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Title

Evaluation of the intranasal administration of novel A_{2A} antagonists in the mice EAE model of chronic multiple sclerosis

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Background

Multiple sclerosis (MS) is an autoimmune and inflammatory disease of the central nervous system (CNS) characterized by oxidative stress, demyelination, and neuronal damage. Adenosine plays an important role in MS as it regulates, via activation of its A_{2A} receptor, the inflammatory and immune response. In the brains of MS patients there is an increased density of A_{2A} receptors that appears to correlate with disease severity (Rissanen et al., 2013). In experimental autoimmune encephalomyelitis (EAE), a mouse model of MS, A_{2A} signaling can produce opposite effects, depending on the level of disease progression. Genetic inactivation of the A_{2A} receptor generates a more severe form of EAE (Yao et al., 2012), while the A_{2A} antagonist SCH58261 administered at an advanced stage reduces neuroinflammation and axon demyelination (Chen. et al., 2019). The aim of this project is to further clarify the role of A_{2A} receptors in MS. To this purpose we will evaluate the pharmacological profile of novel and selective A_{2A} antagonists labelled P400 (Ki = 26 nM) and P625 (Ki= 15.1 nM) (Varano et al. 2016, Varano et al. 2020) in reducing symptoms associate with a chronic MS model.

Methods

The EAE model of chronic MS, a less frequent form that does not respond to therapies, was induced in C57BL/6 mice by the administration of MOG_{35-55}, as previously described (Procaccini et al., 2015). The main MS-related symptoms, such as motor disability (clinical score, rotarod test), pain hypersensitivity (von Frey test, hot plate test, hot plate test) and weight loss, were regularly assessed during the experiment. A_{2A} antagonists, were administered intranasally 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 was removed 30 days after immunization, and sample were used for biochemical analysis.

Results

On day 28 after immunization, animals treated with P400 and P625 10 µg/mice intranasally reduced mechanical allodynia, thermal hyperalgesia, and motor disability associated with the MOG-EAE. Staining with Luxol Fast Blue showed that MOG-EAE animals had a strong loss of myelin, whereas treatment with P400 and P625 resulted in an increase in coloration corresponding to an increase in myelin content. These findings were then confirmed by immunostaining MBP in the spinal cord. No effect was observed on lymphocyte infiltration in the spinal cord following treatment with P400 and P625 compared to the MOG-EAE control group.

Conclusions

These data reveal the interesting pharmacological role of new A_{2A} antagonists in reducing the symptomatology associated with MOG-EAE as well as spinal demyelination.

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Title

Single or repeated exposure to cannabidiol modulates BDNF expression in the rat prefrontal cortex and striatum

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Background

Cannabidiol (CBD) is one of the most abundant phytocannabinoid contained in *Cannabis sativa* L. plant that lacks psychoactive effects. Due to its ability to counteract the psychoactive D9-tetrahydrocannabinol, and its broad-spectrum pharmacological activity, without toxic effects for humans and animals, CBD is considered a valuable potential treatment for several psychiatric and neurodegenerative disorders. Despite the evidence about CBD pharmacological profile and toxicity, a thorough characterization of its brain distribution and of its neuroplastic effects is still lacking. In this study we performed a dose-response treatment with CBD to evaluate 1) its brain distribution and 2) its modulatory effects on Brain-derived neurotrophic factor (BDNF), a neurotrophin critical for synaptic plasticity, focusing our attention on the cortico-striatal pathway.

Methods

For these purposes, male adult rats were exposed to single and repeated CBD treatments at 5, 15 and 30 mg/kg, to investigate both the rapid modulation of the neurotrophin after single treatment, as well as a potential drug-free time point following seven days of CBD treatment. CBD levels were evaluated in the medial prefrontal cortex (mPFC) and in plasma, and all the molecular analysis were performed both in the mPFC and in the Striatum.

Results

We show here, for the first time, that CBD can be found in the rat brain, specifically in the mPFC. In fact, CBD is present in the mPFC of rats treated either acutely or repeatedly with the phytocannabinoid, with a clear dose-response profile. From a molecular standpoint, we found that a single dose (30 mg/kg), but not repeated, of CBD is sufficient to upregulate Bdnf exon IV, the most abundant isoform of the BDNF gene, which is paralleled by a similar increase in cortical mBDNF and TrkB. Conversely, the repeated exposure increased BDNF only in the striatum, with a slight decrease in the mPFC, potentially supporting an increased anterograde trafficking of BDNF from the mPFC to the striatum, mediated by CBD.

Conclusions

Our results show that CBD can be detected in the plasma and in the mPFC, following single or repeated injections, with a dose-response profile. Moreover, we have found that CBD induced a dose-dependent modulation of BDNF, anatomically specific, which adds to the notions so far obtained on the central nervous system effects of CBD.

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References

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Title

Effects of environmental enrichment on alcohol self-administration and mesolimbic dopaminergic neuronal activity in Sardinian alcohol-preferring rats.

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Background

Living in an enriched environment (EE) produces brain structural and functional effects that are associated with changes in behavior, including those motivated by drugs of abuse. However, while several studies support a protective effect of EE against addiction-related behaviors from psychostimulants, the picture appears more complex when referring to alcohol. To further disentangle the relationship between EE and alcohol-motivated behaviors, this study aimed to assess the effect of EE in a validated animal model of alcohol use disorder, the Sardinian alcohol-preferring (sP) rats. In particular, we investigated the influence of an EE exposure on i) operant oral alcohol self-administration and ii) functionality of the mesolimbic dopamine system, key in the mechanisms underlying alcohol consumption and dependence.

Methods

Starting from postnatal day 21 (PND 21), male sP rats were housed in 3 different conditions: impoverished environment (IE; single housing and no environmental enrichment), standard environment (SE; small colony and no environmental enrichment), and EE (large colony and multiple elements of environmental enrichment). From PND 60, rats were subjected to different phases of shaping and training of alcohol self-administration. IE, SE, and EE rats were then compared under (a) fixed ratio (FR) 4 (FR4) schedule of reinforcement for 20 daily sessions and (b) progressive ratio schedule of reinforcement in a final single session. In a separate set of experiments, extracellular electrophysiological recordings from ventral tegmental area (VTA) dopamine neurons were carried out in anesthetized rats (PND 60)

belonging to the different experimental groups (IE, SE, and EE).

Results

We found that, compared to IE rats, EE rats required a higher number of shaping and training sessions to acquire the alcohol self-administration behavior; SE rats displaying intermediate values. A similar ranking order (IE>SE>EE) was also observed in number of lever-responses for alcohol, amount of self-administered alcohol, and breakpoint for alcohol under FR4 and PR schedules of reinforcement. In addition, analysis of the electrophysiological properties of VTA dopamine neurons revealed a higher number of cells/track, coupled with a higher mean spikes/burst, in EE rats compared to SE and IE rats (EE>SE>IE), thus suggesting that exposure to EE was able to increase both tonic and phasic mesolimbic dopaminergic neuronal activity.

Conclusions

Altogether our results indicate that living in enriched environments reduced the reinforcing and motivational properties of alcohol in sP rats. Importantly, the tendency toward an increased in mesolimbic dopamine neuron functionality might provide a mechanism for these behavioral responses.

References

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Title

Gonadotropin-releasing hormone analog treatment in children with idiopathic central precocious puberty: a pharmacovigilance study in a pediatric population.

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Background

Central precocious puberty (CPP) results from premature activation of hypothalamic-pituitary-gonadal axis, which leads to an increased release of gonadotropin- releasing hormone (GnRH). GnRH stimulates the development of secondary sexual features, rapid bone maturation and growth. GnRH agonists (GnRHa) represent the gold-standard therapy in children with CPP.The treatment is generally safe and well tolerated. However, several studies reported side effects, including bruising, pain, injection reactions and sterile abscesses. Furthermore, flushes, headaches, and nausea were observed and are classified as minor menopausal-like side effects. The aim was to identify the side effects occurrence in CPP patients treated with different GnRHa. We also evaluated the predictive factors for the onset of adverse events.

Methods

110 patients (median age 7.35 ± 0.67) affected by CPP were enrolled in this retrospective study, carried out from 2018 to 2020. The enrolled patients were treated with leuprolide (48 patients) or triptorelin (62 patients) and minor menopausal-like side effects appearance during therapy was investigated. Moreover, clinical parameters and radiological changes were monitored to evaluate the possible relationship between **GnRHa** treatment and side effects appearance.

Results

Overall 47 (42,7%) of the studied patients reported minor menopausal-like symptoms. Triptorelin patients had significantly higher LH and peak LH levels than leuprolide patients at baseline. No significant differences in other patient characteristics were observed between groups at baseline. In addition, a significantly greater increase in height was observed in patients on leuprolide. Concerning the drug used, 28 patients treated with triptorelin and 19 patients with leuprolide reported symptoms ($p = 0.558$). The patients treated with triptorelin, or leuprolide showed headache (60,7% vs 42,1%), mood swings (28,6% vs 42,1%), increased appetite (28,6% vs 47,4%) and nausea (3,6% vs 26,3%) respectively. Symptoms were managed with analgesics or over the counter (OTC) medications so that it was possible to keep GnRHa treatment.

Conclusions

Our results suggest the need of implementing pharmacovigilance activity in pediatric patients treated with GnRH agonists to optimize and personalize the treatment. In conclusion, our report highlighted the importance of early treatment of CPP patients to avoided minor menopausal-like symptoms.

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Title

INTERLEUKIN-1 RECEPTOR 8-DEFICIENT MATURE NATURAL KILLER CELLS DO NOT IMPACT ATHEROSCLEROSIS

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Background

Reduced inflammation correlates with decreased cardiovascular risk, thus suggesting that identification of players of metabolic inflammation underlying atherosclerosis could offer novel therapeutic targets for the treatment of patients at increased CVD risk. An important immune modulator is Interleukin-1 receptor 8 (Il-1r8), able to dampen IL-Rs and Toll Like Receptors (TLR) activation. Previously, we showed that *Il-1r8* deficiency results in increased frequency of mature natural killer type 2 cells (mNK2) as well as their hyperresponsiveness, thus improving the resistance to hepatic tumor, metastasis, and cytomegalovirus infection. Our aim was to investigate the role of Il-1r8 and thus mNK2 phenotype in experimental atherosclerosis.

Methods

Il-1r8-/- mice were crossed with *Ldlr*-/- mice to generate an experimental model presenting increased frequency of mature NK2 cells on an atheroprone background (double KO, DKO). 8 weeks old-*Ldlr*-/- and *Il-1r8*-/- *Ldlr* -/- male mice were fed with standard diet (Chow Diet, ChowD) or cholesterol-enriched diet (western-type diet, WTD) for 12 weeks to promote atherosclerosis. The effect of *Il-1r8* deficiency on lipid metabolism and atherosclerosis progression were evaluated through plasma lipid profiling, extensive immunophenotyping by flow cytometry, and histological analysis of the atherosclerotic plaques at the aortic sinus.

Results

Ldlr-/- mice fed for 12 weeks with WTD present a significant increase in circulating mature Natural Killer type 2 cells when compared to ChowD ($p<0,05$). To understand whether increased mNK2 levels result in increased atherogenesis or represent a bystander of the underlying inflammation, we generated an experimental model presenting elevated levels of circulating mNK2 cells on an atheroprone background, by crossing *Il-1r8-/-* mice with *Ldlr-/-* mice. Plasma lipid profile and circulating immune cell distribution resulted comparable in DKO mice compared to *Ldlr-/-* mice, while a decrease in NKs and DCs levels in mediastinal lymph nodes, and an increase in splenic macrophages count were observed. Interestingly, DKO mice fed with WTD for 12 weeks presented a significant increase in circulating mNK2 ($p<0,05$) and monocytes ($p<0,05$) compared to *Ldlr-/-* animals. These differences, however, were not paralleled nor by increased infiltration of these immune subsets in the atherosclerotic plaque of DKO mice, neither by changes in atherosclerotic lesion area, collagen deposition and macrophages content. In addition, plasma cholesterol and triglycerides levels were similar between the two animal models.

Conclusions

These data suggest that *Il-1r8* deficiency does not impact atherosclerosis development and Il-1r8 pathway is redundant in the immune activation observed during atherogenesis.

References

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Title

Effect of PCSK9 inhibitors treatment on serum lipoprotein functions in subjects with familial hypercholesterolemia: a multi-lipid-center real-word evaluation.

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Background

Proprotein convertase subtilisin/kexin type 9 (PCSK9), beyond regulating plasma cholesterol levels, exerts several pleiotropic effects modulating lipid metabolism in extrahepatic cells, including macrophages. Indeed, PCSK9 may directly modulate both macrophage cholesterol efflux and cholesterol uptake. However, macrophage cholesterol homeostasis also strictly depends on serum lipoprotein functions, including the HDL capacity to promote cell cholesterol efflux capacity (CEC) and the serum capacity to promote cell cholesterol loading capacity (CLC). The aim of the present study was to evaluate the effect of PCSK9 monoclonal antibodies treatment on HDL-CEC and serum CLC in a cohort of heterozygous Familial Hypercholesterolemia (HeFH) patients.

Methods

N = 31 patients with a diagnosis of heterozygous FH have been recruited. Blood was collected and serum isolated at baseline and after six months of treatment with PCSK9 inhibitors (evolocumab/alirocumab). HDL-CEC through the main pathways was evaluated with a radioisotopic cell-based assay. Serum CLC was assessed fluorimetrically in human monocyte-derived macrophages THP-1.

Results

After treatment with PCSK9 inhibitors, total cholesterol and LDL-c significantly decreased (-40.3%, p<0.0001 and -50.4%, p<0.0001 respectively), while no changes were observed in HDL-c and TG levels. Total HDL-CEC was not different between patients before and after treatment. Conversely, despite no changes in HDL-c levels between the groups, ABCG1 HDL-CEC significantly increased after treatment (+22.2%, p<0.0001) as well as HDL-CEC by aqueous diffusion (+7.8%, p=0.0008). No significant changes of ABCA1 CEC between the groups occurred after treatment. PCSK9 inhibition significantly decreased serum CLC (-6.6%, p=0.0272). This effect was only partly related to the reduction of LDL-c levels.

Conclusions

Treatment with PCSK9 inhibitors had a positive impact on both quantitative and functional lipid profile as it increased aqueous diffusion and ABCG1 HDL-CEC and reduced the serum CLC. All these effects may contribute to the reduction of CV risk obtained after PCSK9 inhibitors treatment in FH patients.

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Title

Sagrantino cv. leaves extracts: chemical characterization and in vitro evaluation of vasoactive properties.

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Background

The grape harvesting and vine pruning procedures produce a huge number of by-products that represent an important source of secondary raw material, due to their polyphenols content.

Methods

Sagrantino cv. leaves, collected during and after the grapes harvest period, were extracted using an ultrasounds bath at 60°C for 1,5 h in green solvents (water and ethanol at various ratios). Extracts were characterized by 1 H-NMR and HRMS analyses and investigated for their vasoactive properties in *in vitro* experiments on rat thoracic aorta rings (with and without endothelium), contracted by phenylephrine or KCl.

Results

The spectroscopic analyses revealed the presence of different bioactives in the extracts: in particular, high amount of sugars, but also glycosylated flavonoids such quercetin derivatives and flavonols including catechin. Furthermore,

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different amino acids, such GABA and tyrosine have been identified but also cinnamic acids, such as ferulic and caffeic ones.
Water and ethanol extracts enhanced 25 mM KCl-induced contraction, stimulation ranging between 20% and 100% of control, though only at concentrations above 10 µg/ml. In rings with an intact endothelium, pre-contracted by phenylephrine, an hormetic effect was observed, characterised by a maximum 50% relaxation at concentration of 10 µg/ml that, at high concentrations, reverted to control values.
This effect was not observed in the absence of endothelium, where ethanol extracts caused a concentration- dependent increase in the phenylephrine-induced tone.
These data are in line with the presence of known vasorelaxant compounds present in the extract.

Conclusions

These preliminary findings suggest that Sagrantino's leaves can provide potentially vasoactive by-products.

References

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Title

PROPROTEIN CONVERTASE SUBTILISIN/KEXIN TYPE 9 (PCSK9) MODULATES CHOLESTEROL HOMEOSTASIS AND NEUROINFLAMMATION IN CELL MODELS OF ASTROCYTES AND NEURONS

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Background

PCSK9 may be involved in Alzheimer’s disease (AD), although the underlying mechanisms are not fully clarified. This study aims to investigate the influence of PCSK9 on brain cholesterol transport, essential to maintain neuronal functions, and neuroinflammation.

Methods

Human neuroblastoma SH-SY5Y cells overexpressing PCSK9 and astrocytoma U373 cells exposed to exogenous PCSK9 have been used. The incubation with Amyloid β (Aβ) fibrils was used to reproduce AD-like conditions. Cholesterol synthesis, efflux and uptake were evaluated by radioisotopic assays, cholesterol content by a fluorometric assay, gene and protein expression through qRT-PCR and Western Blot (WB).

Results

In PCSK9-overexpressing SH-SY5Y cells, apoE-HDL uptake was reduced ($p<0.001$) as compared to control cells, independently of Aβ. PCSK9 overexpression reduced LDLR and ApoER2 expression ($p<0.05$). The overexpression of

PCSK9 in SH-SY5Y furtherly increased the neurotoxicity induced by Aβ and by cholesterol depletion ($p<0.05$). We recently discovered a family of new small-molecules with inhibitory activity on PCSK9 transcription; treatment of SH-SY5Y with one of these molecules reversed the PCSK9-associated reduction of cholesterol uptake. In U373 PCSK9 reduced intracellular cholesterol content ($p<0.05$), with a strengthened effect when co-incubated with Aβ ($p<0.01$). PCSK9 increased cholesterol biosynthesis ($p<0.05$) and reduced LDLR and apoER2 expression ($p<0.05$).). PCSK9 did not alter ABCA1- and ABCG1-mediated cholesterol efflux. Conversely, ABCA1-efflux and expression were reduced by Aβ ($p<0.001$). Finally, in U373 PCSK9 enhanced the neuroinflammation induced by Aβ, increasing IL-6, IL-1β, TNF-α gene expression and chemokine MCP1 release ($p<0.05$ for all).

Conclusions

PCSK9, cooperating with Aβ, impairs brain cholesterol transport and worsens neuroinflammation, with negative consequences on neuronal survival. A PCSK9 inhibitor seems to be able to restore the last step of brain cholesterol transport. Hence, PCSK9 may be considered a pathogenic factor and a possible pharmacological target in AD.

References

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Title

Improving the prediction of permeability and anti-inflammatory effects of drugs in the colon

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Background

Considering the growing concern about inflammatory bowel diseases (IBD), reliable models that mimic the inflamed gut epithelium are increasingly explored[1]. Colonic 3D organoids (colonoids) are currently the most advanced model of colonic epithelium[2], although the 3D structure hinders the access to the luminal surface[3]. Our general goal is therefore to develop an IBD *in vitro* model based on human colonoid-derived monolayers exposed to the pro-inflammatory cytokines TNF-α and IFN-γ, in order to predict the impact of inflammation on the enteric absorption of drugs and to evaluate their potential anti-inflammatory actions. Specifically, the present work aims at: 1) investigating the factors affecting the establishment of a colonoid monolayer; 2) setting up the inflammatory environment by assessing the effects of TNF-α and IFN-γ on the integrity of Caco-2 barrier as a mean of comparison.

Methods

Colonic crypts, from biopsies of healthy subjects, were embedded in Matrigel® or Cultrex® and grown for 5-7 days. Expansion media of different compositions were used to generate spheroids, that, after a few passages, were dissociated and seeded onto permeable membranes.

Caco-2 cells, seeded at $5x10^4$ cells·cm^{-2} on 24-well inserts, were exposed after 21 days to 25ng·mL^{-1} TNF-α, 25ng·mL^{-1} IFN-γ or a mixture of them for 48h. Changes in the integrity of the epithelial barrier were evaluated as variations of the trans-epithelial electrical resistance (TEER) after 24h and 48h.

Results

The composition of the expansion media, the seeding cell density, and the type of extracellular matrix could greatly affect the outcome of the cultures and the yield in spheroids: in particular, embedding crypts in Matrigel, with a medium rich in specific growth factors of definite concentration, enabled the proliferation of stem cells into spheroids.

As for Caco-2 cultures, after 24h, only the mixture of TNF-α and IFN-γ significantly decreased TEER values with respect to the basal conditions (344.7±44.1 *vs.* 537.7±69.5 $W \times cm^2$, $p<0.001$). After 48h, both the mixture and the single cytokines remarkably reduced the Caco-2 barrier integrity, decreasing TEER values up to about 30%.

Conclusions

These preliminary results suggest that: 1) optimal conditions for the culture of 3D colonoids must be set out before moving onto the establishment of a colonoid-derived monolayer; 2) the simultaneous exposure to TNF-α and IFN-γ for 24h could provide an adequate level of inflammation for the generation of a predictive *in vitro* model of inflamed colonic epithelium.

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Title

Thrombocytopenia induced by panitumumab in a patient with colorectal cancer

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Background

Thrombocytopenia is a known adverse drug reaction (ADR) to chemotherapy, mainly due to the interaction with bone marrow functionality, bu Drug-induced *immune* thrombocytopenia (DITP) is a very rare ADR (definition by MedDRA; Medical Dictionary for Regulatory Activities), with an incidence of 10 cases per 1000000 inhabitants [1]. It is characterized by immune-mediated interaction between a certain drug and platelets, leading to their accelerated destruction [2] [3]. Once identified, it is vital to suspend administration of the causing agent and to avoid future re-exposures; platelet count returns to normal in 7 days after suspension of the agent.

Methods

We report the suspected case of DITP in a woman aged 49, diagnosed with rectal adenocarcinoma in 2018, T3 N2b M1. The chosen first-line treatment was panitumumab 320 mg and chemotherapy (CT) with FOLFOX (oxaliplatin 135 mg, 5-FU, 600 mg bolus and 4000 mg 48-h infusion for a BSA of 1,59 m^2). The patient underwent 9 cycles of therapy with no serious adverse effects (SAEs) reported. At the time of her 10^{th} cycle of therapy, 48h after completion of administration, the patient manifested acute thrombocytopenia. The thrombocytopenia was treated with 1 bag of haemocomponents and corticosteroids (prednisone 60 mg). This adverse event was considered to be caused by chemoterapy, leading to a revision of future cycles of therapy with 5FU and oxaliplatin (a 20% dose reduction for both); panitumumab dose remained unchanged.

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Results

Three weeks later the patient presented for her scheduled 11th cycle of therapy. soon after administration of panitumumab she manifested acute back pain and shivering, which lead to the suspension of upcoming CT before administration. Next morning, she manifested severe thrombocytopenia (platelet count <2000 U/uL) and purpura. She was hospitalized and treated with 1 bag of hemocomponents and corticosteroids; during her hospitalization, all autoimmunity tests resulted negative. After 5 days of observation, her platelet count returned to normal. No other adverse events were reported.

Conclusions

Although DITP from panitumumab is not often reported in clinical setting, and is not reported in its SmPC, the occurrence in this patient of thrombocytopenia after re-exposure to panitumumab alone, before administration of other therapies, and the resolution of the event in 5-7 days, strengthened our assumption that immune-mediated destruction of platelets was drug-related. It is important to note that another case report describing the same event is present in literature [4]. Naranjo's algorithm was used to establish correlation between the use of panitumumab and thrombocytopenia, resulting in a score of 6 ("probable") [5]. Although thrombocytopenia is usually caused by the known effects of chemotherapy on bone marrow, it is important to identify the other causes such as DITP, to enact the correct course of actions as soon as possible to prevent serious risks for the well-being of the patient.

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Title

Effects of the HDL mimetic CER-001 in Familial Lecithin:Cholesterol Acyltransferase Deficiency

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Background

Renal failure is the major cause of morbidity and mortality in familial LCAT deficiency (FLD), a very rare inherited disorder of lipid metabolism characterized by very low levels of HDL-cholesterol. The severe clinical manifestations are strictly related to the dramatic lipoprotein abnormalities; the lipoprotein X, which accumulates in the glomerulus, is the main accountant for nephrotoxicity [1,2]. CER-001, an HDL mimetic, has been recently reported by our group to limit renal damage in a mouse model of LCAT deficiency [3], and it was thus tested for the first time in a FLD patient with extremely fast recurrence of renal disease with the aim of delaying renal disease progression.

Methods

A 49-year-old FLD patient, at his third kidney transplantation, presenting with an unusually rapid decline of renal function was treated with CER-001 in the attempt to slow renal disease progression. CER-001 was infused at the dose of 10 mg/kg for 12 weeks. Plasma lipid/lipoprotein profile, renal function, and kidney histology were evaluated before and after treatment.

Results

CER-001 did not substantially change lipid levels; however, analysis of plasma lipoproteins demonstrated that while LpX was the most prominent lipoprotein at baseline, treatment with CER-001 led to a normalization of lipoprotein profile, with a disappearance of the abnormal LpX in favour of normally sized LDL. The worsening of kidney function was slowed by the treatment and the kidney biopsy showed a reduction of lipid deposits and a stabilization of the disease. In vitro experiments showed that podocytes incubated with plasma collected from patient at baseline produced cholesterol accumulation in cells, while incubation of cells with patient's plasma collected after CER-001 treatment progressively led to less lipid accumulation, confirming that the drug-induced remodeling of plasma lipoproteins is responsible of the reduced cholesterol deposit in cells. In addition, CER-001 showed a direct efflux capacity on LpX-induced lipid deposit in podocytes pretreated with patient's plasma.

Conclusions

CER-001 may represent a therapeutic option to delay renal disease for this genetic disorder presently with no cure and could represent an adjuvant therapy in other kidney diseases characterized by lipid deposit.

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Title

Intraspecific and interspecific empathetic behavioral responses

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Background

At the basis of empathy there are some mechanisms, such as *mimicry* or *emotional contagion*, that are shared by different social species (i.e., mice, rats, voles or apes) [1-3]. From them, the empathic process develops in the form of *emotional empathy* and, at a more rational level, in that of *cognitive empathy* [4,5]. Literature suggests that, belonging to a social species, rodents possess empathetic like capabilities [6]. The most common rats paradigms to study empathy are based on two kinds of phenomenons: *emotional contagion* and *prosocial behavior* [7]. The first one is tested with a rat exposed to a repeatedly defeated cage mate [8]. The second one is tested with a rat free to release an enclosed cage mate [9].

Methods

We elaborated a new paradigm, in which an “actor” rat is free to move along a grid, into a Y-shaped apparatus. Under the grid there are three closed chambers that the rat can observe, smell, hear from above, without any chance to interact with them, a part from the possibility to touch the grid itself. One of the chambers is empty, in another one there is an animal which shows a neutral emotive state and in the third one there is an animal that shows an altered emotive state (positively or negatively altered). Among the parameters, we evaluated the time the actor spent above each chamber and the number of “diggings” the actor made on the grid (as an attempt to interact with the chambers downstairs).

Results

Actor rats spend significantly more time focusing on the negative emotion of a cage mate than on every other condition

(both the one with a positive emotion shown by a cage mate and the one with a negative emotion shown by stranger or a mouse). In addition, also the number of diggings is higher above the chamber with the negative emotion shown by the cage mate.

Conclusions

In accordance with literature [10], results suggest that rats seem to show empathetic like behaviors, preferentially in response to distress of one's own group members. These behavior seem to be less present towards out-group members or individuals of other species.

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Title

Effect of prenatal stress in adolescent rats: potential role of microglia

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Background

Exposure to stress early in life is one of the major risk factors for psychiatric disorders. Prenatal stress (PNS) may produce long-lasting effects on brain function through the modulation of different systems, such as HPA axis, neuroplasticity, and immune-inflammatory changes, with the potential involvement of microglia activation [1]. Indeed, microglia may react to stress by secreting proinflammatory cytokines, chemokines, and free oxygen radicals, inducing an overall brain inflammation, altered synaptic pruning and brain circuitry formation, which may predispose to pathological conditions [2]. Moreover, the relationship between early life stress and microglial function may be particularly relevant during adolescence that represents a critical time window for the manifestation of psychiatric conditions. On these bases, the aim of this work was to evaluate the long-lasting effects of PNS on immune-inflammatory mechanisms and the potential involvement of microglia in these changes.

Methods

Pregnant rats were subjected to PNS during the last week of gestation (immobilization under bright light, for 45 min, three times a day), whereas control pregnant females (CTRL) were left undisturbed. At adolescence (PND44), a subgroup of CTRL or PNS rats were exposed to an acute stress paradigm (5 min forced swim). Different brain regions (prefrontal cortex, PFC; dorsal and ventral hippocampus, dHIP and vHIP) were dissected, and total RNA was isolated and used for real-time PCR analyses. In the present study, we investigated the gene expression for CD11b, iNOS and C3, using GAPDH or b-actin as housekeeping gene. Data were evaluated by a two-way ANOVA with Prenatal condition (PNS vs CTRL) and Acute stress (AS vs CTRL) as independent variables or using t-test analysis (PNS vs CTRL).

Results

We observed a significant increase of iNOS and C3 in the dHIP of PNS male rats (respectively +48%, p=0,0427, and +53% p=0,0154), but not in female animals. On the other hand, no differences of iNOS or C3 were detected in vHIP. Then, we analyzed the expression of *CD11b*, a marker for general microglial activation, comparing its expression in PFC at the baseline and after an AS. While under resting conditions we did not observe any significant change of *CD11b* mRNA levels, we found a significant increase of its expression in rats subjected to both PNS and AS as compared to control (+56% p=0,0129).

Conclusions

Our data suggest that rats exposed to PNS show an increase in pro-inflammatory markers at adolescence, although the effect may be limited to male animals, suggesting that the manipulation during gestation may prime microglia generating long-lasting changes that lead to neuroinflammation in adolescence. Moreover, according to the two-hit hypothesis for mental disorders, we show preliminary evidence that PNS can also prime microglia and immune mechanisms, by increasing the susceptibility or responsiveness to further stressors during adolescence.

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Title

Patients with heart failure eligible to a SGLT2 inhibitor treatment: prevalence, characteristics, and costs from a combination of administrative healthcare and primary care databases

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Background

Recent successful findings (i.e. DAPA HF trial [1]) in patients with heart failure (HF) with/without diabetes treated with sodium-glucose co-transporter inhibitors (SGLT2-i) have fostered in-depth analyses on the feasibility among real-life patients. In this scenario, data from Fondazione Ricerca e Salute's (ReS) administrative healthcare and Health Search's (HS) primary care databases (DBs) were combined in the ReS-HS DB Consortium, to (a) identify patients with HF eligible to SGLT2-i, (b) characterize them and (c) assess direct costs charged to the Italian National Health Service (INHS).

Methods

From ReS (~4.7 million adults) and HS (~1 million adults) DBs, adults with HF (ICD9 and disease waiver claims codes), alive at 12/31/2018 and analysable from 01/01/2013 were identified, then compared to develop an imputation model of specific clinical variables from HS to ReS population. Among them, subjects with reduced (≤40%) ejection fraction (EF) and glomerular filtration rate (GFR) ≥30 ml/min were selected. The index date was 01/01/2018. Gender, age and comorbidities were described. Disease duration (≥4 years) and HF-drug prescribed one year before and after the index date were assessed. Annual healthcare resource consumption costs per capita charged to the INHS were estimated from the ReS DB.

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Results

Patients with HF were 67,369 (1.5% of the adult population) and 13,313 (1.5%), respectively from ReS and HS DBs. Those potentially eligible to SGLT2-i were 2,187 in HS (16.4% of HS HF-adults) and, after the imputation model, 15,145 in ReS (22.5% of ReS HF-adults). Demographics, clinical and pharmacological features are shown in Table 1. In both cohorts: men eligible to SGLT2-i were more than women; prevalence of eligible population increased with age (peak >85 years), in both males and females; mean ages were >75; ~80% were affected by HF from ≥4 years; similar prescription patterns within one previous year. In the subsequent year, both cohorts reported increasing patients treated with β-blockers and diuretics alongside decreasing subjects treated with agents acting on renin-angiotensin system. Diabetes affected 33.4% of ReS and 44.3% of HS HF-patients. Cardiovascular comorbidities were in similar proportions of the cohorts. From ReS DB, the mean annual cost charged to the INHS per patient with HF eligible to SGLT2-i was € 7,122: 68% for hospitalizations, 26% for pharmaceuticals and 7% for outpatient specialist care.

Conclusions

Given the recent European approved indication of dapagliflozin for treating patients with HF and reduced EF (with/without diabetes and without renal failure), combining different types of real-world data is crucial to accurately identify, quantify and characterize patients eligible to the new indication of SGLT2-i. From this analysis, about 1 out of 5 patients with HF was eligible to dapagliflozin. Direct cost findings may foster economic models to assess reimbursement criteria of the upcoming antidiabetics.

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Table 1

	Health Search	Fondazione Ricerca e Salute
Adults analyzable in the database from 01/01/2013 (n)	874,962	4,363,833
Adults affected by heart failure (n; %)	13,313; 1.5%	67,369; 1.5%
Patients with heart failure and eligible for SGLT2-i (n; %) *(prevalence on general adult population)*	2,187; 16.4% *(0.25%)*	15,145; 22.5% *(0.35%)*
Demographics		
Males (%)	83.6%	87.5%
Mean age (±SD)	77±10	75±12
Clinical characteristics		
Disease duration (≥4 years) (% patients)	82.8%	79.5%
Concomitant diseases (% patients)		
Diabetes mellitus	44.3%	33.4%
Arterial hypertension	68.1%	94.8%
Ischemic heart disease	57.1%	40.2%
Atrial fibrillation	31.9%	27.3%
Peripheral artery disease	10.8%	11.1%
Ischemic stroke	10.8%	5.5%
Pharmacological pattern (% patients)		
One year before the index date		
Agents acting on the renin-angiotensin system	80.9%	83.5%
Diuretics, other than aldosterone antagonists	80.5%	75.0%
Beta - blockers	82.2%	72.6%
Aldosterone antagonists	55.9%	35.4%
Digitalis glycosides	16.7%	21.8%
Ivabradine	9.6%	8.2%
One year after the index date		
Diuretics, other than aldosterone antagonists	90.1%	85.0%
Agents acting on the renin-angiotensin system	79.4%	81.4%
Beta - blockers	88.1%	80.6%
Aldosterone antagonists	66.1%	44.8%
Digitalis glycosides	16.9%	22.9%
Ivabradine	19.5%	8.5%

SGLT2-i: sodium-glucose co-transporter inhibitors; SD: standard deviation

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Title

ROLE OF THE ACTIVATED MICROGLIA IN SYNAPTIC DYSFUNCTION ASSOCIATED WITH EXPERIMENTAL MODELS OF NEUROINFLAMMATION

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Background

Microglia cells, the main category of macrophages present in the CNS parenchyma, play a key role in brain development and homeostasis. When pathological inflammatory conditions are induced, microglia take on the pro-inflammatory phenotype (M1) that contributes to synaptic alterations and myelin damage, typical of neurodegenerative diseases, including Alzheimer's disease (AD). In experimental models of AD there is increased activation of microglia CSF1 receptor (CSF1R) which is responsible for pathological microglia activation. Activated inflammatory microglia M1 leads to pruning reactivation and consequent structural damage. Microglia conversion to the M2 anti-inflammatory phenotype may be neuroprotective by facilitating its phagocytic activity against Aβ plaques. In this study, using electrophysiological and immunohistochemical analyses we investigated hippocampal synaptic transmission and plasticity in models of neuroinflammation. We then investigated whether pharmacological manipulation of microglia activation could normalize synaptic dysfunction associated with these models.

Methods

Hippocampal brain slices from C57BL6/J mice were used. Electrophysiological recordings (*field* and *whole cell patch clamp)* were made from the stratum radiatum of area CA1 in response to stimulation of Schaffer collaterals. The readout evaluated are Long-Term Potentiation (LTP) induced by theta-burst stimulation, paired-pulse ratio (PPR), AMPA/NMDA ratio and spontaneous EPSCs (sEPSCs). Lipopolysaccharide (LPS) (10 µg/ml) or Aβ 1-42 oligomers (200 nM) were used as *in-vitro* inflammatory models. To modify the activation of microglia we used Pexidartinib (PLX-3397) (10 µM) and Minocycline hydrochloride (500 nM). For immunohistochemical experiments, free-floating brain sections were incubated with primary antibodies overnight at 4°C, followed by Alexa-conjugated secondary antibodies for 2 hours at room temperature in the dark. Pathological pruning is established by triple staining using VGLUT1, Iba1 and CD68 as synaptic markers.

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Results

In *filed* experiments we observed impairment of LTP in the presence of both inflammatory models and this effect was reversed following application of either the non-specific blocker Minocycline, or the specific inhibitor of activated microglia PLX-3397. Using *whole cell patch clamp* recordings, PLX-3397 was able to restore the Aβ-mediated decrease in glutamatergic transmission, evaluated as amplitude or frequency of sEPSs, AMPA/NMDA ratio and PPR. Finally, imaging experiments showed that Aβ led to an M1 inflammatory microglia polarization while PLX-3397 promoted the transition to the M2 anti-inflammatory and phagocytic microglia phenotype.

Conclusions

Our results demonstrate a functional role of activated microglia in the synaptic transmission and plasticity alterations typical of neuroinflammation suggesting CSF1R as a new pharmacological target for AD treatment.

References

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Title

Identification of shared genetic loci between bipolar disorder, lithium response and IGF-1 levels: a local genetic correlation analysis

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Background

Lithium is the mainstay for the long-term treatment of bipolar disorder (BD). However, the molecular mechanisms involved in its clinical efficacy are still not completely understood. In a previous genome-wide expression analysis, we showed that the insulin-like growth factor 1 (IGF-1) gene was significantly over-expressed in lymphoblastoid cell lines from lithium responders compared with either non-responders or non-psychiatric controls, supporting further investigation on the potential role of IGF-1 as a biomarker of lithium response [1]. Recent studies have shown that shared genetic effects underlie different psychiatric disorders and correlated phenotypes [2]. The identification of shared loci and the evaluation of their biological significance may contribute to dissect the molecular mechanisms involved in complex phenotypes. In this study we used the novel local genetic correlation analysis approach to identify shared genetic loci between IGF-1 levels and BD or lithium response.

Methods

Analyses were conducted on the largest genome-wide association datasets available for the investigated phenotypes: for BD, the latest release from the Psychiatric Genomics Consortium (41,917 cases and 371,549 controls of European origin), for serum IGF-1 levels, data for 363,228 individuals from UK Biobank [3], and for lithium response data from the International Consortium on Lithium Genetics (ConLiGen) for 2,435 BD patients [4]. We analyzed the bivariate local correlation between genetically predicted IGF-1 levels and 1) BD, or 2) lithium response using Local Analysis of

[co]Variant Annotation (LAVA) [5]. We first tested the local univariate association between each phenotype and 2,495 semi-independent genomic blocks defined as described in [5]. Loci associated with at least two phenotypes were tested for bivariate local genetic correlation. Results were adjusted according to Bonferroni based on the number of tested loci.

Results

We identified ten significant bivariate correlations (three negative and seven positive) between IGF-1 levels and BD and two between IGF-1 levels and lithium response. Among the ten loci shared between IGF-1 levels and BD, the most significant was on chr 2 (26894103-28819510, rho = -0.56, r2 = 0.31, p = 3.3 x 10 -9) and includes several genes. Among the two loci associated with IGF-1 levels and lithium response, one showed a positive correlation (chr: 12: 54371449-55416802, rho = 0.95, r2 = 0.90, p = 1.8E x 10 -8) and includes several genes and non-coding RNAs, while the other a negative correlation (chr 16: 5782969-6446081, rho = -0.50, r2 = 0.25, p = 1.3 x 10 -5) and includes only *RBFOX1*. Additional analyses to identify other phenotypes that may moderate these associations, as well as functional analyses and fine-mapping to prioritize relevant genes within each locus, are now under progress.

Conclusions

Our results support the existence of shared biological pathways underlying the clinical efficacy of lithium and levels of the IGF-1 neuroprotective factor.

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pharmadvances

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Title

Complement, a synaptic organizer at glutamate tripartite synapses in the cortex of mice suffering from experimental autoimmune encephalomyelitis: synaptic pruning and transmission derangements.

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Background

“Synaptic organizers” are those molecules that regulate the formation, the development, the functions and the removal of synapses in the central nervous system. Complement belongs to this class since it selectively controls glutamate release from presynaptic nerve terminals (synaptosomes) and astrocytic processes (gliosomes) through a mechanism involving the reversal of the EAAT carrier transport but also cause pruning of weak synapsis.

Methods

Synaptosomes and gliosomes were isolated from the cortex of mice suffering from the experimental autoimmune encephalomyelitis (EAE), an animal model of demyelinating disorder. Release experiments, western blot analysis and flow cytometry were carried out to analyze changes in the composition and the function of these particles.

Results

In EAE mice at the acute stage of the disease (21 ± 1 d.p.i.) changes in the viability and in the apoptosis of both cortical synaptosomes and gliosomes did not emerged when compared to controls, but a significant increase of the C1q and C3 proteins was detected, that was concomitant to microgliosis (quantify as CD11b density), astrocytosis (quantified as GFAP density) and synaptic derangements (measured as SNAP25 and PSD95 density) in cortical homogenates.

Glutamate exocytosis was significantly reduced in EAE cortical synaptosomes and gliosomes, while the complement-evoked releasing activity was almost halved in synaptosomes but increased in gliosomes. Interestingly, EAAT2 density in EAE synaptosomes was significantly lower than that in control while the EAAT1 density in gliosomes was increased.

Conclusions

We propose that the organizer activity of complement at glutamatergic cortical synapses is altered in EAE mice and could account for clinical symptoms such as learning and memory impairment, anxiety and motor incoordination detected during the disease.

References

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Title

Phenotypic characterization of mice lacking the nociceptin/orphanin FQ receptor in two *in vivo* models of migraine

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Background

Migraine is one of the most prevalent disabling disorders. Migraine pathophysiology remains partially unclear and the current therapeutic approaches do not satisfy the majority of the migraineurs or are not well tolerated. Many studies demonstrated the central role of the trigeminal ganglion and nucleus caudalis in migraine onset. These structures are enriched with nociceptin/orphanin FQ (N/OFQ) receptors (NOP) whose activation elicits robust inhibitory effects thus suggesting a potential role of endogenous N/OFQ pathway in migraine. The aim of the present study was to test if the N/OFQ – NOP system is involved in the modulation of migraine by assessing the phenotype of mice knockout for the NOP receptor gene (NOP(-/-)) in two migraine models.

Methods

CD-1 NOP(+/+) and NOP(-/-) male and female mice were used. As migraine triggers nitroglycerin (GTN, 10 mg/kg, i.p.) and CGRP (0.01, 0.03, and 0. 1 mg/Kg, i.p.) were used. Von Frey filament test in the periorbital region was performed to assess the periorbital mechanical allodynia (PMA) as migraine sign.

Results

GTN 10 mg/kg induced significant PMA in male but not in female NOP(+/+) mice. Both male and female NOP(-/-) mice displayed higher sensitivity than NOP(+/+) mice to the GTN effects. The administration of the maximal dose of CGRP induced PMA in all mice with no significant difference between males and females and between NOP(+/+) and NOP(-/-) mice. However, lower CGRP doses were effective in NOP(-/-) but not NOP(+/+) mice.

Conclusions

In conclusion, NOP(-/-) mice displayed higher susceptibility to the development of migraine-like symptoms compared to NOP(+/+) mice. These results suggest the involvement of the NOP receptor in the modulation of migraine and candidateNOP receptor selective agonists as innovative antimigraine drugs. .

References

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Title

Natural P-glycoprotein inhibitors to bypass MDR in acute myeloid leukemia

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Background

Multidrug resistance (MDR) is an obstacle to numerous anticancer drugs and limits the therapeutic choice. One of the best known mechanisms responsible of MDR phenotype is the overexpression of P-glycoprotein, a member of the adenosine triphosphate binding cassette (ABC) transporters, encoded by multidrug resistance protein 1 gene (MDR1 or ABCB1) [1]. This phenomenon appears to be relevant also in acute myeloid leukemia (AML) in which in addition to the standard “7+3” chemotherapy, new therapeutic agents have been approved towards which resistance is already emerging. Most AML patients develops MDR after very short time and in these cases, allogeneic stem cell transplantation becomes the only choice. The discovery of specific P-gp inhibitors to date has not produced results transferable to the clinic, due to the numerous toxic effects that characterize these molecules [2]. The identification of peculiar mechanisms of natural molecules, such as terpenes, curcuminoids, flavonoids, both on the expression and on the function of P-gp, seems to have the advantage of a low toxicity with the same efficacy [3].

Methods

We performed cell growth assay by MTS on an acute myeloid leukemia cell line HL-60 and on its MDR variant HL-60R; in both cell lines after treatment with the compounds was evaluated both DNA binding capacity of the NF-κB p65 subunit using the TransAM NF-κB Kit and P-gp expression at mRNA and protein levels by RT-qPCR and Western Blotting. Accumulation assays were made using cytofluorimetric analysis in HL-60 and HL-60R cells after treatment with molecules. At last, P-gp ATPase activity was performed with Pgp-Glo™ Assay Systems and docking study on P-gp was performed.

Results

We have identified four molecules, all present in natural foods: phytol, curcumin, heptacosane and lupeol, all of which can interfere with P-gp. Furthermore these molecules, except for heptacosane, have the ability to interfere on the NF-κB

pathway. Since P-gp overexpression also depends on NF-κB overactivation, phytol, curcumin and lupeol are able to inhibit efflux pump expression. All molecules, including heptacosane, act like verapamil on P-gp, promoting an intracellular accumulation of doxorubicin, in HL-60R cells. In fact, they are able to directly inhibit its function, acting as competitive antagonist substrates, or by binding to the active site of transporters (drug binding pocket, DBP) and reducing drug efflux.

Conclusions

Our data underline the possibility to use non-toxic inhibitors of ATPase activity and P-gp function in combination with different anticancer chemotherapy drugs to increase their efficacy without producing greater toxicity in AML patients with MDR phenotype.

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Title

A retrospective analysis on anxiolytic, hypnotic-sedatives and antidepressants prescriptions in an Italian hub hospital during the Covid-19 Pandemic

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Background

Most recent findings show that COVID-19 pandemic and the measures implemented as an attempt to stop the coronavirus spread have caused depressive and anxious symptoms, especially in young people [1]. Here we retrospectively evaluated the consumption of anxiolytic, hypnotic-sedatives and antidepressants within the S. Croce and Carle hospital in Cuneo, comparing the pre pandemic period, Mar2018-Apr2019, with the pandemic, Mar2020-Apr2021.

Methods

The data collected, in COVID and NON-COVID wards, during the pandemic waves were compared (I:Mar2020-May2020, II:Oct2020-Dec2020, III:Feb2021-Apr2021) in order to highlight how consumption has changed over time according to variables such as the incidence of hospitalizations and vaccination campaigns.

Results

We documented a statistically significant increase in the consumption of antidepressants (Wilcoxon test $\alpha=0.05$ $W=30$, critical value=40) within COVID departments. In the pandemic period, consumption was 411.31 Defined Daily Dose (DDDs) per 100 beds days compared to 341.71 DDDs per 100 beds days in the pre pandemic period, with a percentage increase over 20%. The peak of consumption occurred during the second wave, and then decreased during the third wave. The increased demand for antidepressants can be explained by the increase in the prevalence of depressive

symptoms also in in-patients who had to cope with new issues, including the social isolation and the lack of contact with relatives during hospitalization [2]. Anxiolytic drugs recorded a non-significant increase in the pandemic period compared to the pre pandemic period, respectively 236.58 DDDs per 100 beds days and 203.23 DDDs per 100 beds days. Similarly, DDDs per 100 beds days for hypnotic-sedative drugs was 372.88 in the pandemic period compared to 344.98 in the pre pandemic period, thus showing an increase which did not reach statistical significance.

Conclusions

We documented a statistically significant increase in the consumption of antidepressants within the COVID wards of the S. Croce and Carle hospital, when compared to non COVID wards. The greatest peak in consumption was recorded during the second epidemic wave, while a reduction occurred during the third wave, coinciding with the decrease in hospitalizations and the start of vaccination campaigns. Surprisingly, we detected only a slight, but not significant, increase in consumptions of anxiolytic and hypnotic-sedative drugs during the epidemic. However, we have to take into account that these drugs are commonly used in sedative and surgical procedures, which were drastically reduced during COVID-19 pandemic, and so during pandemic there was mainly a shift in their therapeutic use, not being affected the total amount of their consumption.

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Title

CIC-39Na reverse thrombocytopenia and muscle damage that characterize Tubular Aggregate Myopathyes

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Background

Store-Operated Ca^{2+}-Entry is a cellular mechanism that governs the replenishment of intracellular stores of Ca^{2+} upon depletion caused by the opening of intracellular Ca^{2+}-channels. Gain-of-function mutations of the two key proteins of Store-Operated Ca^{2+}-Entry, STIM1 and ORAI1, are associated with several ultra-rare diseases clustered as tubular aggregate myopathies[1]. Our group has previously demonstrated that a mouse model bearing the STIM1 p.I115F mutation recapitulates the main features of these gain-of-function disorders: thrombocytopenia and muscle weakness[2]. Similar findings have been found in other mouse model bearing different mutations on STIM1[3,4] (p.R304W). At present, no valid treatment is available for these patients.

Methods

We evaluated the effect of CIC-39Na, a Store-Operated Ca^{2+}-Entry negative modulator owns by a spin-off from Università del Piemonte Orientale, both in *in vivo* and *ex vivo* models of tubular aggregate myopathies. Wild-type and KI-STIM1^{I115F} mouse were treated with CIC-39Na (60 mg/Kg/daily 56days) in constant infusion using micro-pumps intraperitoneally. The efficacy of the compound *in vivo* on the major clinical phenotypes, were evaluated using bleeding test on platelets and functional tests (treadmill and hanging) on muscle.

CIC-39Na was tested for Ca^{2+}-entry using fluorescent probes both in platelets and myotubes deriving from wild-type and KI-STIM1^{I115F} treated mice.

Results

In the present communication, we report that CIC-39Na, restores platelet number and counteracts the abnormal bleeding that characterizes these mice. Subtle differences in thrombopoiesis were observed in KI-STIM1^{I115F} mice, but the main difference between wild-type and mutated mice was observed in platelet clearance and in the cytosolic basal Ca^{2+} levels. Both were restored upon treatment with CIC-39Na.

Moreover, the same compound is able to re-establish the functional motor levels and restore Ca^{2+} to physiological levels in KI-STIM1^{I115F} derived myotubes.

Conclusions

These findings pave the way to a novel pharmacological strategy for thrombocytopenia and muscle weakness characterizing these cluster of rare genetic disorders.

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pharmadvances

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Title

Fluoxetine Ecofriendly Nanoemulsion Enhances Wound Healing in Diabetic Rats: In Vivo Efficacy Assessment

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Background

Impaired diabetic wound healing is a major concern for health care professionals worldwide, imposing an intense financial burden and reducing the quality of life of patients. A dysregulation of this process can be responsible for the development of intractable ulcers and the formation of excessive scars. Therefore, the identification of novel pharmacological strategies able to promote wound healing and restore the mechanical integrity of injured tissue becomes essential.

Methods

In the present study, fluoxetine ecofriendly nanoemulsion (FLX-EFNE) was prepared and its potential efficacy in enhancing wound healing was tested in diabetic rats (streptozotocin-induced). The Box–Behnken response surface design was used to select the optimized formulation that was prepared by the high-shear homogenization-based technique. A Zetasizer was used for the characterization of the optimized formulation. For the in vivo study, a wound

was induced by surgical methods, and diabetic rats were divided into five groups: untreated control, vehicle-treated, FLX, FLX-EFNE, and positive control receiving a commercially available formula. The treatment continued from the day of wound induction to day 21. Then, the animals were sacrificed and skin tissues were collected at the site of wounding and used for biochemical, histopathological, immunohistochemical, and mRNA expression assessments.

Results

The new formulation, developed and optimized using Box–Behnken response surface design, was characterized by a globule size of 199 nm. FLX-EFNE showed enhanced wound healing properties following 21 days of daily topical application compared to the free drug (FLX) and, in most cases, to the commercially available ointment Mebo® (positive control). The potentiated activity of FLX-EFNE in inducing wound closure, and therefore to enhance the wound healing process, is attributable to its ability to decrease fibroblast proliferation and the infiltration of inflammatory cells, and increase re-epithelization. The daily topical application of FLX-EFNE was also characterized by anti-inflammatory (decreased tumor necrosis factor-alpha levels), antioxidant (decreased malondialdehyde and increased GSH, superoxide dismutase, and glutathione peroxidase), and collagen-enhancing (increased collagen, type I, alpha 1 gene expression and hydroxyproline levels) activities. The FLX-EFNE formulation was also found to significantly increase the protein expression of platelet-derived growth factor subunit B, transforming growth factor-beta 1, and vascular endothelial growth factor A, as well as the gene expression of angiopoietin 1, with all factors playing an important role in the tissue recovery linked to the wound- healing process.

Conclusions

Overall, the present findings underline the therapeutic potential of the new FLX-EFNE formulation that is believed to represent an innovative pharmacological tool able to enhance the wound healing process in pathological conditions such as diabetes.

References

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pharmadvances

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Title

Chronic acetazolamide treatment ameliorates brain pathology and restores cognitive functions in a mouse model of Amyloid-β deposition

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Background

Alzheimer's disease (AD) is the most common form of neurodegenerative disease. Despite the substantial economic cost and healthcare burden, the current treatment options are very limited. Recently, a series of observations are shedding light on the involvement of brain carbonic anhydrases (CAs) in neurodegenerative disorders[1]: a higher expression of the CAII isoform was found in the plasma of AD patients when compared to age-matched control subjects[2]; two CA inhibitors (CAIs) prevented Aβ-induced mitochondrial toxicity and cell death *in vitro*[3,4]. Despite these encouraging findings, the impact of CAIs in AD animal models were not yet investigated. Thus, here we aim to evaluate the efficacy of a pharmacological intervention with acetazolamide (ACTZ), a well-known CAI, against the cognitive impairments and neuropathological alterations observed in TgCRND8 mice.

Methods

Male and female wild type and TgCNRD8 mice (expressing the human amyloid precursor protein carrying the Swedish and Indiana *mutations*) were fed with control or ACTZ-enriched diets (100 and 200 ppm) starting at 6 weeks of age. After 6 or 12 weeks of treatment, animals' cognitive function was evaluated in the social discrimination paradigm. The day after the behavioral evaluation, the animals were sacrificed, their brains were collected and processed for neurochemical analysis.

Results

Chronic CA inhibition prevented the short-term social recognition memory deficits observed in TgCNRD8 mice at both ages. A reduction in the number of β-amyloid plaques (Aβ1-42), in the expression of the β-amyloid pyroglutamic derivative (Aβ N3pE) and in Tau phosphorylation (AT8) were also observed in the hippocampus of ACTZ-fed animals compared to the animals fed with control diet. Chronic ACTZ treatment also reduced the levels of caspase-3 in the same area.

Conclusions

Chronic treatment with a ACTZ prevented both behavioral and neuropathological alterations observed in TgCNRD8 animals, suggesting a role for CAs as an innovative target for AD treatment.

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pharmadvances

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Title

Sulforaphane and its metabolites protects PC12 HD-Q74 cells against the neurotoxicity induced by mutant huntingtin through the activation of Keap1/Nrf2/ARE Pathway

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Background

Huntington's disease (HD) is a fatal autosomal dominant inherited neurodegenerative disorder caused by an unstable expansion of CAG trinucleotide repeats coding for polyglutamine in the huntingtin (HTT) gene. HD is characterized by progressive neuronal death and the misfolding of HTT protein into soluble oligomers and insoluble aggregates that accumulate in neurons. Currently, there are no treatments available to prevent the onset of HD or halt or reverse its progression. Compromised oxidative stress defence systems and functional insufficiency of protein degradation machinery have been implicated in HD pathogenesis. Sulforaphane (SFN) is a natural compound originally isolated from broccoli or other cruciferous vegetables and has been shown to be an effective neuroprotective agent in neurodegenerative diseases. Recent studies have reported that SFN enhanced mutant HTT (mHTT) degradation through the ubiquitin-proteasome system pathway as well as reduced mHTT cytotoxicity in *in vitro* and *in vivo* models of HD. The aim of our research was to evaluate the neuroprotective effects of SFN against the neurotoxicity induced by mHTT and the contribute of its most important metabolites sulforaphane-glutathione, sulforaphane-cysteine and sulforaphane-N-acetylcysteine to these effects.

Methods

We used neuronal cell lines derived from rat pheochromocytoma (PC12) that express an inducible enhanced green fluorescent protein-tagged huntingtin exon 1 fragment with 74 glutamine repeats (HD-Q74) that shape the mutant form of HTT. Cells were treated with doxycycline (DOX) for 72 h and SFN or its metabolites (2.5 – 5 µM) were added during the last 24 h of treatment with DOX. We evaluated the neurotoxicity induced by mHTT as well as the activation of the adaptative response through Keap1/Nrf2/ARE pathway in the presence of SFN or its metabolites in HD-Q74 cells. In particular, the neurotoxicity in terms of cell viability and the expression of genes under transcriptional control of Keap1/Nrf2/ARE pathway, including Nrf2, NAD(P)H dehydrogenase quinone 1 (NQO1) and glutathione S-transferase pi 2 (GSTP2) were measured by tetrazolium salt assay and RT-PCR, respectively.

Results

The treatment with SFN or its metabolites at the concentration of 5 μM significantly reduced the neurotoxicity induced by mHTT showing similar neuroprotective effects. In parallel, at the same experimental conditions, the treatment with SFN or its metabolites strengthened the increase of NQO1 and GSTP2 expression induced by mHTT, while the same treatment did not have effects on the expression of Nrf2.

Conclusions

Taken together, these results suggest that the studied metabolites may contribute to the neuroprotective effects of SFN observed in *in vivo* HD models and encourage further research to better explore the neuroprotective potential of SFN and its metabolites as drug or functional food for preventing or treating HD.

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pharmadvances

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Title

Therapeutic drug monitoring to optimize rifampicin dosing in TB patients: a real-life investigation

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Background

Tuberculosis (TB) is caused by *Mycobacterium tuberculosis*. In 2020 an estimated 10 million people fell ill with TB worldwide and 1.5 million people died from TB: TB is the 13th cause of death and the 2nd infectious killer after COVID-19. A distinguishing characteristic of all the *Mycobacteria* is the slow growth rate, which affect also the clearing rate once therapy is established. Considering the increase in both treatment complexity and price of multidrug resistant (MDR) TB, optimization of available treatments by therapeutic drug monitoring (TDM) is of paramount importance.

Methods

The use of rifampicin (RFP) TDM as diagnostic tool to optimize drug dosage in routine clinical practice at TB outpatient clinic at L. Sacco Teaching Hospital started in Jan. 2017. From Jan. 2017 to Dec. 2020 only C_{max} (2 hours after oral RFP intake) were performed; from Jan. 2021 to date drug concentrations 4-6 hours after RFP intake were also collected to estimate drug AUC and, when needed, RFP dosage was subsequently adjusted. In this retrospective investigation, we focused on adequacy of standard RFP doses in reaching TDM-based therapeutic ranges (normal values: AUC/MIC>270 or C_{max}=8-24mg/L). Main demographic and clinical data of all patients (pts) from Jan. '17 to Apr. '22 were also collected.

Results

100 pts were evaluated (49% females). Median age was 48 years (19-93) for males and 41 years (19-89) for females. Pts' nationality was belonging to 4 geographical areas: 37% Europe, 29% Asia, 23% Africa and 11% Central-South America (Table 1a-b).

More frequent risk factors for TB were coming from endemic countries (66 pts) and being a household contact with a TB pt (10 pts). In 78 cases more than one risk factor was present (Table 2).

43 pts had a pulmonary TB only, 36 had an extrapulmonary TB only, and 21 had both conditions.

At the 1st TDM assessment, done 1 week after starting therapy, 39% of pts had suboptimal plasma drug concentration requiring an increase in the RFP dose, 5% reduced RFP dose because of drug overexposure, 1 pt first stopped and then restarted at a reduced dosage RFP due to a drug-related rash. In 38 pts RFP dosage correction due to non-optimal plasma concentration was necessary also at the 2nd TDM determination (performed at 1 month after therapy start).

Conclusions

Nearly 50% of the pts had suboptimal RFP exposure while treated with the conventional drug dose regimens. RFP TDM is of paramount importance in order to obtain the correct drug exposure in TB pts: underexposure can, in fact, lead to drug resistance whereas RFP overexposure can lead to drug toxicity. Future developments to increase the appropriate use of 1st line anti-TB drugs are implementing TDM for the other 3 1st line drugs and NAT2 pharmacogenetics.

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Title

Ultrasonic vocalizations as behavioral correlates of fear memory dynamics in a rat model of Post-Traumatic Stress Disorder

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Background

Stress exposure may lead to the development of post-traumatic stress disorder (PTSD), a chronic psychiatric disease characterized by over-consolidation, generalization and impaired extinction[1]. The return of fear following the acquisition of fear extinction learning is common[2] and often responsible for the PTSD-treatment unfailure[3,4]. While only a small proportion of trauma-exposed individuals develop PTSD, women have a twofold greater risk, prevalence and duration of PTSD than men[5]. We have recently demonstrated that there is a link between fear extinction and 22-kHz USV emission in a fear conditioning paradigm, associated to profound sex differences[6].

Methods

Thus, in the present study we tested the extent to which 22-kHz USVs would mirror freezing behavior in an animal model of PTSD[7], and we examined potential sex differences in fear acquisition and extinction.

Results

Our results indicate that, although during trauma exposure males show higher freezing levels while females exhibit a greater reactivity to trauma, they both emit a similar number of alarm USVs. Moreover, along the extinction sessions, the number of USV emission gradually decreases in both sexes, mirroring the freezing response, although females show a higher extinction rate compared to males, in terms of both freezing and USV. Taking only freezing into account, a single mild footshock exposure induces a conditioned fear response in males only and reinstatement in both male and

female rats. However, reinstatement of USV emission has been observed only in males.

Conclusions

Taken together, the present findings reveal sex differences in trauma response and extinction process in our model of PTSD and underline the importance of 22-kHz USV analysis, in parallel with freezing, to provide a complete index of fear memory.

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Title

Environmental enrichment influences mouse hippocampal neuroinflammatory response

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Background

Many studies have shown the positive effects of environmental enrichment on brain plasticity with significant implications for development, behavior, learning, memory, and recovery from brain damage (1).

Experimental and clinical studies report that one's living environment can modulate cellular and molecular responses in the brain, counteracting cognitive decline, alleviating anxiety, and depressive behaviours, as well as moderating the outcome of pharmacological treatments (2). Neuroinflammation has been well established as an important factor in the aetiopathogenesis and progression of brain disorders. It can affect neural development and alters hippocampal plasticity thus resulting in cognitive impairments. Neuroinflammation is characterized by a dysregulation of the NLRP3 inflammasome activation, an increase in the expression of inflammatory cytokines, and a decrease of neurotrophic factors (3). Behavioral and neurochemical changes, caused by neuroinflammation, have been most frequently investigated through peripheral administration of lipopolysaccharide (LPS) which can, directly and indirectly, affect the central nervous system (4).

Based on these premises, the aim of this study was to explore the molecular effects of the quality of the living environment in modulating the LPS-induced neuroinflammatory response in the hippocampus of wild-type mice

Methods

Male C57BL6J mice (13 weeks-old) were randomly housed in Impoverished (IE) or Enriched Environment (EE) condition for 28 days, then exposed to LPS (0.830 mg/Kg, i.p.) or saline (SAL). Twenty-four hours after injection hippocampi were removed for gene expression analysis performed by means of qRT-PCR. Data from groups were analyzed by Two-way ANOVA followed by Tukey's post hoc test.

Results

The analysis of the environmental effects on the LPS signaling system highlighted the downregulation of the membrane-bound protein LBP, the receptor TLR4 and the co-receptor cluster CD14 expression levels in EE-housed animals compared to their counterparts. The exposure to an EE condition was able to attenuate the LPS-induced increase of TLR4 and NLRP3 inflammasome mRNA levels. Gene expression analysis revealed a significant downregulation of the pro-inflammatory cytokines IL-1β and TNFα levels in EE-housed mice while LPS exposure strongly increased IL-1β and TNFα mRNA levels irrespective of the housing conditions.

Moreover, EE-exposed mice showed a significant upregulation of BDNF hippocampal mRNA levels, although no effects were observed after LPS treatment in both conditions.

Conclusions

Our results displayed the beneficial effect of EE in regulating the expression of inflammatory mediators involved in the LPS-induced response in the hippocampus, a key area for learning, memory, and emotion. These data suggest that living environment may exert a positive and protective role on the brain by reducing susceptibility toward neurodegenerative or neuropsychiatric disorders.

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Title

Store-operated calcium entry modulators as novel pharmacological strategy for Duchenne muscular dystrophy

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Background

Duchenne Muscular Dystrophy (DMD) is a lethal progressive paediatric muscle disorder genetically inherited as an X-linked disease caused by mutations in the dystrophin gene, leading to progressive muscle degeneration and wasting, with life expectancy significantly reduced with death occurring in early adulthood[1]. Although progressive wasting of muscle fibres is a cause of muscle deterioration, the pathophysiology of DMD is still elusive. Recently, It has been reported that the effects triggered by dystrophin mutations are present at the earlier stage and additional pathogenic mechanisms including abnormal store-operated calcium entry (SOCE), *i.e.* the ability of cells to sense a decrease in endoplasmic reticulum luminal Ca^{2+} and induce Ca^{2+}-entry across the plasma membrane[2], are involved in the pathophysiology of this disorder[3,4]. In the present contributions we studied the therapeutic effect of a novel SOCE negative modulator (CIC-39)[5,6] in a specific mouse model of DMD.

Methods

We evaluated the effect of both in *ex vivo* and *in vivo* models of DMD. CIC-39 was tested for Ca^{2+}-entry using fluorescent probes in myotubes deriving from wild-type and DMD mice (*mdx*) and was administered to wild-type and *mdx* mice to evaluate the creatine kinase plasma level and the muscle damage, using grip tests and immunohistochemical analysis.

Results

By calcium imaging, we have demonstrated that CIC-39 is able to restore SOCE to physiological levels in *mdx* derived myotubes. Furthermore, *in vivo* experiments demonstrated that CIC-39 (60 mg/Kg/daily 28 days) was able to: (i) significantly reduce the creatine kinase plasma levels, (ii) clearly improve the parameters evaluated in grip tests and (iii) counteract the apoptosis in quadriceps and gastrocnemius muscles.

Conclusions

Our results demonstrate that negative modulators of SOCE, in particular CIC-39, could represent a novel pharmacological strategy able to ameliorate symptoms and contrast the progression of Duchenne muscular dystrophy. Finally, since the over-activation of SOCE is due by the absence of the cytoskeleton dystrophin regardless of the genetic mutation, the use of SOCE modulators as therapeutic strategy could allow to reach a greater number of patients, overcoming the current issues related to subpopulations not responding to the therapeutic approaches currently in clinical trials.

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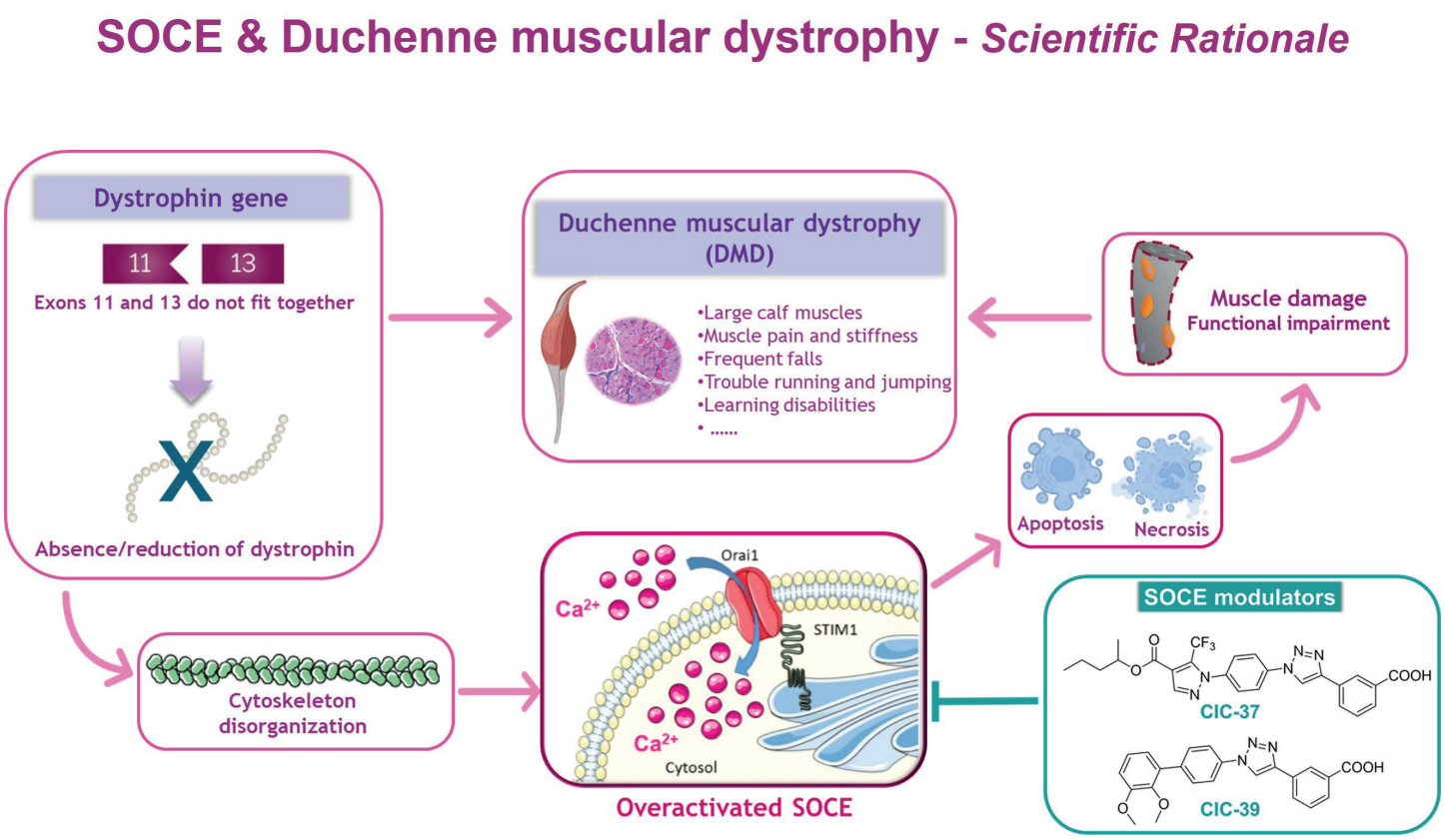

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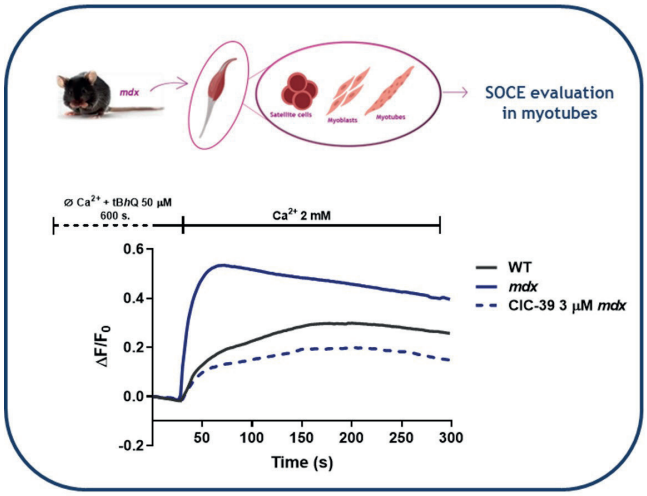
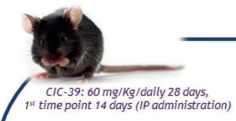
Scientific Data – *in vivo Proof of Concept*

Ex vivo experiments

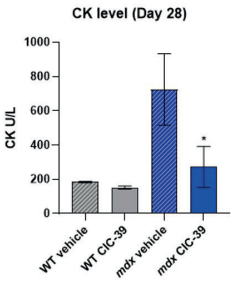


CIC-39 reduces SOCE restoring Ca^{2+} to physiological levels

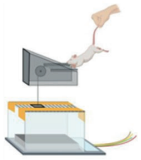
In vivo experiments



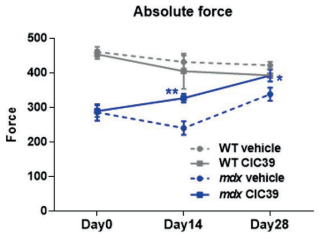
CIC-39: 60 mg/Kg/daily 28 days, 1st time point 14 days (IP administration)



CIC-39 reduces the creatine-kinase plasma level



Grip test



CIC-39 contrasts muscle demage and increases force

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Title

Role of dopamine D3 receptors, dysbindin, and their functional interaction on the expression of key genes for neuroplasticity in the mouse brain

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Background

Cognitive impairment in schizophrenia remains a clinically and pharmacologically unsolved challenge.

Dysbindin (DYS) and dopamine receptors D3 are leading candidate genes for susceptibility to this psychiatric disorder and its pharmacological treatment[1]. Clinical and preclinical studies revealed that the concomitant reduction in D3 and DYS functionality is associated with improved cognitive functions[1]. However, the molecular machinery underlying this epistatic interaction has not been fully elucidated yet. In this context, the neurotrophin BDNF and the Myristoylated alanine rich protein kinase C substrate (MARCKs), with their established role in promoting neuroplasticity[2,3] may be involved in the complex network regulated by the D3/DYS interaction. In fact, decreased levels of DYS lead to a reduced secretion of BDNF[2], whereas the dysregulated interaction between DYS and the subunit B of the nuclear transcription factor NFY causes an abnormal expression of MARCKs, which, in turn, may impair neural transmission and synaptogenesis[3].

By employing mutant mice bearing selective heterozygosis for D3 and/or DYS, we aimed to provide new insights into the functional interactions (single and synergic) between these schizophrenia-susceptibility genes, and the NFY complex, MARCKs, and BDNF in brain areas implicated in the cognitive symptoms of schizophrenia.

Methods

The mRNA levels of BDNF, MARCKs, and the three subunits of NFY (A, B, C) have been analyzed in the prefrontal cortex (PFC), striatum (STR), and hippocampus (HIPP) of wild-type (WT), D3 single heterozygous (D3+/−), DYS single heterozygous (DYS+/−), and double D3 and DYS heterozygous (D3×DYS+/−) male adult mice (N=5 for each genotype).

Data from single areas were analyzed with One-way ANOVA followed by Tuckey's post hoc test.

Results

In DYS+/- mice there was a significant upregulation of NFYB ($p<0.05$ vs D3+/−) and MARKS ($p<0.05$ vs D3+/− and $p<0.01$ vs WT) in the PFC. Moreover, in both the STR and HIPP of D3×DYS+/− mice we found a significant upregulation of BDNF (STR: $p<0.001$vs WT, $p<0.01$ vs D3+/−, $p<0.05$ vs DYS+/−; HIPP: $p<0.001$vs WT, $p<0.0001$ vs D3+/−, $p<0.01$ vs DYS+/−). In contrast, NFYC levels were decreased in both DYS+/− and D3×DYS+/− mice compared to D3+/− and WT ($p<0.05$ both). No significant differences were found in the expression levels of NFYA.

Conclusions

This study provides new insights into the impact of the D3/DYS interaction in regulating mRNA levels of neuroplasticity-related genes in three key brain areas for schizophrenia. Our results may help to clarify the genetic mechanisms and functional interactions involved in the etiology and development of this psychiatric disorder.

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Title

Amygdalar neuroplastic alterations in an experimental model of Anorexia Nervosa: focus on the BDNF system

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Background

Anorexia Nervosa (AN) is a severe psychiatric disorder that mainly affects adolescent females. The core symptoms of the disease are dietary restriction and compulsive exercise that, altogether, generate an out-of-control spiral in which the behaviors adopted to lose weight become rewarding, in turn motivating weight loss and preventing patients from recovering. The critical outcome of AN is worsened by the patient emotional instability, characterized by low self-esteem, fear of gaining weight, altered body-image perception and anxiety. Although the etiology of AN is still poorly known, patients exhibit morphological and functional alterations of the amygdala (Amy), a brain region fundamental for emotional processing. Since amygdala-dependent responses to emotional stimuli were found to be mediated by the neurotrophin BDNF in different psychiatric disorders, our major aim was to investigate the neurobiological alterations of the BDNF system induced by the anorexic phenotype in the Amy of adolescent rats exposed to the Activity-Based Anorexia (ABA) protocol.

Methods

Adolescent female Sprague-Dawley rats were individually housed since postnatal day (P) 35 and divided into four experimental groups: controls (CTRL, food ad libitum-sedentary), FR (food restricted–sedentary), EXE (food ad libitum-exercise) and ABA (food restricted-exercise). On P38, the ABA rats had free access to a running wheel and limited access to food (2h/day) till P42, when they reached the anorexic phenotype. In the acute phase of the phenotype (P42) and after 7-days of body weight recovery (P49), animals were sacrificed and trunk blood and Amy were collected. Total RNA and proteins were extracted and analyzed by means of Real-Time PCR and Western blot, respectively.

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Results

After four days of ABA induction, body weight was reduced in ABA rats more than in FR rats. Over days, wheel activity of ABA rats constantly increased, whereas EXE animals maintained a stable performance. Despite ABA rats showed increased total Bdnf, Bdnf isoform IV and VI gene expression at P42 in the Amy, BDNF protein levels were reduced in the crude membrane fraction, an effect paralleled by decreased activation of BDNF high-affinity receptor TrkB and BDNF-downstream signalling pathway (i.e., Akt). The overall downregulation of the BDNF system persisted even after body-weight recovery at P49. In line with clinical studies reporting decreased BDNF serum levels in anorexic patients, ABA rats showed a trend toward a reduction of BDNF plasma levels at P42, which instead were restored after 7-days of body weight recovery.

Conclusions

These data suggest that the combination of food restriction and hyperactivity determines a long-lasting dysregulation of the BDNF signaling in the Amy, an effect that might contribute to explain the increased vulnerability observed in anorexic patients and to the onset of psychiatric comorbid conditions typical of AN.

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References

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Title

The Role of Nutrition on Parkinson’s disease: a systematic review

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Background

Parkinson's disease (PD) in elderly patients is the second most prevalent neurodegenerative disease. Risk of developing PD is twice as high in men than women, but women have a higher mortality rate and faster progression of the disease.

The pathogenesis of PD is associated with dopaminergic neuron degeneration of the substantia nigra in the basal ganglia, causing classic motor symptoms; one possible source of male-female differences in the clinical and cognitive characteristics of PD is the effect of estrogen on dopaminergic pathways in the brain. Multifactorial interactions between oxidative stress, mitochondrial dysfunction, and neuroinflammation are putative mechanisms, but also nutrition plays an essential role in the pathogenesis and evolution of this disease.

There is growing evidence that PD affects women and men differently. Compared to men, women are diagnosed with PD less often, respond differently to current therapies, have less access to, and lower use of expert care, and are less socially supported. These factors also combine so that women with PD have poorer quality of life than men

Methods

We performed a systematic search in MEDLINE, EMBASE, and WEB OF SCIENCE databases from 2000 until present. Only randomized clinical trials (RCTs), observational case-control studies, and follow-up studies were included. We retrieved fifty-two studies that met the inclusion criteria and most of them investigated the effects of malnutrition and Mediterranean diet (MeDiet) on PD incidence and progression.

Results

Omega-3 and -6, and vitamins supplementation appeared poorly effective in protecting neuron degeneration. Insulin activity is a prevalent factor contributing to brain health, while malnutrition correlated with the higher development of dementia and mortality. Malnutrition activates also a gut-microbiota-brain axis dysfunction, that exacerbates the neurogenerative process. Polyphenols, polyunsaturated fatty acids, and coffee intake could have a potential protective effect; conversely, milk and its accessory products can increase PD risk.

Conclusions

In conclusion, we suggest that nutritional intervention could improve clinical outcomes and reduce the disease progression of PD both in man that women.

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Title

Clinical efficacy and safety of ceftaroline in a pediatric population 0-24 months of age: data from the PUERI study.

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Background

Ceftaroline is a 5th-generation cephalosporin approved for the treatment of complicated skin and soft tissue infections and community-acquired pneumonia from birth to adulthood, which has bactericidal activity against MRSA[1]. Its safety and effectiveness in community-acquired pneumonia in hospitalized pediatric patients (2 months–18 years) has been described in a ?multicenter, randomized, prospective study that compared ceftaroline to ceftriaxone, which demonstrated ?that ceftaroline fosamil was well tolerated[2].

Here we present its effectiveness and safety data in a population aged 0-24 months, within the spectrum of both on- and off-label indications.

Methods

This retrospective study is conducted using data of the population included in the PUERI pharmacokinetic and tolerability study: children aged 0-24 months, an underrepresented population in approval ceftaroline trials.

The study was carried out in Niguarda Hospital, Milan, Italy, January 2020 to December 2021, on hospitalized children of both sexes, receiving systemic treatment with ceftaroline fosamil for a suspected or confirmed infection. Clinical effectiveness and safety was thus evaluated in this population: clinical data was accessed via their eletronic medical records and was then collected in an electronic spreadsheet.

The primary outcomes were the clinical efficacy and safety of ceftaroline in suspected or confirmed infections, while secondary outcomes were mortality, rehospitalization within 30 days, length of hospital stay, days to clinical resolution

of the infection. Categorical variables are summarized as number and percentage, while continuous variables are summarized as geometrical mean and standard deviation. Prematurity was considered a variable of its own, according to which the population was stratified.

Results

The population included both infants born at term (n=28) and preterm newborns (n=3). Females were 54.8% of the population, mean age was 8.1 months. 64% of the population was hospitalised due to infection. Ceftaroline mean dosage was 8.2 mg/kg/dose (±0.8) in infants, 5.3 mg/kg/dose (±1.1) for preterms. Infection was confirmed in 58.1% of cases: among positive coltures 55.5% were Staphylococci, and 32.2% of bacteria were MDRs (25% of term infants, 100% of preterm newborns). Ceftaroline was effective as salvage therapy in 61.3% of cases, while it led to resolution in 83.9% of cases. Rehospitalization occurred in 2 cases (6.4%), of which 1 was for infection (3.2%). Death occurred in 1 patient affected with Fallot tetralogy and pulmonary valve agenesis. Safety was confirmed as only 1 adverse event occurred (urticarial rash and vomit on 4^{th} cycle of ceftaroline).

Conclusions

Data extracted from the PUERI study confirmed ceftaroline's effectiveness and safety in patients aged 0-24 months. The study was limited by the numerosity of patients and by its real-world setting, though it does lay an important step towards the implementation of real-world studies in pediatric patients, even post-marketing.

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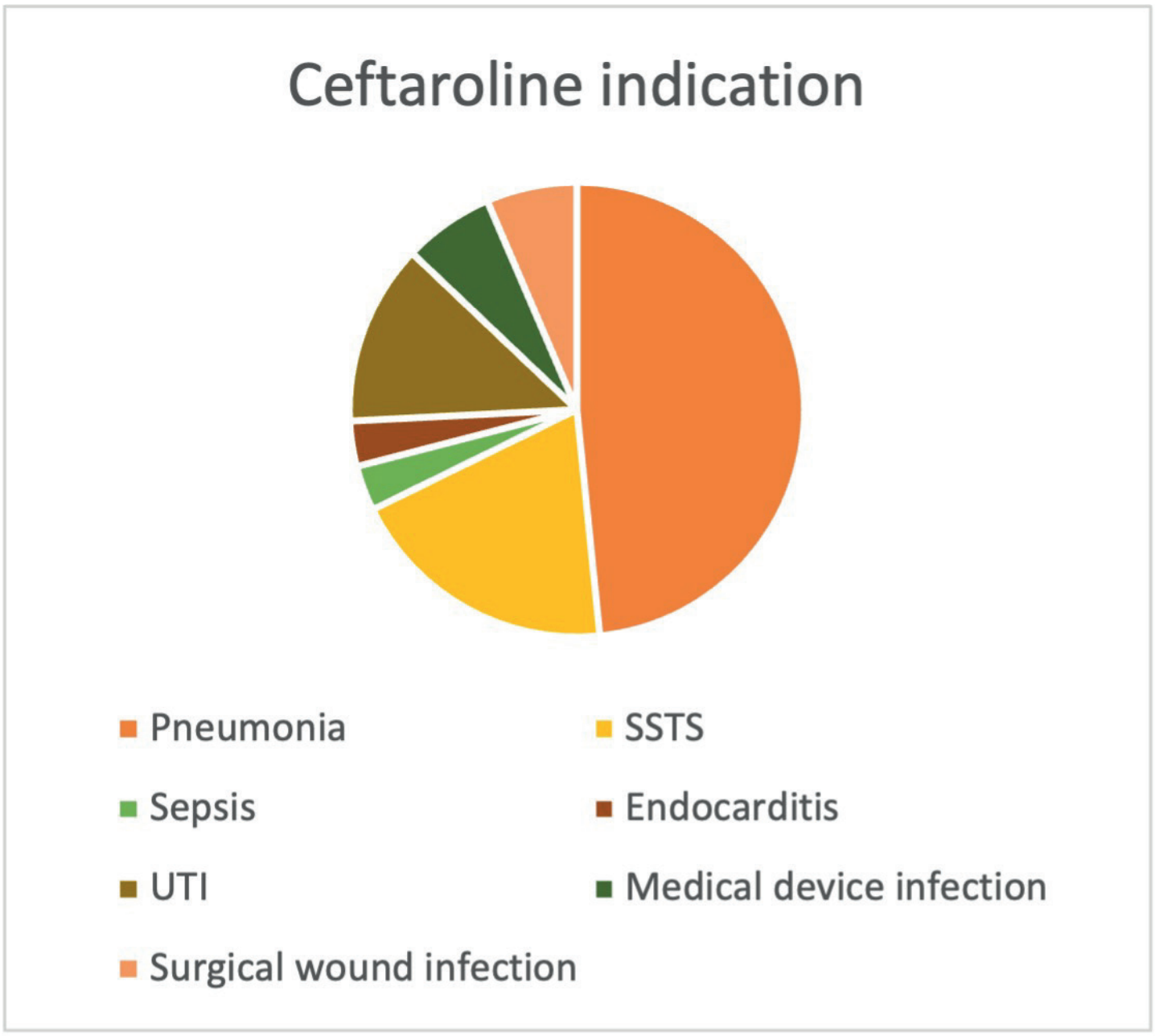

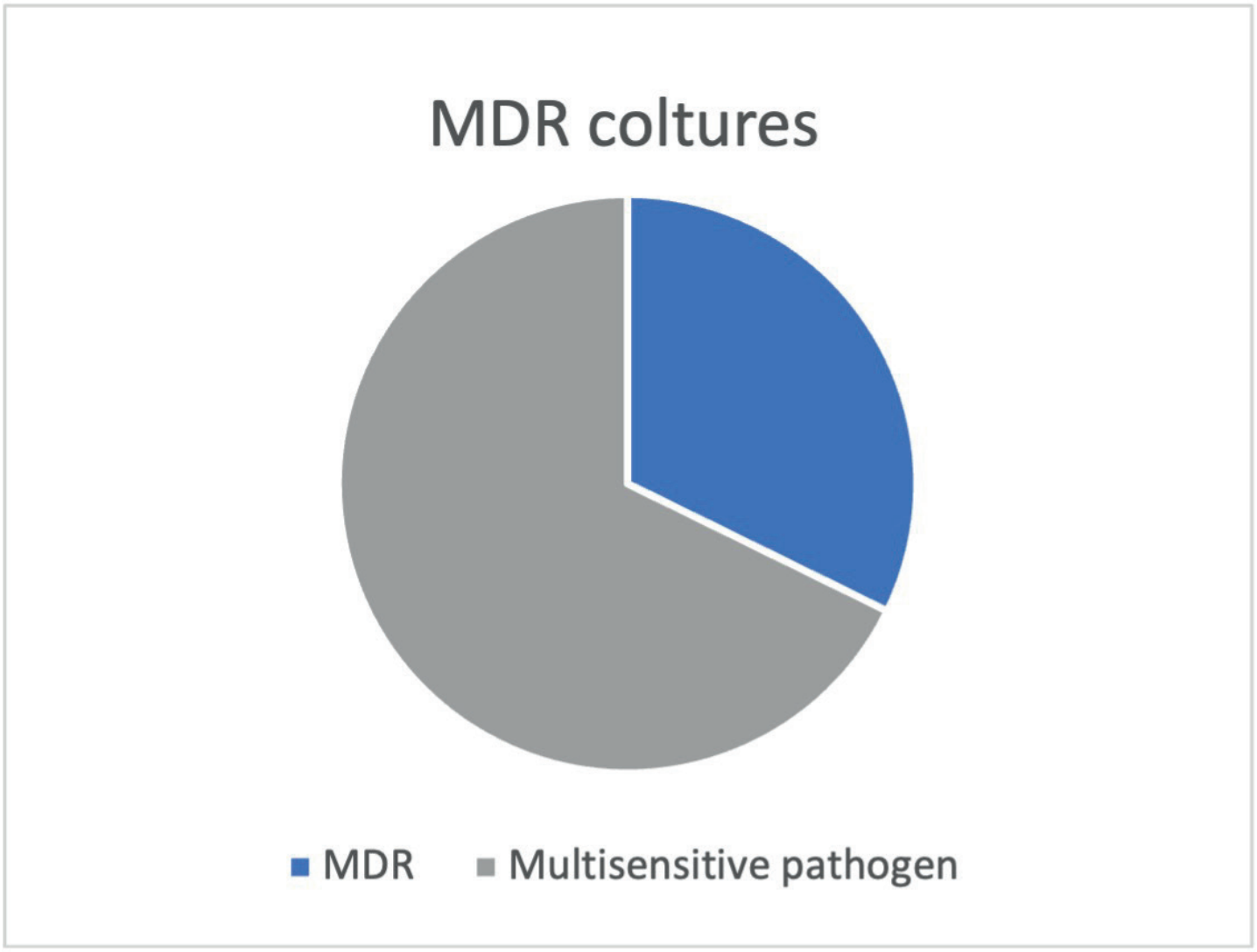
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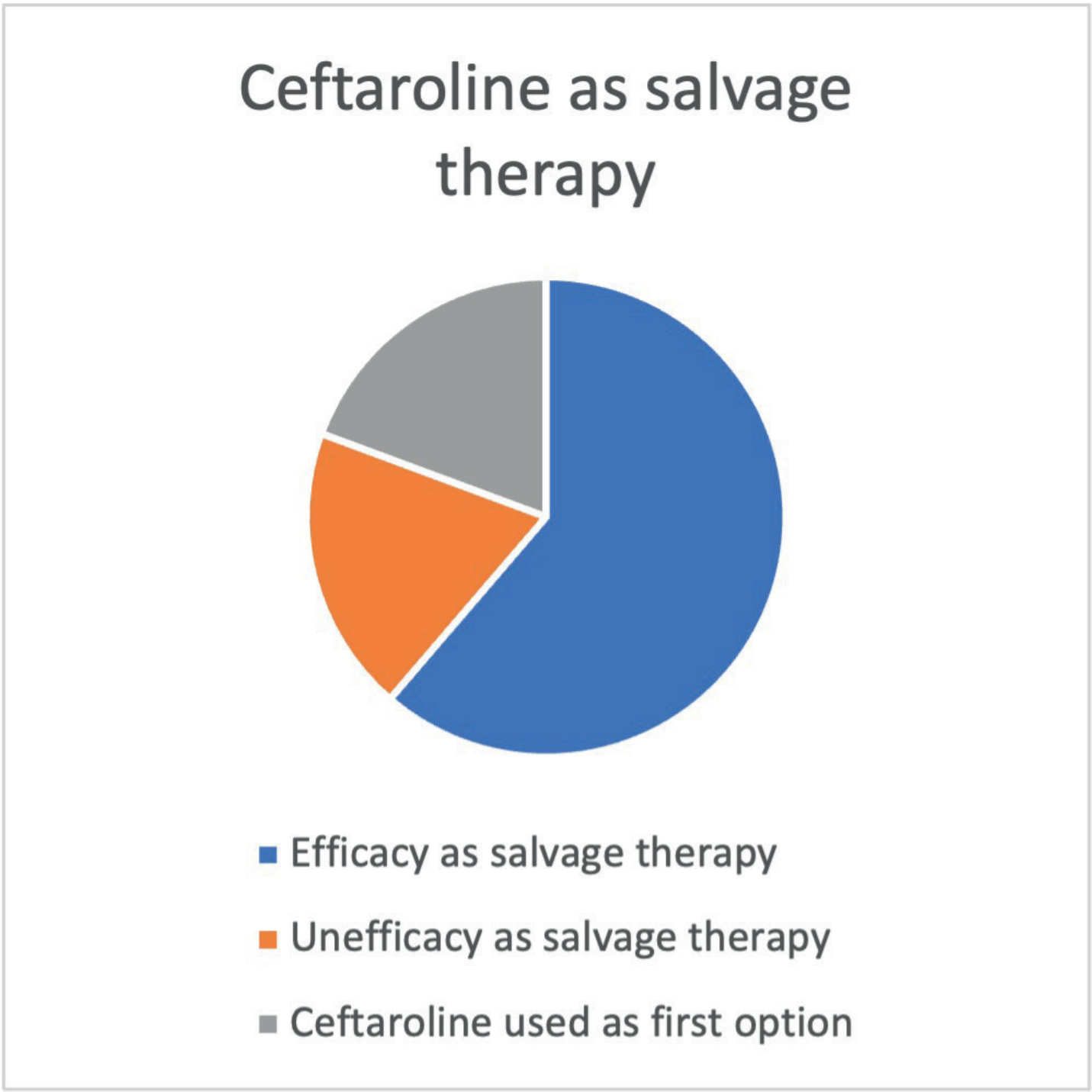

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Title

INVOLVEMENT OF METABOTROPIC GLUTAMATE RECEPTORS IN BEHAVIOURAL EFFECTS OF BERGAMOT ESSENTIAL OIL

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Background

Preclinical studies have recently highlighted that bergamot essential oil (BEO) is endowed with anxiolytic-like/relaxant effects in animal behavioural tasks not superimposable to those of the benzodiazepine diazepam (DZP) (1). Particularly, BEO induces relaxant effects in rats, although the animals are still vigilant, and these are at variance with the effects of DZP. Moreover, flumazenil, a benzodiazepine site antagonist, does not significantly affect behavioural effects of phytocomplex (2), suggesting that neurotransmissions, other than GABAergic, could be involved. Accordingly, we recently reported that 5HT-1A receptor is partially involved in these effects of BEO (3). Since metabotropic glutamate receptors (mGluRs) have been implicated in anxiety-related behaviours (4) and BEO may interfere with mechanisms controlling synaptic levels of glutamate (5), the aim of this study was to investigate the involvement of mGluR2/3 and mGluR5 in anxiolytic-like/relaxant effects of essential oil.

Methods

Anxiety and spontaneous behaviour were measured in male Wistar rats using the elevated plus maze (EPM) and open field (OF) tests after systemic injection of LY341495, a selective mGlu2/3 receptor antagonist, or 2-methyl-6-(phenylethynyl)-pyridine (MPEP), a selective mGlu5 receptor antagonist and BEO. The experimental protocols were authorized by the Ministry of Health (Authorization Number 305/2019 PR; date of approval: 10/04/2019).

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Results

Administration of LY341495 (3mg/kg, i.p.) (n=6), and MPEP (3mg/kg, i.p.) (n=6), differently affects the anxiolytic-like/relaxant effects of BEO (500µl/kg) (n=6) in EPM and OF tests. Particularly, our data indicate that the blocking of mGluR5 rather than mGluR2/3 may interfere with some of the behavioural parameters measured in experimental tasks after BEO treatment.

Conclusions

These findings suggest that mGlu5 receptor is likely involved in the behavioural effects of BEO but that the effects of phytocomplex do not occur through the activation of this receptor only. Moreover, these data confirm that complex mechanisms may be likely implicated in BEO effects and these deserve further investigation. However, the present observations provide further insight to the neuropharmacological profile of BEO and support its rational use in aromatherapy in symptoms of stress-induced anxiety.

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Title

Unmet needs of Parkinson's disease patients in Italy through the COVID-19 pandemic: results of a structured survey

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Background

COVID-19 spread globally, originating a pandemic in 2020. To limit the spreading of the virus, the Italian government declared a lockdown of the whole country lasting about two months, and the introduced restrictive rules heavily impacted patients with chronic neurological diseases because of reduced access to healthcare and community support services. In Parkinson’s disease, studies confirmed lockdown restrictions caused limitations on physical activities, and a lack of clinical assistance, with an impact on patient’s lives even after the lockdown ended (1–4).

Methods

We aimed at investigating the impact of the pandemic during and beyond the lockdown period in Parkinson’s patients, through an online survey. The questionnaire was made available to participants after the lockdown period, between October 2020 and April 2021. Participants were asked to fill in the questionnaire on their own, reporting their own personal experiences.

Results

A total of 387 patients accessed the survey, and 339 completed section A (Opinion and perception of the epidemic), 276 completed section B (Personal experience and involvement in the epidemic), 260 completed section C (Current situation), and 246 completed the whole questionnaire. Our results show participants were worried about the COVID-19 outbreak and social distancing. They considered their risk of contracting SARS-CoV-2 to be higher and thought PD therapy increased their risk of being affected by the virus. They also believed the epidemic prevented them from receiving adequate neurological therapies. Contacting physicians was harder than usual, but such difficulty did not

seem to broadly affect the patients' health conditions. Daily activities and physical activities, including physical therapies, were instead reduced for many of the participants. Moreover, a significant part of patients reported therapy to be less effective on PD symptoms. After lockdown, contacting general practitioners and neurologists was easier and many individuals returned to their normal daily activities, but less than half of them returned to their previous physical activities, including physical therapies. For one in three patients, disease symptoms were harder to control than before the pandemic.

Conclusions

For Parkinson's disease patients, who largely benefit from a multi-disciplinary therapeutic approach (2), restrictions during the lockdown caused the disruption of a delicate balance between the patients' need for healthcare and ability to provide services, counselling, and access to dedicated structures for personal physical activities (4). This balance is still far from being restored, even after months since stricter lockdown restrictions were lifted. We analyse collected data to gain further insights on patients' unmet needs during the current pandemic.

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Title

High Cardiovascular Risk Management in Hypertension Patients in General Practice: A Focus on Inappropriate Drugs Prescriptions

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Background

Hypertension is the most common chronic disease and the leading preventable risk factor for cardiovascular disease (CVD) and premature deaths worldwide

The aim of this study is to evaluate the management of hypertension in clinical practice by analyzing the prescribing behavior of GPs.

Methods

A retrospective cohort study was carried out using the data recorded between 2018 and 2020 in the clinical database of 10 GPs. Hypertension was defined using the International Classification of Diseases (ICD-9 = 401*-404*) or using the laboratory parameters (systolic/diastolic blood pressure >140/90 mmHg), according to ESCH/ESH. The cohort was described stratifying by demographic, clinical and therapeutic characteristics, and laboratory tests.

Patients not treated with antihypertensive drugs, or treated with a beta blocker (BB) without diagnosis of heart failure, angina, post myocardial-infarct, arrhythmias, or in monotherapy that did not achieve the blood pressure target, were considered inappropriate.

Univariate and multivariate logistic regression models were applied to identify predictors of antihypertensive treatment and inappropriate prescriptions.

Results

A total of 3,486 (26.4%) patients out of 13,206 people covered by the medical care were affected by hypertension. Only 2,446 (70.2%) patients had a blood pressure value registered, of them the 18.2% achieved the blood pressure target. Patients on antihypertensive therapy were 2,866 (82.2%), of them 631 (22.0%) were in monotherapy. About half of the cohort of hypertensive patients (49.5%) had never had a cardiological counselling during the study period.

The probability of antihypertensive prescription was increased in the elderly (Adj OR, CI: 1.04, 1.03–1.05), in patients with at least one cardiological counselling (Adj OR, CI: 1.66, 1.02-2.16), with ischemic heart disease (Adj OR, CI: 1.68, 1.09-2.59), with the number of other drugs used (Adj OR, CI: 1.06, 1.02-1.09) and of prescriptions received (Adj OR, CI: 1.01, 1.00-1.01).

Conversely, the probability was significantly reduced in patients with kidney disease (Adj OR, CI: 0.56, 0.43-0.74), chronic respiratory diseases (Adj OR, CI: 0.56, 0.43-0.73), and osteoporosis (Adj OR, CI: 0.68, 0.51-0.93).

The hypertensive patients identified as inappropriately treated were 1,645 (47.2%). In particular, the 40.0% of inappropriateness was due to the use of a BB in patients without indications, the 37.7% patients were not antihypertensive users, the 20.2% were on monotherapy despite they were out of target and the 2.1% were on monotherapy with a BB in the absence of indication or despite they were out of target.

Conclusions

Our results highlighted some critical aspects in the management of patients with hypertension in clinical practice.

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Title

Sex-dependent impacts of an essential amino acid defined-diet on obesity management

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Background

Modifying feeding habits represent a promising anti-obesity therapy. Indeed, food can be considered a cocktail of hormonally-active molecules [e.g., amino acids (AA)] involved in regulating multiple signalling pathways1. The supplementation with a specific branched-chain amino acid-enriched mixture (BCAAem) enhances mitochondrial biogenesis, increases the healthy life span in mice, and ameliorates the physical and cognitive performances of elderly patients2,3. Of note, the BCAAem supplementation rejuvenates the gut microbiota of ageing mice4. Recently we demonstrated that a designer diet in which protein was replaced by a specific free essential amino acid formula (SFA-EAA), mimicking the BCAAem2, shows a strong anti-obesity and anti-diabetic properties, stimulating energy expenditure via brown adipose tissue (BAT) activation (i.e., adaptive thermogenesis), modulating the gut microbiota, and prolonging health span5. However, sex has an important role in metabolic response to diets6. Thus, we extended our observations on female mice to elucidate the sex-based signalling properties of the SFA-EAA diet.

Methods

We investigated the effect of a designer dietary approach in male and female C57BL6/N mice fed with one of 4 diets: 1) SFA: a diet with a high ratio of saturated to unsaturated fatty acids, which leads to obesity and glucose homeostasis

impairments after prolonged consumption; 2) SFA-EAA: a new designer diet, isocaloric, isolipidic, isonitrogenous to SFA, in which the protein component (*i.e.*, casein) was almost entirely replaced by a defined free essential AA (EAA) formula; 3) SFA-CAA: an additional control diet, in which casein was substituted with the free AA designed on the casein profile, and 4) chow diet (Fig.1).

Results

Our results show a marked sex-specificity of the SFA-EAA diet efficacy. This diet prevents obesity development more efficiently in males than females and ameliorates dysregulated glucose homeostasis only in males. The activation of the BAT thermogenic program via sympathetic innervation (SNS) is observed only in SFA-EAA-fed males. BAT of females seems to be resistant due to a lower SNS recruitment than males. Moreover, the SFA-EAA diet shapes gut microbiota profile in a sex-dependent manner (Fig.2).

Conclusions

Our data suggest that the beneficial effects of the SFA-EAA diet are related to a sex-dependent BAT thermogenesis activation. In particular, the sex-based differences in response to the diet could be associated with SNS recruitment. Recent observations revealed a potential role of BAT thermogenesis stimulation in humans, which is more relevant in modulating insulin sensitivity than as an anti-obesity approach7. Therefore, the SFA-EAA mediated BAT thermogenesis activation could be mainly linked to improving T2D rather than the prevention of obesity. The modulation of body weight could be due to other mechanisms, such as gut microbiota changes (Fig.3).

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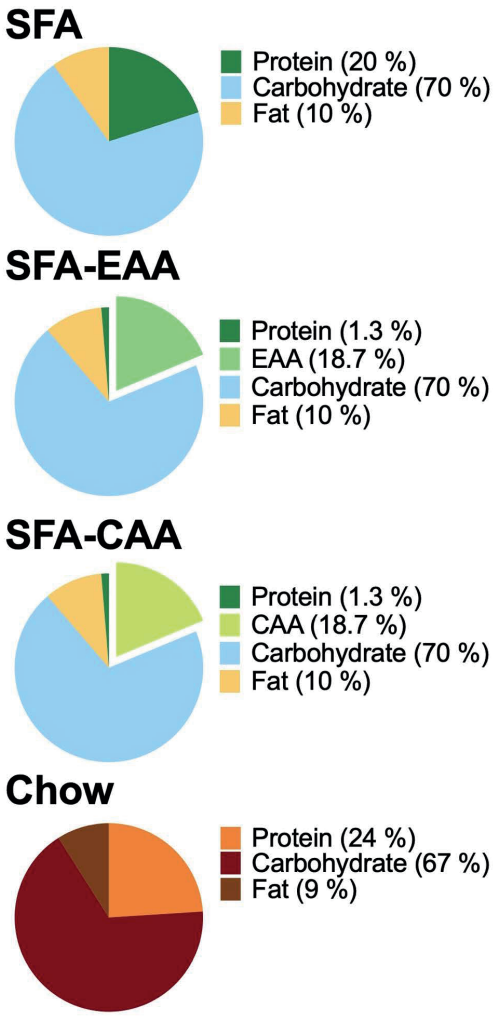

	SUPPLEMENT	DESIGNER DIETS	
	BCAAem*	EAA substitution#	CAA substitution#
Leucine	30,01	58,6	16,8
Lysine (chlorhydrate)	19,58	30,34	14
Isoleucine	15	29,22	8,04
Valine	15	22,9	9,91
Threonine	8,4	16,48	7,67
Cysteine/cystine	3,6	7,12	1,31
Histidine	3,6	7,12	4,86
Phenylalanine	2,4	4,68	8,98
Methionine	1,2	2,44	5,42
Tyrosine	0,72	1,31	0,72
Tryptophan	0,48	0,94	2,24
Alanine	-	-	5,42
Arginine	-	-	6,36
Aspartic acid	-	-	12,9
Glutamic acid	-	-	40,58
Glycine	-	-	3,18
Proline	-	-	18,89
Serine	-	-	10,66

*All values are reported as percentage (g/100 g),
#All values are reported as 200g of protein/1000g diets

Figure 1. BCAAem supplement and designer diet composition.

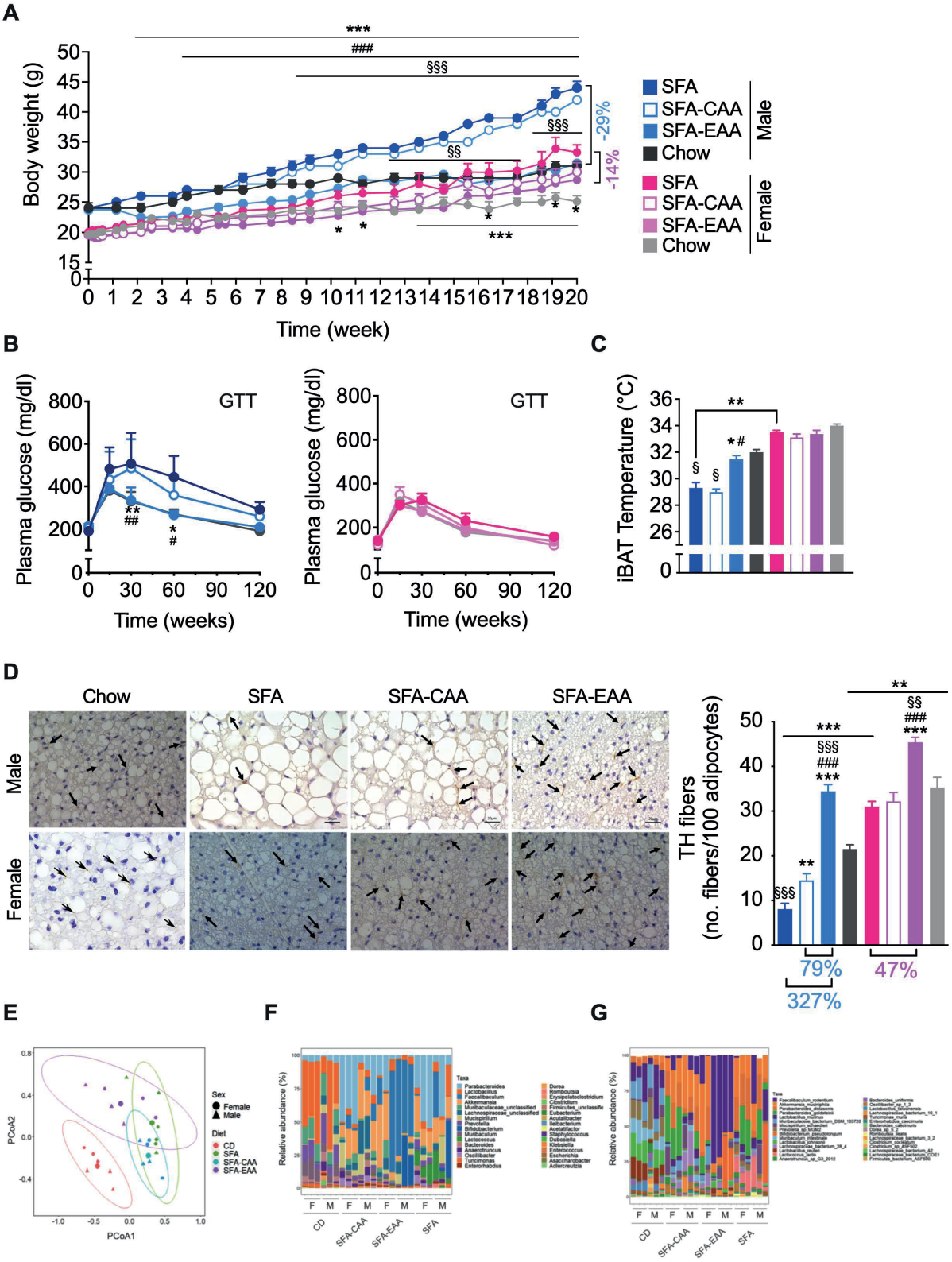

Figure 2. Sex-dependent metabolic effect of SFA-EAA diet. Male and female mice were fed with SFA, SFA-EAA, SFA-CAA and chow diets for 20 weeks. (**A**) Body weight measured once a week; (**B**) Glucose tolerance test (GTT) in mice fed for 15 weeks. GTT were performed after overnight fasting (16–18 h), by i.p. injection of glucose (1.5 g/kg body weight). Glucose levels were measured in tail-vein blood at 0, 15, 30,

60 and 120 minutes after bolus. The results are reported as time course of blood glucose levels. (**C**) interscapular (iBAT) temperature measured with a thermographic FLIR camera in mice fed with diets for 7 weeks; (**D**) Immunohistochemistry of sympathetic fibres was studied using anti-tyrosine hydroxylase (TH) antibody in iBAT of mice fed with diets for 7 weeks. The density of TH-immunoreactive fibres was calculated as the number of fibres per 100 adipocytes. Arrowheads indicate TH-positive fibres. Scale bar, 20 µm. All data are presented as mean ± SEM (n=5 mice/group). One-way ANOVA. *p < 0.05, **p < 0.01 *vs* SFA-fed mice, ***p<0.001; #p < 0.05, ##p < 0.01, ###p < 0.001 *vs* SFA-CAA-fed mice, §§p < 0.01, §§§p < 0.001 *vs* Chow diet-fed mice. (**E-G**) The whole metagenome sequencing analysis on faecal samples of 7 weeks fed mice. (**E**) Principal coordinates analysis (PCoA) plot of Bray–Curtis distances for bacterial communities. Triangle for females and dot for males represent a single sample. The big dots are the centroid, and each point is included in a particular cluster if it is closer to the centroid. (**F,G**) Genera (**F**) and species (**G**) relative abundance (%) (n=3 mice/group). One-way ANOVA (**E**); analysis of similarities (ANOSIM, p=0.001) (**E**).

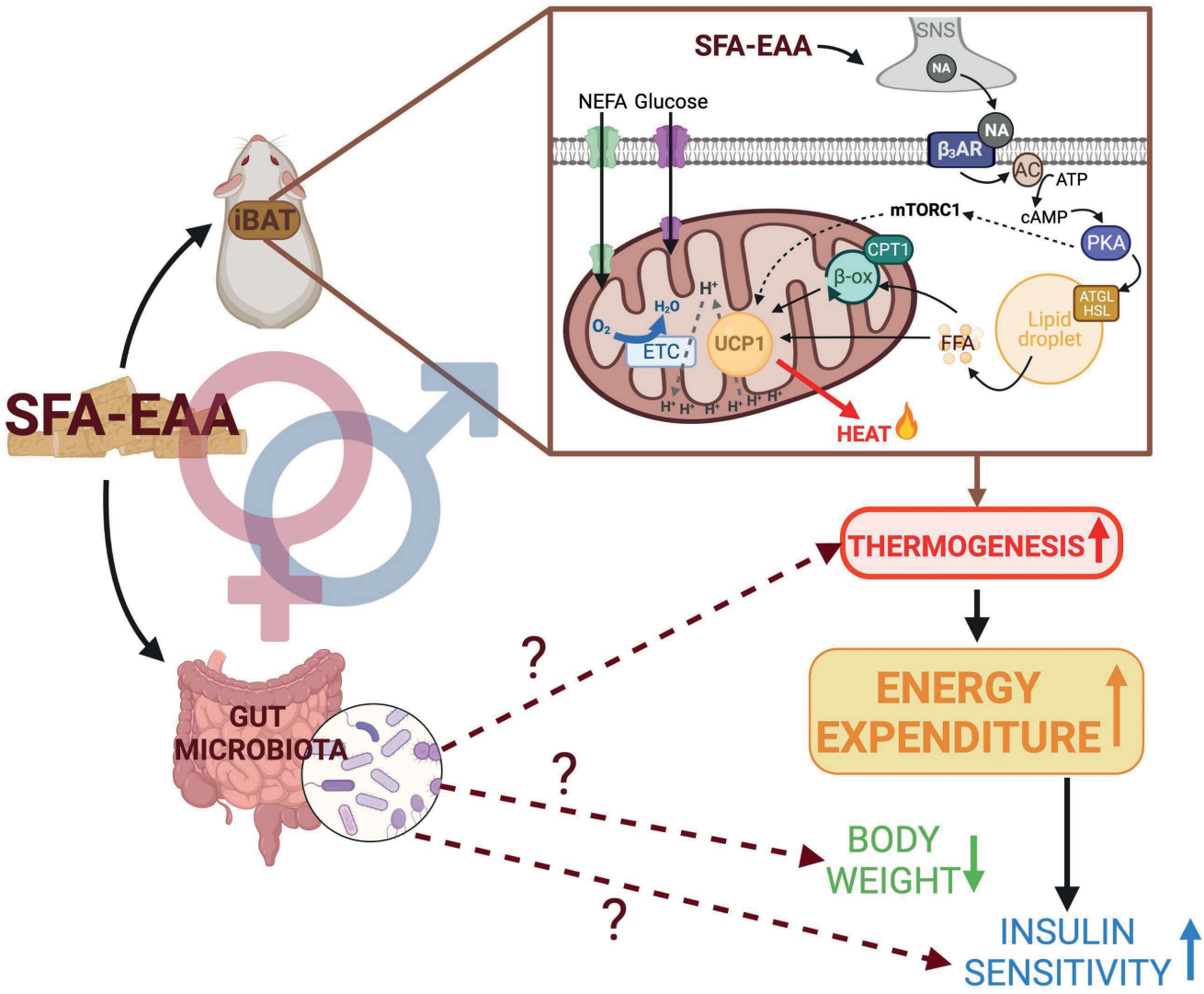

Figure 3. Graphical summary.

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Title

ADVERSE DRUG REACTIONS TO ANTI–TUMOR NECROSIS FACTOR-α THERAPIES IN INFLAMMATORY BOWEL DISEASE

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Background

The arrival of anti–tumor necrosis factor (TNF) therapy has provided an improvement in management of patients with inflammatory bowel disease (IBD). Despite newer available drug, anti-TNF agents remain leading biologics in first-line therapy. As a result of their wide use, continuous monitoring of safety and effectiveness play an key role. In this regard, we performed a prospective pharmacovigilance study with the aim to identify and assess possible adverse events (AEs) in patients naïve treated with anti-TNF during the first-year therapy.

Methods

This observational prospective study included patients naïve at first administration of infliximab (IFX), golimumab (GOL) or adalimumab (ADA), with a diagnosis of ulcerative colitis (UC) or Crohn's disease (CD), in treatment in one of Gastroenterology Centers of Calabria Region. Enrollment was performed from January 2019 to April 2021, considering a follow up period of 12 month from the date of first administration. AEs were collected and analyzed through mapping with the PT (Preferred Term) and SOC (System Organ Class) codes of MedDRA dictionary.

Results

On a total of 296 patients treated with anti-TNF agents during the study period, 252 (85,1%) were naïve: mean age (±SD) was 42,9 (±14,1) years and 38,9% (98) were females; prevalence of UC was slightly higher than CD (55,2% vs 44,8%). IFX was the most used anti-TNF (162 patients; 64,3%), while to lesser extent ADA (65 patients; 25,8%) and GOL (25 patients; 9,9%). During the follow up period, 52 AEs, referred to 38 patients, and only one case of failure of therapy were reported. The major rate of AEs belongs to the class of “general disorders and administration site conditions” (34,1% AEs): it is mainly about asthenia in patients treated with IFX (10 patients) and ADA (1); the remaining were injection site reaction (1 patients with IFX and 1 with GOL), flushing (2 IFX), oedema (2 IFX) and pyrexia (1 GOL). “Nervous system disorders” occurred in percentage of 18,2% in treated with IFX: 5 reports of headache and other of dizziness (1), somnolence (1), confusional state (1). Furthermore, we detected 7 (13,5%) report of “musculoskeletal and connective tissue disorders”, namely arthralgia in patients treated with IFX.

Conclusions

Among IBD patients in anti-TNF therapy, more than half were naïve: indeed these drugs represent the mainstay of treatment, as well as first-line biologic choice, for moderate-to-severe IBD. IFX was frequently associated with the occurrence of AEs, but it was also the most used in our cohort. Most of the AEs occurred (e.g. arthralgia and fever), although belonging to different SOC, can be related to acute/delayed infusion reaction. However, results are in line with the data available in literature; no patients experienced serious AEs during first-year therapy. Pharmacovigilance activities may improve the understanding, recognition and management of AEs in these patients.

References

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Title

SERIOUS ADVERSE DRUG REACTIONS TO ANTINEOPLASTIC DRUGS IN WOMEN: A REGIONAL PHARMACOVIGILANCE ANALYSIS

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Background

Given the growing incidence of cancer in women, use of antineoplastic agents in female population was increased in the last years. It is well known that chemotherapeutic agents have an high potential to cause serious adverse drug reactions (ADRs) in patients. The aim of this study was to detect the ADRs related to antineoplastic drugs in female patients, thus enhancing their management in clinical practice.

Methods

From 01 January 2020 to 31 December 2020All ADRs related to antineoplastic drugs (ATC: L01) administration to female patients and classified as serious submitted by Calabrian healthcare professionals and patients to the Italian Network of Pharmacovigilance (RNF), were examined.

Results

During the study period, 614 ADRs were reported to RNF, of which 308 (50,2%) referred to women; of these, 49 (15,9%) were classified as serious. Among serious ADRs reported in female population, 28 (57,1%) reported an antineoplastic agent as “suspected drug”. The study sample consist in women with a mean age (±SD) of 56,4 (±12,4) and diagnosis of cancer (92,9%) or leukemia (7,1%). ADRs that required hospitalization or prolongation of existing hospitalization were the most common (23; 82,1%), mainly related to administration of agents belonging ATC group L01X (7), followed by: alkylating agents (4), antimetabolites (3), plant alkaloids and other natural products (3), protein kinase inhibitors (3) monoclonal antibodies and antibody drug conjugates (3). Neutropenia (8; 34,8%) and anaemia (5; 21,7%) were the main ADRs requiring hospitalization or re- hospitalization. One 59-years-old female patient affected by chronic lymphocytic leukemia (CLL) treated with ibrutinib reported progression of CLL that led to death. Lastly, 14,3% of serious ADRs detected were classified as “other relevant clinical condition”, specifically gastrointestinal disorders (vomiting and nausea).

Conclusions

As shown by our results, hospitalization or re-hospitalization occurred frequently in women in treatment with antineoplastic drugs, resulting in increase of health-care costs, as well as decrease of patients compliance. Pharmacovigilance activities to aid detection and reporting of serious ADRs may serve to enhance knowledge about their nature and impact.

References

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Title

A designer diet enriched in essential amino acids as a non-pharmacological treatment for cardiac hypertrophy in rodents

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Background

Heart failure with reduced ejection fraction (HFrEF) is a leading cause of mortality worldwide [1]. Current pharmacological therapies for HFrEF aim to control symptoms and increase exercise capacity [2]. However, alteration of metabolic substrate utilization and flexibility in myocardial tissue is a significant hallmark in cardiac hypertrophy [3]; furthermore, a nutritional approach is still lacking: Dietary manipulation aimed to reinstate the compromised metabolic homeostasis could be, therefore, a promising therapeutic approach

Methods

Mice were subjected to transverse aortic constriction (TAC) to induce left ventricle (LV) pressure overload or sham surgery and were fed before (preventive experiment) and after TAC (therapeutic experiment) with a control diet (SFA) or a diet where casein, the main component of rodent diet, was substituted with a specific essential amino acid (EAA) formulation (SFA-EAA). A diet substituted with casein amino acids (SFA-CAA) was also employed as a further control [4]. We assessed global cardiac metabolite levels and gene expression, as well as ultrastructural morphological analysis and mitochondrial activity to investigate the molecular mechanisms of the diets

Results

The SFA-EAA amino acid-substituted diet, but not the SFA-CAA diet, restored the TAC-induced impairment in both fractional shortening (FS) and ejection fraction (EF) in both preventive and therapeutic experiments, as compared to TAC mice fed with control SFA diet. Metabolomic, transcriptomic, and biochemical analysis showed that the designer diet restored the alteration in cardiac substrate utilization and the metabolic inflexibility induced by TAC by improving and boosting mitochondrial fuel oxidation and blunting hyperactivation of cardiac insulin signaling in TAC animals. The cardioprotective effects of the diets were absent in PP2Cm knock-out mice, which are deficient in branched-chain amino acids (BCAA) utilization [5], suggesting that activation of BCAA catabolism, which is altered in TAC, underlies the mechanism of action of the diet.

Conclusions

Our data indicate that the dietary manipulation of EAA by improving cardiac mitochondrial energy metabolism prevents and ameliorates cardiac hypertrophy and could therefore represent a novel nutritional approach for HFrEF in humans

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pharmadvances

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Title

The bispecific DARPin® molecule MP0310 enhances daratumumab-mediated anti-myeloma activit

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Background

Daratumumab is an anti-CD38 mAb with potent anti-multiple myeloma (MM) activity through antibody-dependent cellular cytotoxicity (ADCC) mediated by NK cells. Despite promising results, daratumumab-treated MM patients invariably relapse [1]. MP0310 is a bispecific a DARPin® molecule that simultaneously binds fibroblast activation protein (FAP) and 4-1BB (CD137), resulting in CD137 clustering and signaling. CD137 is a co-stimulatory receptor expressed on activated NK cells [2]. Fibroblasts (FBs) from patients with MM overexpress FAP and contribute to drug resistance by creating a supportive bone marrow (BM) niche where MM cells escape drug treatment by adhering to FBs [3]. Based on these observations, MP0310 may recruit activated NK cells towards FBs fostering daratumumab-mediated NK cell activity. Here, we evaluate the anti-myeloma effect of MP0310 molecule and its putative synergic effect in combination with daratumumab.

Methods

CD137, CD107a and perforin expression on $CD3^{-}CD16^{+}CD56^{+}$NK cells was evaluated by FACS analysis of BM mononuclear cells from MM patients at different clinical stages. The cytotoxic effect of MP0310 on the daratumumab-mediated ADCC was evaluated using the calcein-AM release assay by co-culturing daratumumab pre-treated and untreated BM lymphocytes (BMLs) (effector cells) with MM cells (target cells) in the presence or absence of MM FBs (E:T cell ratio 10:1).

Results

Flow cytometry analysis indicated a low expression of CD137 on MM NK cells. *In vitro* treatment of BMLs:MM cells cultures with daratumumab increased the expression of CD137 as well as of CD107a and perforin on NK cells suggesting that daratumumab activates NK cells. Furthermore, MP0310 increased adhesion of daratumumab-treated NK cells on MM FBs. Analysis of ADCC in BMLs:MM cells co-cultures in the presence of MM FBs showed that MP0310 enhanced NK cell activity of daratumumab pre-treated BMLs.

Conclusions

MP0310 may represent a new therapeutic strategy to further enhance daratumumab-mediated anti-MM activity.

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Title

ROLE OF A_{2A} AND A_{2B} ADENOSINE RECEPTORS IN MYELINATION PROCESSES AND IONIC CONDUCTANCES IN NEURONAL AND OLIGODENDROGLIAL CELLS

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Background

Background: The physiological relevance of oligodendrocytes (OLs) is highlighted by diseases such as multiple sclerosis (MS), in which the myelin sheaths are degraded and the axonal signal transmission is compromised. This condition is characterized by demyelination of white matter in CNS and apoptosis of OLs. In addition, remyelination processes are hindered due to a failure of the oligodendrocyte precursor cell (OPC) differentiation into mature OLs.

Extracellular adenosine increases during ischemia or inflammation, suggesting adenosine receptors (A_1R, A_{2A}R, A_{2B}R, A_3R) as valid therapeutic targets in a variety of pathological conditions, including demyelinating diseases. We previously demonstrated that selective stimulation of A_{2A}Rs or A_{2B}Rs decreases OPC maturation in vitro by inhibiting potassium currents necessary to their differentiation. Here, the functional role of A_{2A}R and A_{2B}R on OPC maturation and on myelin

deposition in purified rat OPC cultures and in OPC/dorsal root ganglion (DRG) co-cultures was investigated.

Methods

Experiments were carried out by patch clamp technique coupled to Real Time PCR and immunocytochemistry. We used selective ligands for A_{2A}Rs or A_{2B}Rs and, for the first time, the newly synthetized multitarget antagonist, P626, able to simultaneously block both receptor subtypes.

Results

We confirmed that the A_{2B}R agonist BAY60-6583 (0.1-30 μM), as well as the A_{2A}R agonist CGS21680 (50-100 nM), reduced outward currents evoked by a voltage ramp protocol (from -120 to +80 mV, 800 ms). The effect of BAY60-6583 was prevented by the A_{2B}R antagonist MRS1706 (10 μM) whereas the effect of CGS21680 was blocked by the A_{2A}R antagonist SCH58261 (100 nM), Both inhibitory effects were also prevented by the new dual selective A_{2A}R/A_{2B}R antagonist, P626 (100 nM). Consistently, the endogenous agonist adenosine (50 μM), applied in the presence of A_1R and A_3R selective blockers DPCPX and MRS1523, respectively (both 500 nM), also inhibited outward ramp currents, an effect that was prevented by the mixed A_{2A}R/A_{2B}R antagonist P626 (0,1-200 nM). Chronic (7-days) application of BAY60-6583 to OPC cultures reduced the expression of mature OL markers whereas A_{2B}R-gene silencing increased mature OL marker expression.

Furthermore, chronic treatment with A_{2A}R or A_{2B}R agonists modulated myelination of DRG axons in DRG-OPC co-cultures, without effects on total MBP expression. Finally, A_{2B}R stimulation enhances action potential firing in DRG neurons.

Conclusions

Our data show that A_{2A}R/A_{2B}R activation prevents OPC differentiation by inhibiting potassium currents. However, in OPC-DRG co-cultures the selective stimulation of A_{2A}R and A_{2B}R increase myelin deposition, possibly by enhancing neuronal firing. These results suggest that activation of "A_2Rs" modulates different functions in oligodendrogliogenesis, depending on their cellular localization and may represent a valuable target in demyelinating pathologies such as MS.

References

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Title

Infliximab treatment and switching in Inflammatory Bowel Disease: a seven years' experience in a Hospital of ULSS2 Marca Trevigiana

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Background

Inflammatory Bowel Disease (IBD) comprehend Ulcerative Colitis and Crohn's Disease and are chronic disorders based on bowel inflammation with debilitating symptoms such as abdominal pain, diarrhea, rectal bleeding. In Italy infliximab is authorized for the first line treatment, ustekinumab and vedolizumab for the second line. After commercialization of Remicade® (2005), several infliximab biosimilars have come out on the market since 2015: Inflectra®, Remsima®, Flixabi®. The purchases made by each hospital depend on regional agreements that put biosimilars with same indications in bioequivalence. The clinical choice must be guided both by prescriptive appropriateness and cost.

Methods

Drug utilization data of infliximab and biosimilars prescribed by Gastroenterology Unit from 2015 to 2021 have been collected from our database. From our data extraction, it appears that originator Remicade® has been available in our hospital from 2005 to 2020; Inflectra® in 2015 and 2016; Remsima® since 2016 and Flixabi® since 2017.

Results

A total number of 49 patients has received at least one dose of an infliximab-based treatment. In total 14 patients have been administered Remicade®, 8 biosimilar Inflectra®, 24 biosimilar Remsima®, 16 biosimilar Flixabi®. In particular, 12 patients have received only Remicade®. Indeed, 2 of the 14 patients who started with Remicade® have been switched to Remsima®. 8 patients who started with Inflectra® have been switched to Remsima®. 11 patients have received only Remsima®. 3 patients who started with Remsima® have been switched to Flixabi®. 13 patients have received only Flixabi®. 7 patients have started the vedolizumab-based second line treatment with Entyvio®. Only 2 patients have started the ustekinumab-based second line treatment with Stelara®. One of them has made several switching: Remsima® in 2017; Flixabi® in 2017 and 2018; Entyvio® in 2019 and 2020; Stelara® in 2020.

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Conclusions

Considering 6 months Remsima® treatment, healthcare costs are equal to €5.561. Instead, the same Flixabi® treatment costs €4.377. This makes evidence of the convenience of the newest biosimilar. On the other hand, considering 6 months Entyvio® treatment, healthcare costs reach €16.125. This marked increase is justified by the clinical benefit, especially in non-responders. To sum up, 13 of 49 patients (26,5%) have been switched at least once and could have developed immunogenicity. Since data on IBD patients switched to biosimilars are lacking, it is necessary to better investigate immunogenicity after switching. Fischer et al[1] evaluated anti-drug antibodies (ADAs) and recorded that 9.8% of 144 enrolled patients developed ADAs after the switch. It could be interesting to evaluate ADAs titer in the aforementioned IBD patients to collect real world evidence. There is an open question to reflect about the switch in the pursue of clinical benefit: is it appropriate to lower the expense in front of the doubt on the relevance of immunogenicity?[2]

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Title

Urinary tract infections in paediatric patients and antibiotic resistance. Interference of antibiotic past and short-term outcomes: a retrospective observational study.

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Background

Urinary tract infections (UTIs) are one of the most common and serious bacterial infections in children. Amoxicillin/clavulanate and third generation cephalosporins are traditionally the empiric treatment of choice; however, antibiotic resistance patterns could be considered. Literature data shows that antibiotic resistance is related in large part to the remote and close pharmacological history of the patients. The aim of this study was to describe the diagnosis and treatment of UTI in children. In addition, we evaluated the modifications of the etiologic agents and the pathogens more virulent in the timing of therapeutic response.

Methods

Antibiotic prescriptions and antibiograms of children with UTI admitted to the University Hospital “G. Martino” of Messina were registered. Data covering a 4 year period (January 2017- March 2021) were collected retrospectively from Pediatric Nephrology and Rheumatology Unit.

Results

The study included 73 patients diagnosed with UTIs, the most common causative organisms were: E. Coli in 49 cases (67.1%), Klebsiella Pneumoniae in 12 cases (16.4%), Pseudomonas Aeruginosa in 6 cases (8.2%) and Proteus Mirabilis

in 3 cases (4.1%).

Among patients, 44 (60.3%) were female, while 29 (39.7%) were male. Infants (from 28 days to <1 year) 29 cases (39.7%), children (from 3 years to <12 years) 21 cases (28.8%), toddlers (from 1 year to <3 years) 14 cases (19.2%) and adolescents (12 to <18 years) 9 cases (12.3%) were the most frequently age group involved. In addition, males were the most frequently in infants (n=17) while females in children (n=14).

Antibiotic resistance was observed in 51 patients, with 36 cases of previous antibiotic treatment.

Considering the early response to antibiotic treatment, 37 patients showed antibiotics resistance, while 18 had no resistance to antibiotics.

UTIs due to Klebsiella pneumoniae were higher in females than males (75.0% versus 25.0%). In addition, this pathogen, caused antibiotic resistance in patients who had previously antibiotic treatment (83.3%).

Conclusions

Our results suggest that previous antibiotic treatment caused antibiotic resistance in patients cared with a new antibiotic therapy. This issue is related to the pathogen organisms, gender, age group and antibiotic therapy used.

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Title

Olipudase Alfa for Adults with Acid Sphingomyelinase Deficiency: Improvements in Crossover Placebo Patients and Further Improvements in Original Olipudase Alfa Patients after 2 Years in ASCEND Trial

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Background

Acid sphingomyelinase de?ciency (ASMD) is a rare debilitating lysosomal storage disease characterized by pulmonary dysfunction, hepatosplenomegaly and dyslipidemia. Olipudase alfa, intravenous recombinant human ASM (Sanofi) was recently approved in Japan and is being evaluated in other markets as the first and only treatment for the non-central-nervous-system manifestations of ASMD. The ASCEND study (NCT02004691), a phase 2/3 placebo-controlled trial in 36 adults with ASMD, completed its primary analysis.[1] At Year 1, olipudase alfa patients compared to placebo patients (1:1 randomization) had statistically significant increases in %-predicted diffusing capacity for carbon monoxide in lung (DL_{CO}) and decreases in spleen and liver volume.[1] Thirty-five patients continued in an open-label trial extension. One placebo patient withdrew during Year 1.

Methods

We report Year 2 results for the former placebo group after 1 year of olipudase alfa (crossover group) and for the original olipudase alfa group patients after 2 years of olipudase alfa. Patients underwent gradual dose-escalation to 3.0 mg/kg/2-weeks. Change from baseline results are presented as least-square (LS) mean ANCOVA % change ±standard error of the mean for %-predicted DL_{CO}, spleen volume, liver volume, platelet count, liver function, and lipid profile. Change in lung high-resolution computed tomography (HRCT) scores for ground glass appearance is presented as LS mean ANCOVA absolute change from baseline.

Results

Of 35 patients who completed Year 1, 33 completed Year 2. In Year 2, improvements in crossover group paralleled the original olipudase alfa group (Table): DL_{CO} increased 28.0±6.2% (n=10); spleen volume decreased 36.0±3.0% (n=11); liver volume decreased 30.7±2.5% (n=11). In the 2-year olipudase alfa group, %-predicted DL_{CO} increased by 22.2±3.4% (n=17) at Year 1 and 28.5±6.2% at Year 2 (n=10); spleen volume decreased by 39.5±2.4% (n=17) at Year 1 and 47.0±2.7% (n=14) at Year 2; liver volume decreased by 27.8±2.5% (n=17) at Year 1 and 33.4±2.2% (n=14) at Year 2. HRCT ground glass appearance score decreased 0.30±0.5 (n=14) at Year 2 for the crossover group and decreased 0.45±0.13 (n=18) at Year 1 and 0.48±0.07 (n=16) at Year 2 for 2-year olipudase alfa group. Improvements in dyslipidemia, liver function, platelet count, liver sphingomyelin clearance, and plasma lyso-sphingomyelin in crossover patients paralleled those seen in the 2-year olipudase alfa patients in Year 1; 2-year olipudase alfa patients maintained these benefits in Year 2. Overall, 99% of treatment-emergent adverse events (AEs) were mild or moderate, with 1 treatment-related serious AE. No patient discontinued due to an AE.

Conclusions

In summary, during Year 2 of ASCEND, crossover patients improved to a similar extent as olipudase alfa patients in Year 1 and patients continuing on olipudase alfa showed sustained or further improvements.

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	Placebo-Olipudase alfa (N=18) (Mean ±SD)			Olipudase alfa-olipudase alfa (N=18) (Mean ±SD)		
Parameter	Trial Baseline	Year 1	Year 2	Trial Baseline	Year 1	Year 2
% predicted DL_{co}	48.5 ±10.8 (n=18)	49.9 ±11.1 (n=17)	61.9 ±11.4 (n=10)	49.4 ±11.0 (n=18)	59.4 ±12.5 (n=17)	66.9 ±15.4 (n=10)
Spleen volume (multiples of normal)	11.2 ±3.8 (n=18)	11.2 ±4.2 (n=17)	7.7 ±2.9 (n=11)	11.7 ±4.9 (n=18)	7.2 ±3.6 (n=18)	6.0 ±2.7 (n=14)
Liver volume (multiples of normal)	1.62 ±0.5 (n=18)	1.6 ±0.5 (n=17)	1.1 ±0.3 (n=11)	1.44 ±0.3 (n=18)	1.0 ±0.2 (n=17)	1.0 ±0.1 (n=14)
Platelet count (10^9/L)	115.6 ±36.3 (n=18)	120.2 ±43.1 (n=16)	140.0 ±50.8 (n=15)	107.2 ±26.9 (n=18)	123.1 ±25.8 (n=18)	133.6 ±29.6 (n=13)
Lung HRCT ground glass appearance score	0.53 ±0.6 (n=18)	0.70 ±0.7 (n=17)	0.22 ±0.4 (n=14)	0.65 ±0.7 (n=18)	0.153 ±0.3 (n=18)	0.13 ±0.3 (n=16)
ALT (IU/L)	44.7 ±30.8 (n=18)	42.2 ±25.4 (n=16)	17.3 ±6.8 (n=15)	40.8 ±28.3 (n=18)	20.5 ±9.9 (n=18)	19.7 ±8.5 (n=12)
HDL cholesterol (mg/dL)	20.7 ±9.8 (n=18)	19.9 ±7.9 (n=16)	30.7 ±12.7 (n=14)	23.8 ±8.4 (n=18)	31.5 ±9.4 (n=18)	38.6 ±11.2 (n=12)
LDL cholesterol (mg/dL)	154.8 ±65.1 (n=17)	137.3 ±34.0 (n=15)	106.5 ±36.8 (n=14)	137.4 ±28.6 (n=18)	99.5 ±27.3 (n=18)	105.5 ±41.9 (n=12)

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Title

Increased stress vulnerability in the offspring of socially isolated rats: behavioural and redox dysfunctions

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Background

Increased vulnerability to stress exposure has been described in the offspring of psychotic patients[1]. In this context, animal models are very useful tool to investigate possible underlying mechanisms. The rat social isolation represents a reliable experimental manipulation to mimick in rodents behavioural, neuropathological and redox dysfunctions observed in psychotic subjects[2-3]. Moreover, social deprivation during highly vulnerable life periods, such as adolescence, has also been described as one of the strongest stressors interfering with key neurodevelopmental processes occurring in this life stage[4]. Here, we investigated the possible development of cognitive, locomotor and redox dysfunctions in the offspring of socially isolated rats, housed in grouped (GRP) or isolation (ISO) conditions until their adolescence and adulthood.

Methods

The male offspring of control and socially isolated females was housed in GRP or ISO condition until adolescence (PND42). At this time point, the open field (OF), the passive avoidance (PA), as well as the novel object recognition (NOR) tests were performed. After the behavioural tests, animals were grown in GRP or ISO condition until adulthood (PND70), when OF, PA and NOR tests were repeated. Prefrontal cortex (PFC) was then obtained in order to assess expression levels of markers related to the redox balance, in terms of both pro-oxidant and anti-oxidant pathways.

Results

A significant decrease of the latency time in the PA task was observed in the adolescent offspring of socially isolated

females, indipendently from the housing condition to which they were exposed until PND42. This alteration was still present at adulthood, whereas offspring grown in group until adolescence and then exposed to isolation until PND70 did not show such a decrease. Adolescent rats, born from socially isolated females, also showed a signifcant increase of the distance travelled in the OF test and in the time spent in the centre of the arena, as well as a reduction in the time spent in the walls. A significant decrease of the exploratory activity of the novel object in the NOR test was found in the offspring of ISO females, indipendently from their housing condition. This was also evident at adulthood in rats born from ISO females, grown in group until PND42 and then exposed to social isolation. Behavioural dysfunctions were accompanied by significant alterations of the redox balance.

Conclusions

Our results highlight the central role of the adolescent period in the development of stress vulnerability following maternal mental disorders, thus identifying this specific time window for possible preventive and therapeutic strategies targeting the redox system.

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Title

The role of glial reactivity in neurodegenerative disorders: from cellular modifications to possible therapeutic approaches

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Background

Alzheimer’s disease (AD) is a multifactorial neurodegenerative disease, histopathologically characterized by deposits of beta-amyloid in the extracellular space and of neurofibrillary tangles inside neurons [1]. Together with aberrant protein deposits, alterations in glia morphology and functions have been observed [2]. It is believed that glial cells acquire a reactive phenotype and foster inflammation upon beta-amyloid deposition or neurodegeneration. However, compelling evidence indicates that reactive gliosis and inflammation occur in the very early phase of the disease before histopathological modifications. Data accumulated so far converge in indicating that glia response in Alzheimer's is intricate. So, using several AD preclinical models we investigated the behavior of glial cells and, in particular, of astrocytes. These cells, indeed, emerge as central elements in AD etiology or progression, mainly because of their ability to maintain brain homeostasis at all levels of organization [3].

Methods

We studied the behavior of glial cells in different in vitro and in vivo models of AD. In particular, by western blot, RT-qPCR, immunofluorescence, and MRI/MRS we studied the expression of markers commonly related to the reactive state of glial cells as well as pro-inflammatory mediators. The effect of different treatments in controlling such alterations has been also tested.

Results

Results obtained in different models of beta-amyloid toxicity demonstrated the presence of marked and long-lasting glial activation of astrocytes and microglia as well as the presence of inflammation. Intriguingly, controlling inflammation and glia over-reactivity resulted in neuroprotection and restoration of cognitive deficits. On the contrary, using a transgenic model of AD we observed that astrocytes appear to be reactive during the early stages of the AD-like pathology while in the late stages they appear atrophic.

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Conclusions

Our data show that glial cells, especially astrocytes and microglia, are involved in AD. Their response is intricate. It is indeed possible to identify both glia reactivity and atrophy. These responses are considered pathological, and both contribute to the perturbation of brain homeostasis leading to neuronal damage and cell death. Understanding the mechanisms underpinning glial response in AD is essential for contributing to understanding the etiology and progression of this disease as well as for developing new therapeutics.

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Title

Real-world evidence supports combination therapy with anti-CGRP mAbs and onabotulinumtoxinA for chronic migraine

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Background

Migraine is a disabling neurovascular disorder with negative impact on functioning and quality of life in working age (1). The onabotulinum toxin A was approved by the FDA in 2010 for the prophylactic treatment of chronic migraine (2) and recommended by the National Institute for Health and Care Excellence (NICE) to patients not responding to at least three prior preventative treatments. Between 2018 and 2020, specific monoclonal antibodies (mAbs) directed towards the calcitonin gene-related peptide (CGRP) ligand (fremanezumab, galcanezumab and eptinezumab) or receptor complex (erenumab) were developed and approved by the FDA. Despite the clinical success of the latter, some 40% non responders is in demand of intervention (3). Here we now report real-world evidence supporting sinergy of combined therapy with onabotulinumtoxin A and anti-CGRP mAbs in the management of drug resistant migraineurs.

Methods

Systematic review and meta-analysis (PRISMA 2020 recommendations (4)) was made up to April 19th, 2022 to gather pooled data of real-world evidence, together with pharmaco-epidemiological analysis in the local Italian setting. The study protocol is registered in the National Institute for Health Research (NIHR) International prospective register of systematic reviews PROSPERO (CRD42022313640).

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Results

The combined treatment affords ≥50% monthly headache days (MHDs)/frequency reduction respect to baseline in up to 58.8% of patients. Our study demonstrates for the first time that the combination therapy of onabotulinum toxin A and anti-CGRP mAbs reduces MHDs of 2.67 days in comparison with onabotulinum toxin A alone with moderate certainty of evidence. In fact, anti-CGRP mAbs reduce MHDs of 1.94 days from baseline and botulinum toxin of 1.86 days.

Conclusions

The present study provides rational evidence for the need of randomized, double-blind, placebo-controlled clinical trials investigating the efficacy and safety of the combination therapy with onabotulinum toxin A and mAbs directed towards the signaling of CGRP in resistant patients.

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Title

Validation of an international pain assessment tool to improve pharmacological treatment of pain and agitation in severe dementia

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Background

By 2030, 78 million people will suffer from dementia and will develop neuropsychiatric symptoms (NPS), remarkably reducing their quality of life. The most resistant NPS is agitation, treated with atypical antipsychotics almost doubling the risk of death for cardiocerebrovascular accidents (1). Agitation is a help-seeking behavior at least in part caused by unrelieved pain and significantly reduced through appropriate analgesic therapy (2). The underdiagnosis and inappropriate treatment of pain originates from assessment difficulties in patients with reduced cognitive and communication capabilities and self-reporting (3). Mobilization–Observation–Behaviour–Intensity–Dementia (MOBID2) tool is unique since it takes into account the common co-occurrence of musculoskeletal and visceral pain and it unravels concealed pain through active guided movements (4). Therefore, the aims of the present clinical trial are: 1) to validate this indispensable tool to allow its use in the Italian setting; 2) to evaluate the effect of analgesia based on proper assessment on agitation and on the reduction of harmful antipsychotics.

Methods

The translation, adaptation and validation of the scale for non-verbal, severe demented patients has been conducted in agreement with current international guidelines in a cohort of 11 patients over 65 with mini-mental state examination ≤12, to obtain the Italian MOBID2 (I-MOBID2). The trial is approved by Calabria Region Ethics Committee protocol No. 31/2017 and follows the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) guidelines.

Results

The patients present an age range of 73-94 years with MMSE from 0 to 10.3, proving lack of communication capabilities. Pain prevalence up to 45% of body surface in pain occurred in 63,6% of patients. The I-MOBID2 proved good scale content validity index (0.89), high construct validity (Spearman rank order correlation Rho=0.748), reliable internal consistency (Cronbach's α coefficient=0.751), good-excellent inter-rater (Intraclass correlation coefficient, ICC =0,778) and test-retest (ICC =0,902) reliability, good inter-rater and test-retest agreement (Cohen's K=0,744) with 5.8 min average execution time.

Conclusions

I-MOBID2 allowed to disclose pain in a cohort of patients suffering from severe dementia treated with antipsychotics, antidepressants and benzodiazepines and not receiving adequate pain treatment. The effect of establishment of appropriate pain treatment on reduction of the Cohen-Mansfield Agitation Inventory score and on as-need agitation rescue medications is under investigation.

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Title

Possible interference between exercise training and antioxidant supplementation

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Background

Exercise training (ET) provides health benefits to patients belonging to several clinical settings [1]. The beneficial effects of ET (mainly aerobic) have been linked to its capacity for triggering an antioxidant response through a transient and moderate increase of oxidant molecules based on the concept of mitohormesis. ET induces Sirtuin 1 (SIRT1), deacetylysases capable of triggering inflammatory and antioxidant response [2, 3]. Athletes commonly used supplements, containing vitamins and other micronutrients, to improve their performance and avoid cellular damage associated with oxidative stress and inflammation [1]. However, the effects of antioxidant supplementation have not yet been elucidated, particularly in athletes performing endurance training [1]. In this study, we investigated the effects of ET on markers of inflammatory and oxidative response in endurance athletes using or not antioxidant supplements.

Methods

An observational study enrolling middle distance runners (MDR) and age-matched sedentary volunteers (CTR) was conducted. Adult MDR performed ET for at least 6 months and signed informed consent before study initiation. MDR using antioxidant supplementation (MDR-S) took 240 mg vitamin C and 15 mg vitamin E together with mineral salts. All athletes allowed blood sample collection, reported information about dietary/consumption habits and ET regimen. RNA samples were isolated by peripheral blood mononuclear cells and then reverse-transcribed to measure mRNA levels of SIRT1, biomarkers of inflammation (cyclooxygenase-2, COX2) and oxidative stress (manganese superoxide dismutase, MnSOD and catalase, CAT).

Results

Thirty-two MDR (18 MDR-S and 14 MDR not taking supplements, MDR-NoS) and 14 CTR were enrolled. The MDR group demonstrated higher levels of SIRT1 mRNA compared to the CTR group ($p = 0.0387$) and, notably, MDR-NoS showed higher levels than CTR ($p = 0.0136$) while no difference between MDR-NoS and CTR was found. MDR showed COX-2 mRNA expression levels lower than CTR ($p=0.038$), while no statistically significant differences between MDR-S and MDR-NoS were found. MnSOD mRNA expression showed an increased trend in MDR compared to CTR. A statistically significant higher expression was recorded in MDR-S compared to CTR ($p=0.047$) and in MDR-S compared to MDR-NoS ($p=0.013$). No differences were observed in CAT mRNA expression.

Conclusions

Endurance exercise provides antioxidative and antinflammatory effects as demonstrated by increase in SIRT1 and MnSOD and decrease in COX2 levels. The antioxidant supplementation seems to hinder the expression of SIRT1 triggered by the exercise. Moreover, it had no effect on COX2 while it was associated to an increase of MnSOD. These results provide insights to further investigate the potential interferences between exercise and antioxidant supplementation.

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Title

Effects of a Histamine H_3 receptor (H_3R) antagonist-Nitric Oxide (NO) donor hybrid compound in a model of retinal ischemia/reperfusion in rabbit

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Background

Ischemia is a pathological condition consisting of a restriction of blood flow to a specific organ. At ocular level, ischemia can depend by various diseases, such as diabetic retinopathy or glaucoma of which, it can be both a cause and a consequence.

There are different pharmacological approaches for ocular ischemia, most of them are addressed to reduce the intraocular pressure (IOP) and ameliorate the hemodynamics of the ophthalmic artery.

Several studies demonstrate that nitric oxide (NO) is involved in vasodilation, IOP homeostasis, and modulation of ocular blood flow. A recent publication demonstrates that a histamine H_3 receptor (H_3R) antagonist can ameliorate ocular blood flow suggesting a role of this amine in controlling ocular vascular tone[1].

Based on this evidence, our project evaluated the capability of a histamine H_3R antagonist-NO donor hybrid compound, ST-1989, to reduce the IOP, to ameliorate the hemodynamics of ophthalmic artery, and to preserve the degeneration of photoreceptors induced by the ischemic damage, in a rabbit model of retinal ischemia/reperfusion (I/R).

Methods

The I/R model was carried out on New Zealand White rabbits through repeated injections of ET-1[2], twice a week for 6 weeks. IOP measurements were performed with a Pneumotonometer. The hemodynamic was evaluated with an Eco Color Doppler and the Pourcelot Resistivity Index was measured. The electroretinogram was used to assess the retinal function. The animals were treated with vehicle or with compound ST-1989 twice a day for four weeks, starting from the third week of ET-1 injections.

Results

Hybrid compound ST-1989 demonstrated to be effective in reducing IOP at the end of treatments. Moreover, this compound was able to ameliorate the vascular tone and to prevent photoreceptors damage induced by ET-1 injections.

Conclusions

In conclusion, this histamine H_3R antagonist-NO donor compound is a promising therapeutic strategy for the prevention of post-ischemic photoreceptors degeneration.

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Title

SIRT1 and COX-2 as biomarkers of preterm birth

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Background

According to the World Health Organization, preterm birth occurs before the 37th week of pregnancy. It is the leading cause of infant morbidity and mortality [1]. Although several genetic and environmental factors have been proposed as risk factors, the underlying mechanisms are not yet fully understood [2]. Recent studies have demonstrated a relationship between preterm birth and increased expression levels of inflammatory biomarkers such as COX-2 and IL-1β [3-4]. These molecules are modulated by Sirtuin 1 (SIRT1), a NAD^+-dependent deacetylase known to be a stress-sensor able to mount a response to stressors, including the oxidative and inflammatory ones [5-6-7]. This study aims to compare SIRT1 activity and mRNA levels of SIRT1-dependent inflammatory biomarkers in preterm and term pregnant women to identify mechanisms and therapeutic targets useful in the prevention of preterm birth.

Methods

Fifteen and ten pregnant women having respectively preterm (PreTM) and term (TM) birth were enrolled at the Gynecology and Obstetrics Units of the University Hospital of Salerno and Naples (Federico II University). Inclusion criteria were age ≥18 years and cesarean delivery. Exclusion criteria were fertilization techniques, obesity, metabolic syndrome, and other comorbidities (e.g. autoimmune diseases, tumors). Blood samples were taken from each participant before (PreB) and after (PostB) birth and Peripheral Blood Mononuclear Cells (PBMCs) were isolated. Fresh nuclei were extracted from PBMCs and SIRT1 activity was measured using a deacetylase fluorometric assay kit. RNA was extracted from PBMCs and reversely transcribed into cDNA. The mRNA expression levels of COX-2 and IL-1β were measured by SYBR Green Real-Time PCR and analyzed using the $2-\Delta\Delta Ct$ method as relative quantification. Beta-actin was used as the internal control.

Results

SIRT1 activity was higher ($p = 0.03$) in PreTM-PreB than in TM-PreB. Similarly, PreTM-PostB showed higher SIRT1 activity ($p<0.0001$) as compared with TM-PostB.

COX-2 mRNA levels were higher in PreTM-PreB than in TM-PreB ($p = 0.0477$) and in PreTM-PostB as compared with TM-PostB ($p=0.0060$). There were no significant differences between PreB and PostB in the PreTM group. PreTM-PostB showed higher levels of IL-1β mRNA as compared with TM-PostB without reaching a statistical significance.

Conclusions

SIRT1 activity and COX2 mRNA levels were higher in the PreTM as compared with the TM group both before and after the delivery. We suggest that in women belonging to the preTM group SIRT1, which works as a stress sensor, remains activated in the effort to mount an adequate response to a permanently high level of inflammation.

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Title

Quantification of Ivacaftor, Tezacaftor and Elexacaftor in plasma of cystic fibrosis patients treated with Kaftrio by a validated liquid chromatography-tandem mass spectrometric (LC-MS/MS) method.

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Background

Ivacaftor (I), tezacaftor (T) and elexacaftor (E) is a novel drug combination for cystic fibrosis (CF) that directly modulates the activity and trafficking of the CF transmembrane conductance regulatory protein (CFTR)[1,2]. During clinical trials unprecedented positive outcomes have been demonstrated on patients bearing one or two copies of the F508del allele. Interpatient variability among patients under treatment with CFTR modulators has been observed in terms of response to treatment [1,3]. More data on PK and on dose-concentration relationship would be therefore useful to better understand the mechanisms of response.

Methods

We have developed and validated a new liquid chromatography-tandem mass spectrometry (LC-MS/MS) method to simultaneously quantify I, T and E in plasma based on a rapid organic extraction and separation on a reversed-phase C-18 HPLC column after addition of deuterated internal standards. Accurate analytes quantification using SRM detection was obtained using a Thermofisher Quantiva triple quadrupole MS coupled to a Ultimate 3000 UHPLC. The method has been validated following EMA guidelines for bioanalytical method validation[4] and was applied on 29 samples obtained at Cmin from 27 patients with CF treated with T, E and I at Giannina Gaslini Istitute. Response assessment was

established measuring Forced Expiratory Volume (FEV1) before and after treatment. Patients were then classified into three response groups based on the increase of FEV1 (ΔFEV1) as follows: non-responders, poor responders and high responders. Comparison of quantitative variables (Cmin) between two groups (non-responders vs. high responders) was made by the Mann–Whitney U test, and among more than two groups (non-responder vs. low responders or high responders) by the nonparametric analysis of variance (Kruskal–Wallis test).

Results

The LC-MS/MS assay is linear over wide concentration ranges (0.12 -12 mg/L) in plasma for I, T, and E, accurate and reproducible in the absence of matrix effects. The application of the LC-MS/MS method allowed us to obtain a very specific, sensitive, and rapid quantification of the three CFTR modulators starting from very low volumes (50 µL) of plasma samples. The stability of analytes in plasma for at least 30 days allows for a cost effective shipment and storage at room temperature. Plasma levels obtained (mean ± SD) were 3.51 ± 1.42 µg/mL for T, 4.77 ± 2.11 µg/mL for E and 0.78 ± 0.38 µg/mL for I. Plasma levels were found to be not significantly different in the 3 groups of patients (P>0.05).

Conclusions

No correlation between Cmin and drug response has been demonstrated, further studies are needed to better understand exposure-response relationships and interpatient variability.

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pharmadvances

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Title

Perampanel in Glioblastoma: an *in vitro* study and clinical perspective

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Background

Glioblastoma (GB) is the most aggressive brain tumor, still with a dismal prognosis[1]. Curative treatment remains elusive due to the extensive intratumoral cellular heterogeneity which renders difficult the identification of specific targets. GB stem cells (GSCs) are deemed responsible for sustaining long-term tumor growth through self-renewal and the generation of more differentiated highly proliferative cell populations (nonGSCs)[2]. Increasing evidence is gathering on GB and brain tumor-related epilepsy (BTRE) sharing a glutamatergic pathophysiological mechanism which drives both tumor progression and seizure onset[3]. Perampanel (PER), a non-competitive antagonist of AMPA glutamate receptors, showed efficacy against drug-resistant epilepsy, BTRE[4,5] and GB growth *in vitro*[6] and *in vivo*[7]. AMPA inhibition with PER in GB could then lead to patient control of both seizure and tumor growth. This study evaluates PER antineoplastic activity against patient-derived GSC cultures and their differentiated nonGSC counterpart.

Methods

Five GSC-enriched cultures (GB1, GB2, GB3, GB4, GB5), obtained from neurosurgical specimens, were plated in stem cell-permissive medium supplemented with bFGF and EGF. To induce cell differentiation, GSCs were shifted to growth factor-deprived medium containing 10% FBS for at least 2 weeks. AMPA receptor expression was verified by single cell RNA-Seq. GSC and nonGSC cultures were exposed to increasing concentrations of PER. Cell viability, proliferation, self-renewal and invasive capacity were evaluated by MTT assay, cell count, spherogenesis and extracellular matrix invasion in 3D GSC cultures.

Results

Transcriptome analysis demonstrated the expression of AMPA receptor subunits in tested GSCs. PER shows statistically significant inhibition of cell viability after 48h. Efficacy at 150µM was at 56% in GSC GB1, 38% in GB2, 56% in GB3, 43% in GB4 and 26% in GB5. Time-dependent response to treatment was only present in line GB3. NonGSCs cultures showed a response at rather lower concentrations compared to their GSC counterparts. In nonGSC cultures efficacy at 25µM was at 28% in line GB1, 16% in GB2, 29% in GB3, 50% in GB4 and 21% in GB5. The average potency (IC50) is 171.7µM for GSCs and 19.8µM for nonGSCs. Cell count assay at 48h revealed a prevailing cytostatic effect of PER. Data from spherogenesis and 3D invasion showed an inhibitory effect on both self-renewal and invasive capacity of GSC culture GB1, following exposure to PER.

Conclusions

PER significantly reduces proliferation in both GSCs and their nonGSC counterparts, with a predominant cytostatic effect, even able to control invasive capacity. Since different potency and efficacy was observed in cultures from individual patients, further analyses are issued to clarify the molecular pathways which could be involved in the personalization of patient response to treatment.

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pharmadvances
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Title

Urine-derived stem cells and derived skeletal muscle cells as a functional model to study calcium homeostasis perturbation

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Background

Muscular diseases are characterized by a wide genetic diversity and the Ca^{2+}-signalling machinery is often perturbed[1]. Therefore, an appropriate and a personalized cellular model is required to study these diseases. Muscle biopsies are the best approach but are invasive for the patient and difficult to justify if it is not for diagnostic purposes. To circumvent this, interest is mounting in urine-derived stem cells that can be differentiated into skeletal muscle cells[2]. We optimised the method to obtain urine stem cells from healthy volunteers and to differentiate them into skeletal muscle cells, as well as we characterized the calcium toolkit.

Methods

We isolated stem cells from urine (USC) samples of healthy donors and differentiated into skeletal muscle cells (USC-SkMC) by MyoD lentiviral vector transduction. We assessed the expression of myogenic markers and calcium homeostasis players by qPCR and western blot, and we evaluated the calcium transient by Fura-2AM probe in live imaging microscopy.

Results

We demonstrated a successful differentiation by the different pattern of expression of stem and skeletal muscle markers in USCs and USC-SkMCs. Therefore, undifferentiated and differentiated cells differed in the expression of key

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proteins involved in Ca^{2+}-homeostasis and also displayed different Ca^{2+}-responses to external stimuli, confirming that during differentiation there was a transition from a non-excitable to an excitable phenotype. In USCs, the main mechanism of calcium entry was IP3 dependent, suggesting a major involvement of receptor-operated Ca^{2+} entry, while in USC-SkMCs both store- and receptor-operated calcium entry were active. Furthermore, a caffeine challenge led to Ca^{2+} release, suggesting the presence and functionality of ryanodine receptors in USC-SkMCs. Lastly, the voltage-operated calcium channels are operative in USC-SkMCs, unlike in USCs.

Conclusions

Our results demonstrated that, starting from easy-to-collect urine samples, it is possible to obtain good quality multipotent stem cells. Therefore, this study opens an avenue to establish patient-derived disease models where perturbations of Ca^{2+} homeostasis in muscular disorders can be evaluated. In particular, this may lead to a non-invasive method to determine the functionality of new mutations identified in patients and a strategy for personalized medicine.

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Title

Cdk5 as a potential new target for the improvement of testicular non-seminoma cisplatin response: a preclinical study

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Background

The majority of patients with testicular germ cell tumours (GCTs) can be cured with cisplatin (CP)-based chemotherapy, however, when it arises, CP resistance is a clinical challenge [1,2], thus novel therapeutic approaches are needed. It has been reported that Cyclin dependent kinase 5 (Cdk5) is involved in the progression of different types of cancer [3,4] and linked to chemotherapy resistance [5]. Here, we investigated the possible role of Cdk5 in CP sensitivity in non-seminoma testicular cancer (TC) cell models, evaluating whether the Cdk5 inhibitor dinaciclib could be promising as a single/combined agent for the treatment of advanced/metastatic TC.

Methods

Wild-type (wt) NT2/D1 and NCCIT cell lines were purchased by ATCC (Manassas, VA) and cultured as indicated. The CP-resistant subclones NT2/D1–R and NCCIT–R were kindly given by Prof. Bremmer (Gottingen, Germany) and cultured as suggested [6]. Dinaciclib and/or CP effect on NT2/D1/-R and NCCIT/-R cell viability and proliferation was evaluated by MTT assay and direct count. Gene expression was studied by qRT-PCR. The protein expression was assessed by western blot. Flow cytometry cell-cycle analysis was performed as indicated [7]. The *in vivo* experiments were conducted in AB

zebrafish embryos xenografted with TC cells.

Results

Cdk5 gene and protein expression was firstly measured, showing a significant increase in protein expression in CP-resistant models (Cdk5/α-tubulin: NT2/D1/-R: 0.51 ± 0.1; 0.86 ± 0.07*; NCCIT/-R: 0.23 ± 0.02; 1.09 ± 0.13$^{\#}$; *P<0.05; $^{\#}$P<0.001 vs wt cells). Dinaciclib and CP as a single drug reduced the cell viability: the IC_{50} value for each cell model is reported in the table 1. The cell proliferation rate was as well reduced by both dinaciclib and CP. The *in vivo* data in zebrafish embryos xenografted with the different cell lines confirmed the *in vitro* results (Fig.1). In drug combination experiments, we observed that in NT2/D1-R dinaciclib enhances CP efficacy (viable cells: CP15μM: 62.10% ± 2.56%; CP15μM+IC_{25}/IC_{50} dinaciclib: 43.14%±0.13%, 48.58%±1.15% P<0.05), while in NCCIT-R dinaciclib enhances CP potency (IC_{50} CP: 6.78μM; CP+IC_{25}/IC_{50} dinaciclib: 4.14μM P<0.05, 2.86μM P<0.005). Preliminary data on cell cycle analysis revealed that in NT2/D1 dinaciclib induced a cell increase in G0 (ctrl: 0.44%±0.17%, treated: 1.19%±0.18%; *P<0.05*) while in NCCIT was observed an increase in G0 and G2 (G0: ctrl:0.65%±0.13%, treated:2.93%±0.48%; *P<0.01*. G2: ctrl: 21.12%±0.68%, treated: 25.49%±1.02%; *P<0.01*). The effect of combined treatment on cell cycle distribution is still under investigation.

Conclusions

Dinaciclib, when combined with CP, could be useful to improve non-seminoma TC response to CP.

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Title

Preclinical effects of progesterone as a new pharmacological tool in human adrenocortical carcinoma cell lines.

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Background

Adrenocortical cancer (ACC) is a rare malignant neoplasm with a dismal prognosis, in particular in advanced and metastatic disease. For patients with metastatic ACC, the proposed treatment includes mitotane, platinum-based chemotherapy, and locoregional strategies. [1, 2]. However, the progression of advanced disease occurs almost invariably after less than 18 months and there are no defined second and following lines of treatment. Unfortunately, in the past 20 years, the therapeutic scenario has not changed substantially [3]. There is a need for therapeutic approaches for advanced ACC, particularly targeting the metastatic process signaling pathways. We demonstrate that progesterone (Pg) was able to reduce cell viability in ACC cell models [4, 5], in line with results demonstrating the role of this hormone as an anti-tumoral drug in different cancers [6, 7]. Pg can as well inhibit breast cancer cell invasion and migration [8]. Here, we investigated the molecular mechanism underlying the cytotoxic effect of Pg, and we studied whether Pg could influence ACC cell invasiveness and metastasis formation.

Methods

NCI-H295R, MUC-1, and TVBF-7 ACC cell lines were employed. Cell viability was tested by MTT assay. Cell apoptosis and cell cycle were analyzed by flow cytometry. Cell migration and invasiveness were studied using the scratch assay and ECMatrix invasion assay. Cell tumor xenografts in Danio rerio embryos were performed for each cell line, measuring both the tumor mass areas and the number of embryos with metastasis. The protein levels were evaluated by Western blot and metalloprotease 2 (MMP2) activity by zymography.

Results

Pg exerted a concentration-dependent cytotoxic effect that was maintained after the drug is withdrawn (Fig. 1). Pg induced apoptosis in NCI-H295R and MUC-1 cells, inducing changes in the cell cycle distribution. The maturation of microtubule-associated protein 1A/1B-LC3B indicated the activation of autophagy in all three cell lines. In the experimental model of Danio rerio embryos, Pg significantly reduced the xenograft tumor area of each ACC cell line (Fig.2), and the metastasis formation in Danio rerio embryos injected with MUC-1 cells(75.87 ± 6.7% to 10,8 ± 0,85%), confirming the in vitro results where migration (Fig.3) and invasion (Fig.4) ability was significantly suppressed by Pg like also MMP2 levels (decreased vs control of 72 ± 2.9% and 55 ± 14.4% in MUC-1 and TVBF-7 cells respectively).

Conclusions

Pg inhibited the invasiveness and metastasis processes, a dramatic feature of ACC. These results supported the bases for to design of a clinical study to explore the efficacy of Pg in the clinical setting. Interestingly, Pg and its derivative are already part of the cancer-supporting care, thus giving the opportunity to have another pharmacological tool over the usual systemic therapy.

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Title

Adherence trajectories to biological treatments in patients with inflammatory bowel diseases: a pilot study from Sicily region (Italy)

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Background

Inflammatory bowel diseases (IBD) are a group of disorders characterized by a chronic or remitting/relapsing inflammation of the gastrointestinal tract [1] and with increased mortality, and reduced quality of life [2;3]. Alongside conventional drugs, biological drug treatments have been introduced. However, non-adherence to treatment is one of the major obstacles to the achievement of patient's health goals [4].

The aim of this study is to identify and describe trajectories of adherence to biologics in patients with IBDs.

Methods

A retrospective cohort drug-utilization study was conducted during the years 2010-2020 using Sicily claims databasesfrom the Italian VALORE project network, covering ~ 5 million inhabitants.

In order to identify and describe trajectories of adherence to biological drugs in patients with IBDs, incident users (i.e. first dispensing of biologics during the study period without any other biologics dispensing in the previous year) of infliximab, adalimumab, golimumab, ustekinumab, or vedolizumab with a diagnosis of Crohn's disease (CD) or Ulcerative Colitis (CU) any time prior to the first dispensing of biologics were identified.

Both cohorts were characterized at baseline and the adherence was evaluated monthly through the Medication Possession Ratio by observing users during the first year of biologic treatment. In this study were identified and described trajectories of adherence to biologics in patients with IBDs.

Results

Among 4,697 biological drug users identified during the study years, 1,692 (36%) were incident users with a diagnosis of IBD. The male-female ratio was 1.3 and the median age was 43 (IQR: 28-55) years in the Sicily region. The most frequent observed comorbidities were hypertension (25.1%) and diabetes (6.8%) in both cohorts. The most frequently used concomitant drugs were drugs for the treatment of peptic ulcer and gastro-oesophageal reflux disease (GORD) (76.5%), followed by antithrombotic agents (22.1%). CU patients were older (median (IQR): 44 (29-55)) than CD (median (IQR): 41 (26-54)) and had higher frequencies of hypertension (27.0%), diabetes (7.8%) and use of GORD drugs (79.7%) compared to CD (23.7%, 5.4% and 75.7%, respectively).

Three trajectories of adherence to biological drugs were identified. Almost two/thirds (72%) of the biologics users were identified with ‘high adherence’ trajectory (T1) while ‘medium’ (T2) and ‘low adherence’ trajectories (T3) includes 24% and 4% of users respectively.

Conclusions

Three different trajectories of biologics approved for IBD were identified. Patients with the lowest adherence trajectories (i.e. T3) were more frequently concomitant drug users. Adherence trajectories could be a reliable methodology to identify patient profiles in the use of biologics with a view to personalized medicine.

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Title

Hippocampal dysregulation of membrane-associated glucocorticoid receptors and structural plasticity in the activity-based anorexia rat model

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Background

Anorexia Nervosa (AN) is a complex psychiatric disorder defined by the combination of restricted eating behavior with an intense exercise regimen, which can progress into an out-of-control spiral. This disease mainly occurs in adolescent females and, despite the poor knowledge of its etiology, evidence in anorexic patients has shown dysregulation of the hypothalamic-pituitary-adrenal axis (HPA), a system that plays an important role in stress response. The major aim of our work was to explore stress-related mechanisms and structural alterations induced by the anorexic phenotype in the hippocampus, a brain area strictly involved in the negative feedback of HPA, in a well-established model of AN, the activity-based anorexia (ABA) rat model.

Methods

Female adolescent rats were individually housed and divided into four groups at postnatal day (P) 35: *control* (CTRL, food *ad libitum* and sedentary), *food-restricted* (FR, food limited 2h/day and sedentary), *exercise* (EXE, food *ad libitum* and access to an activity wheel) and ABA (food limited 2h/day and access to an activity wheel). On P38, FR and ABA rats were food-restricted until P42, when ABA reached the acute phase of the pathology. A group of animals was sacrificed at P42, while another group after 7-days of body weight recovery (P49).

Results

ABA rats dramatically reduced their body weight, significantly more than FR rats, and exponentially increased wheel activity over days. Corticosterone plasma levels were increased at P42 in both FR and ABA rats, whereas after 7-days of recovery corticosterone levels were decreased only in ABA rats. At the molecular level, in the crude membrane fraction of the hippocampus, the combination of food restriction and hyperactivity reduced glucocorticoid receptor levels and phospho-synapsin1, an indirect index of neurotransmitter release. In parallel, we found a reduced expression of molecular markers of cytoskeletal stability, such as caldesmon, n-cadherin and neuroligin-1, selective for ABA rats. Remarkably, these molecular impairments were strengthened by structural and morphological analyses that revealed reduced spine density and decreased number of mushroom-shaped spines, along with an increased number of filopodia in the hippocampus of ABA rats. Notably, such structural reorganization occurred both at P42 as well as at P49.

Conclusions

Taken together, the herein molecular and structural data reveal an AN-induced maladaptive plasticity in the hippocampus that persists even when body weight is recovered. These effects might be the trigger for an atypical processing of food reward and a sensitive aspect for relapse.

References

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Title

Medication adherence trajectory methods in pharmacoepidemiology: a scoping review

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Background

Adherence is a dynamic behavior varying over time that needs longitudinal methods to be analyzed. These methods focus on identifying latent trajectories and allow to determine subgroups within a given population to better understand individual variability in health outcome patterns over time [1]. Furthermore, they enable the identification of the characteristics associated with medication adherence and, thus, improve the effectiveness of treatments. The aims of this study are: (1) to identify the available ‘real-world' studies related to the use of trajectory modelling techniques; (2) to explore various trajectory modelling approaches applied to different research topics; and (3) to compare their applications and differences.

Methods

We conducted a scoping review of original articles on secondary data, English-based concerning pharmacological treatments [2]. The MEDLINE and EMBASE databases were queried to identify the studies published until 13 November 2020. Research strings included the following terms combined through AND/OR Boolean operators: trajectory/e, drugs (i.e. prescriptions, medications), drug use (i.e. drug use, pattern of use, drug adherence, monitoring programs) and pharmacoepidemiology.

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Results

Out of 1,839 articles, after the removal of 435 duplicates and 1,280 articles deemed irrelevant, 124 works were evaluated for eligibility. The application of the inclusion criteria led to the selection of 69 articles: 42% was released between 2019 and 2020, more than half in the US and 27% in Europe; 46% of the articles included samples with 15,000+ participants, 24% between 5,000 and 15,000, and 30% with less than 5,000 participants. The following interest groups were selected: subjects aged <18 years (3 articles on drugs for the metabolic system), subjects aged 18+ years (37 articles; 18 on drugs for the nervous system and 9 on cardiovascular drugs), subjects with 65+ years (12 articles; 11 on cardiovascular drugs), pregnant women (9 studies; 7 on drugs for the nervous system) and women with breast cancer (5 articles on oncological drugs). The most used predictive methods were *group-based trajectory modelling (GBTM)* (54 articles), *latent class analysis (LCA)* and *latent transition analysis (LTA)* (8 studies), whereas the descriptive methods included *K-means for Longitudinal data (KLM)* (5 studies).

Conclusions

In recent years trajectories are assuming an increasingly important role in the assessment of medication adherence. Therefore, this revision may represents a useful tool for future research, providing methodological support to the application of these methods in a 'real-world' context.

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pharmadvances

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Title

Novel mechanism for a fixed combination of Serenoa repens and Urtica dioica in Benign Prostatic hyperplasia therapy

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Background

Benign prostatic hyperplasia (BPH) [1] is a pathology that frequently occurs in ageing men and causes lower urinary tract symptoms (LUTS). This chronic disorder is characterized by the hyperproliferation of prostatic epithelial and stromal cells, driving to prostate enlargement. In the last decades, BPH has been strictly correlated with inflammation. In particular, the etiology and the progression of this disease have been linked with the persistence of an inflammatory stimulus which could be induced both by NF-κB activation and ROS production. Thus, it appears that the inhibition of these pathways could be a good strategy for the clinical treatment of BPH.

Methods

Human BPH-1 and PC-3 cell lines were used to perform all the experiments. Cell viability was determined through crystal violet assay. IL-6 and IL-8 levels were evaluated through ELISA and their gene expression exploiting RT-PCR, while confocal microscopy was used to determine the translocation of NF-kB

Results

The focus of this project was the comparison of the antioxidant and anti-inflammatory activities of a combined formulation of *Serenoa repens* and *Urtica dioica* (SR/UD) in an *in vitro* human model of BPH, with *Serenoa repens* (SR) alone. SR/UD demonstrated both the antioxidant and the anti-inflammatory effects. In details, the combined formulation was able to decrease ROS production, confirming the antioxidant activity. In addition, SR/UD decreased both IL-8 and IL-6 production, NF-κB translocation inside the nucleus, and NLRP3 inflammasome gene transcription, confirming the anti.-inflammatory activity. In addition, since the onset of prostate cancer is linked to the progression of BPH, the effect of SR/UD was also tested in a PC human cell line. SR/UD was able to reduce NF-κB translocation.

Conclusions

Considering these results, it appears that the combined formulation of *Serenoa repens* and *Urtica dioica* is a good strategy to prevent and treat the inflammatory status BPH-related.

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Title

Differential responses to redox stress in brain cortex and spinal cord astrocytes from the SOD1^{G93A} mouse model of amyotrophic lateral sclerosis

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Background

Motor neuron (MN) degeneration in amyotrophic lateral sclerosis (ALS) represents a non-cell-autonomous process also affected by astrocytes. Accordingly, the enhanced content of reactive oxygen species (ROS) in these glial cells concurs to the neuronal damage in ALS patients and animal disease models. The evaluation of redox stress in the central nervous system is complicated by the lack of noninvasive methods. However, our recent studies reported a direct link between [18F]-fluorodeoxyglucose (FDG) uptake and oxidative stress (1). The tracer retention is linked to the reduction of NADP to NADPH operated by a specific pentose phosphate pathway (PPP). In ALS patients, FDG uptake is increased in the spinal cord (SC) and decreased in the motor cortex (MC), suggesting that the disease might differently involve the two nervous districts with different mechanisms or time sequences (2, 3). The present study aimed to investigate the different metabolic patterns of MC and SC ALS astrocytes.

Methods

MC and SC astrocytes were cultured from SOD1^{G93A} mice to study ROS levels by flow cytometry; activity of hexose-6P dehydrogenase (H6PD), glucose-6-phosphate dehydrogenase, and malondialdehyde levels by spectrophotometry; antioxidant capacity and NADP/NADPH ratio by commercial assay kits; oxygen consumption and extracellular acidification by Seahorse®; FDG kinetic uptake by LigandTracer®; mitochondria and endoplasmic reticulum (ER) ultrastructure by transmission electron microscopy.

Results

We highlighted an increase in redox stress, lower antioxidant capacity, and relative mitochondria respiratory uncoupling, in MC SOD1^{G93A} astrocytes. By contrast, SC mutated cells showed a higher ability to respond to oxidative damage by increasing the antioxidant defenses and preserving the respiratory function. FDG uptake reproduced the metabolic response previously observed in-vivo in ALS patients by PET analysis (2, 3); in fact, only mutated SC astrocytes showed a selective enhancement of tracer retention, matching the activity of the reticular PPP and, thus, of H6PD. Finally, MC and SC SOD1^{G93A} astrocytes were characterized by a dramatic ultrastructural enlargement of ER and impairment in ER-mitochondria networking, more evident in mutated MC than in SC cells.

Conclusions

These results indicate that the oxidative damage associated with the SOD1^{G93A} mutation is not counterbalanced by the activation of cytosolic PPP. By contrast, despite the notable alteration of ER typical of SOD1^{G93A} disease model and ALS patients, the activation of the ER-PPP provides protection. The activation signals of this pathway in SC astrocytes remain to be elucidated; however, their comprehension might help define the ALS progression mechanisms.

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Title

Alteration of osteoclast activity in childhood cancer survivors: role of iron and of CB2/TRPV1 receptors

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Background

Childhood cancer survivors (CCS) are predisposed to the onset of osteoporosis (OP) [1]. Iron accumulation promotes bone resorption [2]. We previously demonstrted that iron overload induces osteoclasts (OCs) overactivity and that iron chelators can decrease osteoclast (OC) activity [3-4]. The Cannabinoid Receptor type 2 (CB2) and the transient receptor potential vanilloid type-1 (TRPV1), are potential therapeutic targets for OP [5].

Methods

We isolated OCs from peripheral blood of CCS and healthy donors (CTR) and investigated OC markers expression by western blotting and OC activity by TRAP assay and Bone Resorption assay. We evalueted iron metabolism measuring iron concentration in OCs by iron assay and the expression of molecules involved in its regulation by western blotting. Moreover, we analyzed the effects of CB2 and TRPV1 stimulation in combination with the iron chelator, DFX on OC activity and iron metabolism.

Results

We observed in CCS an OC hyper-activation as demonstrated by the overexpression of TRAP and Cathepsin K, and by the increased activity. We also observed an increase of iron concentration in OCs derived from CCS together with an increase of DMT1 and Transferrin receptor 1 (TfR1) responsible for iron import, and a reduction of Ferroportin (FPN-1) suggesting a role of iron in CCS-OC overactivation. We observed an up-regulation of the pro-osteoporotic TRPV1 and a reduction of the protective CB2 in OCs from CCS. As expected, CB2 and TRPV1 stimulation induce a reduction of OCs activation affecting OC markers expression and activity. Interestingly, the effects of CB2 and TRPV1 modulation, after the iron chelation induced by DFX, are stronger, confirming the involvement of iron in OC

activation.We also observed a reduction of DMT1, TfR1 and an increase of FPN-1 after CB2 and TRPV1 modulation. After CB2 and TRPV1 modulation in combination with DFX we observed a more evident reduction of DMT1 and TfR1 and a greater increase of FPN-1.

Conclusions

In conclusion, we observed an OC hyperactivation in CCS suggesting a role for iron in its development. Moreover, we confirmed the role of CB2 and TRPV1 in bone metabolism, suggesting the receptors as possible markers of bone damage. Moreover, we demonstrated a promising synergism between compounds stimulating CB2 or inhibiting/desensitizing TRPV1 and DFX, in counteracting OC overactivity in CCS.

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Title

ADOLESCENCE AS A CRITICAL TIME-WINDOW FOR NEUROINFLAMMATION IN THE MOUSE: WHY SEX MATTERS

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Background

Early life immune challenges are risk factors for neurodevelopmental disorders and adolescence is a life-stage especially prone to the emergence of these diseases [1]. Growing evidence shows substantial gender differences in their prevalence [2], but, to date, relatively few studies have focused on the role of sex in adolescent vulnerability to psychiatric disorders. Systemic injection of lipopolysaccharide (LPS) is widely used to mimic peripherally induced neuroinflammation in rodents, a process known to be an important feature in the pathogenesis and progression of these diseases [3]. In the current study, we evaluated whether an acute immune challenge experienced during pre-puberty (postnatal day 21 (PND21)) or adolescence (PND35) may differentially affect the expression levels of inflammatory markers in the hippocampus in male or female mice.

Methods

Male and female PND21 or PND35 C57BL6J mice were injected with LPS (0.1 mg/kg i.p.) or saline (ctrl) and sacrificed after 6 or 24 hours (n=7-8 per group). By means of qPCR, we performed gene expression analysis on the hippocampus. Data were analyzed with a One-way analysis of variance (ANOVAs) followed by Tukey.

Results

We analysed the main pro-inflammatory and anti-inflammatory cytokines: IL-1β, IL-6, TNF-α and IL-4. While Hippocampal IL-6 mRNA levels were not affected in our experimental conditions, we found an upregulation of IL-1β and TNF-α in this area in LPS-treated animals (PND21 TNF- α: $F=9.96$, $p<0.0001$; PND35 TNF-α: $F=17.77$, $p<0.0001$. PND21 IL-1β: $F=22.38$, $p<0.0001$; PND35 IL-1β: $F=9.28$, $p<0.0001$) both in PND21 and PND35 mice. In particular, TNF-α and IL-1β mRNA levels were significantly increased 6 hours after the treatment in females injected at PND21 or PND35 ($p<0.0001$ vs ctrl, vs LPS-24h). This effect was present also in their male counterparts but was less pronounced and it reached statistical significance only for IL-1β on PND21 ($p<0.05$) and TNF-α on PND35 ($p<0.05$ vs ctrl, vs LPS-24h). In general, transcription returned to basal levels 24 hours after the immune challenge in both male and female animals irrespective of their age. IL-4 mRNA levels were upregulated just in PND35 males 24 hours after the LPS treatment, no effect was shown for young female animals irrespective of their age ($F=2.350$, $p=0.0351$).

Conclusions

Our data suggest that sex represents an important discriminant of the transcriptional effects of an immune challenge in the hippocampus during such a critical phase of development such as adolescence. In fact, females seem to be more responsive to the immune challenge already in early adolescence than their male counterparts. Considering that this time window is especially sensitive to stressors, understanding the short- and long-term consequences of neuroinflammation may help to better understand this crucial postnatal period and its impact in shaping the adult behaviour.

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Title

The dual role of VEGF-A in mediating pain and neuroprotective signaling in rat organotypic spinal cord slices

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Background

The Vascular Endothelial Growth Factor (VEGF) family is the main responsible for pro-angiogenic action, but its involvement in pain signaling has also recently emerged. VEGF-A, the most studied family member, is known to influence neuronal and glial biological processes. *In vivo* studies conducted in our laboratory demonstrated that the VEGF-A/VEGFR-1 signaling pathway mediates chemotherapy-induced neuropathic pain and that the anti-VEGFR-1 mAb D16F7 endowed of anti-hypersensitivity effect (Micheli L. et al. 2021). This study aims to investigate the dual pro-algic and neuroprotective effects of VEGF-A, with special attention to the role exerted by VEGFR-2 in an *in vitro* model of oxaliplatin-induced toxicity.

Methods

Spinal cord slices were prepared from 4 PND rat pups kept in culture for two weeks. The slices were incubated with increasing concentrations of oxaliplatin (1-100 µM) for 1, 3, 6 and 24 hours and then, the toxicity was evaluated by propidium iodide (PI, 5µg/mL) ?uorescence as well as GFAP and NeuN immuno?uorescence staining. The effect of co-treatment with VEGF165b, the main VEGF-A isoform, and of specific blocking antibodies of VEGFR-1 (D16F7) and VEGFR-2 (DC101) was also evaluated after oxaliplatin treatment by measuring cell viability and pro-algic markers, such as calcitonin gene-related peptide (CGRP) and Substance P (SP) release, and the expression of glutamate transporters (EAAT1 and EAAT2).

Results

From the toxicity studies, we observed that oxaliplatin causes dose-dependent neurotoxicity, which is mitigated by the co-treatment with VEGF165b. To investigate the molecular mechanism underlying this neuroprotective e?ect, we analyzed the role of VEGFR-1 and VEGFR-2 by using D16F7 and DC101 as selective receptor blockers. Treatment with both oxaliplatin (10 µM) and VEGF165b (100 ng/mL) enhanced the release of CGRP and SP compared to control and this effect was prevented by the co-treatment with D16F7 (300 ng/mL), which allows the binding of VEGF-A to VEGFR-2, but not by the co-treatment with DC101 (10 ng/mL). The expression levels of EAAT1 and EAAT2 were reduced by VEGF165b treatment, as well as by oxaliplatin, and improved by D16F7. Quantitative analysis of PI fluorescence showed that VEGFR-2 is involved in VEGF-A-mediated neuroprotection. Finally, to demonstrate the astrocytic origin of VEGF-A, slices were treated with fluorocitrate (80 µM), a glial inhibitor, that induced a reduction of VEGF-A release both in control and oxaliplatin treated slices, causing an exacerbation of oxaliplatin-induced-neurotoxicity. The addition of exogenous VEGF165b reduced the toxicity caused by oxaliplatin and fluorocitrate after 24 hours of treatment.

Conclusions

In conclusion, our study shows that astrocytic VEGF-A plays a dichotomic role as pro-algic and neuroprotective factor, depending on the receptors bound. In particular, VEGFR-1 is involved in the mediation of pain-related signals, while the activation of VEGFR-2 exerts tissue-protective actions.

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Title

Hydroxycarboxylic acid receptor 2 activation and ocular pathologies: focus on diabetic retinopathy

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Background

The hydroxycarboxylic acid receptor 2 (HCA_2) is a Gi protein coupled receptor (GiPCR) mainly involved in the regulation of anti-lipolytic effects on adipocytes [1]. However, it seems to exhibit anti-inflammatory and anti-oxidative properties on immune and epithelial cells [2-4]. Moreover, HCA_2 activation by its endogenous ligand β-hydroxybutyrate (BHB), the predominant ketone used as glucose source during starvation, has showed protective effects in ocular pathologies, both in pre-clinical and clinical settings [5-8]. However, the role of the hydroxycarboxylic acid receptor 2 (HCA_2) in the diabetic retinal damage is still pionering. Therefore, we here investigate in diabetic C57BL6J mice the expression of retinal HCA_2 receptor and its putative anti-inflammatory effects when activated by the HCA_2 endogenous ligand β-hydroxybutyrate [1].

Methods

Seven-to-10-week-old C57BL6J mice were intraperitoneally injected with streptozotocin (75 mg/kg of body weight) and monitored over a 10-week period from the initial diabetes assessment. Mice showing fasting blood glucose levels higher than 250 mg/dl for 2 consecutive weeks after streptozotocin were treated with intraperitoneal injections of 25-50-100 mg/kg β-hydroxybutyrate twice a week.

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Results

HCA_2 receptor was overexpressed in diabetic retina. Its activation with 50 mg/kg and 100 mg/kg BHB reduced the retinal endoplasmic reticulum stress markers (pPERK, pIRE1, ATF-6α), which were elevated in diabetic C57BL6J mice. These showed also high levels of NLRP3 inflammasome activity markers (NLRP3, ASC, caspase-1) and proinflammatory cytokine levels (IL-1β, IL-18), all reduced by BHB 50 mg/kg and 100 mg/kg. These doses also reduced the apoptotic cell number in the retinal outer nuclear layer (ONL) in diabetic mice and increased connexin 43 expression in ONL, by improving retinal permeability and homeostasis.

Conclusions

Systemic treatment of diabetic C57BL6J mice with BHB seems to activate retinal HCA_2 and mediates protective effects on diabetic retinal damage. Ongoing studies are evaluating the effects of HCA_2 activation by BHB on retinal neuroprotection and autophagy in hyperglycemic conditions.

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Title

Nutraceutical approaches in Duchenne Muscular Dystrophy: in vivo and ex vivo effects of L-citrulline supplementation in combination with prednisolone in the dystrophic *mdx* mouse model.

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Background

Duchenne Muscular Dystrophy (DMD) is a severe muscle wasting disease caused by the absence of functional protein dystrophin. Dystrophin is a subsarcolemmal protein that is pivotal for sarcolemmal stability and is involved in various intracellular signaling. Therefore, the absence of dystrophin leads to a complex pathogenic cascade which triggers, and in turn exacerbates, alteration in muscle metabolism. In recent years, the use of dietary supplements in DMD boys is increasing, in order to support the recovery of defective metabolic pathways. The amino acid L-citrulline (L-cit), precursor of L-arginine, is physiologically required for protein turnover and nitric oxide production, this latter being a deregulated modulator of mitochondrial biogenesis in muscle and blood vessels of dystrophic subjects. Based on the reduced level of L-Cit observed in dystrophic mdx mouse muscles, we hypothesized that a dietary supplementation with L-Cit could exert beneficial effects in mitigating the early necrotic phase of DMD. The aim of this study was hence to evaluate the effects of L-cit supplementation in dystrophic *mdx* mice.

Methods

L-Cit was administered as part of a nutrient-defined diet (AIN93G) to 4-5-week-old *mdx* mice at a final dose of 2 mg/g per day (16.5 g L-citrulline/kg diet) for 8 weeks. AIN93G without L-Cit was in parallel administered to control groups of

wild type (WT) and *mdx* mice. To evaluate any synergistic or antagonistic interaction of L-Cit with standard therapy, other two groups *mdx* mice were used receiving α-methylprednisolone (PDN, s.c, 1 mg/kg, 5 days/week) administered alone or in combination with L-Cit added diet. The outcomes were evaluated by using a multifunctional approach, including in vivo indices of muscle function and ex vivo functional, biochemical and histological readouts.

Results

At the end of treatment, maximal forelimb strength was improved, vs T0, in all treated groups vs. untreated *mdx* mice (+13%), particularly by L-Cit alone (+25%) or in combination with PDN (+24%). Ex vivo, all groups of treated *mdx* mice displayed a significant increase of specific isometric twitch and tetanic force of diaphragm (DIA), with minor effects in extensor digitorum longus muscle. Additionally, L-Cit, alone or in combination with PDN, reduced fibrosis and inflammation in gastrocnemius and DIA muscles, and decreased plasma levels of lactate dehydrogenase, an index of metabolic sufferance. Early RT-PCR experiments showed no significant effect of treatment on the expression of key genes involved in metabolism, inflammation and fibrosis.

Conclusions

Our results confirm the effects of gold standard PDN in ameliorating *mdx* pathology at both functional and structural level and support the hypothesis that L-cit supplementation could be useful in mitigating dystrophic symptoms also when combined with corticosteroids (supported by NL-DPP).

References

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Title

Non-Overt Disseminated Intravascular Coagulation Associated with Obinutuzumab: a Signal Detection Case Study

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Background

Disseminated intravascular coagulation (DIC) is a rare disease of coagulation that have different etiologies, including drug treatment (1). The Agenzia Italiana del Farmaco (AIFA), during a scheduled periodic assessment meeting, investigated on a new possible signal based on 4 individual case safety reports (ICSRs) that reported obinutuzumab and non-overt DIC (2).

We described the process of signal detection for obinutuzumab-associated non-overt DIC managed by the AIFA in collaboration with Pharmacovigilance (PV) Regional Centers.

Methods

Periodic assessments of signal detection for drugs are conducted twice per year in Italy. ICSRs are organized in file named “Reaction Monitoring Report” (RMR) in which all the drug-event pairs collected in the National Pharmacovigilance Network (Rete Nazionale Farmacovigilanza, RNF) database in the specific six-months period of the analysis were listed. For each drug-event pair, the reporting odds ratio (ROR) is calculated as a measure of disproportionality. As the number of ICSRs is ≥3 and the lower limit of the 95% confidence interval of ROR is ≥1, a signal is generated. Priority is given to unexpected and important medical events. Based on medical literature

information possible signals are presented and discussed. ICSRs of interest are also checked in Eudravigilance. If a signal is considered enough robust, an assessment report is submitted to the rapporteur country for a possible evaluation by the Pharmacovigilance Risk Assessment Committee (PRAC) at the European Medicine Agency.

Results

Four cases of obinutuzumab-associated DIC were considered as a possible signal during the scheduled periodic signal detection assessment meeting in December 2020 (ROR 213.6 and IC025 77). About 150.000 ICSRs are contained at the time in the RNF. Patients (2 men and 2 women, age 67-77) were in treatment with obinutuzumab for chronic lymphocytic leukemia. Patients are also in treatment with drug for age-related diseases. Within 48 hours from the administration of obinutuzumab was detected a subclinical DIC. All the 4 cases were reported by hematologists as suspected adverse drug reactions of obinutuzumab, once excluding alternative causes. The DIC spontaneously resolved in all cases. In EudraVigilance database were reported 3 more ICSRs. In medical literature were published only one-case report on a woman who developed DIC after the administration of obinutuzumab (3). A pro-inflammatory response with the increase of interleukins and tumor necrosis factor after the administration of obinutuzumab was showed by some authors (4)(5). Rituximab, another anti-CD20, is rarely related to DIC so a rare class effect cannot be excluded (3). The evidence was robust enough to present the signal to the PRAC.

Conclusions

The PRAC identified non-overt DIC as a possible adverse reaction to obinutuzumab and recommended to update the summary of product characteristics (SPC) with this new adverse reaction with “uncommon” frequency.

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Title

Stress resilience and vulnerability: do glial cells play a role?

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Background

A traumatic event could lead vulnerable subjects to develop psychiatric conditions or, even, diseases. The short and long-term effects of acute stress exposure can vary from adaptive to maladaptive depending both on the adverse event experienced and on the characteristics of the individual exposed, such as genetic background, education, and environment (1). The brain orchestrates the physiological neuroendocrine and behavioral responses to stress but, even when behavior appears to have recovered from stress, the brain retains some changes, resulting in structural remodeling (2). To date, most studies focused on the effects of stress on neurons, especially of the glutamatergic system, considered the most important cell type for proper brain functioning (3). However, glial cells are pivotal for keeping tissue homeostasis and shaping tissue architecture (4), thus they could be reasonably involved in mechanisms underlying brain stress responses and recovery. Therefore, here we studied the impact of acute stress on glial cell functions, comparing resilient and vulnerable animals in a validated model of traumatic stress exposure (5). We focused our analysis on the prefrontal cortex since this model showed glutamate system changes there, as well human imaging studies highlight reduced cortical functional connectivity associated with post-traumatic stress (6)

Methods

Adult Sprague-Dawley rats were exposed to a single footshock stress session (5) and then classified as resilient or vulnerable rats based on their anhedonic-like behavior, measured by sucrose intake test. Rats were sacrificed 24h or 48h after traumatic stress to study both short and prolonged changes in gene and protein expression of markers of glial cell morphology and functions, by RT-PCR, western blotting, and immunofluorescence.

Results

Vulnerable rats displayed a robust and prolonged anhedonic-like behavior after exposure to footshock different from resilient animals and control, not stressed, rats. Signs of reactivity of astrocytes and microglia and inflammation were present 24h after stress. Resilient rats showed increased expression of various astrocytic proteins involved in glutamate metabolism and uptake compared with vulnerable rats. Microglia was still reactive in vulnerable rats 48h after stress. Also, different expressions of the dendritic marker MAP2 suggest that acute stress promoted dendritic remodeling in resilient, but not vulnerable, rats.

Conclusions

This study suggests that stress affects glial cells and that their responses vary between resilient and vulnerable rats. Prolonged alteration in glial functions in vulnerable subjects affects neurons and glutamate homeostasis. So, glial cells may result crucial in determining vulnerability to traumatic stress, thus they might become an innovative target for therapy.

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pharmadvances

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Title

Safety and tolerability of PaliperidonePalmitate

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Background

Currently, SGAs available in a LAI formulation are Risperidone, Olanzapine, Aripiprazole and Paliperidone. Paliperidone Palmitate (PP) is the only LAI available both in monthly (PP1M) and 3-monthly (PPM3) injection. This paper consists in a case report of PP1M accidental overdose: to our knowledge, such occurrence is reported only once in scientific literature. [(1)] We followed the CARE guidelines in writing this case report.

Methods

The patient is a 55-year-old Italian male. He met criteria for Schizophrenia .During the first contact with our service, he complained of sexual side effects and lower limb tremors, asking to be switched to a different medication. For that reason, the patient was given oral Paliperidone that was well tolerated; he was then switched to PP1M. After he was given the first 150 mg injection and a second 100 mg injection 7 days later, he was put on a 4-week schedule of 100 mg of PP for 6 months. During this time the patient showed a good response to medication and maintained stable psychiatric conditions. After the outbreak of the COVID-19 crisis and the enactment of restrictive measures across Italy, the patient complained of irritability and severe anxiety. Therefore he was given an adjunctive anxiolytic intramuscular therapy (Delorazepam) and, in correspondence of the seventh-month injection, the psychiatrist made the decision to increase PP dose to 100 mg monthly. Five days later the patient visited our mental health center again, asking to be given adjunctive anxiolytic therapy. However, due to a mistake in the interpretation of the prescription order, he was given a 100 mg injection of PP instead. The mistake was promptly recognized and this allowed a careful follow-up: the patient was rapidly evaluated by a physician that performed a complete physical examination and, in the following hours, he was periodically evaluated by a nurse who took vital signs resulting within the normal range all time. The psychiatrist requested an ECG registration with QTc measurement that was performed on the day following the accidental overdose, resulting in a normal trace and a QTc interval of 425 milliseconds. Moreover, a complete blood count, an electrolyte, liver and renal panel were performed. Results were all within range. Clinical conditions were monitored on a daily basis through visits conducted both in person and by telephone. The patient never reported any side effect and denied to experience symptoms such as stiffness, anxiety or agitation. The psychiatrist administered a new PP injection 4 weeks after the accidental overdose

Results

Conclusions

Our experience, supported by data from Literature, suggests that PP is generally well tolerated and safe, especially when used as monotherapy. However, as in some cases Paliperidone side effects can be fatal, a close clinical follow-up during the hours and days following an accidental overdose is recommended.

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Title

SAFETY PROFILE OF BIOLOGICAL DRUGS IN PSORIASIS: REAL LIFE DATA FROM THE ACTIVE CALABRIA BIOLOGICS PHARMACOVIGILANCE PROGRAM (CBPP)

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Background

Psoriasis (Ps) and psoriatic arthritis (PsA) are chronic conditions with multifactorial genesis, characterized by clinical pictures of extremely variable complexity with an often-compromised quality of life. In the last two decades, clinical practice has been revolutionized following the development and marketing of several biologic disease-modifying antirheumatic drugs (bDMARDs) resulting in a dramatic improvement in the management of refractory patients.

Methods

All patients treated with almost one biologic drug from January 2018 to March 2022, in Calabria Center of Dermatology and satisfying inclusion criteria, were enrolled. Demographic and clinical characteristics of patients, type of treatment used, therapy discontinuation, failures, switch/swap to another biologic, and possible onset of adverse events (AEs) were collected.

Results

, A total of 733 patients (303 females; male 430, mean 54,7±13,4) started on treatment with biologic drugs for active Ps 48,1%, PsA 51,9%. Adalimumab (ADA) was the most commonly administered biologic 31.3%, followed by etanercept (ETN) 29.8%, ustekinumab (UST) 12.8%, infliximab (IFX) 6.5%, secukinumab (SEC) 8.8%, golimumab (GOL) 4.3%, ixekizumab (IXE) 2.4%, guselkumab (GUS) 1.2%, risankizumab (RZE) 0.9%, brodalumab (BRO) 0.8%, tildrakizumab (TIL) 0.4%, tocilizumab (TCZ) 0.1%, and abatacept (ABT) 0.1%. Patients naive for biologic treatment were 77.0%. Overall, 427 patients (58,2%) have not developed AEs or therapeutic failures, whereas patients 22,7% experienced at least one AE and 20,1% had at least a primary/secondary failure. During follow-up, we reported 25,5% AEs and 0,66% serious AEs, for each drug, AEs/SAEs have been reported with GOL 34.3%, BRO 33,3%, TIL 33,3%, ADA 29.5%, IXE 27,7%, ETN 24.6%, IFX 22.9%, SEC 18,4%, RZE 14,2%, UST 11.7%, no AEs have been occurred with GUS, ABT and TCZ. The most common AEs were asthenia, injection site reactions, skin disorders and gastrointestinal disorders, all the AEs observed were expected. Five patients experiencing serious AEs: 1 case of severe pneumonia with IFX, 3 cases with ETN (benign respiratory tract neoplasm, new-onset lupus-like syndrome and a hemorrhagic cystitis) and a case of severe splenomegaly leading to hospitalization with ADA.

Conclusions

Nevertheless, the acquisition of clinical practice should be provided useful data on widely used biologic drugs and their tolerability, discontinuation rate and the incurrence of severe adverse events. Aim of this active post-marketing study is to monitor and analyses AEs occurring with biologic drugs using the data from the dermatologic area of the active CBPP.

References

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Title

Potential role of PKR2/MRAP2 physical interaction in obesity

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Background

Adipokines, cytokines secreted by adipocytes, regulate food intake and energy homeostasis by acting on hypothalamus and controlling feeding behaviors, weight gain, and obesity development [1]. Prokineticin 2 (PK2) is a novel adipokine that can reduce food intake and adipose tissue via binding of prokineticin receptor 1 (PKR1) and activation of STAT3 and ERK signaling pathways in mice [2]. Indeed, PKR1 knockout mice (KO) develop an obese phenotype at the age of 40 weeks and are used as a model for obesity [3]. The trafficking and the signal transduction of several G-protein coupled receptors critical for the control of energy homeostasis, including those of PKRs, are modulated by the melanocortin receptor accessory protein 2 (MRAP2) [4]. MRAP2 acts as a depressor of PKR1 signaling favoring food intake and weight gain [5] and it inhibits glycosylation of Asparagine at position 27 of the N-terminal extracellular region of prokineticin receptor 2 (PKR2) preventing the correct localization of PKR2 in the plasmatic membrane [6]. In this work, we evaluate how MRAP2 prevents glycosylation of PKR2 and the consequence of MRAP2/PKR2 crosstalk. In the C-terminal region of MRAP2 there is an Arginine residue in position 125 essential for MRAP2 function and mutation of this residue causes a severely obese phenotype in humans [7]. We characterize obesity-associated MRAP2 mutant, R125H, in the regulation of PKR2 activity.

Methods

-Biochemical techniques (GST-pull down, immunoprecipitation analysis) were used to analyze specific regions involved in the interaction of PKR2 with MRAP2.

-Chinese hamster ovary (CHO) cells stably expressing human PKR2, transfected with MRAP2 or MRAP2 R125H, and stimulated with PK2 were used to assess activation of the PKR2 transduction pathway (STAT3 and ERK1/2) by Western blot.

-Expression of MRAP2 was assessed in hypothalamic explants from wild-type (wt) mice and adipose tissue from wt- and obese PKR1-ko mice by RT-PCR and Western blot.

Results

We demonstrated a physical interaction between PKR2 and MRAP2 that occurs via the N-terminal region of PKR2 and the C-terminal region of MRAP2. This PKR2/MRAP2 binding prevents PKR2 glycosylation and, consequently, its membrane localization in the plasmatic membrane. In this way, MRAP2 significantly reduces PK2/PKR2-mediated phosphorylation of STAT3 and ERK in CHO cells. MRAP2 R125H decreases PK2-induced Erk activation more effectively than MRAP2. In hypothalamic explants of wt-mice, incubation with PK2 reduces MRAP2 expression levels: treatment with a STAT3 inhibitor prevents the PK2-induced decrease of MRAP2 suggesting that the STAT3 pathway is involved in the regulation of MRAP2 expression. Conversely, MRAP2 expression is greatly increased in adipose tissue of obese PKR1-ko mice at both mRNA and protein levels.

Conclusions

Our data indicate that not only the absence of PKR1 but also the interaction of PKR2 and MRAP2 may be involved in the failure of PK2 anorectic activity and the development of obesity.

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Title

Algorithm to detect type 2 diabetes mellitus disease using claims data from a general population from Southern Italy

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Background

In Italy, type 2 diabetes (T2DM) accounts for over 90% of DM cases, with a prevalence in the general population of about 5.8%, which is higher in Southern Italy than in Northern Italy (6.5% vs 4.5%). Type 1 diabetes mellitus (T1DM) represents 2-3% of DM cases, while the remaining are gestational diabetes mellitus (GDM), Latent Autoimmune Diabetes in Adults (LADA) and Maturity-onset diabetes of the young (MODY), accounting for about 1-2% of all known diabetes cases [1, 2]. Distinguish between T2DM and other DM types using administrative claims is challenging: a lot of different algorithms are found in literature with a non-optimal performance most of the time [3]. The aim of the study is to identify T2DM patients by distinguishing them from T1DM and gestational diabetes mellitus patients in a general population from Southern Italy, using administrative data from Caserta Local Health Unit (LHU) during 2011-2021.

Methods

Firstly, patients with diabetes mellitus were identified from Caserta LHU claims database looking for: ≥1 DM-related ICD9-CM code (250*) OR ≥2 antidiabetic drugs dispensing (ATC=A10*) OR ≥1 co-payment exemption code (013*). Then, in order to exclude T1DM and GDM patients the following criteria were applied: patients with T1DM were identified by exclusive consumption of insulin (ATC: A10A*) AND [Age less than 20 years OR hospital discharge diagnosis, as primary/secondary causes of hospital admissions, related to T1DM (ICD9-CM: 250.*1/250.*3) OR exclusive consumption of rapid insulin (ATC: A10AB*)]. Patients with GDM were identified looking for diagnosis of GDM (ICD9-CM: 648.8*). The remaining patients were considered as T2DM patients. Demographic characteristics (e.g. sex and mean age) of T2DM patients were explored.

Results

Among almost 1.2 million inhabitants from Caserta LHU during the years 2011-2021, 109,917 (9.2%) diabetic patients were identified; of these, 5,351 (4.9%) were exclusively treated with insulin during the study period. Applying the exclusion criteria, 2,391 (2.2% of DM patients) patients with T1DM and 170 (0.2% of DM patients) GDM patients were identified. Specifically, among T1DM patients, 720 (13.5%) were less than 20 years old, 1,432 (26.8%) received hospitalization for T1DM, and 600 (11.2%) patients were exclusively treated with rapid analogues of insulin. Finally, T2DM patients were 107,356 with a total prevalence of the T2DM disease of 9,0% in the Caserta LHU. An equal sex distribution (female-male ratio = 1.0) was observed and the mean age of T2DM patients was 60.9 ± 13.4 years.

Conclusions

Demographic characteristics in combination with drug-utilization patterns and hospital discharge diagnoses can be used to discriminate T2DM from other types of diabetes mellitus. Algorithm validation studies could be helpful to identify type 2 diabetes mellitus patients from claims databases.

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Title

PHARMACOLOGICAL CHARACTERIZATION OF NEW COMPOUNDS AIMED AT INHIBITING EPH-EPHRIN INTERACTION IN GLIOBLASTOMA

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Background

Eph receptors are the largest family of receptor tyrosine kinases in human and together with ephrin ligands play a critical role in the development of many tumours [1]. Different research groups in the world are focusing their efforts on the optimization of pharmacological tools able to interfere with Eph-ephrin interactions. Here we show the pharmacological characterization of new small molecules designed to interfere with Eph-ephrin binding. These compounds derive from modifications introduced in meta and in para position of the terminal phenylsulfonyl portion of UniPR1449, an EphA2 antagonist with affinity in the low micromolar range.

Methods

Displacement curves of the biotinylated ligand ephrin-A1-Fc (used at its K_D) from the recombinant EphA2-Fc receptor were constructed to calculate the IC_{50}, in the presence of increasing concentrations of the compounds. Saturation curves of biotinylated ephrinA1-Fc on EphA2-Fc in the presence of increasing concentrations of the molecules under study were constructed to calculate Ki values.

Non-specific toxicity of compounds was evaluated by measuring the release of enzyme lactate dehydrogenase (LDH) from U251 glioblastoma cells in the cell culture medium. The antiproliferative effect of the compounds on U251 was carried out through MTT assay. All compounds were tested at 30 and 10µM, after 24h, 48h and 72h of incubation. To evaluate the antagonist activity, U251 were incubated for 20 minutes with the compounds under study and then stimulated for 10 minutes with ephrin-A1-Fc. The phosphorylation level of the EphA2 receptor was determined through an ELISA assay.

Results

Except from the fluorine derivative UniPR1454, para-substitution on the phenylsulfonyl portion is tolerated by EphA2 receptor but does not lead to a significant improvement of in the compound activity compared to the parent antagonist UniPR1449.

Compounds tested as antagonists inhibit the phosphorylation of the EphA2 receptor at 30 μM and have antiproliferative activity on U251 cells avoiding non-specific toxicity up to 30 μM concentration when incubated for 2 hours with U251 cells.

Conversely, meta-substitution on the phenylsulfonyl portion resulted detrimental on EphA2 activity compared to para substitutions.

Conclusions

In this work we pharmacologically characterized a new series of molecules synthetized starting from the EphA2 antagonist UniPR1449. In the future, the most promising compounds will be investigated in pathological processes such as tumour angiogenesis and maintenance of stem properties of cancer cells in which Eph-ephrin interactions play a key role.

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pharmadvances

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Title

Impairment of self-care behavior is associated with differences in the transcriptomic profile of the Habenula in adult rats exposed to prenatal stress

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Background

Stress experienced early in life produces widespread changes on brain development and has long-lasting effects on physical and mental health, predisposing the individual to develop psychopathology later in life. Indeed, clinical, and preclinical studies have shown that exposure to stress during gestation or early postnatally can produce different behavioural alterations, although not all the exposed animals show a vulnerable phenotype. With this respect, recent studies pinpointed the habenula as a potential key brain region mediating the effect of stress by modulating depressive like-behaviours and sociability [1,2], although the underlying mechanisms are still poorly understood.

Methods

Sprague Dawley pregnant rats underwent a prenatal stress experimental paradigm (PNS) during the last week of gestation. After birth, male offspring (n=48) were left undisturbed until early adulthood (PND68) when selected tests were performed to evaluate specific behavioral domains: self-care, social interaction, and cognition. One week after the end of the behavioral assessment, animals were sacrificed, and different brain areas were microdissected. RNA-Sequencing analysis was performed on the habenula, and genes differentially expressed were identified by using DeSEq2, whereas pathways analyses were performed by using Ingenuity Pathways Analysis software.

Results

An impairment in self-care behaviour was observed in PNS males, as shown by an increase in the latency time (expressed as mean ± SD) in the splash test. Specifically, based on the behavioural score PNS rats were divided into vulnerable (VULN) and resilient (RES), with VULN animals showing an increased latency in starting the grooming behaviour (VULN: 154s ± 11s; RES: 107s± 22s; p <0,001). By using RNA-Sequencing analysis, we found that 1784 genes were differentially expressed in VULN as compared with RES rats (p < 0,05; FC ± 1,2), and among them 177 genes survived the FDR correction (q-value < 0,1). The pathways analysis showed increased activity of several pathways in VULN animals, such as the IL-18 Signalling (p<0,001; z-score 3,8), CREB signalling (p<0,001; z-score 3,4), Synaptogenesis Signalling (p<0,001; z-score 2,7) Phagosome Formation (p<0,001; z-score 2,8), and Chemokine Signalling (p<0,05; z-score 2,3).

Conclusions

Exposure to PNS produced a significant impairment of the self-care domain in adult male rats. Moreover, transcriptomic analysis showed an increased activation of pathways associated with inflammation and synaptogenesis in the habenula of vulnerable PNS rats, as compared with resilient animals, which may contribute to some of the long-lasting defects produced as a consequence of stress exposure during gestation.

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pharmadvances
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Title

Drugs for melanoma. Recommendations and epidemiological impact models as clinical Governance tools in the Veneto Region.

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Background

New oncology drugs exert a high pressure on the Regional Health System, due to high costs and the possibility to offer additional drug lines to patients. Over the last years, immunotherapies (pembrolizumab and nivolumab) and the target therapy dabrafenib+trametinib have been reimbursed for the adjuvant treatment of melanoma, leading to the necessity of re-define the place in therapies of drugs already available for metastatic disease. Thus the Regional Working Group on Oncology drugs have produced Recommendations on melanoma drugs, defining the target population.

Methods

Recommendations were produced by the Working Group on Oncology drugs with GRADE method, considering clinical needs, therapeutic value and quality of evidence, with the support of HTA Unit of Azienda Zero in defining the place in therapy in accordance with reimbursement criteria and the epidemiological model.

Results

In adjuvant setting, immunotherapy is indicated regardless both type of melanoma and mutations; considering the available evidence, pembrolizumab is attended to be used in cutaneous melanoma. Adjuvant target therapy is indicated

in BRAF-mutated cutaneous melanoma, for which the choice between immunotherapy and target therapy should be discussed with patients. Available evidence do not permit to establish differences in terms of efficacy and safety between pembrolizumab and nivolumab. Thus, if clinically possible, it is recommended to take into account costs in the choice between the two immunotherapies.

In metastatic setting, the choice of drugs should take into account adjuvant treatments previously received by patients. Since clinical studies on drugs for metastatic disease included patients not treated in adjuvant setting, Recommendations on the treatment of metastatic melanoma in patients previously treated with adjuvant therapies were not produced.

Considering place in therapy and regional epidemiology, about 296 patients were estimated to be elegible every year to adjuvant therapies, out of which about 196 will receive immunotherapy and about 100 will receive target therapy. In the metastatic setting, about 174 patients per year will be eligible to receive an immunotherapy and about 48 the target therapy.

Conclusions

Adherence to Recommendations will help to promote quality, uniformity and equity of access to care, rationalizing resource allocation. The number of patients treated with melanoma drugs will be monitored as indicator of adherence to Recomendations and will help to determine the impact of drugs on pharmaceutical expenditure. Audit could be organized if anomalies will be detected.

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Recommendation n. 35 on Oncology drugs in the Veneto Region - Decree n. 116 of 14.10.2021

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Title

Forecasting model for drug utilization and expenditure in support of clinical Governace in the Veneto Region. The example of drugs for chronic lymphocytic leukemia.

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Background

New onco-hematology drugs exert a high pressure on the Regional Health System, due to high costs and the possibility to offer additional drug lines to patients. Acalabrutinib and venetoclax+obinutuzumab are the most recent new therapies for chronic lymphocytic leukemia (CLL). Definition of place in therapy of such drugs and of their impact on pharmaceutical expenditure is necessary to plan resource allocation.

Methods

HTA Unit of Azienda Zero supported the Working Group on onco-hematology drugs in critically evaluating new therapies and defining place in therapy in accordance with reimbursement criteria, if already available. A forecasting model was developed assuming that each new drug will be used according to place in therapy criteria.

Results

Acalabrutinib is a Bruton Tyrosine Kinase inhibitor (BTKi), as well as ibrutinib, and is reimbursed as monotherapy in first line and relapsed-refractory setting. Venetoclax+obinutuzumab is a new first line therapy (reimbursed criteria not yet available at the time of the analysis). Incremental impact of new therapies was estimated for three years, as follow: (i) first year: low impact (below €1 million), due to acalabrutinib market entry, which, at the time of the analysis, had the same cost as ibrutinib, with a broader reimbursed indication (all ages for acalabrutinib vs reimbursement age restrictions for ibrutinib); (ii) second year: about €1,6 million, due to venetoclax+obinutuzumab market entry and to acalabrutinib (new patients and patients continuing therapy from previous year); (ii) third year: impact below €1 million,

edra

due to competition between BTKi and venetoclax+obinutuzumab; the latter is a fixed-duration therapy lasting for a maximum of one year, differently from BTKi, that are chronic therapies given for years until disease progression. The usage of venetoclax+obinutuzumab will decrease the number of patients treated for more than one year.

Conclusions

Place in therapy definition optimizes drug utilization, permits to define target population of new therapies, taking into account their competitors already on the market, and leads to pharmaceutical expenditure forecast supporting resource allocation. Forecasting models need to be updated over the time with new reimbursed drugs and/or new reimbursement criteria of therapies already on the market. Prescrition and expenditure monitoring will permit to verify forecast accuracy. *Audit* could be organized if anomalies will be detected.

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Report on acalabrutinib and venetoclax+obinutuzumab for chronic lymphocytic leukemia produced in the Veneto Region - Decree n. 66 of 11.05.2022

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Title

Drugs for ovarian cancer. Recommendations and epidemiological impact models as clinical Governance tools in the Veneto Region.

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Background

PARP inhibitors (olaparib, niraparib, rucaparib) have been approved as maintenance therapy after platinum-based chemotherapy for ovarian cancer and have changed treatment strategy in this settimg. In order to manage the introduction of these therapies, the Regional Working Group on Oncology drugs have produced Recommendations, defining the target population.

Methods

Recommendations were produced by the Working Group on Oncology drugs with GRADE method, considering clinical needs, therapeutic value and quality of evidence, with the support of HTA Unit of Azienda Zero in defining the place in therapy in accordance with reimbursement criteria and the epidemiological model.

Results

At the end of 2021, Recommendations were produced on all maintenance monotherapies available at that time: olaparib (first-line and relapsed-refractory settings), niraparib (relapsed-refractory setting) and rucaparib (relapsed-refractory setting).

Considering place in therapy and regional epidemiology, about 78 patients/year with BRCA-mutated ovarian cancer were estimated to be eligible to olaparib as first line maintenance therapy; in relapsed-refractory setting, a maximum of 66 patients/year were estimated to be eligible to a PARP inhibitor as maintenance monotherapy, if not previously

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received.

Conclusions

Adherence to Recommendations will help to promote quality, uniformity and equity of access to care, rationalizing resource allocation. The number of patients treated with PARP inhibitor will be monitored as indicator of adherence to Recomendations and will help to determine the impact of drugs on pharmaceutical expenditure. *Audit* could be organized if anomalies will be detected. Epidemiological estimates are currently under revision to include therapies newly reimbursed.

References

Recommendation n. 34 on Oncology drugs in the Veneto Region - Decree n. 115 of 14.10.2021