients the outcome was death.

Conclusions

The disproportionality analysis showed a significant risk of AHA reporting associated with COVID-19 vaccines. Several good-quality reports of AHA were identified showing no alternative causes to immunization with COVID-19. Although the unexpected frequency of AHA in the population could be explained by detection bias, one worthy of further investigation was identified.

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Title

Role of the endocannabinoid system in a preclinical model of autism spectrum disorder induced by maternal immune activation (MIA)

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Background

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by persistent deficits in social communication and interaction and restricted-repetitive patterns of behavior, interests or activities. These symptoms typically occur at early childhood and produce clinically significant developmental impairments[1].

The prenatal immune environment has a key role in the onset of neurodevelopmental disorders and its delicate equilibrium can be disrupted by perturbations in the maternal immune system[2].

Recently, some studies have pointed to a strong inflammatory state associated with ASD[3] which correlates with immune system dysfunctions[4]. Furthermore, the recent demonstration that microglia, the resident immune cells of the CNS, contribute not only to inflammatory events but also to neural development[5] has raised new hypotheses regarding a microglial role in the etiology of ASD.

The endocannabinoid system (ECS) is a neuromodulatory system with a major role in brain development and functioning and it is involved in the regulation of emotional responses, cognitive performance, social interaction and immune modulation[6]. Furthermore, the ECS plays a role in ASD[2], although the exact mechanisms involved are still debated.

Methods

In the present work we first aimed to shed light on the mechanisms involved in the development of ASD following MIA, by characterizing a preclinical model of autism based on prenatal exposure to Lipopolysaccharide (LPS) in Wistar rats (100µg/kg i.p. to pregnant rats at GD 9.5). Next, we tested the hypothesis that drugs targeting endocannabinoid neurotransmission could improve the altered phenotype displayed by LPS-exposed rats.

Results

Our results support the hypothesis of an impaired neural development in offspring exposed to MIA, highlighting a deficit of these animals in more than a behavioral task.

Conclusions

Although the mechanisms underlying the role of the ECS in ASD and its correlation with inflammation need to be clarified, this study contributes to a better understanding of the etiology of ASD and to the identification of new therapeutic targets for this complex disorder.

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Title

Association of genetic polymorphisms in DNA repair genes with the risk of radiation-induced fibrosis in breast cancer patients

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Background

The standard of care for early breast cancer is conservative surgery followed by radiotherapy [1]. Breast cancer patients benefit from adjuvant radiotherapy in terms of recurrence risk and breast cancer-related deaths. However, a significant number of long-term survivors experience late radiotherapy-induced fibrosis (RIF), which can affect patients' quality of life [2]. Multiple factors influence the risk of RIF, including individual genetic background, whose contribution still remains elusive [3,4]. In the present study, we aimed to investigate the role of 10 single nucleotide polymorphisms (SNPs) in 9 DNA repair genes as risk factors for RIF in breast cancer patients.

Methods

XRCC5 rs3835, LIG4 rs1805388, XRCC4 rs1805377, PRKDC rs2213178, BRCA1 rs799917, BRCA1 rs16942, BRCA2 rs1801406, RAD51 rs1801320, XRCC2 rs3218536, XRCC3 rs861539 were genotyped by real-time polymerase chain reaction in 285 Italian breast cancer patients who received radiotherapy after breast conserving surgery. RIF was scored according to the Late Effects of Normal Tissue-Subjective Objective Management Analytical (LENT-SOMA) scale. Univariate and multivariate Cox regression analyses were performed to calculate the hazard ratio (HR) and the 95% confidence interval (CI) to evaluate the influence of clinical variables and genotypes on the risk of grade ≥ 2 RIF.

Results

Overall, 51 out of 286 breast cancer patients (17.9%) experienced moderate to severe RIF (LENT-SOMA ≥ grade 2). In the univariate Cox analysis, the clinical variables related with grade ≥ 2 RIF were breast diameter (HR, 1.13; 95% CI, 1.03-1.23, p=0.009), BMI (HR, 1.06; 95% CI, 1.00-1.12, p=0.048) and radiation type (HR, 0.21; 95% CI, 0.05-0.90,

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p=0.036). The Cox regression analysis adjusted for confounding clinical factors revealed that XRCC4 rs1805377 was associated with a higher risk of grade ≥ 2 RIF under either the additive (HR, 2.45; 95% CI, 1.39-4.34, p=0.002) and the dominant model of inheritance (HR, 2.54; 95% CI, 1.42-4.56, p=0.002). Analogously, XRCC3 rs861539 was found to confer a higher risk or RIF of grade ≥ 2 under the dominant model of inheritance (HR, 2.28; 95% CI, 1.16-4.46, p=0.017).

Conclusions

XRCC4 rs1805377 and XRCC3 rs861539 emerged to be associated with the risk of RIF in breast cancer patients. However, large replication studies are needed to confirm the present results.

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Title

In vitro spike protein production of SARS-CoV-2 mRNA vaccines in the real-world setting: efficacy and safety implications

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Background

By the end of the winter of 2022, the number of patients infected with SARS-CoV-2 and especially those suffering from severe CoViD-19 has declined sharply in Europe. This is mainly due to the protective effect of anti-SARS-CoV-2 vaccines. Currently, the most widely used vaccines in Europe are Comirnaty (BNT162b2) and Spikevax (mRNA-1273), both based on mRNA technology. The vaccine contains mRNA that codes a full-length, stabilized viral Spike protein (S), that includes the COOH-terminal transmembrane region.[1,2]

Their safety is very good, however, some extremely rare adverse events (AEs) have been described and Spikevax demonstrates a higher frequency of AEs than Comirnaty.[3] It is not known whether AEs are due to the vaccine-dependent production of S protein or to other components of vaccines. Furthermore, in Spikevax recipients, the anti-S antibody titer is higher than in Comirnaty recipients.[4] Both data may suggest the S protein production of vaccines is different.

The aim of our study was the evaluation of SARS-CoV-2 S protein production by anti-SARS-CoV-2 mRNA-based vaccines.

Methods

Thanks to the agreement with two vaccination centers in Perugia, the vaccine available in the vials after preparing the doses to be administered to people was used in the project. The human cell lines K562 and Jurkat were treated with 1 ul (0.2% of the dose), 10 ul (2% of the dose), and 100 ul (20% of the dose) of each vaccine.

After 24 h, S protein expression was evaluated on the cell surface (flow cytometry) and in the cell culture supernatant (ELISA). In order to prevent any possible degradation of vaccines, we paid particular attention to the use of fresh vaccines; the experiments were performed immediately after opening the vials and preparing the doses for the people. In addition, the experiments were repeated three times using different batches of each vaccine.

Results

The study found that both vaccines are capable of producing detectable amounts of S protein, in a dose-dependent manner. However, Spikevax's production of S protein was far superior to that of Comirnaty, both in terms of the amount of protein detected on the cell membrane and in the amount released into the culture medium. Similar results were obtained with both cell lines.

Conclusions

The data provided a direct comparison of the level of S protein production by SARS-CoV-2 mRNA-based vaccines, showing that the Spikevax vaccine produces more S protein than the Comirnaty vaccine in the real world. As far as we know, no such comparison has ever been made.

Surprisingly, we also showed that S protein is not only detected on the cell membrane but also released by cells, suggesting that it could be present in the bloodstream in the hours following vaccination.

These findings may help to explain the different efficacy and safety of Comirnaty and Spikevax vaccines.

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pharmadvances

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Title

The neuroprotective effect of carnosine is mediated by insulin-degrading enzyme

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Background

L-Carnosine (Car) is an endogenous dipeptide with metal chelating, antioxidant, anti-aggregating, anti-inflammatory, and neuroprotective properties. Amyloid-β (Aβ) represents the key peptide in the pathogenesis of Alzheimer's disease (AD). Reactive microglia, the brain-resident immune cells, co-localize with Aβ within the neuritic plaques observed in AD brain. Microglia can promote Aβ clearance through different mechanisms including the secretion of enzymes able to degrade Aβ such as insulin-degrading enzyme (IDE), a major enzyme responsible for the degradation of insulin and a well-recognized common pharmacological target between AD and diabetes. In the present study, we wondered whether Car can exert neuroprotective effects against Aβ oligomers through the modulation of IDE activity.

Methods

By employing trypan blue viability assay, we ?rst investigated the toxic potential of Aβ1-42 oligomers, in the absence or presence of Car and/or a highly selective IDE inhibitor (6bK), in primary mixed neuronal cultures. Once the neuroprotective activity of Car was established to be IDE-mediated, we then investigated Car/IDE interaction and the molecular mechanisms underlying the protective effects of Car. For this purpose, we have applied high performance liquid chromatography-mass spectrometry (HPLC-MS), surface plasmon resonace (SPR), dynamic light scattering (DLS) and fluorescent methods to determine the effect of Car on IDE activity, oligomerization and cooperativity.

Results

Our results show that Car is protective against Aβ1-42-induced toxicity and also that the neuroprotective activity of Car is lost in the presence of 6bK. DLS measurements show that Car alters the average hydrodynamic radius of the enzyme, hinting to higher oligomeric forms induced by the presence of Car. SPR measurements applied to calculate the Hill coefficient gave a clear indication that Car directly affects the enzyme cooperativity, increasing the value of the Hill coefficient. Last but not least, HPLC-MS experiments clearly show an increase in IDE activity towards both insulin and Aβ peptides in the presence of Car. On the contrary, the IDE degradation of a smaller fluorogenic substrate (substrate V) does not seem to be affected by the presence of Car.

Conclusions

All results point at an IDE activating role of Car due to an increase in the oligomerization and in the cooperativity of the enzyme, which increase the enzyme capability to degrade long substrates such as insulin and Aβ, but not shorter one such as substrate V. This specific regulatory mechanism indicates that Car does not bind to the IDE catalytic site, being a heterotropic modulator, as it is able to regulate the enzyme activity by binding to the exosite or to other not identified sites, causing a different interaction between the enzyme and long substrates, changing their reciprocal affinity and, in turn, IDE catalytic activity. These results open a new path to explore the therapeutic potential of Car in AD.

References

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Title

Melatonin modulates microglia polarization: effects on neuronal vulnerability to β-amyloid

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Background

Microglia, the resident immune cells of the CNS, play a crucial role in the inflammatory response that occurs in neurodegenerative diseases such as Alzheimer's disease (Heneka et al., 2014). Specifically, during the early acute phase of inflammation, microglia are characterized by an anti-inflammatory (M2-like) phenotype that switches towards pro-inflammatory (M1-like) when inflammation becomes chronic (Merlo et al., 2020).

Microglia regulates also protein homeostasis. Specifically, in M1-like microglia the activity of the ubiquitin-proteasome-system, a central proteolytic pathway involved in protein clearance, is differently regulated leading to accumulation of ubiquitinated proteins that exacerbate neuronal damage (Orre et al., 2013). We investigated how melatonin, a neurohormone exerting anti-inflammatory functions (Chen et al., 2020), can modulate microglial activation after long-term exposure to β-amyloid protein (Aβ) and how these events impact on neuronal vulnerability to A

Methods

The human microglial HMC3 and neuroblastoma SH-SY5Y cells were used in the study. Protein expression/ubiquitination were analyzed by Western blot and mRNA levels by real-time PCR. Proteasome activity was investigated with appropriate enzymatic assays. Neuronal vulnerability to Aβ was assessed by WB of synaptic proteins and analysis of neuritic length.

Results

HMC3 cells were exposed to Aβ42 (200 nM for 96 h) in the presence of melatonin (MEL, 1 μM) added since the beginning (MELco) or for 24 h after 72 h of exposure to Aβ42 (MELpost). In both conditions, MEL promoted microglial anti-inflammatory responses by maintaining high SIRT1/BDNF levels and preventing CASP1 and phospho-ERK up-regulation. MEL was also able to partially rescue proteasome function that was altered in M1-like microglia, reestablishing both 20S and 26S chymotrypsin-like activity. SH-SY5Y cells were exposed to Aβ42 (200 nM for 24 h) in non-conditioned medium (CM) or in the presence of CM collected from microglia exposed for 6 or 96 h to Aβ42 alone or in combination with MELco or MELpost. The results showed that Aβ reduced the mean neuritic length and the expression of pre-synaptic proteins synaptophysin and VAMP2. These effects were rescued by CM-M2 or CM-M1 from microglia treated with MELco and MELpost. Pre-treatment with SIRT1 inhibitor EX527 counteracted the rescue of neuritic length, confirming SIRT1's involvement in melatonin's effects.

Conclusions

Melatonin delays microglial pro-inflammatory switch by supporting the SIRT1/BDNF axis and by modulating proteasome function, with a beneficial effect on neurons.

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pharmadvances

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Title

Contraceptive drug, Nestorone, enhances stem cell-mediated remodeling of the stroke brain by dampening inflammation and rescuing mitochondria

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Background

Ischemic stroke has a high incidence worldwide and the available treatments for ischemic stroke are extremely limited[1]. It has been reported that the contraceptive drug Nestorone® (segesterone acetate) resulted neuroprotective in various central nervous system disorders[2], however, the underlying mechanism is unidentified.

Methods

In this work, we demonstrated that Nestorone was effective as stand-alone treatment or in combination with human amniotic fluid-derived stem cells (hAFSc) both *in vivo* and *in vitro* models of stroke.

Results

Indeed, rats receiving stand-alone or combined treatments of Nestorone and hAFSc showed an amelioration in behavioral tests, peri-infarct and infarct cell loss. In addition, significantly reduced levels of pro-inflammatory signals combined with increased levels of stem cell proliferation and differentiation in both brain and spleen of treated animals were reported. In concert, the *in vitro* oxygen-glucose deprivation stroke model showed that neural stem cells treated with Nestorone exhibited increased stem cell proliferation and differentiation parallel with rescue of the mitochondrial

respiratory activity characterized by decreased mitochondrial reactive oxygen species, raised ATP, higher mitochondrial deacetylase Sirtuin 3, and a normalized ratio of acetyl-superoxide dismutase 2 /SOD2, implying the key role of mitochondrial metabolism and oxidative protection in Nestorone's therapeutic effects in stroke.

Conclusions

The present data offer novel pathways of neuroprotection on which to build upon strategies to abrogate mitochondrial dysfunction via stem cell-based and drug-aided (i.e., Nestorone) stroke therapeutics.

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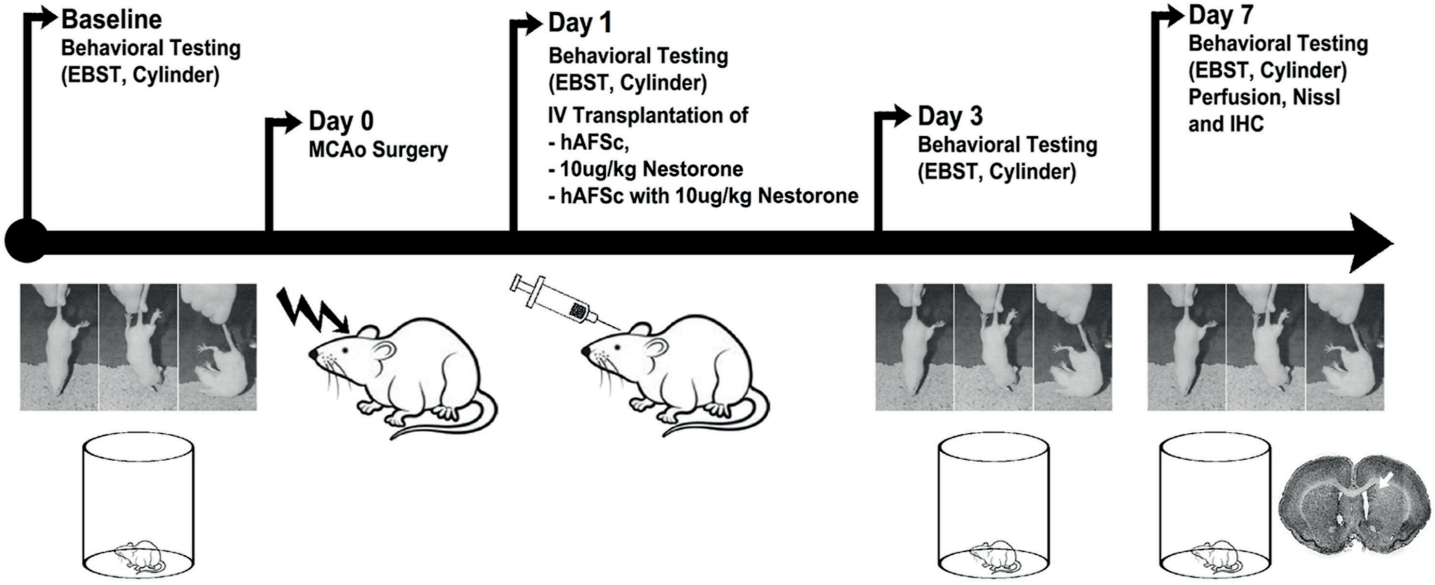

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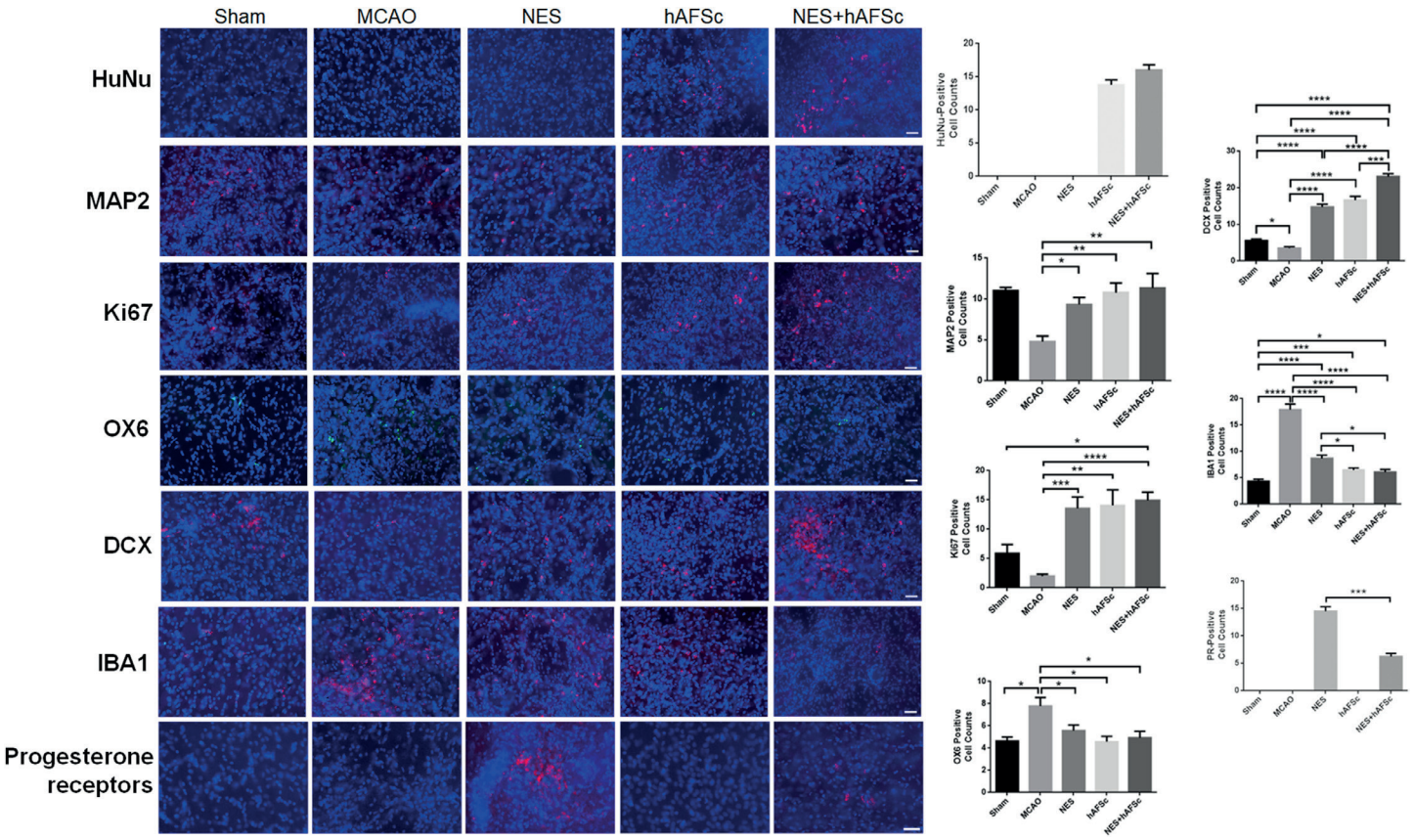

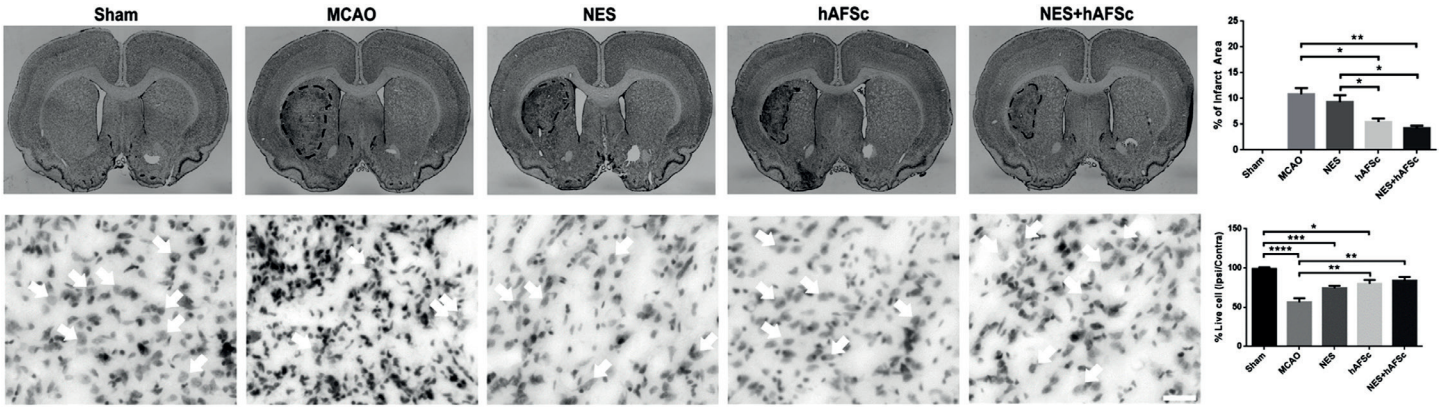

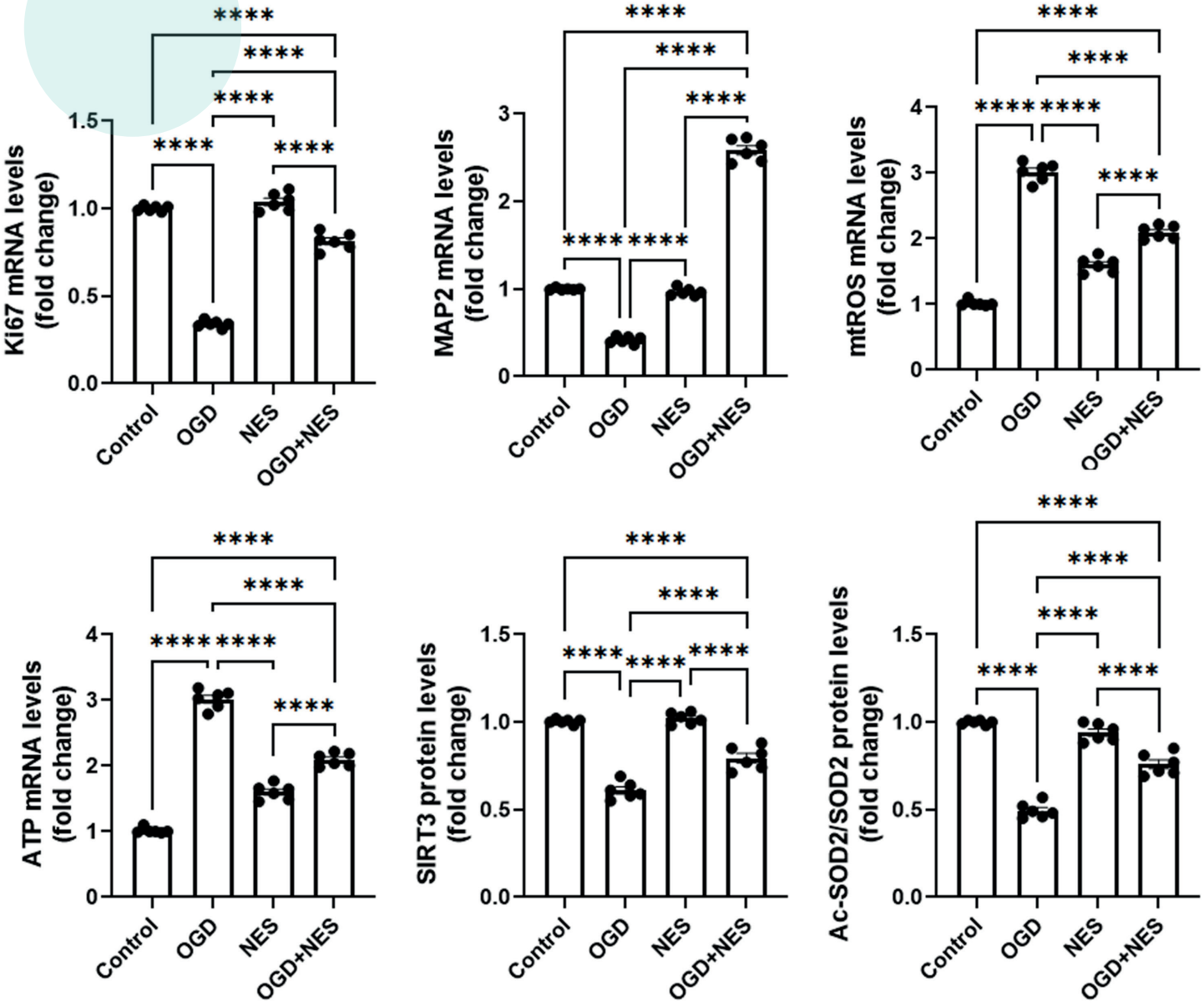
Ki67 mRNA levels (fold change)
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Ac-SOD2/SOD2 protein levels (fold change)
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Title

Electrophysiological characterization of wild-type and dystrophic myogenic cells: ion channels profile in Duchenne Muscular Dystrophy during myogenesis.

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Background

Ion channels expression and activity are pivotal to drive muscle cells differentiation into myofibers. Several ion channels have an active crosstalk with the dystrophin complex, which is disrupted in Duchenne muscular dystrophy (DMD). However, little is known about their involvement in myogenesis, in particular in DMD settings.

Methods

We performed a pilot study to characterize the electrophysiological asset of myoblasts and myocytes by using two immortalized mouse satellite-derived cell lines: the wild-type 2B4 and the dystrophic SF1. We evaluated both inward and outward currents at different time points of differentiation by whole-cell patch clamp. Besides the proliferative state (myoblasts), we considered 4 time points of differentiation: the 2^{nd}, the 4^{th}, the 6^{th} and the 11^{th} day of differentiation for each cell line.

Results

2B4 cells showed an increment of inward currents as the days of differentiation progresses. In SF1 myocytes, inward currents increased up to the 6th day of differentiation, like in 2B4 cells. However, day-11 SF1 myocytes showed 50% lower inward currents compared to day-11 2B4 myocytes (5.2 nA vs 2.8 nA at -20 mV). High concentration of tetrodotoxin blocked these inward currents in both day-6 and day-11 2B4/SF1.

Outward currents were clearly detectable in day-11 2B4 cells but very small in day-2/4/6 2B4 myocytes. On the other

hand, SF1 outward currents reached the highest value at day-6, being 3-fold higher than 6-day 2B4 cells (733 pA vs 283 pA at +60 mV). However, day-11 SF1 myocytes showed 44% lower outward currents, compared to day-11 2B4 cells (722 pA vs 1283 pA). Moreover, $BaCl_2$ decreased outward currents in day-6 2B4/SF1 cells and in day-11 2B4 myocytes.

In addition, we assessed resting membrane potentials which became more negative as the differentiation program progresses in both cell lines. However, day-11 SF1 myocytes had a more depolarised membrane potential, compared to day-11 2B4 myocytes.

Conclusions

This preliminary data suggests that during myogenesis, intrinsic impairments in ion channel development are disclosed by dystrophic conditions, likely in relation to the primary defect.

The underlying mechanism and gene expression of ion channels are currently under investigation, along with the potential impact of these biophysical alterations on the efficiency of the progression of the myogenic program. Furthermore, electrophysiological characterization of human wild-type and dystrophic myoblasts/myocytes is on-going.

In conclusion, the better characterization of the ion channels affected by dystrophin loss could be pivotal to further unravel DMD pathophysiology.

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Title

A novel LC-MS method to quantify Cenobamate in plasma samples of young adult patients at the University Hospital of Salerno.

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Background

Cenobamate (CNB) is the newest antiseizure drug (ASD), approved by FDA in 2019, to reduce uncontrolled partial-onset seizures in adult patients. Its mechanism of action has not been fully understood yet; however, it is known that it inhibits voltage-gated sodium channels and positively modulates aminobutyric acid (GABA) ion channel[1]. CNB shows 88% of oral bioavailability and is responsible to modify the plasmatic concentration of other co-administered ASDs, such as lamotrigine and carbamazepine. Indeed, CNB increases the levels of phenytoin, phenobarbital and clobazam active metabolite[2]. It also interferes with CYP2B6 and CYP3A substrates. Nowadays, few methods are reported to dose CNB in human plasma. To date, in the Unit of Child Neuropsychiatry of Salerno University Hospital (Italy), CNB is prescribed on a compassionate basis in young adult patients (over 18 years of age) that do not respond to other antiepileptic therapies.

The aim of this research was first to develop and validate an analytical method using ultra high performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS) for CNB plasma dosage and then to provide a preliminary application of our methodology on clinical samples.

Methods

UHPLC-MS/MS analyses were carried out using an Ultimate 300 UHPLC coupled with a TSQ-Endura ESI-Q-q-Q mass spectrometer (Thermo-Fisher). Multiple reaction monitoring transitions were set to detect CNB and lamotrigine $13C_3$-D_3, used as internal standards (IS). Starting from 50 µL of plasma sample, CNB and IS were extracted using a single step protein precipitation in acetonitrile. Chromatographic separation was achieved on a Kinetex 2.6 µm Pentafluorophenyl (PFP) 100Å (2.1 x 50 mm) column by gradient elution using water and acetonitrile to 0.1% of formic acid as mobile phases.

Results

The method has been validated according to the most recent guidelines[3] and used to measure plasma concentrations of CNB in two young adult patients.

Following the U.S. Food and Drug Administration (FDA) prescribing information the dosage has been initially set at 12.5 mg per day and was increased every two weeks until maintenance dose of 200 mg[4]. Plasma levels were monitored for about two months. Preliminary data showed that a linear increase in plasma CNB concentrations was observed, in both patients, in agreement with the increase in CNB dosage[5]. Despite a relevant difference in plasma level concentrations observed between the two patients (Fig.1), a minimum dose of 50 mg per day lead to a reduction in seizures for both patients. A seizure-free state was reported from both patients at a dosage of 150 mg per day.

Conclusions

Currently, there is a lack of studies on CNB pharmacokinetics in young adult patients in concomitant treatment with other ASDs and benzodiazepines[6]. Our work may allow a more accurate evaluation of the effects of CNB on this population thus assisting the clinician in the decision-making process involving this new ASD.

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Title

Oxytocin regulation of KCC2 in neurodevelopmental disorders

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Background

In the last decade, it has been firmly established that the neuropeptide oxytocin (OT) is released not only in the periphery but also in the central nervous system (1) , where OT acts as a master regulator of the social brain; this has led to propose OT as a drug to ameliorate social deficits in a number of neuropsychiatric conditions. OT has been also shown to regulate key neurodevelopmental events, suggesting that this peptide can modify the onset and progression of these conditions if administered postnatally or in early childhood. To fully exploit the therapeutic effects of OT in neurodevelopmental disorder, we are working to identify the molecular targets and time window of action of OT in the developing brain in different mice models of neurodevelopmental disorders (22q11DS, Magel2, Dysbindin, Oprm1, MeCP2 and Oxtr KO mice). Here we have investigated, in a Rett syndrome model, the action of OT on the expression of KCC2, the K+/Cl- cotransporter involved in the polarity transition of the GABAergic response critical for brain development and maturation. Loss of function mutations in Mecp2 male mice recapitulates many symptoms of Rett Syndrome, including early developmental dysregulation in excitatory-inhibitory (E/I) balance and altered synaptic plasticity. Recombinant human insulin-like growth factor-1 (rhIGF-1) treatment ameliorates the phenotype of MeCP2 KO mice and normalizes the E/I balance by restoring KCC2 expression. Because we have previously shown that KCC2 can also be modulated by OT through its specific receptor OXTR (2), we hypothesized that a IGF-1/OT signaling cross-talk could modulate KCC2 in MeCP2 KO mice.

Methods

To test the possible OT/IGF-1 cross-talk, we mapped and quantified KCC2, OXT receptor (OXTR), and IGF-1 receptor (IGF-1R) levels in the olfactory system and in the hippocampus of MeCP2 KO male mice treated with OT, recombinant human IGF-1 (rhIGF-1), or vehicle and compared their levels to littermate control mice.

Results

MeCP2 KO mice displayed region-specific alterations of IGF-1R and OXTR levels in all the regions analyzed; these changes were accompanied by alterations in KCC2 levels that were also region-specific. Even more strikingly, we found that the effects of OT and GF-1 treatments on KCC2 levels in MeCP2 KO are brain region-specific.

Conclusions

We reveal a region-specific IGF-1 and OT signaling on KCC2 expression that could critically contribute to early brain development (3). The strong region-specificity of KCC2 alterations and of rhIGF-1 and OT effects suggests that region-selective interventions could be designed as innovative therapeutic strategies. This approach will allow normalizing the E/I balance only in key brain regions subtending the RTT symptomatology. Our data also strongly support the hypothesis that the administration of OT can modify specific clinical manifestation observed in neurodevelopmental disorders.

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Title

Glymphatic system and Inflammatory Bowel Diseases: evidence of brain clearance impairment in a mouse model of colitis-induced visceral pain

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Background

Signaling events from the gut can modulate brain functionality through the gut-brain axis. Growing evidence suggests a link between inflammatory bowel diseases (IBDs) and neurodegenerative diseases. Recently, a correlation between neurodegenerative diseases and alterations of the glymphatic system has also been reported. Glymphatic system is an astrocytic AQP4-dependent transport implied in the waste-clearance from the brain parenchyma. An impaired clearance leads to accumulation of neurotoxic products in the brain and therefore contributes to neurodegeneration. The aim of the present study was to highlight the alterations that affect the glymphatic system in an *in vivo* model of colitis-induced visceral pain in mice, to address glymphatic system impairment as a pivotal mechanism of central damage in IBDs.

Methods

Intestinal damage was induced by exposing 8-week-old male C57BL6/N mice to 2.5% DSS solution for 5 days, followed by 3 days of wash out. Visceral pain was assessed by Abdominal Withdrawal Reflex (AWR) and Visceromotor Response (VMR) to Colorectal Distension (CRD). To evaluate glymphatic system alterations, we injected two fluorescent tracers,

Fluorescein-dextran (Fd, 40 kDa) and Texas red-dextran (Trd, 3 kDa), into the cisterna magna of control and DSS-treated animals. Mice were sacrificed 15, 30 and 45 minutes post-injection to collect brain tissues for fluorescence microscopy analysis.

Results

Both AWR and VMR to CRD were significantly higher in DSS-treated animals compared to controls, proving the validity of DSS insult as a model of painful colitis. The fluorescence intensity of the larger tracer, Fd 40 kDa, was significantly reduced in DSS-treated animals, and the reduction was consistent at 15, 30 and 45 minutes. Furthermore, we observed a time-dependent increase of fluorescence intensity both in control and DSS compared to the respective 15 minutes slices, indicating that at 30 and 45 minutes the tracer is in a phase of gradual penetration into the brain parenchyma. Therefore, these results suggest a delay in the penetration of the larger tracer into the brain of DSS-treated animals. The fluorescence intensity of the smaller tracer, Trd 3 kDa, displayed no relevant differences between control and DSS. The size-dependency is an index of glymphatic system involvement, as only larger solutes (40 - 200 kDa) rely on this type of transport.

Conclusions

Our results highlight an impairment of the glymphatic system in an *in vivo* model of visceral pain induced by colitis. Dysfunctionality of glymphatic transport can lead to neurotoxicity due to waste product accumulation into the brain parenchyma, as well as water and ions homeostasis dysregulation, neuronal excitability imbalance and altered signaling along the gut-brain axis.

References

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Title

Paliperidone Palmitate Real World Tolerability and Safety: A Publicly Available EudraVigilance Data Analysis

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Background

Long-acting injectable antipsychotics (LAIs) have proven to be effective in the maintenance treatment of patients with chronic schizophrenia. Paliperidone palmitate (PP) is the only second-generation LAI (SGA-LAI), available in both one- (PP1M) and 3-month (PP3M) formulations. Safety and tolerability profiles often represent a key factor in choosing a LAI [1]. However, real-world studies regarding PP1M and PP3M on this topic are still few and mostly conducted on small samples. In this context, our aim was to better define the safety and tolerability profile of PP, with a focus on PP3M.

Methods

We retrospectively analyzed the Individual Case Safety Reports (ICSRs) data publicly available on EudraVigilance. ICSRs uploaded between 2011 and 2021, presenting PP1M and PP3M as suspected drugs were evaluated. ICSRs relative to at least one SGA-LAI other than PP (i.e., LAI formulations of aripiprazole, olanzapine and risperidone), reported between 2003 and 2021, were also examined as reference group. ICSRs derived from literature or presenting as suspected drugs vaccines were excluded from the analyses. Adverse drug reactions (ADRs) were classified according to the Medical Dictionary for Regulatory Activities (MedDRA). Data were evaluated with a descriptive analysis, then, as disproportionality measures, crude reporting odds ratio (ROR) and 95% confidence interval (CI) were calculated. Chi^2 tests were also performed to compare ICSRs characteristics between PP1M and PP3M.

Results

A total of 7.836 ICSRs met the inclusion criteria, 77.8% (n = 6.095) of those presented as suspected drug PP1M, 21.1% (n = 1.655) PP3M, while 86 cases indicated both PP1M and PP3M. The majority of ICSRs regarded male patients (n = 4.616; 58.9%) with the 18-64 years age group being the most prominent (n = 5.079; 64.8%). A total of 19.048 specific ADRs were observed. After grouping ADRs into System Organ Class (SOC) terms, the most frequently observed one was "Psychiatry Disorders" (n = 2.817; 19,5%) followed by "General disorders and administration site conditions" (n = 2.493; 17,3%) and "Nervous system disorders" (n = 1.867; 12,9%). The disproportionality analysis showed significant values for the SOC "Reproductive system and breast disorders" (ROR = 1.17; 95%CI 1.04-1.30) and for the SOC "Endocrine disorders" (ROR = 1.45; 95%CI 1.21-1.73). The sub-analysis conducted using Chi^2 showed increased frequencies of reporting for PP3M over PP1M regarding the SOCs "Psychiatric disorders" [n = 772 (27.9%) vs n = 2.011 (17.5%); p<0.001] and "General disorders and administration site conditions" [n = 602 (21.8%) vs n = 1.862 (16.2%); p<0.001].

Conclusions

Our analysis indicates that the tolerability and safety profile of PP is in line with what is known for the other SGA-LAIs. However, differences regarding endocrine system ADRs have been noticed. Furthermore, our data seem to point out some variations in the ADRs reporting pattern between PP1M and PP3M.

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pharmadvances

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Title

SGLT2-Is and GLP-1 RAs in T2DM patients: Preliminary Data from An Ordinary Clinical Practice Study

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Background

Type 2 diabetes mellitus (T2DM) remains today one of the main causes of chronic kidney disease (CKD), blindness and cardiovascular (CV) complications [1]. The results of several CV outcome trials in patients affected by T2DM highlighted significantly beneficial effects for two innovative therapeutic options, the glucagon-like peptide 1 receptor agonists (GLP-1 RAs) and the sodium-glucose cotransporter 2 inhibitors (SGLT2-Is) [2]. The consequently expected increase in the use of these drugs could bring major changes in their prescriptive patterns. Considering this, our objective was to evaluate T2DM patient's characteristics when treated with SGLT2-Is and GLP-1 RAs in an ordinary clinical practice setting.

Methods

Starting from January 2022, patients followed by the unit of Internal Medicine of A.O.U. Policlinico G. Martino, with a diagnosis of T2DM, and initiating a therapy with SGLT2-Is or GLP-1 RAs were included into the study. Data related to patient's characteristics, therapy regimen, clinical and laboratory examinations were collected at the time of treatment initiation and regularly thereafter. Data were evaluated with a descriptive analysis and reported as means with standard deviations (SD) and absolute frequency and percentages, for continuous and categorical variables, respectively.

Results

To date, 75 patients have been enrolled into the study. Of them, the majority were males (n=46; 61.3%) with an

observed mean age of 65.7 years ± 8.7 SD. Most patients (n = 47; 62.7%) were treated with GLP-1 RAs, while, 21 (28%) with SGLT2-Is and 7 patients with both an GLP-1 RA and a SGLT2-I. CV risk was evaluated in accordance with the EASD/ESC guidelines [3], with 85.1% of GLP-1 RAs patients showing a very high CV risk (n = 40), 12.8% a high CV Risk (n = 6) and one patient showing a low CV risk. For SGLT2-Is, 20 patients (95.2%) had a very high CV risk and one a high CV risk, while all GLP-1 RAs + SGLT2-Is patients had a very high CV risk. The mean pre-treatment glycated haemoglobin (HbA1c) values were of 7.4 mmol/mol ± 1.2 SD for the GLP-1 RAs group, 8.2 mmol/mol ± 1.2 SD for the SGLT2-Is one and 8.9 mmol/mol ± 1.3 SD for the GLP-1 RAs + SGLT2-Is group. In patients who had already completed 3 months of therapy (n = 41) mean HbA1c values of 7.0 mmol/mol ± 0.9 SD; 8.4 mmol/mol ± 1.2 SD and 8.4 mmol/mol ± 0.6 SD were observed for the GLP-1 RAs, SGLT2-Is GLP-1 RAs + SGLT2-Is groups, respectively. Of these patients, 9 (32.1%) out of those treated with GLP-1 RAs (n = 28) were below their therapeutic HbA1c targets as well as one of those treated with SGLT2-Is (n = 9) and one of the 4 treated with a combination therapy.

Conclusions

These preliminary data seem to suggest some differences in terms of CV risk status and therapeutic target achievement between the examined patient groups. However, more data are necessary to draw any firm conclusion and to further define the efficacy, safety, and tolerability profile of these drug classes in this setting.

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Title

Cohort Event Monitoring of COVID-19 Vaccine safety in Special Cohorts in Italy

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Background

Marketed COVID-19 vaccines showed an overall favourable safety profile in pivotal trials. However, those trials did not include high risk/fragile patient categories; hence, close monitoring of COVID-19 vaccine safety in those special cohorts in real-world setting is needed. The European Medicines Agency-funded "Covid-Vaccine-Monitor" project was set up to prospectively measure the incidence of solicited/unsolicited adverse events following COVID-19 vaccination in several European Countries as reported by vaccinees through web-based questionnaires. Italy is one of the Countries participating in the project as a large network named "ilmiovaccinocovid19 collaborating group". The aim of this study was To measure the incidence of vaccinee-reported adverse events following immunization (AEFIs) with marketed COVID-19 vaccines in pregnant and lactating women, pediatrics, immunocompromised, people with history of allergy and people with prior SARS-CoV-2 infection in Italy.

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Methods

We performed a prospective cohort study of the above-mentioned categories of vaccinees, who were recruited at multiple vaccination centers within 48 hours from either the first or booster COVID-19 vaccine dose administration. After providing informed consent and get registered into the web-app, vaccinees were asked to fill in an electronic baseline questionnaire and 6 follow-up questionnaires at different time points, within 6-month from vaccine administration, in which information on potential vaccine-related AEFIs were collected. We described and compared the frequency of local and systemic solicited adverse events following first or booster dose for each special cohort and vaccine brands.

Results

Overall, 1,331 vaccinees (40.4% first dose and 59.6% booster dose) were included in the analysis. Of these, 8.4% were immunocompromised, 32.0% had history of allergy, 25.5% had prior SARS-CoV-2 infection, 23.4% were children/adolescents, 6.2% were pregnant and 4.6% were lactating women (non-mutually exclusive cohorts). Of subjects belonging to at least one cohort, 52.3% reported at least one AEFI following either the first or booster dose. Among all special cohorts, injection site pain was the most frequently reported solicited local AEFI, after both the first (37.5%) and booster (39.5%) dose. As solicited systemic AEFIs, headache (19.0%) was the most frequently reported after the first dose, while malaise (23.7%) and fatigue (23.7%) after the booster dose. People with history of allergy, with prior COVID-19 and lactating women reported overall the highest reactogenicity following both first and booster dose as compared to other special cohorts. The frequency of severe AEFIs was very low following both first dose (0.8%) and booster dose (0.6%).

Conclusions

Overall, this study confirmed the favourable safety profile of COVID-19 vaccines also in the above-mentioned special categories who have not (or marginally) been included in pivotal trials.

References

N/A

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Title

ALDH1A1 OVEREXPRESSION PROMOTES TUMOR ANGIOGENESIS BY ACTIVATING IL-8/NOTCH SIGNALING

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Background

Aldehyde dehydrogenase 1A1 (ALDH1A1) is a cytosolic enzyme upregulated in tumor cells, involved in detoxifying cells from reactive aldehydes and in acquiring resistance to chemotherapeutic drugs. Its expression correlates with poor clinical outcomes in cancers, including melanoma[1]. In melanoma, progression and resistance to therapies are driven by the close interplay among tumor microenvironment (TME) cells and plasticity of cancer cells[2]. In particular, the communication between tumor cells and the endothelial cells (ECs) is essential in regulating angiogenesis and instrumental for the spread of metastasis. Previously, we demonstrated the involvement of ALDH1A1 in regulating tumor angiogenesis in preclinical model of breast cancer[3]. The present study investigated the link between ALDH1A1 expression in melanoma cells and the acquisition of pro?angiogenic phenotype of normal endothelium as component of TME.

Methods

A375 and WM?266?4 melanoma cells silenced for ALDH1A1 (ALDH1A1KD) or overexpressing ALDH1A1 (ALDH1A1+) were used. In a transwell apparatus we co-cultured melanoma cells and endothelial cells (HUVEC), with the medium between compartments being diffusible. Under these conditions we measured HUVEC proliferation, migration (scratch assay), tube formation and permeability.

3D multicellular system, obtained co?culturing melanoma cells with stromal cells, including ECs, was performed to study the recruitment of ECs. To evaluate the angiogenic factors released from melanoma cells harbouring different level of ALDH1A1, a cytokines plate array was performed. By using a genes array, modulation of Notch cascade gene expression in ECs cocultured with melanoma cells was investigated. Finally, *in vivo* melanoma cells-derived xenografts were used to study microvessel density.

Results

We showed that the co-culture with melanoma cells overexpressing ALDH1A1 promoted in ECs functions related to angiogenesis. These were associated with a significant increase of proangiogenic factors release from melanoma cells, including IL-8. Addition of IL-8 neutralizing antibody to the exposure blunted these effects. In the 3D multicellular system, melanoma cells overexpressing ALDH1A1 induced the recruitment of ECs into the core of the tumorspheres. Notch cascade gene expression in ECs from 3D multicellular spheroid revealed a rearrangement of this pathway, dependent by expression of ALDH1A1 in melanoma. Finally, *in vivo* when subcutaneously implanted in immunodeficient mice, melanoma tumors overexpressing ALDH1A1 displayed a higher microvessel density.

Conclusions

The present study demonstrates the existence of a relationship between ALDH1A1 expression in melanoma and tumor angiogenesis, mediated by the release of several pro?angiogenic mediators, including the chemokine IL?8.

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Title

rhNGF enhances the regenerative processes in a zebrafish model of retinal degeneration

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Background

Nerve growth factor (NGF) is the best-characterized neurotrophin and it is known to play an important role in ocular homeostasis[1]. In fact, both NGF and its receptors TrkA and p75 are expressed in ocular tissues[2] and are crucial in physiologic and pathologic conditions of the eye. Here we developed a retinal degeneration/regeneration paradigm in zebrafish adults based on constant light irradiation. We used these approaches to assess the potential effect of intravitreal administration of recombinant human NGF on the recovery of damaged retinae.

Methods

The expression of p75 and TrkA was analyzed by whole-mount in situ hybridization (ISH) in adult zebrafish. The severity of the retinal injury and its recovery rate were analyzed through zpr1 and PCNA immunostaining in cross-sections of enucleated eyes. The effects of rhNGF treatment on down streaming pathways were analyzed by RT-PCR and western blot.

Results

Adult zebrafish retinae exposed to 60 hours of light irradiation (60 h LID) displayed an evident reduction of outer nuclear layer (ONL) thickness and cell number with the presence of apoptotic cells. Retinal histologic evaluation at different timepoints showed that IV therapeutic injection of rhNGF resulted in an increase of ONL thickness and cell number at late timepoints after damage (14 and 21 days post-injury), ultimately accelerating retinal tissue recovery by driving retinal cell proliferation. At a molecular level, rhNGF activated the ERK1/2 pathway and enhanced the regenerative potential of Müller glia gfap- and vim- expressing cells by stimulating at early timepoints the expression of the photoreceptor regeneration factor Drgal1-L2.

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Conclusions

Our results demonstrate the highly conserved nature of NGF canonical pathway in zebrafish and thus support the use of zebrafish models for testing new compounds with potential retinal regenerative properties. Moreover, the pro-regenerative effects of IV-injected NGF that we observed open the way to further studies aimed at evaluating its effects also in mammals, in order to expedite the development of novel rhNGF-based therapeutic approaches for ophthalmological disorders.

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Title

Autophagy inhibitors PIK-III and SAR405 sensitize resistant cancer cells to cisplatin treatment

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Background

Despite the common use of Cisplatin (CDDP) to treat a multitude of tumors including sarcomas, ovarian and cervical cancers [1], its clinical efficacy is severely limited by the frequent onset of resistance phenomena, whose molecular mechanism and not fully elucidated. Mitochondria solve a central role in cellular physiology being implicated in metabolic processes and cell apoptosis and death. Mitochondria-dependent apoptosis is counterbalanced by their selective degradation via autophagy, a process called mitophagy (mitochondrial selected autophagy) [2], possibly furnishing an escape route to cell death induced by stressful conditions such as chemotherapy cytotoxicity [3]. In this work, we identify BNIP3 (BCL2/adenovirus E1B 19 kDa interacting protein 3) as a key factor implicated in cisplatin resistance and we show that inhibition of mitophagy can be a promising strategy to counteract platinum resistance.

Methods

Ovarian carcinoma (2008) sensitive and (C13) cisplatin-resistant cells and osteosarcoma U2OS and U2OS-PT cells were used as a cell model of platinum resistance. Cell viability was measured by Trypan Blue exclusion assay. Immunofluorescence and TEM have been used to evaluate mitochondrial morphology and shape. RT-PCR and Western blot analysis furnishes mRNA and protein expression evaluation. Mitophagy flux by using mito-keima imaging has been evaluated by using Operetta® High Content Imaging System.

Results

Results shows that in cisplatin-resistant cells, mitochondria are more fragmented, presenting a higher level of fission proteins. Fragmented mitochondria are usually targeted for degradation by autophagy, and in line with this, resistant clones present higher levels of bona fide autophagosome cargoes LC3 and p62 increase in flux of autophagy and higher expression of one of the main mitophagic regulators. Overexpression of BNIP3 has been confirmed in ovarian cancer patient samples platinum-resistant and by a bioinformatic analysis in ovarian patients. The downregulation of BNIP3 in cells further supports its role in mediating mitophagy in cisplatin resistance, being able to reduce the mitophagy flux induced by cisplatin specifically in resistant cells. In the end, it has been demonstrated that BNIP3 silencing, per se, is sufficient to chemosensitize resistant cells to platinum treatment and that pharmacological approach by using a combination of cisplatin and autophagy inhibitors PIK-III and SAR405, induced a significant reduction of cell survival and cell colony formation[4].

Conclusions

In this work, we highlight that BNIP3-dependent mitochondrial autophagy is exploited by resistant cells to escape CDDP cytotoxicity. Data indicate that BNIP3, and more in general mitophagy, can be exploited as a target for the development of new therapeutical strategies to counteract cisplatin resistance.

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Title

Environmental monitoring for the evaluation of professional exposure to antineoplastic agents in healthcare workers of the University Hospital San Giovanni di Dio e Ruggi d'Aragona.

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Background

Handling of antineoplastic agents (AA) in healthcare settings is associated with severe health risks, due to carcinogenicity, mutagenicity, teratogenicity and reproductive toxicity[1]. The number of healthcare workers potentially exposed to antineoplastic drugs is growing, as the use of these molecules for treating non-malignant diseases has also recently increased. It is therefore crucial for healthcare professionals to validate the efficiency of safety procedures by periodic biological and environmental monitoring[2]. Here, we describe a Wipe sampling protocol for UHPLC-MS/MS analysis for the environmental monitoring of six antiblastic compounds including methotrexate (MTX), cyclophosphamide (CFA), ifosfamide (IFA) doxorubicin (DXR), irinotecan (IRT), paclitaxel (PTX). Analytical method was optimized and validated following the current guidelines[3], then used for the environmental monitoring of the units for cytotoxic drug preparation (UCDP) at the University Hospital "San Giovanni di Dio e Ruggi d'Aragona" in Salerno (Italy)

Methods

UHPLC-MS/MS analyses were carried out using an Ultimate 3000 UHPLC coupled with a TSQ-Endura ESI-Q-q-Q mass spectrometer (Thermo-Fisher) in Single Reaction Monitoring transitions mode. Surface sampling kit was a prototype

kindly provided from Matox S.r.l. (Salerno, Italy). The sampling operations were carried out according to manufacturer, on the monitored surfaces, over an area of ??3x3 dm^2

Results

The method was used to evaluate possible contamination affecting different working areas in the UCDC[4]. Two working rooms were examined: the laboratory and the anteroom. Several samples were withdrawn from different potentially contaminated surfaces in both rooms. The first cycle of analyses showed that significant amounts of CFA and IFA were present on the two hood workstations, even after the surfaces standard cleaning procedures, thus highlighting that these were largely ineffective. After these first campaign, the laboratory and the anteroom were deep cleaned and the working procedures at the UDPC were reorganized based on the criticisms shown by the environmental monitoring. In addition, the efficacy of different surface cleaning methods was evaluated and two washing approaches and three different removal supports were tested. Both organizational and cleaning modifications concurred in gradually decreasing AA concentrations on all work surfaces, as evident from following monitoring campaigns

Conclusions

Our experience confirm the need to monitor workplaces in healthcare facilities, where potentially toxic substances for exposed workers are handled. The analysis of the surfaces highlighted criticism both in the manipulation and cleaning operations. Corrective actions, based on the results obtained with the environmental monitoring of the working surface areas, have led to a decrease of the residual AA levels on the surfaces[5],thus providing a healthier and safer work environment

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Title

Resistance to immunotherapy in advanced non-small cell lung cancer patients does not correlate to the development of anti-Drug Antibodies

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Background

Immunotherapy has revolutionized the treatment landscape of non-small cell lung cancer (NSCLC). The expression of an immune checkpoint, such as programmed cell death protein ligand 1 (PD-L1), can be targeted by the administration of immune checkpoint inhibitors (ICIs), such as monoclonal antibodies (mAbs), which block PD-1-related immunosuppression (1). However, a consistent proportion of PD-L1 positive patients are not responsive to the treatment due to primary and acquired resistance (2). The aim of this research was to investigate whether NSCLC patients receiving Pembrolizumab developed anti-drug antibodies (ADAs) impacting on drug efficacy and effectiveness.

Methods

Advanced (non-resectable, stage IV) NSCLC patients treated with Pembrolizumab monotherapy (n=7) or Pembrolizumab plus chemotherapy (pemetrexed and platinum-based agents) (n=31) were considered. ADAs levels were measured in serial plasma samples collected at baseline (T0) and every cycle (Tn) until progression disease (PD) and correlated with progression-free survival (PFS). Moreover, the levels of IgG1 and complement complex C3a were analyzed.

Results

Advanced NSCLC patients treated with Pembrolizumab monotherapy did not develop ADAs. Instead, patients who were treated with Pembrolizumab plus chemotherapy showed increased ADA levels at the first (T1) and at the second (T2) cycle of treatment compared to the baseline (T0) (19 out of 24 patients, 79.2%). Interestingly, patients treated with Pembrolizumab plus chemotherapy, who showed an increase of ADAs at T1, had lower survival rate than those who did not develop ADAs at T1. In order to understand the nature of drug-induced immunoglobulin developed by these patients at T1, we performed ELISA assays. IgG1, but not IgG4, levels were significantly increased at T1 in both patients

treated with Pembrolizumab alone (6 out of 7, 85.7%) or Pembrolizumab plus chemotherapy (20 out of 31, 64.5%). Surprisingly, patients treated with Pembrolizumab plus chemotherapy showed a significant reduction of the circulating levels of the complement C3a at T1 and PD (79.2% and 75% of total, respectively) compared to the levels at T0. Patients treated with Pembrolizumab plus chemotherapy, who showed a decrease of C3a levels at T1, had lower survival rate than those who did not. Instead, patients treated with the sole Pembrolizumab had a tendency of increase of C3a levels, which was associated to lower survival rate.

Conclusions

No patients had as such levels of ADA as expected after treatment with the sole Pembrolizumab. Instead, among patients treated with Pembrolizumab plus chemotherapy, 79,2% had higher titre of ADAs at T1, that correlated to lower survival rate than those who had lower levels. These data suggest that neutralizing antibodies occur at early time points, but more studies are needed to understand their activity on long-term clinical outcome.

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Title

Benzodiazepine dependence: A new silent pandemic

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Background

Benzodiazepine (BDZ) represent the most prescribed and used pharmacological class in Italy and currently they are still considered a good option for the treatment of both acute and chronic anxiety disorders, although recent reviews have shown that SSRIs and SNRIs should be considered as first choice in the treatment of these conditions. However, despite the high therapeutic index, prolonged use of BZD is strongly correlated with cognitive deficits, road and domestic accidents, work injuries and the onset of addiction.

Methods

We performed a literature research on PubMed using the keywords “benzodiazepine dependence”, “benzodiazepine withdrawal” and “benzodiazepine detoxification”.

Results

Clinically we can identificate four patterns of consumption: 1) episodic use that lasts 2-4 weeks in acute anxiety and insomnia disorders, the only correct indication for these drugs; 2) prolonged use beyond the recommended limits but at therapeutic doses (Long Term Users, LTU), that is often related to an high risk of addiction; 3) occasional abuse of high doses, that frequently involves subjects addicted to illicit drugs and alcohol; 4) prolonged use of excessively high doses (High Dose Users-Long Term Users, HDU-LTU).
The gold standard of LTU detoxification is to convert a short or medium halflife BDZ (in Italy especially Lormetazepam, Alprazolam and Loazepam), to a long half-life one, like Diazepam. Subsequently, according to the clinical presentation, a gradual suspention of the drug is made. At this stage, it may be useful to insert an SSRI or SNRI or a sedative drug like Mirtazapine, Amitriptyline or Trazodone into therapy. Frequent clinical evaluations using CIWA-B scale are essential to dictating the timing of detoxification and psychological support and cognitive behavioral therapy are important are important both in the initial stages and after the cessation of use. Prognosis with a slow detoxification is generally good, with two-thirds of patients reaching full cessation. Unfavorable prognostic factors are: previous failed attempts, lack of caregivers, incorrect clinical management, alcohol dependence, depression and personality disorders.

Conclusions

Benzodiazepine dependence represent a serious public health problem due to increased prescriptions and erroneous indications of use: knowledge of this pandemic and its clinical management represent a new challenge for the clinicians.

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Title

The potent alpha$_2$-adrenoceptor antagonist RS 79948 also inhibits dopamine D$_2$-receptors

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Background

Alpha2-adrenoceptors antagonists have been successfully used as adjunctive therapy to improve the pharmacotherapy of antipsychotic drugs. The beneficial effect results in their ability to enhance dopamine (DA) output and transmission in the prefrontal cortex (PFC), rescuing the dopaminergic hypoactivity that typically characterizes the negative symptoms of schizophrenia. However, the mechanism by which alpha2 receptor blockade leads to increased DA output in the PFC is debated. Our laboratory provided evidenced that noradrenergic terminals are the primary source of alpha2-mediated DA release in the mPFC, as noradrenergic denervation suppressed the increase of DA induced by the selective alpha2 antagonist atipamezole. RS 79948 is classed among the most potent and selective alpha2-adrenoceptor antagonists, with negligeable affinity for alpha1- adrenoceptors. However, differently from atipamezole, we found that the RS 79948-induced elevation of extracellular DA was not abolished after noradrenergic denervation. This finding led us to investigate whether RS 79948 might induce DA release other than from noradrenergic also from dopaminergic terminals, this property being disclosed after noradrenergic denervation, as in the case of D2-receptor inhibitors.

Methods

Receptor binding, microdialysis, electrophysiological and behavioral studies were used to assess whether RS 79948 inhibits D2- other than alpha2-adrenoceptors. We compared the effect of RS 79948 with that of atipamezole and raclopride in tests sensitive to alpha2 and D2 receptors modulation, to evaluate how its double action would interfere with noradrenergic or dopaminergic-system dependent behaviors.

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Results

Ligand binding and adenylate cyclase activity indicate that RS 79948 binds to D2 receptors and antagonized D2 receptor-mediated inhibition of cAMP synthesis at nanomolar concentrations. Results from microdialysis confirmed that RS 79948 releases DA also from dopaminergic terminals, as similarly to the D2 antagonist raclopride, but unlike atipamezole, RS 79948 increased extracellular DA and DOPAC in the caudate nucleus. Electrophysiological results indicate that this compound shared with raclopride the ability to activate the firing of VTA DA neurons, while atipamezole was ineffective. At the behavioral level, RS 79948, but not atipamezole, prevented D2-autoreceptor mediated hypomotility produced by a small dose of quinpirole; it potentiated, more effectively than atipamezole, quinpirole-induced motor stimulation, and antagonized, less effectively than atipamezole, raclopride-induced catalepsy.

Conclusions

Our results indicate that RS 79948 inhibits other than alpha2- also D2 receptors and modulates cortical and striatal functions in opposite, independent and cooperative manner. Future studies should clarify if the dual alpha2- and D2-receptor antagonistic action might endow RS 79948 with potential therapeutic relevance in the treatment of schizophrenia, drug dependence, depression, and Parkinson’s disease.

References

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Title

Investigating the role of calcium homeostasis and SOCE alterations in sarcopenia: potential benefits of supplementation with BCAA-based formulation in aged mice

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Background

Alterations in Ca^{2+} homeostasis and store-operated-calcium-entry (SOCE) without a change in the mRNA expression of STIM1/Orai1, the main proteins involved in SOCE mechanism, have been proposed in sarcopenia and muscle weakness during aging (1). However, other factors may be of physiological importance in the SOCE mechanism in skeletal muscle. In particular, Mitsugumin29(MG29), a muscle synaptophysin-related protein, seems to be involved in Ca^{2+} signaling because MG29 ablation leads to dysfunctional SOCE in neonatal skeletal muscle (2). Furthermore, Mitsugumin53(MG53), a tripartite motif (TRIM)-family protein involved in cell membrane injury repair, could play roles in muscle Ca^{2+} movements (3). We recently demonstrated that branched-chain amino acids (BCAAs), which regulate metabolism and protein turnover, ameliorated muscle mass and function in a mouse model of atrophy (4,5). On this basis, our study aims to evaluate the mechanisms underlying Ca^{2+}-related alterations of aged muscle, and to assess the potential benefit of BCAA-based diet supplementation.

Methods

17-months-old male C57BL/6J mice received a 12-weeks-treatment introducing BCAAs alone, or boosted with two equivalents of L-Alanine(2-Ala) or with dipeptide L-Alanyl-L-Alanine(Di-Ala), in drinking water (4, 5). Aging/treatment outcomes were evaluated *ex vivo* on calcium homeostasis using Fura2 cytofluorimetry and on the expression of genes and proteins involved in SOCE compared to adult 6-months-old male C57BL/6J mice.

Results

Ca^{2+} imaging analysis performed on FDB muscles confirmed an increase in resting Ca^{2+} concentration accompanied by a decrease in SOCE activity in aged *vs* adult mice. Interestingly, gene expression analysis performed on gastrocnemius muscles confirmed no change in STIM1/Orai1/TRPC1 mRNA levels, while unexpectedly we found a significant MG29/SERCA1/Ryr1 reduction together with an increase of MG53 in aged *vs* adult mice. All observed alterations were restored by BCAAs+2-Ala treatments. MG29 reduction, MG53 increase, and BCAAs+2-Ala restoration were confirmed by western blot analysis. Interestingly, we also found a reduction in the protein expression of TRPC3, a non-selective sarcolemmal/t-tubule cation channel that interacts with MG29, which was restored by BCAA treatments.

Conclusions

In aging**,** Ca^{2+} homeostasis and SOCE dysregulation result from dysfunction of signaling downstream of the known SOCE components, pointing towards MG29/MG53 which may be proposed as promising therapeutic targets. In this frame, the beneficial effects of BCAAs highlighted their interest in counteracting the sarcopenia-related alterations.

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Title

Evaluating the disease activity of rheumatoid arthritis patients at initiation and discontinuation of biologic DMARDs in Tuscany

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Background

Rheumatoid arthritis (RA) clinical guidelines recommend starting biologic disease-modifying anti-rheumatic drugs (bDMARDs) upon uncontrolled disease and tapering or discontinuing bDMARDs upon remission.[1,2] In a previous investigation on drug utilization of bDMARDs, most patients belonged to the continuous users' trajectory involving the fluctuations from full to no coverage.[3] This study is aimed at evaluating the disease activity associated with the bDMARD initiation and discontinuations.

Methods

The PATHFINDER study (EUPAS29263)[4] is a retrospective population-based study carried out on data collected in the Tuscan administrative healthcare database (TAHD). We included the same sample selected from TAHD and used for the validation analysis,[5] RA patients having a first-ever bDMARD dispensation (index drug) and a RA visit at the Pisa University Hospital between 2013 and 2016. Information about the Disease Activity Score 28 (DAS28) was retrieved from the medical charts, and linked with data on drug supplies obtained from TAHD. In the 3 years follow-up period,

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discontinuations were identified by the end of bDMARD coverage, measured on the basis of the Defined Daily Dose, and the number of doses supplied plus a grace period of 60 and 30 days in the main and sensitivity analyses, respectively. The DAS28 recorded before (T0) and after (T1) the first bDMARD dispensation, and that before (TD0) and after (TD1) discontinuations were analyzed. The “off-target” or “in-target” disease was classified by DAS28>3.2 and DAS28≤3.2, respectively. We tested for differences patients with at least 3 DAS28 available and those without, patients with at least one discontinuation and those continuing among patients with at least 3 DAS28 assessments. Finally, we described the disease activity trends at bDMARD initiation and at discontinuation events in patients with DAS28 recorded at both T0 and T1 and at TD0 and TD1, respectively.

Results

We included 95 RA patients initiating bDMARDs. Females were 73 (76.8%), and the mean age was 59.6 standard deviation, SD, 12.1. In the main analysis, among 70 patients with at least 3 DAS28 values available, we estimated 91 discontinuations and 33 patients (47%) had at least one discontinuation also. An off-target condition was observed in 28 patients (40%) at T0, while the in-target RA was found in 38 (54%) at T1. When DAS28 was recorded both at T0 and at T1 (37 patients), disease stability or improvement was displayed in 31 patients (84%). As regards discontinuations, an in-target RA was found at TD0 in 38 events (42%) and at TD1 in 32 (35%). By focusing on the 37 discontinuations with both DAS28TD0 and DAS28TD1, 28 (76%) showed disease improvement or stability. The sensitivity analysis confirmed these results.

Conclusions

This study pointed out that both initiation and discontinuations of bDMARDs occurred in line with that recommended by RA clinical guidelines.

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Title

A Palmitoylethanolamide Producing *Lactobacillus paracasei* Improves *Clostridium difficile* Toxin A-Induced Colitis

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Background

Clostridium difficile infection (CDI) represents the leading cause of nosocomial diarrhea, which induce disruption of gut resident flora. The bacterial virulence depends on releasing two exotoxins, *C. difficile* toxin A and B (TcdA and B), involved in epithelial barrier disruption, inflammation, and neutrophil infiltration [1].

Palmitoylethanolamide (PEA) is an endogenous molecule involved in the regulation of several physiological processes, including analgesia, neuroprotection, and inflammation. PEA is safe and virtually free from side effects but requires high doses to produce significant effects [2]. To overcome this limitation, we developed a probiotic-based delivery system, with an engineering probiotic *Lactobacillus paracasei subsp. paracasei* F19 with the human NAPE-PLD gene (NAPE-LP) enzyme involved in the synthesis of PEA in order to achieve an in-situ delivery of PEA, under the boost of exogenous palmitate [3].

The aim off this study was to explore the efficacy of a newly designed PEA-producing probiotic (pNAPE-LP) in a mice model of *C. difficile* toxin A (TcdA)-induced colitis.

Methods

Wild type and PPARα KO mice received a daily prophylactic gavage administration of either pNAPE-LP (10^9 CFU) or pLP (10^9 CFU) 200 µl suspensions with sodium palmitate (0.0003 µg/ml). At day 7, animals received a single intrarectal instillation of TcdA (50 µg/ml). Animals were euthanized 4 h later and PEA quantification and other molecular/histological analyses were thus carried on postmortem isolated colonic tissue or related samples.

Results

- pNAPE-LP under palmitate dose has released PEA level peaked at 12 h; NAPE-PLD protein expression increased in a time-dependent manner in pNAPE-LP bacteria *in vitro.* pNAPE-LP and palmitate increased PEA release and the expression of NAPE-PLD *in vivo*, after TcdA challenge, in WT and PPARα KO mice.

- pNAPE-LP and ultralow dose of palmitate, improved the histological damage score with a reduction of macrophage and neutrophils infiltration in WT but failed in PPARα KO mice.

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- pNAPE-LP and palmitate have reduced expression of pro-inflammatory signaling molecules induced by TcdA challenge in WT, no significant changes were detected in PPARα KO mice.

- pNAPE-LP and ultralow palmitate restored colon-barrier caused by disruption of TcdA toxin exposure, through the up-regulation of ZO-1 and occludin proteins in WT, instead this effect was unmodified in PPARα KO mice.

Conclusions

Our study demonstrates the beneficial effect of the probiotic pNAPE-LP and for the first time the anti-inflammatory effect of PEA in CDI. Therefore, we have shown that pNAPE-LP has a potential therapeutic effect in CDI, inhibiting inflammation of the colon and restoring the expression of proteins at tight junction.

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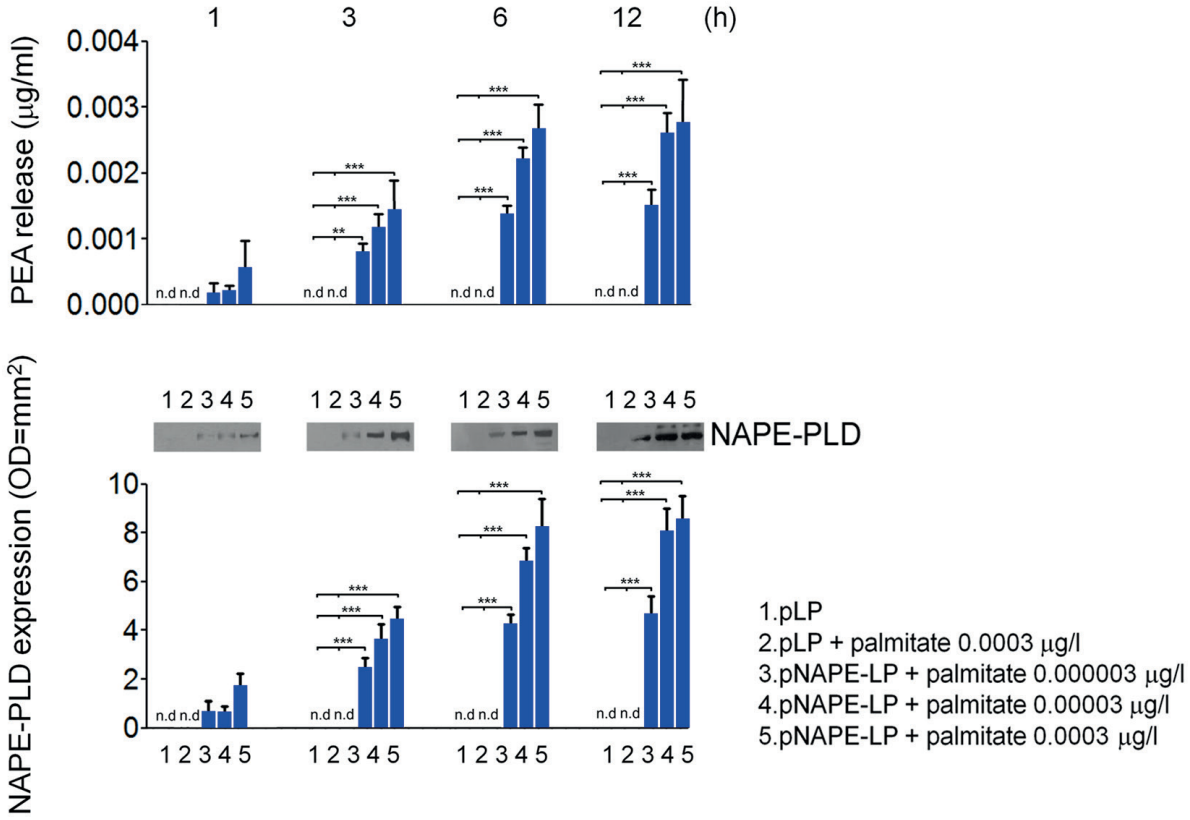
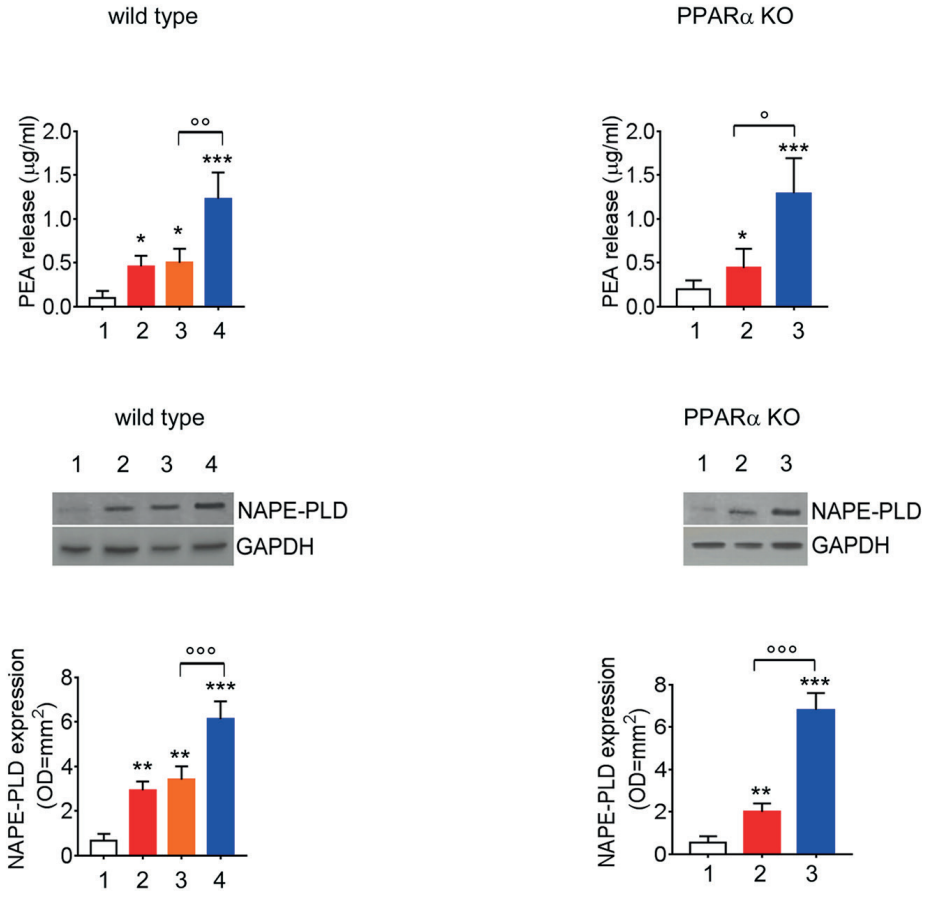
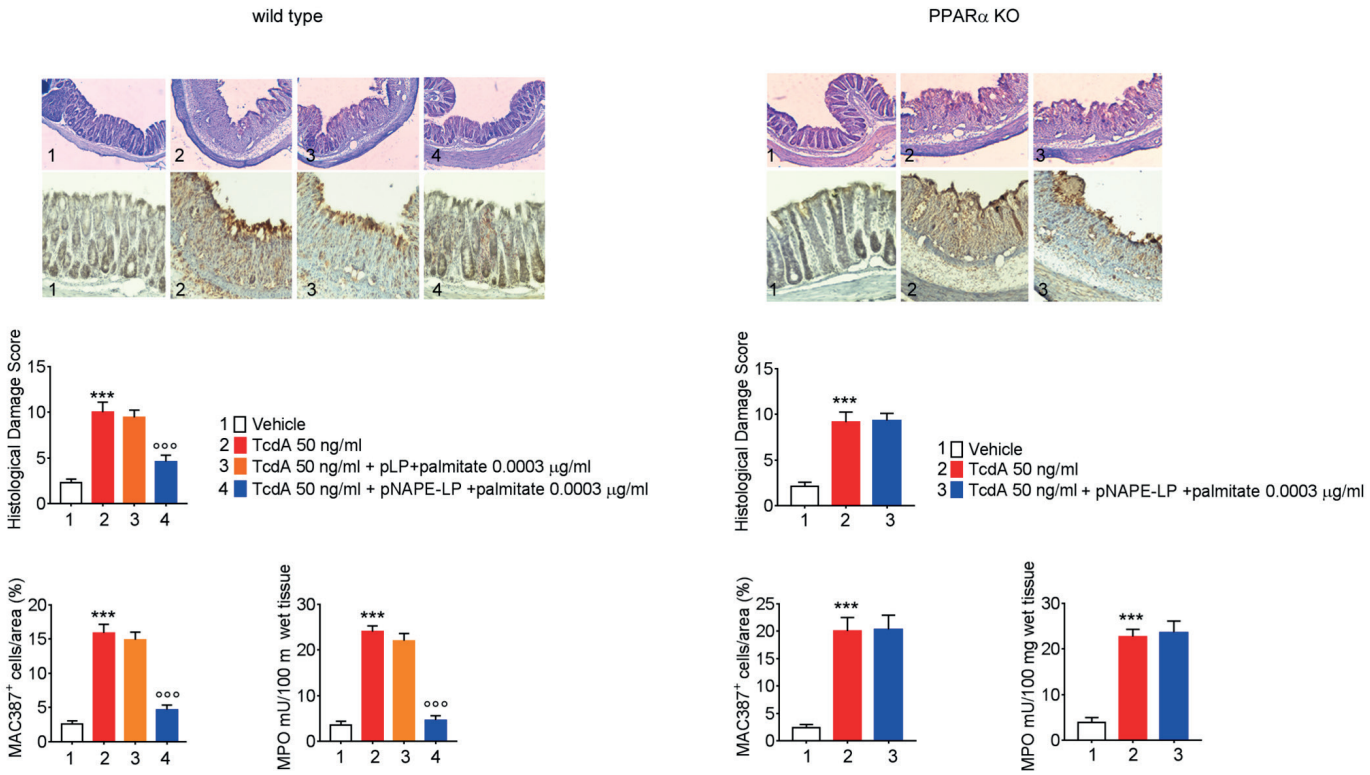
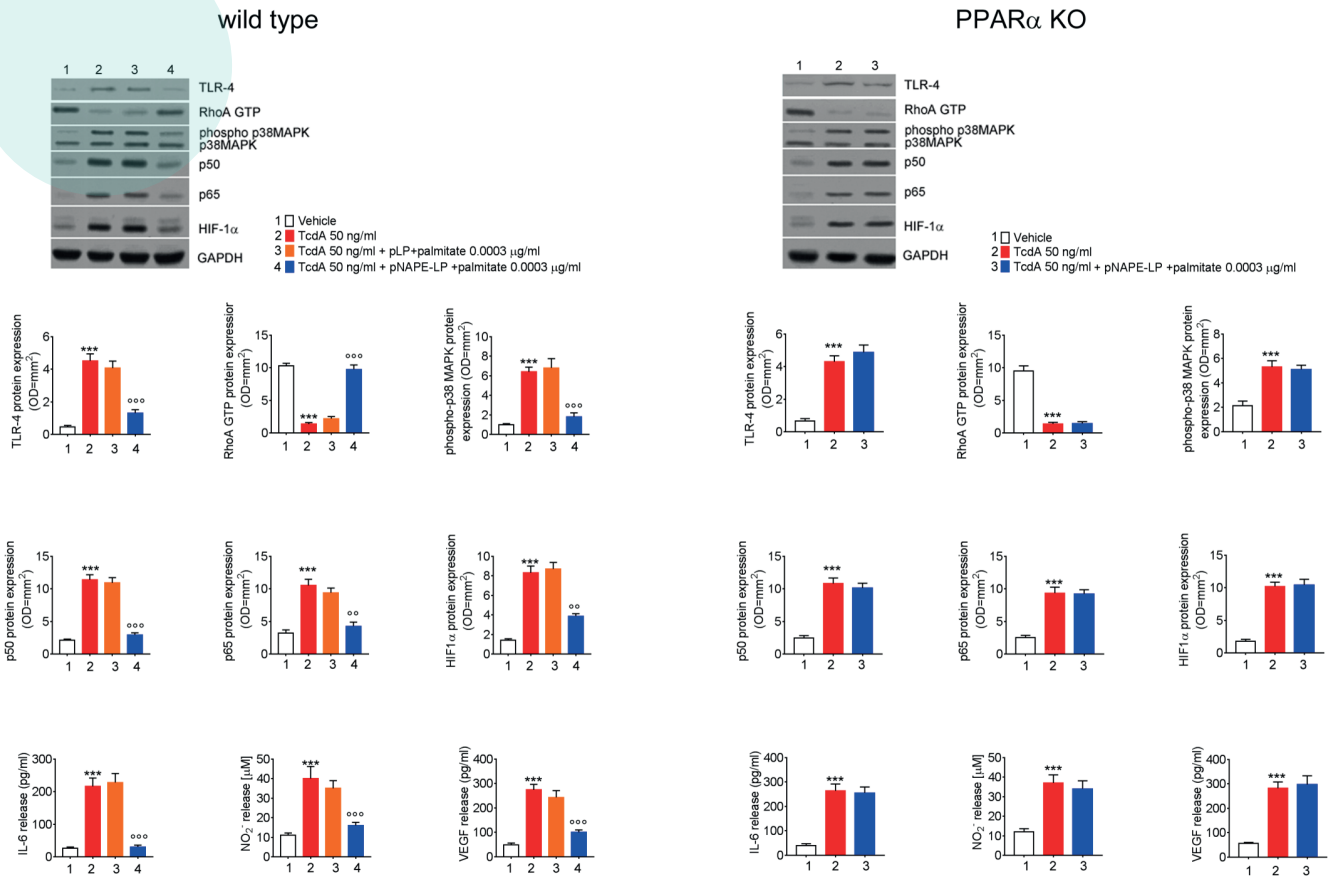
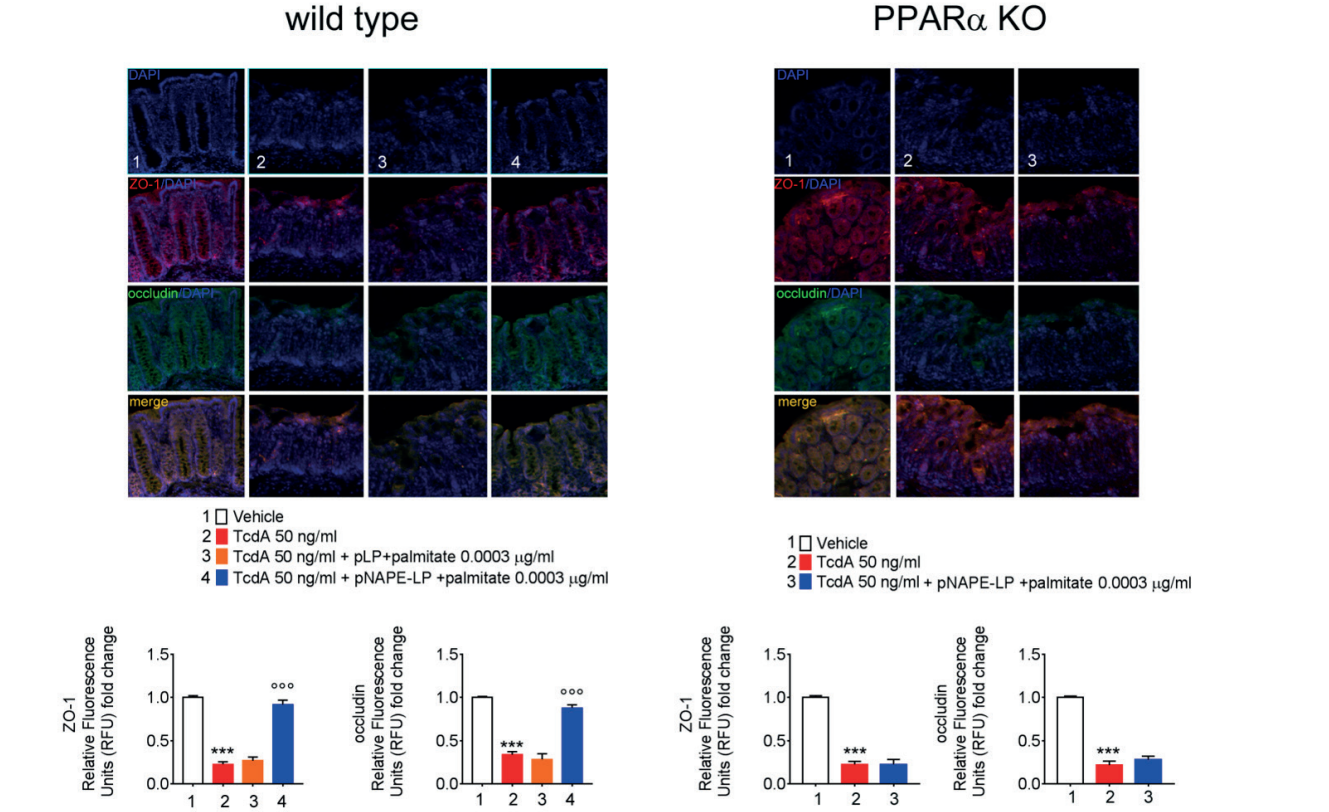







wild type
PPARα KO
TLR-4
RhoA GTP
phospho p38MAPK
p38MAPK
p50
p65
HIF-1α
GAPDH
1 Vehicle
2 TcdA 50 ng/ml
3 TcdA 50 ng/ml + pLP+palmitate 0.0003 μg/ml
4 TcdA 50 ng/ml + pNAPE-LP +palmitate 0.0003 μg/ml
TLR-4 protein expression (OD=mm²)
RhoA GTP protein expression (OD=mm²)
phospho-p38 MAPK protein expression (OD=mm²)
p50 protein expression (OD=mm²)
p65 protein expression (OD=mm²)
HIF1α protein expression (OD=mm²)
IL-6 release (pg/ml)
NO2- release [μM]
VEGF release (pg/ml)


wild type
PPARα KO
DAPI
ZO-1/DAPI
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merge
1 Vehicle
2 TcdA 50 ng/ml
3 TcdA 50 ng/ml + pLP+palmitate 0.0003 μg/ml
4 TcdA 50 ng/ml + pNAPE-LP +palmitate 0.0003 μg/ml
ZO-1 Relative Fluorescence Units (RFU) fold change
occludin Relative Fluorescence Units (RFU) fold change

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Title

Tuberomammillary nucleus histaminergic neurons modulates stress susceptibility and resilience in a murine model of chronic social stress

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Background

Chronic stressors are defined as persistent events which require an individual to make adaptations over an extended period of time. Exposure to stress represents a major condition conferring risk for different psychiatric disorders, characterized by dysfunctions of cognitive, emotional, and social domains[1,2]. However, not everyone who experiences an adverse or stressful event succumbs to negative outcomes and enters a pathological state. Indeed, some individuals are highly vulnerable to the pathological consequences of stress exposure, whereas others appear to be resilient[3]. Neuromodulators linked to susceptibility/resilience have been identified in multiple-stress related brain structures such as limbic areas and the hypothalamus. However, whether different substrates are engaged in the initial response or in more durable resilience/susceptibility responses remains poorly understood. The hypothalamic histaminergic system is critical in modulating the effects of protective agents against stress-induced maladaptive consequences[4,5]. However, our current knowledge of the role of brain histamine (HA) in stress susceptibility and resilience is lacking.

Methods

To understand the involvement of the HA neurons activation or silencing in the development of stress-induced impairments, we used an ethologically valid rodent model of chronic stress, the chronic social defeat stress (CSDS). HA cells were activated or silenced through different techniques: chemogenetic, pharmacological and genetic manipulations. Mice were then subjected to the stress procedure and tested for different behavioural domains.

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Results

We showed that manipulation of HA neurons impacts on the instatement of a specific stress-induced phenotype. HA activation led to the instatement of resilient phenotype, whereas its silencing induced susceptibility.

Conclusions

The results obtained revealed the full implication of the brain HA system in promoting a coping behaviour toward stress-related dysfunctions. This suggests that interventions targeting HA system can promote coping and long-term resilience and ultimately normalizing the affected systems.

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Title

A role for metabotropic glutamate receptors 2/3 in the modulation of BBB function

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Background

The blood-brain barrier (BBB) is a highly specialized structure with the main role of preserving the central nervous system. The endothelial cells at the BBB are organized as a continuous intercellular layer forming tight junctions and are supported by astrocytes, that contribute to specific barrier properties. Recent evidence suggests a role also for microglia in the control of BBB function. In inflammatory conditions, astrocytes and microglia may either affect barrier stability or stabilize endothelial cells at the BBB. The class 2 metabotropic glutamate receptors (mGluRs), mGluR2 and mGluR3, are highly similar Gi coupled receptors, with different cell-type distribution and capable of eliciting different responses, sometimes opposite. Our aim was to evaluate the effects of the modulation of mGluR2 and mGluR3 on BBB proprieties in an in vitro model in inflammatory conditions.

Methods

The BBB in vitro model was constituted by three human cell lines. A human microvascular endothelial cell line (TY-10) was cultured alone or in co-culture with human astrocytes (hAst) or in a triple-culture with astrocytes and a human microglial cell line (HMC3). Cultures were exposed to an inflammatory insult (TNFα, 10UI + IFNγ, 5UI, T&I) in the presence of a mGluR2/3 agonist (LY379268, 1 μM, LY37) and a mGluR2 negative allosteric modulator (NAM; VU 6001966, 3 μM, VU). To investigate BBB properties, the tightness of endothelial junctions was tested by measuring both transendothelial electrical resistance (TEER) and barrier permeability to a dye-conjugated sugar (FITC-dextran). The expression of the junctional protein claudin-5 was evaluated using western blot analysis and immunocytochemistry. Gene expression of inflammatory cytokines and chemokines was evaluated on astrocytes and microglia.

Results

We analyzed the BBB's properties on the three models. Endothelial exposure to T&I caused impairment of barrier properties, with increased permeability to dextran and reduced values of TEER. Claudin-5 expression was reduced and was mostly redistributed in the cytosolic compartment. When cultures were pre-exposed to LY37, the reduction of TEER values and the increase of dextran permeability were partially prevented. In addition, expression of claudin-5 was preserved. All these effects were partially contrasted by the addiction of VU to LY37. In the endothelium/astrocyte co-cultures, LY37 prevented T&I effects on BBB properties, but VU did not significantly modify LY37 actions. In triple-co-cultures, LY37 increased the maintenance of BBB properties, effects prevented in treatment with VU+LY37. In addition, LY37 reduced T&I-induced expression of inflammatory cytokines on microglia cells, and co-treatment with VU contrasted this action.

Conclusions

mGluR3 and mGluR2 protect the BBB functions in the presence of an inflammatory insult, acting directly on the endothelial component and, indirectly, limiting the astrocytic and microglial inflammatory response.

References

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Title

In search of early diagnosis of acromegaly: machine learning on drug prescriptions and other healthcare services

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Background

Acromegaly is a rare endocrine disease characterized by an excessive production of growth-hormone and insulin-like growth factor 1, typically resulting from a GH-secreting pituitary adenoma [1,2]. This study was aimed at developing and validating machine-learning algorithms to detect the diagnosis of acromegaly in Sicilian population using administrative claims databases.

Methods

Cases were defined as those subjects who had claims suggestive of acromegaly in ≥ 2 different databases (i.e., hospital discharge records, co-payment exemptions registry, pharmacy claims and specialist visits/laboratory tests registry). For each identified case, the date of the first occurrence of ≥ 1 suggestive claim was considered as the index date (ID). Cases were matched with up to 10 controls by date of birth and gender, assigning them the same ID of the corresponding matched case. Potential acromegaly predictors were identified by evaluating the number of pharmaceutical prescriptions and specialist examinations recorded within 2 years before the ID (hospital discharge and exemption codes at any time prior to the ID) using a sequence of different machine-learning algorithms, such as the multivariable classical logistic model with “lasso” penality (LASSO), Recursive PArtitioning and Regression Tree (RPART), Random Forest (RF) and Support Vector Machines (SVM). The diagnostic accuracy of each algorithm was evaluated through c-statistic, sensitivity (Se), specificity (Sp), positive and negative predictive values, Youden Index and F-score.

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Results

Among the algorithms providing an estimate of the individual probability of having acromegaly, the one that achieved the highest discriminatory accuracy was the RF (c-statistic: 0.81). Also when forced to produce a binary classification of the individuals (as having acromegaly or not), the RF algorithm yielded the highest discriminatory accuracy (Se = 72.5; Sp = 71.9 and Youden Index = 0.44). Despite its high performance, this algorithm had the considerable disadvantage of not being able to be exploited to identify an optimal classification rule, but only a list of the variables mostly contributing to the prediction of the diagnosis. At present, the number of the most important predictors was greater than 40. In contrast, the LASSO model identified a minimal set of 10 predictors, including dispensing of: drugs for acid related disorders, cabergoline, thyroid therapy, antineoplastic agents, immunosuppressants and anti-inflammatory drugs and the following specialist examinations or lab tests: general medical examination, azotemia, complete blood count and venous blood sampling, achieving however a moderate discriminatory accuracy (c-statistic: 0.67).

Conclusions

Machine learning techniques using electronic healthcare data can help establish an early diagnosis of acromegaly. This could help clinicians anticipate the diagnosis and initiate early treatment.

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Title

Effect of oral sodium butyrate supplementation on paclitaxel-induced behavioral and intestinal dysfunctions

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Background

Paclitaxel (PTX) is one of the most widely used chemotherapeutic agents with a good efficacy in several tumor types including ovarian, breast, and non-small cell lung cancer (1). However, its use is limited by debilitating side effects, involving both gastrointestinal (2) and behavioral dysfunctions (3); therefore, there is relationship among behavior, central and peripheral immune activation, and microbiota in PTX side effects (4). Recent findings show that among SCFA, butyrate, in addition to show intestinal and immuno-modulatory functions (5) is also able to modulate neurodevelopment and behavioral dysfunctions (6). This study aimed to identify a possible therapeutic approach, using sodium butyrate, with beneficial effects on PTX-induced brain and gut comorbidities.

Methods

Mice were pre-treated with sodium butyrate (BuNa) dissolved in daily drinking water for 30 days before receiving one cycle of PTX (8mg/kg). After 14 days, mice underwent to behavioral analysis, biochemical investigations at colonic, plasma and hippocampal level and microbiota composition.

Results

PTX significantly reduced fecal levels of butyric acid suggesting a correlation between chemotherapeutic agent and the reduction in SCFAs normally preserving gut homeostasis. In addition, we found that PTX treatment induced moderate

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sickness-like behavior, altered microbiota and gut permeability leading to systemic inflammation in mice. The preventive treatment with BuNa was able to repristinate microbiota, intestinal permeability and eubiosis in PTX-treated mice. At central level, the reduction of neuro-inflammation mediated by BuNa was associated with an improvement in behavioral changes confirming its antidepressant and anxiolytic action in PTX-treated mice also associated with molecular and functional changes in the hippocampus.

Conclusions

We conclude that microbiota and intestinal perturbation induced by PTX leads to a systemic inflammation and depressive- and anxiety- like behavior. The use of BuNa improves both central and peripheral side effects associated with PTX. On this basis, BuNa should be considered in clinical practice, suggesting a possible involvement of the gut-brain axis in BuNa effects.

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Title

Differential expression of let-7e and miR-126 may predict response to anti-TNFα biologics in patients with Crohn's disease

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Background

Crohn's disease is a form of inflammatory bowel disease that recognizes a multifactorial etiology. Although the advent of therapeutic monoclonal antibodies against TNFα (e.g., infliximab or adalimumab) has significantly improved the clinical management of CD patients, a high percentage of patients show no clinical improvement after initiation of therapy or lose their ability to respond to these drugs over time. Therefore, the identification of new biomarkers able to predict drug response represents an unmet medical need. MicroRNAs (miRNAs) are endogenous small non-coding RNAs that play crucial roles in the pathophysiology of various diseases, including immunological homeostasis and inflammatory processes. The current study was aimed at testing whether several selected miRNAs CD patients could be used as potential predictive biomarkers of response to anti-TNFα biologics.

Methods

This study was carried out on serum samples from CD patients previously treated with infliximab (n. 23) or adalimumab (n. 17), stored in the Gastroenterology Unit of the University Hospital of Pisa. The tested miRNAs were: miR-10A, miR-301A, miR- 146b, miR-126, and Let-7e. Purification was performed using miRNeasy mini kit (Qiagen, Hilden, Germany), and expression (normalized to that of miR-484) was determined by Taqman microRNA Reverse Transcription Kit and Taqman miRNA Assays using the Quant studio Real-Time PCR System (Applied Biosystems). CD patients were categorized as responders or non-responders based on the following treatment outcomes: clinical response at week 14,

clinical remission at weeks 14 and 54, and endoscopic healing at week 54. Comparison of the expression levels of selected miRNAs and statistical analyses were carried out using MedCalc and GraphPad Prism software. Receiver operating characteristic (ROC) curve analyses were then computed to assess the predictive performance of potential biomarkers.

Results

In patients who were in clinical remission at week 14, the expression levels of let-7e were lower than those observed in non-responders (-53%; p=0.0128). The expression levels of miR-126 were significantly lower in responders than non-responders (-64%; p=0.0411), when considering the clinical remission at week 54 as a treatment outcome. The expression of miR-126 was also reduced in patients with endoscopic healing at week 54 as compared to non-responders, although the difference between the two groups did not reach the limit of statistical significance (data not shown). Finally, the ROC curve analysis allowed us identifying the optimal cut-off values for let-7e (4.24, p= 0.003; sensitivity = 92.9%, specificity = 61.1%) and for miR-126 (13.31, p=0.02; sensitivity = 62.5%, specificity = 83.3%).

Conclusions

These findings provide evidence that the analysis of the expression levels of let-7e and miR-126 at baseline could predict response to anti-TNFα drugs in CD patients.

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Title

Generation of a patient specific hiPSC-derived neuronal model for Congenital Central Hypoventilation Syndrome (CCHS)

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Background

Congenital Central Hypoventilation Syndrome (CCHS), is a rare neonatal disorder of the autonomic nervous system (ANS), characterized by a deficient control of autonomic ventilation and a global autonomic dysfunction (1). Indeed, the disease-defining gene, *PHOX2B* encodes a master transcription factor whose role is essential during development of the neural lineages of the ANS (2).

Polyalanine repeat expansion mutations (PARMs), occurring within the sequence stretch coding for the 20-alanine tract in exon 3 of *PHOX2B*, have been identified in ~ 90% of patients affected by CCHS.

Importantly, *in vivo* and *in vitro* models of the disease that have been generated so far provide a limited representation of human pathophysiology (3). Therefore, the use of human-induced pluripotent stem cell (hiPSC) technology is essential to obtain patient-specific cell type models relevant to the disease that could be otherwise unobtainable.

Methods

To clarify the pathogenesis of CCHS, we have generated a hiPSC-derived autonomic neuronal model that fully recapitulates the patient's entire genetic profile.

Using a non-integrating Sendai Virus (SeV), we reprogrammed fibroblasts from two CCHS patient's carrying the same genetic mutation but with different clinical manifestation of the disease.

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Using a specific autonomic neural differentiation protocol, iPSC lines have been differentiated to neural crest stem cells (NCSCs), which bear the potential to develop into different lineages, among which PHOX2B positive peripheral autonomic neurons that carry the patient's specific mutation.

Results

Here we show a complete characterization of both patient-derived iPSC lines by karyotyping, morphology study, immunocytochemistry, and qPCR analysis to confirm the presence of gene and protein expression of markers of pluripotency (e.g., Nanog, Oct4 and SSEA4) (4). Moreover, we derived PHOX2B+ sympathetic neurons from both patient-derived iPSC lines.

Conclusions

This new personalized disease-in-a-dish model of CCHS opens numerous possibilities to identify molecular and cellular defects induced by the mutations as well as modelling for drug discovery/screening for therapeutic perspectives.

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Title

Effect of Sodium Butyrate on peripheral and central alterations induced by paclitaxel-based chemotherapy

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Background

Paclitaxel (PTX) is one of the most widely used chemotherapeutic agents with a good efficacy in several types of solid tumors[1]. However, it is often associated with debilitating neuronal and affective side effects. In particular, it has been reported that paclitaxel-associated intestinal changes could be involved in important central conditions, involving development of neuropathic pain[2] and mood disorders such as depression and anxiety behaviors[3]. Previous studies underline the beneficial effect of Sodium butyrate (BuNa), a gut microbial metabolite, in several acute and chronic pain conditions, inflammation and central nervous system diseases through different mechanisms[4,5]. Therefore, the goal of our study was to investigate the effects of the intake of BuNa in attenuating paclitaxel-induced negative symptoms in mice.

Methods

Mice received four intraperitoneal injections of PTX at the dose of 8 mg/kg on alternate days, as previously described[6]. BuNa was administered daily in drinking water starting from 30 days before the first injection of paclitaxel. After treatments, we performed different *in vivo* animal behavioural tests in order to evaluate pain and mood. Then, *ex vivo* experiments were conducted on different tissues (brain, spinal cord, serum) for biochemical analyses.

Results

Mice treated with PTX showed a significant inflammation state both at peripheral and central level; this is accompanied with peripheral neuropathy, and depressive- and anxiety-like phenotype. Preventive BuNa treatment significantly reduced mechanical hyperalgesia(Randall-Selitto) and allodynia(Von Frey). These effects were mediated by cannabinoid (CB1) and opioid (μ) receptors as demonstrated by their up-regulation in spinal cord BuNa mice with respect to vehicle

mice. Moreover, BuNa decreased serum (TNF-α, IL-1β, IL6) and spinal cord (COX-2, iNOS) inflammation mediators suggesting an important anti-inflammatory activity. On the other hand, chronic BuNa administration in PTX-treated mice decreased depressive- and anxiety-like behaviors as assessed in specific behavioural tests. BuNa was able to modulate the expression of a biomarker for mood disorders, increasing significantly the protein levels of BDNF and its receptor, tropomyosin receptor kinase B (TrkB) in the hippocampus.

Conclusions

For the first time we revealed the efficacy of Sodium butyrate in paclitaxel-induced pain and mood disorders. In conclusion, we hypothesized that preventive supplementations of BuNa could be a new potential therapeutic strategy for the clinical prevention of chemotherapy-induced toxicity, with the potential to change clinical practice.

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Title

Overview of the safety of biologic therapies for severe asthma: an analysis of VigiBase®, the WHO pharmacovigilance database

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Background

Treatment of severe asthma has deeply improved since the advent of novel biologic therapies. Currently approved biologics for type 2 asthma phenotype are: anti-IgE (omalizumab), anti-IL5 (mepolizumab, reslizumab, benralizumab), and anti-IL4 (dupilumab). These drugs are usually well tolerated, but information on adverse effects, especially in vulnerable subjects as pediatric patients, is still limited. Spontaneous reporting data could be extremely relevant to better explore their safety profiles in clinical practice. The aim of this study was to evaluate the post-marketing safety profile of asthma biologics using VigiBase, the WHO global pharmacovigilance database, with a focus in pediatrics.

Methods

We selected all Individual Case Safety Reports (ICSRs) including adverse drug reactions (ADRs) related to asthma biologics in VigiBase, up to May 8th, 2022. Descriptive frequency analyses have been conducted. Reporting odds ratio and the Information Component with 95% confidence intervals were used as measures of disproportionality, using all other suspected drugs in VigiBase as reference group. Pediatric subgroup analysis was carried out for three biologics approved in this age group (dupilumab, mepolizumab, omalizumab).

Results

Up to May 8th 2022, 147,467 ICSRs related to asthma biologics were retrieved in VigiBase (0.5% of total ICSRs). Among these, 58.3% indicated dupilumab as suspected drug, 27.9% omalizumab, 8.8% mepolizumab, 4.8% benralizumab, and 0.3% reslizumab. Most ICSRs concerned adults (39.4%) and women (54.8%). Asthma biologics showed a lower frequency of serious ADR reporting than the reference group (24.5% vs 32%; $p<0.001$). The most reported ADRs included in the important medical events (IME) list were pneumonia (n=2,706), anaphylactic reaction (n=2,102) and angioedema (n=794). Asthma biologics were disproportionally associated with unexpected ADRs, such as malignancies, pulmonary embolism, sarcoidosis, acquired haemophilia A with omalizumab, herpes infections with dupilumab and mepolizumab.

Focusing on pediatrics (n=7,044), 72.5% ICSRs concerned dupilumab, 25.4% omalizumab and 2.1% mepolizumab. Administration procedural complications, cutaneous disorders, general and administration site conditions were mainly reported. Serious pediatric ICSRs, including anaphylactic reaction (n=132), pneumonia (n=50), and syncope (n=35), accounted for 17.7% ICSRs (vs 24.2 pediatric serious ICSRs in overall database; $p<0.001$). Safety signals in pediatric patients will be further investigated.

Conclusions

The most reported adverse effects related to asthma biologics in VigiBase concerned general, cutaneous and respiratory disorders. Potential safety signals, including malignancies, herpes infections, pulmonary embolism were detected, requiring further assessment. A low frequency of serious ADRs was found in children and adolescents; however, safety signals in pediatrics will be further analyzed.

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VigiBase, the WHO global database of individual case safety reports (ICSRs) is the source of the information; the information comes from a variety of sources, and the probability that the suspected adverse effect is drug-related is not the same in all cases; the information does not represent the opinion of the UMC or the World Health Organization.

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Title

PCSK9 deficiency affects systemic metabolism and cardiac function

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Background

PCSK9 loss of function polymorphisms is associated with lower LDL-Cholesterol levels due to increased lipoprotein catabolism and lipid uptake but also with higher plasma glucose levels and increased risk of developing T2D. Vice versa pharmacological therapies against PCSK9 did not result in an increased risk of new-onset diabetes. The aim of this work was to evaluate the systemic to cellular lipid metabolism crosstalk under PCSK9 deficient conditions and their implications on glucose metabolism and cardiac lipotoxicity.

Methods

WT, Pcsk9KO, liver selective KO and Pcsk9/Ldlr doubleKO (DKO) were fed with SFD diet. Exercise intolerance, muscle strength, cardiac structure, ITT, GTT and tissue morphology were evaluated.

Results

Pcsk9 KO displays glucose intolerance but not systemic insulin resistance with a reduced running endurance associated with echocardiographic alterations. PCSK9 deficiency is affecting both cardiac and pancreatic metabolism leading to cardiac hypertrophy and pancreatic beta-cell enlargement both due to cholesterol accumulation. Interestingly, DKO show an increased LVPWT, thus excluding a contribution for LDLR on heart damage observed in Pcsk9KO mice but a normal pancreatic phenotype thus confirming the crucial role of PCSK9/LDLR axis in beta cell but not in heart function. Moreover, carriers of the R46L variant with loss of function for PCSK9 showed a reduced HOMA-BC with an increased left ventricular mass, but a similar ejection fraction compared to control subjects.

Conclusions

PCSK9 deficiency, modulating other receptors than the LDLR, can influence cardiac lipid metabolism, thus favoring mitochondrial damage and the development of HFpEF

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Title

THE ROLE OF THE CLINICAL PHARMACOLOGIST IN THE MANAGEMENT OF PATIENTS WITH GASTROINTESTINAL CANCER TREATED WITH FP-BASED CHEMOTHERAPY

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Background

Fluoropyrimidines (FP) are commonly used to treat solid tumors. Five polymorphisms in *DPYD* gene encoding Dihydropyrimidine Dehydrogenase, a key enzyme in the FP catabolism, strongly predict the risk of severe Adverse Drug Reactions (ADRs)[1]. Wild-type patients can suffer from severe ADRs with a frequency, which remained undetermined mainly because they are not monitored for a sufficient number of therapy cycles. Drug-drug interactions are also involved in FP-toxicity. *TYMS-TSER* 28bp-2R/*2R* in the main FP-target (thymidylate synthase) and *GSTP1*-313AA genotypes (essential for oxaliplatin detoxification) could predict FP/oxaliplatin-toxicity[2]. This study aimed to evaluate ADRs frequency in patients wild-type for the recommended *DPYD* polymorphisms and to found other factors influencing FP-safety.

Methods

Naïve Patients for FP were enrolled at the University Hospital of Salerno. Demographic and clinical data were collected and FP-ADRs were categorized according to the *Common Terminology Criteria for Adverse Events* V.5. *DPYD* and *TYMS-TSER* polymorphisms were analysed. To verify whether drug interactions could be cause or worsen ADRs, a recognition of drugs and supplements was carried out and 5 drug-interaction checkers were consulted.

Results

Eighty-five patients diagnosed with gastrointestinal cancer were enrolled. Forty-three, 39 and 3 patients underwent FOLFOX, CAPOX and FOLFIRINOX therapy regimen, respectively. All were followed for a mean of 7 therapy cycles. Patients carrying *DPYD* polymorphisms were excluded. Fifty-seven (67%) patients experienced hematological ADRs, 21% were severe. Sixty-two (72%) patients experienced gastrointestinal ADRs, 7% were severe. GSTP1-313AA genotype (30.5%) was associated to increased risk of mild-moderate hematological ADRs (p=0.018) and the patients carriers of *TYMS-TSER*-2R combined with GSTP1-313A alleles (52.9%) were more prone to develop mild/moderate gastrointestinal ADRs (p=0.023).

A pharmacological anamnesis was recorded in 53/85 subjects. Of them, 81.4% were treated with 5-Fluorouracil and 18.6% with capecitabine. Two *TYMS-TSER* -2R2R patients treated with capecitabine received a dosage of more than 2000 mg/m2, the maximum tolerated dose in the case of a concomitant folate-based supplementation. Both patients experienced severe Hand Foot Syndrome and diarrhea. In one case, capecitabine was reduced while folate supplements were not suspended. In the other case, capecitabine and folates were stopped and capecitabine was restarted. Following these changes, severe toxicities were resolved. All drug interaction checkers could have foreseen the capecitabine-related worsening toxicity associated with capecitabine/folates administration.

Conclusions

The screening of polymorphisms other than those in *DPYD* and a systematic recognition of drugs and supplements used by oncological patients allows avoiding ADRs, including life-threatening ones. The role of the Clinical Pharmacologist in this field is crucial.

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Title

The Influence of Sex, Gender, and Age on COVID-19 Data in the Piedmont Region (Northwest Italy): The Virus Prefers Men

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Background

Several important sex and gender differences in the clinical manifestation of diseases have been known for a long time but are still underestimated. The infectious Coronavirus 2019 (COVID-19) disease pandemic has provided evidence of the importance of a sex and gender-based approach; it mainly affected men with worse symptomatology due to a different immune system, which is stronger in women, and to the Angiotensin-converting enzyme 2 and Transmembrane protease serine 2 roles which are differently expressed among the sexes. Additionally, women are more inclined to maintain social distance, smoke less, and are more skilled with personal hygiene [1].

Methods

Analysis of data on the infectious COVID-19 disease testing from people admitted to the Amedeo di Savoia Hospital, a regional referral center for infectious diseases, has been applied to the whole of 2020 data (254,640 records). The data analyzed were not attributable to identity data (name and surname); each record was encoded with a specific identification code. The statistical software used for analysis was R (R Core Team 2017) and its text-mining (TM) packages [2].

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Results

A high percentage of data in the dataset was not suitable due to a lack of information or entering errors. Among the suitable samples, records have been analyzed for positive/negative outcomes, matching records for unique subjects (N = 123,542), to evaluate individual recurrence of testing. Data are presented in age and sex-disaggregated ways. As a central point of the huge work conducted on four different identified periods of 2020 year, interesting information on the disease outcome obtained from a general analysis of COVID-19 symptoms referred in a sex, age, and outcome test-disaggregated way, showed that male sex and older age were risk factors for more severe disease. Analyses of the suitable sample also concerned the relation between testing and hospital admission motivation and symptoms.

Conclusions

Sex and gender medicine does not exist [3]. What should definitively exist is a medical approach tailored on the variables and characteristics of each subject needing clinical assistance. Our analysis indicated that a sex and gender-based approach is mandatory for patients and the National Health System's sustainability. Statistical models like ours developed could be applied in general for human diseases, giving the opportunity to better understand the mechanisms underlying pathologies in the interest of the whole community.

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pharmadvances

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Title

Targeting androgen receptor degradation, in spinal and bulbar muscular atrophy, through PROteolysis TArgeting Chimeras: development of an AF2 domain-oriented degradation enhancer.

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Background

Spinal and bulbar muscular atrophy (SBMA), is a rare disease characterized by the expansion of a CAG trinucleotide repeat (polyQ) in the androgen receptor gene (AR). Nuclear accumulation of ubiquitinated polyQ-AR upon ligand binding is a key step in SBMA pathogenesis, resulting in progressive lower motor neuron degeneration and muscle loss [1]. In addition to the ligand binding, the involvement of intra/intermolecular interactions with the AF-2 domain and the rectruitment of coregulators upon DNA binding are mandatory in the pathogenetic commitment of polyQ-AR [2]. Importantly, apart from the muscle phenotype, SBMA patients do not always develop clear signs of hypogonadism [3]. Anti-androgenic drugs are currently under evaluation for the treatment of SBMA but the exposure risk to the long-term hypogonadism is a matter of concern. PROteolysis TArgeting Chimeras (PROTAC) molecules are gaining interest to obtain the therapeutic knockdown of proteins of interest (POI). PROTACs structurally involve an E3-ligase moiety and a POI binder-warhead joined by a linker chain. In this study we aimed to develop a proof-of-concept panel of PROTACs targeting the AF2 domain of AR in order to enhance the specific degradation of pathogenetically committed polyQ-AR, while preserving a protective androgen sensitivity

Methods

A screening on Protein Data Bank repository (https://www.rcsb.org/) was performed to identify suitable AF2 domain-binding warheads. Provisional AF2-PROTAC prototypes were designed on the von Hippel-Lindau (VHL)-E3 Ubiquitin Ligase binder VH032, by changing the length, in terms of carbon atoms, of a hypothetical linker chain consisting of only methylene units. In order to identify the optimal linker length, prototypes were then submitted to PRosettaC, a free webserver computational protocol for the prediction of PROTAC-induced ternary complexes [4, https://prosettac.weizmann.ac.il/]. A panel of readily synthesizable AF2-PROTAC was then generated on the basis of the reactants available on the market.

Results

3,3',5-triiodothyroacetic acid (Triac)[5] was identified as a recognized AR ligand, suitable for the design of a AF2 domain-oriented PROTAC. Eleven AF2-PROTAC prototypes, differing for linker length (C_2 to C_{11}) and anchor site to Triac, were designed and submitted to PRosettaC. The top-score prototype, according to PRosettaC results, had a linker length of C8. Accordingly, a panel of 3 readily synthesizable AF2-PROTAC candidates was proposed.

Conclusions

AF2-PROTAC candidates will be tested on a cell model of SBMA, involving PC12 cells stably expressing highly expanded polyQ-AR [6], in order to address an efficient reduction of the ligand dependent-nuclear accumulation whilst preserving the expression of AR-responsive genes.

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Title

The clinical risk associated with look-alike/sound-alike (LASA) drugs: results of a survey proposed to the Care Units of the University-Hospital of Padova.

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Background

Look-alike/sound-alike (LASA) drugs are defined as medicinals that can be confused with each other due to the high graphic and/or phonetic similarity of the name or of the packaging and name [1]. The estimated burden attributed to LASA drugs accounts for up to 25% of all medication errors for name confusion and 33% for packaging and labeling confusion [2]. According to current estimates, the potential harm due to LASA drugs accounts to roughly 260,000 people annually in the sole United States [3]. Several proposals of intervention focus on the drug packaging or labeling such as: use of color-coded labels, changes in information layout, changes in prints font size. At the University Hospital of Padova, a pilot trial of color code labeling for LASA risk drugs has been initiated by the Hospital Pharmacy Unit. The aim of this study is to estimate the extent of the clinical risk associated with LASA drugs in care Units.

Methods

A specific six-items questionnaire was conceived, including information on the operator involved in the pharmacovigilance report, the characteristics of any LASA drug pairs present in the pharmaceutical dispensary, and the documented case history of drug exchanges. Questionnaires were transposed into google modules form and shared with the operating units by mail.

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Results

Sixteen out of 82 Ward Care Units included in the mailing list, provided a response to the survey at the time of writing this abstract. Specifically, six were Internal Medicine Care Units, four were Critical Care Units and six were Surgical Care Units. In all cases, the head nurse was in charge of pharmacovigilance. Globally, 60 couples of medicinal specialties were reported to be at LASA risk, 57% of which at look-alike risk, 14% at sound-alike risk and 29% at both. Importantly, the 34% of LASA-risk drugs couples involved a different specialty active ingredient and dosage. Finally, despite non-significantly different, reports of LASA drug couples were more numerous for Critical Care Units (4.5±2.5) compared to Internal Medicine and Surgical Care Units Units (respectively 4.3±1.6 and 3.2±1.3)

Conclusions

Our results show a not negligible risk of medicinal administration errors associated with LASA drugs, particularly in Critical Care Units where further additional stressors can constitute precipitating elements. The nursing staff represents the major professional target to be involved in the implementation of a color code labeling procedure.

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Title

Anaplastic lymphoma kinase receptor: possible involvement in anorexia nervosa

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Background

Anorexia nervosa (AN) is a severe eating disorder characterized by a reduction of food intake to achieve body weight loss. Although the pathophysiology underlying AN has not been fully elucidated, biological factors appears to be involved in its progression. The anaplastic lymphoma kinase (ALK) is a receptor tyrosine kinase known for its oncogenic potential and involved in the development of the peripheral and central nervous system. Recently, genetic deletion of ALK has been found to increase energy expenditure and confers resistance to obesity (1,2). Since no research has been conducted on the possible involvement of the ALK in the etiology of AN, we investigated the expression of this receptor and the downstream intracellular pathways in animals subjected to the activity-based anorexia (ABA) model, which reproduces important features of human AN including body weight loss and hyperactivity. By the virtue of the alterations that have been observed in AN patients and apoptosis and as ALK reduction could be mediated by the apoptotic pathway, we also focused on the caspases activation.

Methods

Animals were divided into four groups and subjected to the ABA protocol: (1) 'Control' rats: sedentary + food ad libitum; (2) 'Restricted' rats: sedentary + food restriction (food limitation for 1.5 h/day); (3) 'Exercise' rats: activity wheel access + food ad libitum; (4) 'ABA' rats activity wheel access+ food restriction (food limitation for 1.5 h/day). At the end of ABA induction, animals were euthanized, the hypothalamus rapidly dissected and frozen in dry ice for the subsequent molecular analyses by western blotting.

Results

In line with previous studies (3), ABA rats developed hyperactivity accompanied by a massive body weight loss. In the

hypothalamic lysates of these animals, we found an enhanced reduction in ALK receptor expression, a down-regulation of Akt phosphorylation, and no change in the extracellular signal-regulated protein kinases 1 and 2 (ERK1/2) phosphorylation. No changes were observed in the other experimental groups. During the recovery from body weight loss, ALK levels in ABA rats returned to the control baseline values. Moreover, our data demonstrated that the ALK receptor protein reduction is independent of apoptosis.

Conclusions

Taken together, our results demonstrate a possible involvement of ALK receptor in the in AN pathophysiology.

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Title

ESTIMATION OF PATIENTS WITH PSORIASIS POTENTIALLY ELIGIBLE TO BIOLOGIC AGENTS AND NOT CURRENTLY TREATED WITH THESE THERAPIES: ANALYSIS OF REAL-WORLD DATA IN ITALY

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Background

Psoriasis (PSO) is an immune-mediated inflammatory skin disease with a chronic and progressive course. The current guidelines recommend the use of topical drugs in mild forms and systemic treatmens for moderate-severe forms. In patients not achieving an adequate response to conventional treatments, biological drugs are recommended. The present analysis aimed to estimate the number of patients with a diagnosis of PSO potentially eligible for biological therapies in the Italian clinical practice setting.

Methods

An observational analysis was performed based on administrative databases of a sample of Italian Entities, covering 11.3% of population. The study included all patients with PSO diagnosis identified by: (a) at least one prescription of topical antipsoriasic drugs (ATC code D05A), OR (b) exemption for PSO (code 045.696.1), OR (c) at least one PSO hospitalization (discharge diagnosis code ICD-9-CM 696.1). The index-date was defined PSO diagnosis date. Patients' eligibility for biologics was evaluated during all available period before the index-date (characterization period) by the following criteria: *Criterion A*, failure of at least one systemic conventional treatment, *Criterion B*, patients with onset of psoriatic arthritis (PsA) before or after PSO diagnosis. The occurrence of co-morbidities, indicated as contraindications for conventional treatments [1], were evaluated during the characterization period.

Results

The prevalence of PSO was 2% for the overall and 2.4% for the adult population. Among 161,650 patients diagnosed with PSO, 160,124 (99%) were treated: 153,753 (96%) with conventional therapies and 6,371 (4%) with biologics. Considering overall PSO patients, 1,526 (1%) were untreated. Among untreated patients or those treated with conventional therapies, 6,098 (corresponding to 3.8% of overall PSO patients) met at least one criterion for eligibility to biologics. Specifically, 25% met the eligibility criterion A (a failure to conventional treatments), and 68% met the eligibility criterion B (co-diagnosis of PsA), and 7% met both eligibility criteria. Among patients potentially eligible for biologics, 26% and 24% had 1 or 2 comorbidities, respectively, and 30% presented 3 or more comorbidities.

Conclusions

The present analysis of real-world data in Italy estimates the epidemiology of PSO and the number of patients potentially eligible for biological drugs. Overall, the prevalence of PSO was 2%, in line with literature data reporting a prevalence in Italy ranging between 1.8% and 3.1% [2]. Almost 4% of PSO patients, not-treated with biological medications, presented one or more criteria of eligibility for biologic therapies, mainly related to the concurrent diagnosis of PsA. Moreover, among these patients, almost 30% were characterized by a complex clinical profile presenting multiple co-morbidities.

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Title

Shared transcriptomic signature in Alcohol use disorder and Post traumatic stress disorder

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Background

One-third of individuals with lifetime Post traumatic stress disorder (PTSD) also meet criteria for lifetime Alcohol use disorder (AUD)[1]. While previous studies on the PTSD and AUD comorbidity focused mostly on shared genetic architecture and epigenetic mechanisms, little is known about their possible common brain transcriptomic signatures. The study of transcriptomic data may unveil shared functional pathways targetable with pharmacological treatments.

Methods

An extensive data-search on AUD and PTSD human transcriptomes was performed on Google, PubMed and Gene Expression Omnibus (GEO). Collected studies were divided into two databases: the main one based on RNA-sequencing (RNA-Seq) data, while the other one was based on microarrays for further validation. Leveraging the high specificity of RNA-Seq, we analyzed only the studies using tissues extracted from the fear/addiction neural circuitry[2]. Thus, we retrieved RNA-Seq data from prefrontal cortex (PFC) and amygdala (AMY) tissues for AUD[3,4] and PTSD[,5,6]. Recorded different expressed genes (DEGs) were meta-analyzed according to the area and condition they were associated to. Metanalysis results were crossed to find those DEGs common to both conditions. Then further statistical analysis, such as gene set based enrichment analysis (GSEA) on QIAGEN IPA (QIAGEN Inc., USA) [7] were performed.

Results

Crossing metanalyses by condition revealed 386 upregulated shared DEGs on the whole PFC area. Shared DEGs found on the whole AMY area were 64 and they were all upregulated. These results were further validated by comparing them with those obtained from the metanalysis of each available single tissue. This reduced the list of DEGs to 187 and 17 for PFC and AMY, respectively. A common inflammatory pathway encompassing cytokine receptors, signal transducers and activators of transcription 3 (STAT 3) was defined by IPA across the two conditions.

Conclusions

Our preliminary results point towards an inflammatory pathway that is consistent with previous literature on the role of stress as a strong explanatory candidate of mechanisms underlying the association between PTSD and AUD[8]. Larger sample sizes are required to achieve a more solid picture of the shared transcriptomic signatures between AUD and PTSD. Nevertheless, we are planning to collect more data that will allow us to determine the gene pathways involved in the shared physio-pathological mechanisms underlying the co-morbid condition.

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Title

Deep Learning approach for the recognition of behaviours in Forced Swim Test

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Background

The Forced Swim Test (FST) was developed by Porsolt in the 1970s [1] to assess anti-depressive properties of drugs in rodents and over the years it has been also used to evaluate coping strategies [2]. In FST animals can perform three kinds of behaviors: swimming, climbing, and immobility [3]. So far, animals' behaviors have been evaluated by an operator - either recording the duration of each behavior by a chronometer or selecting the predominant behavior over each 3/5-sec period - or by using automatic systems that extrapolate the degree of mobility of the animal and just distinguish among three levels of it. The first method has the problem of subjectivity and the second one doesn't reveal the specific kind of behavior. To overcome these limits and better characterize rat's behavior the machine learning (ML) approach was used.

Methods

A dataset of 503-min videos was used to train the ML algorithm and an experiment with the antidepressant desipramine was then performed to assess the robustness of the trained model. Desipramine is a tricyclic antidepressant well validated in FST: it significantly reduces immobility and increases swimming behavior compared to control rats [4]. For the analysis, each video was split in sub-videos of 3-sec fixed length. The cylinder was selected by hand and processed by Python's module openCV to subtract the background, resize it to a standardized dimension. For each sub-video the main behavior was selected. To build up the dataset the videos were classified by two independent trained operators to decrease the human bias and any mismatch was evaluated by them together. Different ML models were trained and that reaching the best accuracy (88%) on the test set was then trained on all the dataset. For the experiment 10 male Wistar rats (5 per group) were used. Two sessions were conducted: an initial 15-min pretest followed 24 h later by a 5-min test. Both sessions started placing rats individually in transparent plexiglass cylinder filled with 30-cm water at 23-25° C and recording their behaviors from a camera placed in front of the cylinders. Desipramine (20 mg/kg) or vehicle were administered subcutaneously in a volume of 4 ml/kg, 23.5 h, 5 h, and 1 h prior the test [4].

Results

The ML trained model was used to measure the behaviors on the drug experiment and was compared by human observation. Both analyses showed a significant increase ($p < .01$) of climbing and reduction of immobility ($p < .05$) in rats treated with desipramine.

Conclusions

The proposed ML method was self-consistent over time and able to classify different behaviours in FST with a sufficient accuracy (88%). The ML model efficacy is currently under the validation of other antidepressants.

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Title

An immature cellular prion protein isoform activates the canonical Wnt/β-catenin pathway to sustains glioblastoma cell stemness

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Background

Prion protein (PrP^C) is a ubiquitous extracellular membrane-anchored glycoprotein, whose physiological role is not yet fully understood. Recent data highlights PrP^C role in the development and progression of different human tumors. In glioblastoma (GBM), the expression of PrP^C is required to preserve stemness and tumorigenicity of cancer stem cells. Here, we analyzed the molecular mechanisms activated by PrP^C to control GBM stem cell (GSC) proliferation and differentiation[1]

Methods

Five GSC-enriched cultures were obtained from neuro-surgical specimens by cell selection in stem cell-permissive medium [DMEM-F12/Neurobasal (1:1), supplemented with 1% B27, 2 mM L-glutamine, 10 ng/ml bFGF and 20 ng/ml EGF[1]]. Stable cell lines with reduced expression of PrP^C (GBM-KO) were obtained by transfection of specific shRNAs[1]. 3D GSC cultures were obtained by growing GSC spheroid, in Matrigel droplets, as reported[2].

Results

GBM-KO from all the 5 GSC cultures show impairment of proliferation and migration rates, reduced survival in 3D cultures and downregulation of stemness-related gene (Sox2, OCT4, NANOG, Nestin). These effects are dependent on the impairment of Wnt/β?catenin signaling, whose activity was impaired due to down-regulation of the receptor/ligand components, resulting in lower the expression of target genes. The relationship between PrP^C expression and Wnt/β?catenin signaling was demonstrated re-transfecting GBM-KO with PrP^C, which restored Wnt/β?catenin activity and

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GSC features. To get insights about the differential role of PrP^C in GBM and normal CSC cells, we analyzed PrP^C structure and cellular topology in GSCs and normal astrocytes. In particular, treating GSCs and normal astrocytes with phosphatidylinositol-specific (GPI) phospholipase C that cleaves GPI anchors and releases membrane-bound extracellular proteins, we checked for the presence of a transmembrane immature form of PrP^C (Pro-PrP) identified in solid tumors and virtually absent in CNS cells. We observed that PrP is retained in GSCs while it is lost in astrocytes. This result is compatible with a prevalent expression of transmembrane Pro-PrP isoform in GSCs and a prevalent extracellular mature form of PrP^C in astrocytes.

Conclusions

We propose that the persistence of Pro-PrP within plasma membrane determines an aberrant activation of Wnt/β-catenin pathway, which mediates GSC malignant features. Thus, immature Pro-PrP form interaction with the Wnt/β-catenin pathway may represent a novel therapeutic target to counteract GBM cell proliferation.

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Title

Genetic deletion of calcineurin B1 in astrocytes reduces amyloid burden, neuroinflammation and improves memory in acute and chronic mouse models of Alzheimer's disease

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Background

Alzheimer's disease (AD) is the major cause of dementia in the elderly, affecting ~50 mln people worldwide. Despite numerous clinical trials designed to clear Aβ and to revert ongoing neuropathology revealed unsuccessful, encouraging deeper investigation of AD pathogenesis and identification of novel druggable targets[1,2] . Reports show that Ca2+/calmodulin-activated phosphatase calcineurin (CaN), highly expressed in the CNS, represents a promising target: i) AD animal models treated with CaN inhibitors showed an improvement of memory and neuropathology[3]; ii) a population study found a reduction of dementia incidence in transplanted patients chronically treated with CaN inhibitors[4]. However, CaN inhibitors are characterized by severe side effects[5]. Furthermore, CaN activation in neurons is required for synaptic plasticity and memory[6]. It has been suggested that activation of CaN expressed in astrocytes causes reactive gliosis and neuroinflammation[7]. We hypothesized that the deletion of CaN specifically in astrocytes at early neuropathology stages could mitigate the development of AD.

Methods

To test this hypothesis we have generated two AD models, acute and chronic, with the deletion of CaN specifically from astrocytes (Astro-CaN-KO). In the acute AD model, bearing conditional Astro-CaN-KO, β-amyloid oligomers (AβO) were injected i.v. at 2 mo of age. In the chronic AD model, inducible Astro-CaN-KO was generated on the background of 3xTgAD mice (3xTgAD-indAstro-CaN-KO and 3xTgAD-Ctr) and the KO was induced from 12 to 16 mo of age. In both models, neuropathology, neuroinflammation and memory was assessed using biochemical (WB, qPCR), histochemical (IHC) and behavioural (novel object recognition test, NORT and Barnes maze) approaches.

Results

In the acute AD model, constitutive Astro-CaN-KO fully prevented AβO-induced neuroinflammation and memory loss in NORT test 48 h after AβO infusion. In the chronic AD model by 16 mo of age, 3xTgAD-Ctr mice developed characteristic Aβ plaques and neurofibrillary tangles and had increased GFAP and Iba1 immunoreactivity. Induction of Astro-CaN KO at 12 mo of age, 5 mo after injection fully reverted Aβ plaques deposition, tau hyperphosphorylation and GFAP and Iba immunoreactivity in the brain of 3xTgAD-indAstro-CaN-KO mice compared to 3xTgAD-Ctr mice. mRNA levels of pro-inflammatory Il1β and Cox2 were significantly lower in 3xTgAD-indAstro-CaN-KO brains. Assessment of spatial memory using Barnes maze paradigm showed that 3xTgAD-indAstro-CaN-KO mice have significantly improved cognitive flexibility compared with 3xTgAD-Ctr mice.

Conclusions

Our results suggest that the astrocytic CaN represents a promising target for development of anti-AD therapy. Targeted inhibition of CaN in astrocytes may be achieved through gene therapy or targeted delivery of approved CaN inhibitors.

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Title

Role of MTHFR-mediated dysmetabolism and endothelial progenitor cell (EPCs) dysfunction on correlation between atrial cardiomyopathy and predictive biomarkers of cryptogenic stroke

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Background

Pre-persistent Atrial Fibrillation (AF) and paradoxical embolism do not explain the wholeness of embolic strokes, therefore suggesting the existence of Atrial Cardiomyopathy (AC). Pathogenic mechanisms underlying AC are still largely unknown. Folate cycle disorders are a dysmetabolism partly explained by methylene tetrahydrofolate reductase (MTHFR)-inherited defects [1]. We hypothesize that folates dysmetabolism could hinder both endothelial and circulating endothelial progenitor cells (EPCs) functioning, therefore providing a unifying explanation to both atrial stasis and endothelial dysfunction [2]. This implies the cardiac-bone marrow (BM) networking as a potential pathogenic mechanism of AC, subsequent AF and cryptogenic stroke predisposition.

We aim to study whether: i) AF patients would show dysfunctional EPCs and ii) atrial fibrosis (AFib) would relate to folate cycle disorders (MTHFR C677T inherited mutations and BM function disorders-erythropoiesis diversions)

Methods

We studied 59 patients (Cardiology Unit, General Hospital “F.Miulli”), with preserved EF and subjected to AF ablation,

and 30 hypertensive patients as controls (Internal Medicine, University of Bari Medical School). AFib was quantified by bipolar peak-to-peak voltage at each acquired point, measured and defined through the relative percentage of low-voltage areas (<0,5 mV) with respect to the wholeness of the picked voltage points. Blood count cell was evaluated at the admission. MTHFR C677T genotypes were elucidated by real-time PCR. Serum folates were measured by a commercial laboratory test. EPCs isolation and characterization were performed by Ficoll-Hypaque gradient and following flow cytometry analysis for cell surface antigens: CD45, CD34, CD133, Vascular Endothelial Growth Factor Receptor2 (VEGFR2) and KDR (Kinase Insert Domain Receptor). EPCs functional wound healing assay determined the number of migrating EPCs, measuring the percentage of relative wound closure compared with matched-controls.

Results

In the AF group, number and migration capacity of EPCs was significantly reduced with respect to controls. The AFib percentage significantly differed between C677T MTHFR homozygosis patients (n=15) with respect to non-C677T MTHFR homozygosis patients (n=44). Subsequent multivariate analysis highlighted highest fit once merged RBC, RDW-SD and folates values were inputted. Either RBC, RDW-SD and folates coefficient reached significance in AF patients compared to controls.

Conclusions

Our findings support the hypothesis that genetically determined folates dysmetabolism (MTHFR dysfunction) promotes AFib via a complex cardiac-BM networking involving circulating EPCs and unraveled by erythropoiesis diversions, thereby contributing to AC development and representing novel biomarkers and potential pharmacological targets.

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pharmadvances
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Title

Cardiovascular risk in patients with Takayasu arteritis directly correlates with diastolic dysfunction and inflammatory cell infiltration in the vessel wall: a clinical, ex vivo and in vitro analysis

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Background

Takayasu Arteritis (TAK) increases vascular stiffness and arterial resistance. Atherosclerosis leads to similar changes. Abnormal immune response is a crucial factor in the pathogenesis of TAK. Regulatory T lymphocytes (Tregs) are central mediators of peripheral tolerance. Recent evidence suggests that Tregs can differentiate into Th1, Th2 or Th17 cells, leading to a shift from an immunosuppressive function to a role in the pathogenesis of autoimmune diseases [1]. The potential role of Tregs and their associated cytokine secretion in TAK patients is under active investigation to expand the horizon for more effective therapies. We investigated possible differences in cardiovascular remodeling between TAK and atherosclerosis and whether the differences are correlated with immune cells expression.

Methods

Patients with active TAK arteritis were compared with age- and sex-matched atherosclerotic patients (Controls). In a subpopulation of TAK patients, Treg/Th17 cells were measured before (T0) and after 18 months (T18) of infliximab

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treatment. Echocardiogram, supraaortic Doppler ultrasound, and lymphocytogram were performed in all patients. Histological and immunohistochemical changes of the vessel wall were evaluated as well.

Results

TAK patients have increased aortic valve dysfunction and diastolic dysfunction. The degree of dysfunction appears associated with uric acid levels. A significant increase in aortic stiffness was also observed and associated with levels of peripheral T lymphocytes. $CD3^{+}CD4^{+}$ cell infiltrates were detected in the vessel wall samples of TAK patients, whose mean percentage of Tregs was lower than Controls at T0, but increased significantly at T18. Opposite behavior was observed for Th17 cells. Finally, TAK patients were found to have an increased risk of atherosclerotic cardiovascular disease (ASCVD).

Conclusions

Our data suggest that different pathogenic mechanisms underlie vessel damage, including atherosclerosis, in TAK patients compared with Controls. The increased risk of ASCVD in TAK patients correlates directly with the degree of inflammatory cell infiltration in the vessel wall. Infliximab treatment restores the normal frequency of Tregs/Th17 in TAK patients and allows a possible reduction of steroids and immunosuppressants. On this basis, assessment of Tregs and Th17 populations could serve as a potential biomarker to monitor treatment efficacy and as a novel therapeutic target to reduce cardiovascular risk in TAK patients.

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Title

The synergic stimulation of adenosine A_{2A}/A_{2B} receptors reduces cortical and striatal damage induced by middle cerebral artery occlusion in a rat model of transient brain ischemia

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Background

Cerebral ischemia is a multifactorial pathology characterized by different events evolving in time. Acute injury, characterized by a massive increase of extracellular glutamate levels, is followed by a secondary inflammatory damage that develops from hours to days after ischemia (Dirnagl et al., 1999). After ischemia adenosine by acting on its receptors (A_1, A_{2A}, A_{2B} and A_3), exerts a crucial role in post-ischemic damage (Pedata et al., 2016). Previous data obtained by our laboratory demonstrated that the adenosine A_{2A} receptor agonist CGS21680, as well as the adenosine A_{2B} receptor agonist BAY 60-6583, chronically administered for 7 days after transient ischemia, are protective by contrasting the secondary inflammatory damage (Melani et al., 2014; Dettori et al., 2021). Therefore, we hypothesize that the simultaneous activation of both A_2Rs may be a valid pharmacological approach in this pathological condition.

On these basis, we investigated, for the first time, the effects of the newly synthetized A_{2A}/A_{2B} receptor mixed agonist, MRS3997, chronically administered (0.1 mg/kg, i.p., twice/day for 7 days) after transient (1 hour) focal cerebral ischemia induced by middle cerebral artery occlusion (MCAo) in the rat.

Methods

After induction of ischemia in vivo, behavioural, histological and immunohistochemical experiments were performed to evaluate damage parameters.

Results

Chronic treatment with MRS3997 significantly reduced the neurological deficit, up to 7 days after tMCAo ($P<0.03$). At this time, the A_{2A}/A_{2B} receptor mixed agonist significantly reduced the volume of ischemic brain damage in the cortex and striatum ($P<0.03$) and reduced microglial activation and astrogliosis in ischemic (at least $P<0.05$) and perischemic areas (at least $P<0.01$).

Conclusions

These results show that synergic stimulation of A_{2A} and A_{2B} receptors reduces the ischemic brain damage and improves the neurological deficit. Protection might be ascribed to central and peripheral effects. Interestingly, A_{2A} and A_{2B} receptors in most cases are co-expressed on hematic cells and might work synergistically to exert anti-inflammatory effects (Yang et al., 2006). Further experiments are needed to confirm the hypothesis that the simultaneous activation of A_{2A} and A_{2B} receptors located both on brain cells and on blood cells could potentially be more protective than A_{2A} and A_{2B} receptor agonists administered individually.

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pharmadvances

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Title

Early life stress exposure: a pattern of adaptation or vulnerability to the development of stress-related psychopathologies?

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Background

Stressful experiences in childhood are among the most important risk factors for the development of a whole spectrum of mental and physical illnesses in adulthood [1]. The plasticity of the brain is particularly pronounced in the early stages of life; therefore, aversive conditions can have conspicuous and long-lasting effects during early development.

Methods

We assessed whether prenatal stress (PNS) exposure could confer susceptibility or resilience to the development of psychopathological conditions in adolescence following a second stressful experience in (single prolonged stress, SPS) and whether these alterations were sex-specific in rats. Behavioral alterations in the emotional and cognitive domines were examined in adolescent rats of both sexes exposed to PNS (at gestational day 14) [2] or SPS (at postnatal day 23) [3] or both in a battery of tests.

Results

Our results in males showed behavioral alterations in the Open field following exposure to PNS and alterations of social

play behavior in the PNS or SPS alone groups, as compared to controls. Furthermore, exposure to both stressors reduced auditory conditioned fear acquisition and recall in males only. Exposure to PNS or SPS increased pre-pulse inhibition, while it did not alter the total amplitude of a startle response, in males. Overall females remained largely unaffected by the adverse exposures only showing increased grooming behavior in the Open field task following exposure to the PNS+SPS relative to controls, possibly indicating increased anxiety-like behavior.

Conclusions

Taken together, these results suggest that early life stress alters emotional and cognitive behavior in adolescence and lay the foundations for more mechanistic investigations to allow the identification of new prophylactic and therapeutic targets for the treatment of psychopathologies at a young age.

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Title

Drug off-target proteins identified on a proteome-wide scale by the innovative SPILLO-PBSS software and experimental validations.

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Background

The property of a drug to interact with multiple molecular targets is usually a major drawback both in the drug development process, where off-target interactions often lead to safety-related failures, and in medicine, where therapeutic agents often lead to side effects that can cause dose limitation or even treatment discontinuation. Representative examples are finasteride, widely used to treat benign prostatic hypertrophy and androgenetic alopecia, which can give rise to sexual dysfunction and psychological and physical disorders[1], and bortezomib, a first-line drug to treat multiple myeloma, which in about 50% of patients causes peripheral neuropathy often responsible for discontinuation of therapy.[2] The use of innovative *in silico* methods, such as the *SPILLO potential binding sites searcher* (SPILLO-PBSS) software, may prove helpful for such challenging problems.[3][4]

Methods

Two unbiased 3D proteome-wide scale *in silico* screenings were performed using SPILLO-PBSS. This tool has unique capabilities in identifying targets and off-targets of any small molecule through a direct identification of their (often previously unknown) binding sites, which can be detected even when hidden or completely closed, and therefore not identifiable by traditional approaches (e.g., molecular docking simulations, QSAR, etc.). The identified off-targets and the proposed biomolecular mechanisms were validated by *in vitro*, *in vivo* and/or NMR experiments.

Results

As for finasteride, the 'phenylethanolamine N-methyltransferase (PNMT)' enzyme was found 5th out of 17900+ proteins analyzed. It catalyzes the conversion of noradrenaline to adrenaline and, therefore, it is potentially correlated to the considered adverse effects of finasteride. Importantly, the binding site of finasteride on PNMT was found to overlap with its catalytic site, thus suggesting a competitive inhibition of the enzyme activity by finasteride. Both the interaction with PNMT and the inhibitory nature of the binding were then confirmed *in vitro* and *in vivo*.[5]

As for bortezomib, tubulin was identified as one of its potential off-target proteins (4th out of 26100+ proteins analyzed). In this case, it was possible to hypothesize an inhibition of the GTPase activity of tubulin by bortezomib and a reduction of the 'microtubule catastrophe' as a possible cause of the neurotoxicity of this drug. Both the inhibition of GTPase activity and the reduction of 'microtubule catastrophe' have been experimentally confirmed *in vitro*, while the direct interaction with tubulin has been confirmed by NMR binding studies.[6]

Conclusions

The results obtained, in addition to representing a step toward a deeper understanding of the molecular mechanisms potentially responsible for the adverse effects of the drugs considered, further confirm SPILLO-PBSS's great potentialities in identifying targets and off-targets of any small molecule on a proteome-wide scale and its manifold applications in the drug R&D.[5][6][7][8]

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Title

Immunoregulatory properties of breast milk: role of mesenchymal stromal cells and cannabinoid receptor type 2

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Background

Breast milk is a biological fluid containing bioactive substances, growth factors and different cells, among them mesenchymal stromal cells (MSCs) [1, 2]. MSCs are multipotent cells able to modulate survival, maturation and differentiation of immune system cells by releasing several soluble factors, such as cytokines [3-6]. Currently, MSCs are isolated from patients bone marrow through an invasive biopsy [4]. We previously demonstrated that MSCs isolated from bone marrow express all compounds of the Endocannabinoid System (EC) and we have suggested Cannabinoid receptor type 2 (CB2) as a mediator of MSCs anti-inflammatory and immunoregulatory properties [7]. This study aims to evaluate EC system expression in MSCs obtained from human breast milk and its possible involvement in MSCs anti-inflammatory and immunoregulatory activity regulation to propose them as an alternative non-invasive source of MSCs.

Methods

We isolated MSCs from bone marrow of healthy donors and from breast milk of lactating women and characterized them by flow cytometry. We collected MSCs supernatants, mRNA, and proteins to evaluate EC system compounds expression and pro-inflammatory and anti-inflamamtory cytokines release. Supernatants were used to analyse interleukin (IL)-4, IL-10, interferon (IFN)-γ, tumor necrosis factor- α (TNF-α), and IL-6 release with enzyme-linked immunosorbent assay (ELISA). mRNA and proteins were used to evaluate EC system compounds expression levels through Real Time PCR and Western Blotting, respectively.

Results

We confirmed the presence of MSCs in breast milk and demonstrated that they express all EC system compounds. MSCs obtained from breast milk showed an increased release of pro-inflammatory (IL-1β, IL-6, IFN-γ and TNF-α) and anti-inflammatory (IL-4 and IL-10) cytokines compared to MSCs isolated from bone marrow.

Conclusions

The presence of CB2 could modulate the anti-inflammatory and immunomodulatory properties of MSCs obtained from breast milk and explain the remarkable protective power conferred to breastfeeding.

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Title

Ischemic preconditioning affects cerebral and blood expression of the immunoregulatory protein tumor necrosis factor-stimulated gene-6 (TSG6) in mice subjected to transient focal cerebral ischemia

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Background

Cerebral and peripheral immune responses play a pivotal role in ischemic pathobiology, exerting detrimental or protective functions, depending on the specific molecules or cell phenotypes involved. Preliminary evidence suggests that the immune system also participates to brain tolerance induced by ischemic preconditioning (IPC), although the mechanisms implicated in immune polarization are still poorly understood [1]. Here, we assess whether the immunoregulatory protein tumor necrosis factor-stimulated gene-6 (TSG6) [2] is involved in IPC in mice.

Methods

Adult male C57Bl6 mice were randomly allocated into 4 experimental groups: SHAM surgery, MCAo, 1h middle cerebral artery occlusion followed by 6, 24 or 48h reperfusion; IPC, 15min MCAo followed by 72h reperfusion; IPC+MCAo (24h). Brain infarct size and edema were assessed in cryostat-cut coronal brain slices stained with cresyl violet; whereas neurological deficit was evaluated by a dichotomized composite neuroscore [3]. TSG6 expression was determined by western blotting in plasma and brain cortex homogenates. Quantitative real-time PCR analysis was performed on microRNA (miR) extracted from blood samples by miRNeasy Serum/Plasma Kit (Qiagen).

Results

In the brain, protein expression of TSG6 was elevated (2.5-fold vs SHAM, $P<0.05$) in the ischemic cortex of mice subjected to MCAo followed by 24h of reperfusion, while IPC further potentiated this effect (6.3-fold elevation vs SHAM,

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P<0.05 vs MCAo, ANOVA, n=4-6). In plasma, we detected a time-dependent modulation of TSG-6, being protein levels reduced, as compared to SHAM, in the acute phase [15% reduction after 6h of reperfusion; 24% reduction after 24h of reperfusion (P<0.01 vs SHAM)]. Interestingly, IPC alone did not affect plasma levels of TSG-6, whereas it abolished TSG-6 reduction observed after 24h of reperfusion. By reverse target prediction analysis of the major genes regulating TSG6, we selected miR-23a and 23b, to demonstrate that both are elevated after MCAo in plasma (P<0.05 vs SHAM), while only miR-23a elevation was prevented by IPC (relative miR levels/fold change vs SHAM: IPC, 1.27±0.10; MCAo, 1.95±0.24; IPC+MCAo, 1.25±0.42). Thus, by re-establishing miR-23a/TSG6 circulating levels, IPC results in significant (P<0.001, t-test, n=12) neuroprotection (infarct volume: MCAo, 92.9±3.9; PC+MCAo, 70.6±3.8 mm^3 - edema: MCAo, 31.2±1.4; PC+MCAo: 22.94±1.5 mm^3) and 10-points amelioration of general neurobehavioral outcome.

Conclusions

IPC abrogates the reduction of TSG6 and the elevation of miR-23a produced by MCAo in plasma, strongly suggesting their implication in the humoral immune modulation underlying ischemic tolerance and highlighting the relevance of TSG6-mediated pathways as novel and promising therapeutic targets for ischemic stroke therapy.

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pharmadvances

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Title

THE LOCAL PHARMACY AS A STUDY CENTRE FOR EPIDEMIOLOGICAL COLLECTION AND ANALYSIS DATA: THE PHARMACIST EXPERIENCE DURING PANDEMIA AT ASL BT, PUGLIA.

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Background

The COVID-19 pandemic has represented a health emergency for the entire world population. Vaccines are biological drugs whose purpose is to prevent infectious diseases through the stimulation of immune system with the production of antibodies. During the emergency COVID19, pharmacies have carried out an important assistance activity, not only for the execution of rapid antigenic buffers for the detection of SARS-cov-2 antigen but also a significant contribution is given during the vaccination campaign.

Methods

In this context, we have conducted a monocentric observational study about vaccine surveillance to evaluate the pharmaceutical service provided by a local Pharmacy of ASL BT (dott Cannone). We have collected anamnestic data and possible post-vaccine adverse reactions. We have vaccinated adult patients (N=329), elderly patients (N=38) and paediatric patients (N=33) for a total of 400 patients. All data were retrospectively analysed and reported according to age, which is a risk factor for populations.

Results

Vaccination has been well tolerated in all 3 population groups and the two most widely administered vaccines have been Comirnaty and Spikevax. The incidence of moderate immune-allergic reactions was almost two-fold in samples

treated with Spikevax compared to samples treated with Comirnaty and these reactions affected above all female sex. More specifically, in most cases of adult patients, vaccination has caused mild and moderate reactions at the injection site as expected. In some patients, other adverse reactions which did not require hospitalization or medical intervention were reported in the data sheet. Uncommon reactions of moderate degree with an incidence of 0.88% and 1.96% with Comirnaty and Spikevax, however expected, such as skin rush, fever and oedema were observed. We have not observed moderate and severe reactions with other vaccines due to the rarity of the event and the low number of samples. The elderly and paediatric populations were less represented in our sample and showed a slight prevalence of patients vaccinated with Comirnaty than other vaccines. Elderly patients were mostly affected by cardiovascular disease and metabolic comorbities with sporadic cases of benign prostatic hyperplasia and cancer.

Conclusions

So, a large part of population has joined to the vaccination campaign, responding in a positive way relatively to the proposed pharmaceutical service and pharmacies have played a crucial role in the management of the COVID 19 pandemic, thus working as a proximity network and meeting population's needs. Their intervention has strengthened the National Health Service. About COVID-19, pharmacies have exceeded the expectations of patients, with high levels of satisfaction.

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Title

EXPOSURE TO CHRONIC SOCIAL STRESS LEADS TO ANXIETY- AND DEPRESSIVE-LIKE BEHAVIOURAL AND NEUROCHEMICAL ALTERATIONS IN RATS

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Background

Increasing lines of evidence show that exposure to chronic stress is one of the most important risk factor for the development of mental illnesses. In particular, a pathological link among chronic stress, anxiety and depression has been described. Moreover, these two psychiatric disorders are higly comorbid with psychosis. Indeed, people diagnosed of this mental disturbance frequently suffer from anxiety and depression, especially following stressful events. However, mechanisms that underlie this comorbidity are not fully understood. In this context, animal models obtained by the chronic exposure to social stress are essential to clarify the molecular link between stress-induced neurobiological alterations and the above-mentioned comorbidities. In particular, the rat social isolation is a non-pharmacological animal model of psychosis in which neuropathological and behavioural alterations, reminiscent of psychotic symptoms in humans (Bakshi et al., 1999; Lapiz at al., 2003; Fone et al., 2008), are induced by animal exposure to a chronic social stress.

Here, we investigated whether social isolation induced behavioural and neurochemical anxious- and depressive-like alterations in rats.

Methods

To this aim, rats were reared in social isolation condition for 7 weeks after weaning (Schiavone et al., 2016). At the end of the isolation period, both controls (GRP) and isolated (ISO) animals were exposed to the Forced swimming test (FST) (Cryan et al., 2005). In another set of GRP and ISO rats, we performed the Elevated Zero Maze (EZM) test (Tucker, 2017). Moreover, we quantified levels of noradrenaline (NA), 5-hydroxytryptamin (5-HT) and 5-hydroxy-indolacetic acid (5-HIAA), glutamate (GLU) and γ-aminobutyric acid (GABA) in the prefrontal cortex (PFC), amygdala (AMY) and hippocampus (HIPP) of GRP and ISO rats.

Results

In the FST, ISO rats showed an increase in immobility frequency and a decrease in swimming frequency compared to controls. Furthermore, in the EZM test, ISO rats showed a significant reduction in the time spent in the open arms and an increase in the time spent in the closed arms, compared to GRP. Isolation-induced anxiety- and depressive-like behavior was accompanied by a reduction of NA, 5-HT and GABA concentrations in AMY, where, instead, 5-HT turnover and glutamate levels were increased. Social isolation also induced a significant reduction of GABA amount and enhanced glutamate levels in HIPP of ISO rat compared to controls. Moreover, decreased 5-HT levels were detected in the PFC of ISO compared to GRP animals.

Conclusions

In conclusion, our results suggest that exposure to chronic social stress induces behavioural and neurochemical anxiety- and depressive-like alterations in rats. This provides novel insights in the understanding of the mechanisms underlying the comorbidity among psychosis, anxiety and depression in order to identify new pharmacological treatments for these mental disorders.

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Title

Effect of early social isolation on the vulnerability to develop alcohol-related behaviors in genetically-selected Marchigian Sardinian alcohol-preferring rats and non-preferring Wistar rats

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Background

Stress experienced during the postnatal period represents one of the main negative environmental factors enhancing the risk to develop alcoholism [1]. Maternal separation (MS) in rodent pups represents the most widely used stressor in the context of alcohol seeking[2]. However, the majority of prior studies have used MS at very early time points and stressors experienced by rodents during this developmental period do not properly mimic similar stressors experienced by humans at comparatively developmental stages[3].

Methods

Here, to enhance the translational value of our research to understand the long-term consequences of early life stress exposure on later vulnerability to develop alcoholism, we exposed preweaning male and female Marchigan Sardinian alcohol preferring (msP) and Wistar rats to mild repeated social deprivations during the third postnatal week. Operant responding for alcohol under fixed ratio 1 (FR1) and progressive ratio (PR) schedule of reinforcement were then determined starting from adolescence. The effect of the pharmacological stressor yohimbine in increasing alcohol self-administration (SA) as well as the vulnerability to relapse after yohimbine injection were also evaluated.

Results

Operant responding and motivation for alcohol were not altered by our environmental manipulation either in Wistars or in msP rats. Administration of the pharmacological stressor yohimbine (0.0, 0.312, 0.625 and 1.25mg/kg) increased alcohol SA in both rat lines independently from early social isolation (ESI). Following extinction, yohimbine (0.625 mg/kg) reinstated alcohol seeking in female rats only, where ESI resulted in a higher level of reinstatement in adult female msPs.

Conclusions

Overall, results indicate that repeated mild social deprivations experienced during the third postnatal week did not affect later susceptibility to increase the motivation for alcohol in male and female msP and Wistar rats. However, in female msP rats, ESI increased alcohol seeking triggered by the pharmacological stressor yohimbine.

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Title

Role of diabetes in the development and progression of calcific aortic valve disease

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Background

Calcific aortic valve disease (CAVD) is the most common valvulopathy in the general population, with a prevalence of 12% in the elderly (over 75 years), but no effective pharmacological therapy has proven to halt or delay its progression [1]. The development of the disease involves the differentiation of valve interstitial cells (VICs), the main population of the aortic valve, towards a pro-calcific phenotype. Diabetic subjects are at higher risk of developing cardiovascular complications, including CAVD [2]. Moreover, diabetes contributes to the progression of CAVD, but the pathophysiological mechanisms are still not completely understood.

This research project aims to assess the molecular mechanisms leading to diabetic CAVD. In particular, we investigated the effects of a hyperglycemic treatment on the progression of CAVD, using both *in vivo* and *in vitro* models.

Methods

$LDLr^{-/-}ApoB^{100/100}$ mice were fed a diabetogenic or control diet for 6, 12 and 26 weeks as previously described [3,4]; RNA was extracted from the aortic valve for RNA sequencing followed by gene expression analysis to assess the dysregulated genes and pathways in this diabetic model of CAVD. For the *in vitro* system, non-human primate VICs were treated with low- or high-glucose culture media (5.6 mM or 25 mM, respectively) and inorganic phosphate (Pi, final concentration 2.6 mM) to induce their osteogenic differentiation. The total RNA was extracted for gene expression analysis (RT-PCR) at 3 different timepoints (1-3-5 days of treatment) and calcium deposition was determined through a colorimetric assay after 5 days of treatment.

Results

The gene expression analysis detected upregulation of several genes in inflammatory and immunity pathways (such as chemokines and cytokines signaling pathways) and a downregulation of genes involved in cardiac differentiation (including NPPA, TBX5, IRX4, GATA5, NKX2.5) and smooth muscle contraction in the aortic valve of diabetic LDLr$^{-/-}$ ApoB$^{100/100}$ mice compared to non-diabetic ones. The *in vitro* exposure of VICs to higher glucose levels in presence of a concomitant osteogenic treatment had no effect on calcium deposition.

Conclusions

Based on our findings, hyperglycemic condition does not directly affect calcium deposition on isolated VICs but can dysregulate several genes and pathways in both *in vitro* and *in vivo* models of diabetic CAVD. Our ongoing experiments using the *in vitro* system will assess whether the pathways and genes discovered with the gene expression analysis are also present and dysregulated in isolated VICs. This will allow us to perform supplemental mechanistic studies with potential targets for diabetic CAVD.

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Title

Potential Effects of Nutraceutical Agents in Venous Chronic Insufficiency Patients

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Background

Chronic venous insufficiency (CVI) is characterized by morphological and functional anomalies of the venous system and represents a risk factor for cardiovascular events. This study aims to evaluate the beneficial effect of a nutraceutical containing Baicalin 190mg, Bromeline 50mg and Escin 30mg in patients with CVI treated with graduated compression stocking.

Methods

A retrospective cohort study was carried out using the computerized medical records of outpatient affected by CVI in U.O.C. of Internal Medicine of the AOU "G.Martino" of Messina in the period 2019-2020. Patients with clinical class between C1 and C4 according to the CEAP (Clinical Etiologic Anatomical and Pathophysiological) classification and on standard treatment with second class graduated compression stocking were selected. Patients without any treatment were defined non-users, while patients on nutraceutical agents containing Baicalin, Bromeline and Escin were defined users. A descriptive analysis of demographic and clinical characteristic was carried out. A modified and complementary Venous Clinical Severity Score (VCSS) has been calculated considering the following clinical criteria pain, skin pigmentation (SP), inflammation and vessels induration (VI). All the variables considered were evaluated at baseline (t0), after 30 (t1) and 90 (t2) days.

Results

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The study population was of 62 patients, with a median age (IQR) = 72 (67-80), 37 (59.7%) females, and 30 (48.4%) were users. No significant difference was observed for all variables considered between users and non-users. A significantly lower value of VCSS was observed in nutraceutical users compared to non-users at t2 (median [Q1-Q3]: 7,0 [4,0-9,0] vs 9,0 [5,0-10,0]; p=0.025, respectively). A significant improvement in SP and the level of VI was observed in both groups at t2. Particularly, a significatively greater improvement of VI was observed in users than non-users (53.3% vs 18.8%, p=0.004; respectively), while no difference was observed for SP (36.7% vs 21.9%, p=0.200; respectively). In addition, a significant improvement in pain and inflammation was observed in users at t2. Particulalrly, in 9.4% of non-users and in 43.3% of users an improvement of pain was observed (p=0.002). In addition, an improvement in inflammation was observed only in users (33.3%).

Conclusions

The results suggested that adding this supplement to the therapeutic standard could improve the clinical status of the patient with CVI in all its symptoms.

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Title

The regulatory framework in the definition of borderline products under the Regulation (EU) 745/2017 and MDCG 2022-5 guideline

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Background

Medical devices (MD), especially medical devices made of substances (MDMS), and medicinal products have many similarities in their nature or specific intended purposes. Even if they should exert a different mode of action, however the defined borderline products are those where it is not clear from the outset whether they fall under the Regulation (EU) 2017/745 on medical devices (MDR) or the Directive 2001/83/EC on the Community code relating to medicinal products for human use (MPD). Several provisions to establish the demarcation between the two legal frameworks have been laid down in the guideline MDCG 2022-5 "*Guidance on borderline between medical devices and medicinal products under Regulation (EU) 2017/745 on medical devices*", not legally binding, that replaces the borderline guidance MEDDEV 2.1/3 Rev3, originally endorsed for the EU Medical Device Directive (MDD).

Methods

This analysis aims at gaining insight into the borderline between MDMS and medicinal products in this new regulatory framework. For this scope, the MDCG 2022-5 guideline and the MDR are analysed, and compared to MDD.

Results

A product cannot be qualified as MD if the product's principal intended action is achieved by pharmacological, immunological or metabolic means. Taking the regulatory perspective, any MD that incorporates a substance which, if used separately, would be considered a medicinal product, and that has an ancillary action to that of the MD, shall be assessed and certified in accordance with the MDR. As opposed to its old analogous MDD rule 13, with the introduction of MDR rule 14, these products are now considered class III MD, regardless of whether the medicinal substance acts directly on the body or not, of the quantity of the substance in the MD and of the method or route of administration. Moreover, the determination of whether the substance has an ancillary action to that of the device is

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scientifically objective and does not depend on the intention envisaged by the manufacturer. According to the last MDCG 2022-5, taking that the herbal substances such as *Chamomile, Calendula Officinalis, Mallow, Lavandula Angustifolia*, are also registered as herbal medicinal products therefore, when included/incorporated in a MD, this may lead to classification as a class III MD according to rule 14 of MDR, if exerted an ancillary action. In such cases the manufacturer must demonstrate that the action is ancillary to the principal intended action of the device.

Conclusions

The MDR and the MDCG endorsed have been a strong impact on the regulatory definition and framework about MDs and especially of MDMS. Looking at the last MDCG 2022-5, also long-standing, strongly consolidated MDs have been influenced, laying doubts about their assessment as MDs or medicines, so a revision of the MDCG 2022-5 is strongly recommended, especially regarding the ancillary substance and the regulatory assessment of the MDs, considered independent from the quantitative aspects of the substances.

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- MDCG 2022-5 Guidance on borderline between medical devices and medicinal products under Regulation (EU) 2017/745 on medical devices;
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Title

β-amyloid impairs the astrocyte-oligodendrocyte crosstalk required for myelination: preclinical evidence with co-ultramicronized palmitoylethanolamide/luteolin

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Background

Oligodendrocytes are fundamental for brain functions as they form the myelin sheath and feed axons (1). The fulfillment of this role is possible thanks to the cooperation with astrocytes (2). The astrocyte/oligodendrocyte crosstalk needs numerous mediators and receptors. For example, the fibroblast growth factor 2 (FGF2) and the transforming growth factor-β (TGF-β) are released by astrocytes to sustain oligodendrocyte precursor cells (OPCs) differentiation and survival (3, 4). Similarly, peroxisome proliferator-activated receptors (PPARs) agonists promote OPCs maturation in myelinating oligodendrocytes (5, 6, 7).
In the Alzheimer's disease (AD) brain, the deposition of beta-amyloid (Aβ) has been linked to several alterations, including astrogliosis (8, 9, 10, 11) and changes in OPCs maturation (12). However, very little is known about the molecular mechanisms involved. To contribute to filling this gap, we investigated the maturation of OPCs co-cultured with astrocytes in an in vitro model of Aβ1–42 toxicity and tested the effect of the anti-inflammatory and neuroprotective composite palmitoylethanolamide and luteolin (co-ultra PEALut), which is known to engage the PPAR-α.

Methods

Primary OPCs were cultured on permeable membranes inserted in wells containing primary astrocytes, thus allowing secreted soluble factors to diffuse while preventing physical contact between cells. Using RT-qPCR, Western Blot, and immunofluorescence experiments, we tested the possible beneficial effect of co-ultra PEALut treatment in counteracting $A\beta_{1-42}$-induced toxicity by studying the modifications of the astrocytic functions and the maturation and morphology of co-cultured OPCs. The involvement of PPAR-α was verified by using GW6471, a selective PPAR-α antagonist.

Results

Our results show that $A\beta_{1-42}$ triggers astrocyte reactivity and inflammation and reduces the levels of FGF2 and TGF-β. The maturation of co-cultured OPCs increases after $A\beta_{1-42}$ challenge but differentiated oligodendrocytes show a lower cell surface area and fewer arborizations with respect to control cells. Co-ultra PEALut counteracts the $A\beta_{1-42}$-induced inflammation and astrocyte reactivity, preserving the morphology of co-cultured oligodendrocytes through a mechanism that in some cases involves PPAR-α.

Conclusions

This study provides the first evidence of the negative effects exerted by $A\beta_{1-42}$ on the astrocyte/oligodendrocyte crosstalk and discloses a never explored co-ultra PEALut ability in restoring oligodendrocyte homeostasis. These findings open new opportunities for the adjuvant treatment of AD, as co-ultra PEALut is a promising composite, already licensed for human use, capable of exerting beneficial effects in both experimental and clinical settings.

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Title

Peripheral pharmacological sympathetic denervation elicits neuromuscular dysfunctions in mouse small intestine

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Background

Irritable bowel syndrome (IBS) is one of the most common functional disorders of the gastrointestinal (GI) tract, with a prevalence estimated to be about 11% in the general population. Persistent alterations of autonomic responsiveness, affecting gut-brain communications and bowel inflammatory responses, are likely to play a role in altered bowel habits (Gros et al., 2021; Zalewski et al., 2018). Thus, we aimed to evaluate the influence of peripheral sympathetic denervation with 6-hydroxydopamine (6-OHDA) on the morphofunctional integrity of mouse enteric nervous system (ENS).

Methods

Wild-type (WT) male C57BL/6J mice (8±2 weeks old; N=5 animals/group/experiment) were subjected to chemical sympathectomy by intraperitoneal (i.p.) administration of 6?OHDA (80 mg/kg/day; treated=6-OHDA) or vehicle (controls=CNTR) on three consecutive days. To sustain sympathectomy, mice were injected with 6-OHDA (80 mg/kg i.p.) every 10 days thereafter until day 20 (Willamze et al., 2019). GI transit was assessed by measuring the in vivo distribution of nonabsorbable-FITC-labeled dextran. Changes in ileal muscle tension were isometrically recorded following: cumulative addition of carbachol (CCh; 0.001–100 μM); increasing electrical field stimulation (EFS, 0–40 Hz); EFS 10 Hz in non-adrenergic, non-cholinergic (NANC) conditions (1 μM atropine, 1 μM guanethidine), with or without 0.1 μM 1400W (inhibitor of inducible nitric oxide synthase, iNOS), or 100 μM L-NAME (pan-NOS inhibitor). In ileal longitudinal muscle myenteric plexus preparations (LMMPs), the immunoreactivity of the pan-neuronal marker HuC/D, and of the glial markers S100β, GFAP, and PLP-1 was analyzed by confocal immunofluorescence.

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Results

Peripheral sympathetic denervation increased GI transit time. In 6-OHDA mice a marked reduction of carbachol-mediated contraction (Emax= -55% vs CNTR; P<0.01) and EFS-induced excitatory responses (Emax= -45% vs CNTR, P<0.01) was observed together with a significant increase of NANC-mediated relaxation (Emax=+180% vs CNTR, P<0.001), mainly dependent on nNOS-derived NO. In 6-OHDA LMMPs, a higher immunoreactivity of S100β, GFAP and PLP-1 was detected (+16%, +26% and +50%, respectively; P<0.01) associated to a reduction of HuC/D^{+} neurons (-15%, P<0.001), suggesting perturbed neuroglial activity.

Conclusions

These findings suggest that peripheral sympathectomy compromised excitatory neuromuscular responses, provoking marked glia activation and enteric neuronal injury. These neuromuscular morphofunctional alterations are highly indicative of the essential regulatory control sustained by catecholaminergic transmissions in guaranteeing an effective homeostatic neuroimmune interaction in the gut.

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Title

Toll-like receptor 4 deficiency alleviates neuromuscular dysfunction and damage to myenteric neurons in a mouse model of DNBS-mediated ileitis

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Background

Changes in enteric neurotransmissions and alterations in Toll-like receptor 4 (TLR4) expression are related to the onset and severity of inflammatory bowel diseases (IBD) in patients and related animal models.[1,2] We aimed to assess the crosstalk between enteric neuronal pathways and TLR4 signaling in a mouse model of dinitrobenzene sulfonic acid (DNBS)-induced small intestine inflammation.

Methods

Male C57/Bl6 (WT) and TLR4$^{-/-}$ mice (9±2 weeks old) were intrarectally treated with 2.5% DNBS. Pro-inflammatory cytokines (IL-6 and TNF-α) were measured in ileal samples. Changes in ileal contractility were isometrically recorded following: i) carbachol cumulative addition (CCh; 0.1-100 μM); ii) 10-Hz electric field-stimulation in non-adrenergic-non-cholinergic (NANC) conditions (1 μM guanethidine + 1 μM atropine) with or without 10 μM 1400W (a inducible nitric oxide synthase (iNOS) inhibitor), or 0.1 μM Nω-nitro-L-arginine methyl ester (L-NAME; a pan-NOS inhibitor).[3] Immunoreactivity (IR) of neuronal (HuC/D, ChAT), glial (S100β) and nitrergic neuron (i.e. nNOS^{+} and iNOS^{+}) markers was determined in longitudinal muscle-myenteric plexus whole-mount preparations (LMMPs) by confocal microscopy.

Results

DNBS administration significantly increased ileal IL-6 and TNF-α mRNA levels only in WT mice. In both genotypes, DNBS-induced ileitis determined a 2-fold increase of CCh-mediated contractions, enhanced ChAT immunoreactivity and number of $ChAT^+$ neurons. In WT mice experimental ileitis determined a 1.5-fold increase in NANC relaxations, sensitive to 1400W and L-NAME, suggesting the enhancement of both iNOS- and nNOS-derived NO release. DNBS-induced ileitis did not affect nitrergic-mediated responses in $TLR4^{-/-}$ mice, suggesting that TLR4 activation compromises inhibitory myenteric pathways. Experimental ileitis induced a significant reduction of the total number of HuC/D^+ neurons (−35% in WT and –25% in $TLR4^{-/-}$ mice), and a proportional increase in $nNOS^+$ neurons. In WT DNBS LMMPs, iNOS-IR and S100β-IR increased (+111% and 23%, respectively), whereas in $TLR4^{-/-}$ DNBS LMMPs, S100β-IR decreased to -12%, whereas iNOS-IR remained unchged with respect to WT untreated animals, highlighting a potential involvement of TLR4 signaling in the development of neuroplasticity and reactive gliosis.

Conclusions

Small intestine inflammation impairs myenteric neuroglial network functionality via TLR4-mediated responses, affecting both excitatory and inhibitory neurotransmission. These findings further strengthen the relevance of characterizing myenteric neuroimmune interactions for identifying the molecular mechanism/s representing putative pharmacological targets for the development of more efficacious IBD treatments.

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Title

Investigation of the two-faced role of IFN-γ in pain promotion and neuroprotection in rat organotypic spinal cord slices

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Background

Neuropathic pain is promoted by neuronal and glial cells activation, which release inflammatory mediators and support pain maintenance[1]. Interferon-γ (IFN-γ) is a pleiotropic cytokine, released by nervous cells, and a crucial modulator of central and peripheral responses[2]. Several studies report a pro-inflammatory action of IFN-γ in the nervous system but data on its neuroprotective activity have also been collected[3]. Its study arose from an RNA-sequencing conducted by our laboratory that showed the deregulation of IFN-γ expression and its pathway in astrocytes isolated from oxaliplatin-treated mice. This work aims to clarify protective functions and the mechanisms by which IFN-γ exerts neuroprotection in oxaliplatin-induced neurotoxicity on rat organotypic spinal cord slices

Methods

Spinal cord slices were prepared from 4 PND rat pups kept in culture for two weeks and then morphologically and functionally analyzed. The slices were incubated with oxaliplatin (10 μM) for 6 and 24 hours. Toxicity was assessed by measuring LDH release, GFAP and NeuN fluorescence intensity, and the expression levels of glutamate transporters (EAAT1 and EAAT2). The expression of neuronal activation marker, c-FOS, pro-algic mediators, like as Substance P (SP) and Calcitonin gene-related peptide (CGRP), and neuroprotective factors, e.g. Glial cell-Derived Neurotrophic Factor (GDNF) and Brain-Derived Neurotrophic Factor (BDNF) was measured by RT-PCR or ELISA. IFN-γ receptor 1 (IFNGR1) and Interferon regulatory factors (IRFs) expression was also observed. The effect of co-treatment with IFN-γ (65 ng/ml) was also evaluated

Results

We observed by toxicity studies that oxaliplatin-induced neurotoxicity was reverted in slices co-treated with IFN-γ. Also, IFN-γ co-treatment rescued neuron count and mitigated astrogliosis, promoted by oxaliplatin exposure, as indicated by NeuN and GFAP staining. IFN-γ-mediated neuroprotective effect was evaluated by observing the expression and release of pro-inflammatory, pro-algic, and neuroprotective mediators. The expression levels of c-FOS, CGRP and SP were enhanced by oxaliplatin or IFN-γ, as well as glutamate transporters production decreased, confirming an inflammatory and excitotoxic scenario, improved in co-treated slices. IFN-γ co-treatment reduced GDNF and BDNF expression, exacerbated by oxaliplatin. Finally, we analyzed the expression of IFNGR1 and IRFs, particularly Irf-1, trying to individuate molecular actors underlying neuroprotection and we observed that co-treatment with IFN-γ enhanced a decrease in IFNGR1 but not in Irf-1 expression levels, candidate this target as a potential neuroprotective player

Conclusions

Our results show a general amelioration of several parameters analyzed in organotypic spinal cord slices treated with oxaliplatin and IFN-γ and thus the next steps will be aimed at clarifying the mechanisms by which IFN-γ is able to exert its neuroprotective role, focusing on its receptor, its downstream targets or collateral activated pathways

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Title

New Eph antagonists discriminating between Eph-As and Eph-Bs classes

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Background

Eph receptors are tyrosine kinase receptors activated by membrane proteins, named ephrins. Both the receptors and ligands are divided in two families, -As and -Bs. It is well demonstrated the key role of Eph-ephrin system in several human cancers supporting tumorigenesis, cancer progression, invasiveness and metastasis (Xi et al. 2012) without a clear distinction between the contribution of -As or -Bs subtypes.

Since 2011, our interest has been captured by the development of small molecules able to prevent Eph-ephrin interactions. However, the main problem of all discovered molecules concerns the absence of selectivity versus a receptor subfamily to the detriment of the other one (Lodola et al. 2017). Here, we identified UniPR1447, a derivative of cholenic acid conjugated with L-β-homotryptophan, as a potential Eph-As selective antagonist. Next, docking simulations suggested that the functionalization of the indole group could lead to a better selectivity versus Eph-As. Our research was focused on the pharmacological characterization via *in vitro* studies of UniPR1447 and of its phenyl-sulfonyl derivate, UniPR1449, in order to confirm our computational previsions.

Methods

ELISA binding assays were performed to determine IC_{50}/Ki values of molecules on EphA2-Fc receptor in presence of ephrin-A1-biothinylated using one-site competition non linear regression analysis with Prism software (GraphPad Software Inc.).

Non-specific toxicity was evaluated through Lactate DeHydrogenase quantification and cell viability was evaluated

using the MTT colorimetric assay on U251 human glioblastoma cells.

The ability of the newly synthesised compounds to inhibit EphA2 or EphB4 phosphorylation in U251 cells was determined through an ELISA assay. Briefly, cells were pretreated for 20 minutes with the vehicle (0.3% DMSO) or the compounds at 30μM and 10μM and then we induced receptors phosphorylation by treatment for 20 minutes with ephrin-A1-Fc or ephrin-B2-Fc.

Results

Both UniPR1447 and UniPR1449 showed a competitive inhibition of EphA2-ephrin-A1 binding sharing Ki values in the low micromolar range. When tested on EphB1, -B2, -B3 and -B4, UniPR1447 maintained its activity whereas UniPR1449 showed poor or no activity.

When tested on Eph phosphorylation, both the compounds showed an antagonism behavior.

Conclusions

The collected data suggest the possibility to synthesize new antagonists able to discriminate between EphAs and -Bs. These findings could be useful to dissect EphAs and -Bs role in different pathophysiological events.

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pharmadvances
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Title

Drug-utilization and healthcare facilities use in patients with ulcerative colitis treated with biologic therapy. Data of healthcare administrative database of Tuscany: results from MICHELANGELO study

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Background

Biologic drugs for ulcerative colitis (infliximab, adalimumab, golimumab and vedolizumab) are recommended when conventional therapy fails[1]. Real-world studies may provide relevant information for the optimization of care. The objective of the study was to describe drug-utilization and Regional Health System use in patients with ulcerative colitis new users of biologics.

Methods

This is a descriptive, retrospective cohort study (EUPAS40896). We used data collected from the Tuscan healthcare administrative databases: the drug-reimbursement database and the registries of emergency department (ED) admission, hospital discharges, and specialist visits. The study cohort included patients with the date of the first dispensation of a biologic drug (index date) recorded between 01/2015 and 12/2019. We created one cohort for each drug of interest. We included patients with: age ≥ 18; at least five years of data recorded before the index date (look-back period); at least one year of follow-up; UC diagnosis OR UC co-payment exemption in the look-back or in the

edra

follow-up OR a gastroenterological visit in the year before the index date. The history of conventional therapy use, treatment coverage and switching events were evaluated. We described the number of patients with at least one admission to ED or hospitalization and the time free from the first event recorded.

Results

We analyzed four cohorts of patients with ulcerative colitis who were new-users of adalimumab (N=239), infliximab (N=175), golimumab (N=110) and vedolizumab (N=107). Almost all patients were treated with conventional therapy prior to biologics. Adalimumab cohort showed the lowest proportion of patients with history of other biologics (N=37; 15%), while vedolizumab cohort had the highest (N= 69; 65%). The mean coverage of biologics exceeds 100% of treatment's days, except for infliximab (94%). Twenty-four patients (22%) new users of golimumab switched to another biologic drug, mostly infliximab. Among the other cohorts, switch events occurred in 22 (13%) infliximab patients, and in 26 (11%) and 8 (8%) adalimumab and vedolizumab patients, respectively. Vedolizumab and infliximab cohorts showed the highest proportion of patients with at least one ED access (N=43; 40%) and hospitalization (N=61; 35%), respectively. Overall, we didn't find relevant difference between the cohorts in the use of healthcare facilities. The time-free from the first ED admission ranged from 140 days (±104.04) for infliximab cohort to 176 days (±107.71) for adalimumab. The time-free from the first hospitalization resulted 139 days (±111.39) for golimumab patients and 166 days (±106.25) for vedolizumab cohort.

Conclusions

The use of biologics seems to be in line with clinical recommendation. Adalimumab resulted to be the most widely used as first choice. The occurrence of hospitalizations and ED accesses was almost similar among patients with ulcerative colitis who were new users of biologic drugs.

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pharmadvances

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Title

ICOS-Fc as innovative immunomodulatory approach to counteract inflammation and organ injury in sepsis

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Background

Inducible T cell co-stimulator (ICOS), a costimulatory immune checkpoint expressed on activated T cells and its ligand, ICOSL, which is expressed on antigen-presenting cells and non-hematopoietic cells have been extensively investigated in the immune response. Recent findings show that a soluble recombinant form of ICOS (ICOS-Fc) can act *in vivo* as an innovative immunomodulatory drug acting as antagonist of ICOS and agonist of ICOSL, modulating cytokine release and cell migration to inflamed tissues. Although the ICOS-ICOSL pathway has been poorly investigated in the septic context, a few studies have reported that septic patients have reduced ICOS expression in whole blood, and that reduced ICOS levels are associated with organ dysfunction. Moreover, septic patients display increased serum levels of osteopontin (OPN), that is another ligand of ICOSL. Thus, we aimed to investigate the role of ICOS-ICOSL in the pathogenesis of sepsis and the potential beneficial effects of its immunomodulation by administering ICOS-Fc in a murine model of cecal ligation and puncture (CLP)-induced sepsis.

Methods

Five-month-old male wild-type (WT) C57BL/6, ICOS$^{-/-}$, ICOSL$^{-/-}$ and OPN$^{-/-}$ mice underwent either CLP or Sham surgery and 1 h after the surgical procedure, mice were randomly assigned to receive once ICOS-Fc, ^{F119S}ICOS-Fc (100 µg each) or vehicle (PBS) intravenously. Organs (liver and kidney) and plasma were collected 24 h after surgery for analyses.

Results

When compared to Sham mice, WT mice subjected to CLP developed a higher clinical severity score and a reduced body temperature 24 h after surgery. This was paralleled by systemic cytokines storm (TNF-α, IL-1β, IL-6, IFN-γ and IL-10) and increased levels of markers of liver (AST and ALT) and kidney (creatinine and urea) damage. Administration of ICOS-Fc to WT CLP mice protected from the sepsis-induced abnormalities, due to a reduced renal and liver leukocyte infiltration. By contrast, the effect was absent using a mutated form of ICOS-Fc (^{F119S}ICOS-Fc), unable to bind ICOSL. We also documented significant inhibitory effects of ICOS-Fc on sepsis-induced local activation of FAK (focal adhesion kinase), P38 MAPK, as well as the NLRP3 inflammasome complex. ICOS-Fc seemed to act at both sides of the ICOS:ICOSL interaction since the protective effect was lost in CLP-induced sepsis in knockout mice for the ICOS or ICOSL genes, whereas it was maintained in OPN knockout mice.

Conclusions

Our data show for the first time the beneficial effects of pharmacological modulation of the ICOS-ICOSL pathway in counteracting the sepsis-induced inflammation and organ dysfunction.

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Title

Semaphorin 3A overexpression activates neuroinflammatory pathway in human microglia

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Background

Semaphorins are a large family of cell surface and secreted guidance molecules [1]. In particular, among all members, Semaphorin 3A (Sema 3A) is involved in neuronal axonal repulsion and dendrite polarity [2] as well as regulation of some essential stages of inflammation [3] and immune response [4]. On note, increased Sema 3A expression levels are found in some neurologic diseases in which neuroinflammation appears to play a critical role [5,6]. As microglia is one of the key mediators of neuroinflammatory processes during the development [7] we investigate whether Sema 3A overexpression affects microglia polarization and activity.

Methods

Human microglia were transiently transfected with GFP-tagged Sema 3A as well as GFP-empty vector control. After 48h, cells were analysed by western blot or immunofluorescence.

Results

Sema 3A overexpression promotes the microglia switch into the M1 pro-inflammatory phenotype, and increases the expression of inflammatory mediators, such as iNOS and TNFα. In addition, a large amount of microglia overexpressing Sema 3A die, suggesting a role of Sema 3A in promoting cell death.

Conclusions

Sema 3A might represent a possible druggable target in neuroinflammation-related mental disorders.

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pharmadvances

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Title

The correlation between Prostaglandin E2 and ERK5 modulates the behavior of NSCLC cells.

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Background

ERK5, the latest discovered member of the mitogen-activated protein kinase (MAPK) family, is involved in modulating many features of cancer (Rovida et al., 2019). The link between inflammation and cancer is now part of an accepted paradigm of carcinogenesis. Prostaglandin E2 (PGE2) is an inflammatory mediator with tumor activity, implicated in regulation of the tumor microenvironment and resistance to chemotherapy (Donnini S. et al., 2012; Donnini S. et al., 2016). PGE2 activates MAPK family members, promoting the progression of various solid tumors including non-small cell lung cancer, NSCLC (Krysan et al., 2005). Based on this evidence, the purpose of this study was to investigate whether PGE2 induced ERK5 activation in a model of NSCLC and the role of this signaling in tumor functions, such as growth, migration, and tumor angiogenesis

Methods

A549 cells wild type (WT) and silenced for ERK5 expression (ERK5-) were used. ERK5 protein expression was evaluated with western blot assay. MTT and proliferation assays were performed to evaluate the proliferation rate of A549 WT and ERK5- under treatment with PGE2. The role of PGE2- ERK5 signaling pathway in migration and invasiveness were tested by scratch assay and Boyden chamber assay, respectively. Clonogenicity was performed to assess the ability of A549 WT and ERK5- to form clones in response to PGE2. Vascular mimicry assay was performed on Matrigel matrix to deeply investigate the involvement of PGE2 and ERK5 in tumor angiogenesis and progression

Results

PGE2 [1µM] induced phosphorylation and activation of ERK5 in A549 WT. PGE2 exposure significantly promoted the proliferation, clonogenicity, migration and invasiveness of A549 WT. Gene knock-down of ERK5 impaired PGE2-mediated proliferation of A549 cells and reduced the ability of the cells to form clones and migrate. Finally, A549 on Matrigel could mimic a vascular network, a feature known as vascular mimicry, which PGE2 was able to empower and ERK5 silencing to prevent both under basal conditions and in response to PGE2

Conclusions

Collectively, these data demonstrate a close relationship between PGE 2 and ERK5 in NSCLC. Therefore, the modulation of PGE2 could be considered a potential strategy to hit the ERK5 activity and its downstream effect on cancer progression and aggressiveness

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Title

Study of angiogenic profile in patients with NSCLC

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Background

Non-Small Cells Lung Cancer (NSCLC) is the most common cause of cancer death worldwide (WHO).Among the therapies used against NSCLC progression immune checkpoint inhibitors (anti-PD1, ICIs) have proven to positively modulate overall survival, progression free survival and objective response rate in cancer patients.However, the diagnostic use of PD-L1 expression for the treatment of NSCLC patients with ICIs has not been found to be a predictive biomarker of effective response.Tumor angiogenesis with disorganized tumor vessels impairs the distribution of drugs, and their efficacy (Filippelli A. et al., 2020).The aim of this project has been to study the expression profiles of molecular determinants of angiogenesis as potential new biomarkers of the efficacy of ICIs.The study has been done in plasma (in platelets) of NSCLC patients before and during ICIs treatment and at disease progression.The final aim will be to correlate these data with clinical data of response to the treatment

Methods

To date, 53 patients with diagnosis of non?operable advanced stage NSCLC, without history of previous or concomitant other neoplastic disease, candidates to treatment with ICIs as first or second line have been accrued by UOC Oncology and Radiotherapy, AOU Careggi, Florence. Blood was collected at baseline, at 2- and 4-months during treatment, and at the time of progression.The poor plasma platelets (PPP) and platelet?rich plasma (PRP) were generated by centrifugation and collected.Multiplex ELISA assay was performed to characterize platelets lysate by the quantification of main markers involved in angiogenesis and/or vessel normalization. Particularly, the concentration of Ang 2, IL-8, VEGF, FGF, and PDGF were assessed

Results

At the moment, a cohort of women (n=22) and men (n=31) with a mean age of about 65 years was enrolled in this clinical trial. For 18 patients, only blood samples were collected at baseline (T0), while 28, 4 and 6 samples were obtained at 2 months (T2) and 4 months (T4) during treatment and at the time of progression (Tp), respectively. For the first 19 patients, whose samples were available at T0 and T2, a multiplex ELISA assay was performed to assess the concentration of Ang 2, VEGF, FGF, PDGF, and IL-8 in platelet lysate. Preliminary data confirmed that VEGF is the main angiogenic marker carried by platelets. The level of PDGF seems to increase with the onset of progression. On the other hand, IL8 and Ang2 showed very low expression in platelet, and no uniform trend has been assessed between patients in the two periods analyzed

Conclusions

The preliminary data will be integrated with molecular (TMB and cfDNA), histological and clinical data by using multi omics data platform to validate the role of platelets as potential biomarker of angiogenesis and response to immunotherapy. The proposed approach would allow to identify a reliable circulating and tissue new markers as predictive factor of activity and efficacy of ICIs in patients with advanced NSCLC

References

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Title

Understanding Metformin: has the penny dropped? A case report.

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Background

Amongst the wide range of treatments used for type 2 diabetes (T2D), metformin is one of the most commonly used both for its efficacy and safety. Nonetheless, one of the most common and often unacknowledged adverse effects is metformin-associated lactic acidosis (MALA), which has a frequency of 2-9:100000 in the USA. The pathogenesis of this condition resides in the pharmacodynamics of the drug itself, which leads to an accumulation of lactic acid in the organism. It shows high mortality (up to 30%) and the most commonly associated symptoms are nausea and vomit, while more severe cases are associated with sensory alterations, coma, and acute kidney failure.

Methods

The reported data were obtained after consent collection. All the data here shown come from the Internal Medicine division of SM delle Croci Hospital, Ravenna, Italy.

Results

An 82-year-old diabetic woman entered the ER for abdominal pain, anuria, and sensory alterations. Blood tests showed a severe kidney failure (11.46 mg/dL creatinine) with an associated metabolic acidosis (7.054 pH, 2.99 mmol/L lactates, 8,6 mmol/L HCO3). At first, she was treated with a total amount of 5000 ml of saline solution iv, 250 mg of furosemide iv, and 400 ml of an 8.4% bicarbonates solution. Subsequently, she was hospitalized and admitted to the Internal Medicine division. It emerged that she was administered 2000 mg of metformin daily as a home therapy for T2D. Suspecting a drug accumulation and the subsequent drug-based metabolic acidosis, metformin was immediately

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terminated. A nephrological consult showed no necessity for dialysis, both for bad timing and for the vast amount of fluids administered, and the diagnosis of MALA was confirmed. On the second day, while the kidney failure was improving (7.05 mg/dL creatinine), she was transferred to the ICU because of the increasing respiratory acidosis (7.05 pH, 60 mmHg pO2, 20 mmol/L HCO3) and non-invasive ventilation (NIV) was performed. Subsequently, while the patient never regained consciousness, she died on the tenth day in ICU because of urosepsis by E. Faecalis.

Conclusions

Given the wide use of metformin as an oral hypoglycemic drug, it is necessary to stress the importance of the adverse effects of this treatment. In fact, recent studies have shown a clear efficacy of early dialysis in case of MALA, with overall survival of 70%. It must be stressed the importance of the presence of Pharmacology and Toxicology practitioners in emergency and internal medicine divisions, so that conditions and situations like the one shown will be further limited and earlier detected.

References

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