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TABLE OF CONTENTS

EDITORIAL

pharmadvances

Volume **5**, special issue **2023**

DEMENTIA THERAPEUTICS AND COGNITIVE REHABILITATION AN INTERNATIONAL MEETING

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The global impact of dementia is increasingly worrisome and up to 90% of dementia patients in low- and middle-income countries is not diagnosed with further delays in diagnosis due to the pandemic during which these fragile population pays the highest price.

Alzheimer's disease (AD) is the most common accounting for about two-thirds of all cases, but mixed forms of dementia are being increasingly recognized, making dementia a public health priority. These different forms may at least in part be explained through progressive alterations of the epigenome that may contribute to the decrease of cognitive abilities with advancing age.

Indeed, there is little doubt that AD is a multifactorial disease, which involves diverse pathogenic mechanisms and will probably require combinatorial therapies. Although many pathological factors, such as accumulation of protein aggregates of amyloid-beta (Aβ) and tau, have been identified, their role in combination with other factors, at different disease stages, requires further clarification. A comprehensive understanding of the complex disease mechanisms, the identification of early disease markers aided by ultrastructural magnetic resonance imaging are necessary to develop more effective treatments.

Although multiple failures of phase III trials with agents targeting Aβ had initially been disappointing, the accelerated approval of aducanumab has recently renewed the interest in targeting Aβ. The gain of information from the real-world data on aducanumab may anticipate that further development of clinical trials in AD will be prompted.

Actually, current treatments of AD provide only limited symptomatic effects and development of neuropsychiatric symptoms (NPS), other than cognitive symptoms, do affect some 97% dementia patients and they are managed with potentially harmful antipsychotics. The latter NPS are at least in part linked to unrelieved pain remarkably reducing quality of life. Hence, studies for the management of NPS are needed and novel opportunities are offered by complementary analgesic and non-BDZ anxiolytic effects of natural products such as phytocomplexes engineered to be trialed in double blind studies also for cognitive rehabilitation.

All the above illustrated topics and more, concerned with cognitive rehabilitation, are the subjects of this proceeding issue of PharmAdvances that hosts abstracts of invited lectures and poster communications of the meeting "***Dementia Therapeutics and Cognitive Rehabilitation***" held at the University of Calabria (Unical), Rende (Cosenza), Italy, November 21st-23rd, 2022. The meeting has been organized under the egidas of the Italian (SIF) and Japanese (JSP) Society of Pharmacology, the Italian Society of Neurologic Rehabilition (SIRN) and the Sant'Anna Institute for Serious Brain Injuries (Kroton, Italy) and with financial support of SIF for that one of us is in-

debted with the Past President, Prof. Giorgio Racagni and the whole Council of the Society. The continuous support of the Head of the Department of Pharmacy, Health Science and Nutrition (Unical), Prof. Maria Luisa Panno, and of all the members of staff of the administration is gratefully acknowledged.

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Volume **5**, special issue **2023**

TARGETING SYNAPTIC DYSFUNCTION IN ALZHEIMER'S DISEASE

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OBJECTIVE: Alzheimer's disease (AD) is the most prevalent and socially disruptive illness of aging populations. Genetic and pathological evidence strongly supports the amyloid cascade hypothesis, which states that Amyloid β (Aβ) has an early and crucial role in AD. Aβ is liberated from the amyloid precursor protein (APP) by BACE and γ-secretase activity. Alternatively, APP is cleaved within the Aβ domain by ADAM10, which prevents Aβ formation. In addition to Aβ, synapse loss has a central role in AD pathogenesis, rather than just a consequence of cell death. Synapse loss represents an early insult that advances with disease and dendritic spine loss is seen in several AD models. We have previously identified ADAM10 as an enzyme positioned at the crossroads between the amyloid cascade and synaptic loss. ADAM10 trafficking and enzymatic activity are controlled by protein partners that regulate its membrane levels. These mechanisms finely tune ADAM10 activity towards APP and synaptic substrates and are impaired in AD. Here, we aimed at developing new tools to target ADAM10 trafficking mechanism in order to rescue synaptic failure in AD.
MATERIALS AND METHODS: we used *in vitro* and *in vivo* models of AD to (i) develop and validate the cell permeable peptides designed to target ADAM10, (ii) identify and characterize novel protein partners of ADAM10. Biomarker study was performed using cerebrospinal fluid (CSF) samples analyzed using ELISA assays.
RESULTS: to upregulate the synaptic availability of ADAM10, we developed cell-permeable peptides (CPPs) that are capable of interfering with the mechanism controlling ADAM10 endocytosis. In particular, the CPP target ADAM10 interaction with AP complex. The results indicated that administration of a CPP capable of inhibiting ADAM10 endocytosis rescued cognitive and synaptic function when administered during the early disease stage in AD model mice, with no evidence of associated safety issues. Given that the regulation of ADAM10 activity depends on the interaction of protein partners, we carried out a two-hybrid screening to identify novel interactors of ADAM10. We identified the actin-binding protein CAP2 as binding partner of ADAM10 and we demonstrated that CAP2 is implicated in ADAM10 endocytosis. Interestingly, CAP2 is recruited to the synapses upon synaptic plasticity events to contribute to the remodeling of spines. Remarkably, CAP2 levels are specifically increased in the CSF of AD patients compared to control subjects or to frontotemporal dementia patients, thus suggesting that CAP2 can be a biomarker of synaptic failure
CONCLUSIONS: overall, our results demonstrate that the dissection of the mechanisms implicated in ADAM10 trafficking is critical to develop tools targeting AD synaptic dysfunction and to identify novel biomarkers of AD synaptic failure to track disease progression.

NERVE GROWTH FACTOR RECEPTOR (NGFR/P75NTR) GENE POLYMORPHISMS IN EARLY VS LATE ONSET SPORADIC ALZHEIMER'S DISEASE

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OBJECTIVE: Alzheimer's Disease (AD) is the most widespread neurodegenerative disorder. AD is commonly categorized as either early onset (EOAD) or late onset (LOAD) based on an age cutoff of 65 years. It has been shown that the genetic variability of Nerve Growth Factor Receptor (NGFR/p75NTR) gene could represent risk factors for AD. However, to date only a few studies have investigated this relationship with conflicting results. The general aim of this study was to better characterize the association between NGFR/p75NTR SNPs in EOAD and LOAD patients.
MATERIALS AND METHODS: this study was conducted on 168 AD patients (60 EOAD and 108 LOAD) recruited at the Regional Neurogenetic Centre (CRN) – ASPCZ of Lamezia Terme (CZ, Italy). Nineteen tag-SNPs were selected and genotyped using TaqMan SNP genotyping assays on DNA extracted from blood samples. The associations between tag-SNPs and AD were assessed by linear regression models after adjustment for gender, APOE genotype and level of education.
RESULTS: the variability of rs2072446 and rs734194 polymorphisms was associated with the onset of LOAD. LOAD patients' carriers of the G allele at the rs734194 had a mean age of onset of about 2.5 years higher than those who were homozygous for the T allele ($p = 0.024$). A similar effect was also detected for the rs2072446 polymorphism for which T allele carriers showed a delayed age of onset of about 3.5 years than homozygotes for the C allele ($p = 0.048$). The variability of three investigated NGFR polymorphisms was also associated with the MMSE performances in EOAD patients. In particular, carriers of the rare alleles of the rs741071, rs2072446 and rs734194 polymorphisms showed a lower MMSE scores ($P < 0.05$).
DISCUSSION AND CONCLUSIONS: our results suggest that polymorphisms in NGFR/p75NRT gene may affect the age of onset and the severity of AD revealing a new role of NGFR/p75NTR in both EOAD and LOAD from a genetic perspective. Further studies are needed to verify if NGFR/p75NTR polymorphisms also determinate an increased risk for developing AD.

CANNABINOID MECHANISMS IN MODELS OF NEURODEGENERATIVE DISORDERS *IN VITRO*

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OBJECTIVE: the interest on the use of compounds present in *Cannabis Sativa* in neurodegenerative diseases such as epilepsy, ischemia, multiple sclerosis and Alzheimer, has massively increased in the past few years. The goal of this study is to examine the role of selected cannabinoids in the mechanisms of neuronal death in models of ischemia and epilepsy.
MATERIALS AND METHODS: we investigated the effects of the selected cannabinoids, Δ9-tetrahydrocannabinol (THC), cannabidiol (CBD) and cannabigerol (CBG) in rat organotypic hippocampal slices exposed to oxygen and glucose deprivation (OGD) or kainate, *in vitro* models of ischemia and epilepsy, respectively. Cell death in the CA1 and CA3 hippocampal subregions was quan-

tified by propidium iodide fluorescence. Morphological analysis and tissue organization were examined by immunohistochemistry and confocal microscopy and microglia activation and polarization were evaluated using flow cytometry and morphological analysis.
RESULTS: when present in the incubation medium, CBD dose-dependently reduced CA1 and CA3 injury induced by OGD and kainate. Conversely, incubation with THC exacerbated hippocampal damage. The neurotoxic effects of THC were dependent on CB1 receptors. While the neuroprotective effects of CBD were blocked by TRPV2 and 5-HT1A antagonists. Confocal microscopy confirmed that CBD but not THC had a significant protective effect against neuronal damage and tissue disorganization caused by OGD and kainate. In the *in vitro* epilepsy model CBD blocks the microglia activation from the M0 to M1 phenotype observed in the kainate treated slices, pushing toward a transition from M0 to M2.
CONCLUSIONS: our results suggest that CBD mitigated neuronal damage induced by OGD and kainate with a safe profile. These data support the idea that CBD may become a valid and safe therapeutic intervention in the treatment of acute neurological diseases.

NANOPARTICLE-MEDIATED DELIVERY OF A NEW TSPO LIGAND SUPPRESSES INFLAMMATION IN LPS-STIMULATED MICROGLIA IN A MOUSE MODEL OF ALZHEIMER'S DISEASE

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OBJECTIVE: the translocator protein 18kDa (TSPO) is a conserved outer mitochondrial membrane protein, implicated in inflammation, cell survival and proliferation. In the central nervous system, the expression of TSPO is markedly upregulated in activated microglia during various disease states such as Alzheimer's Disease. Synthetic as well as endogenous ligands with agonistic or antagonistic properties modulate the function of TSPO. Thus, TSPO ligands can act as putative therapeutic agents during neuroinflammatory processes.
In the present study, we examined the effectiveness of a new TSPO ligand, named TEMNAP, in mitigating inflammatory processes associated to dementia. Lipopolysaccharide (LPS)-activated microglial cells were used as an *in vitro* model to study the anti-inflammatory effects of TEMNAP.
MATERIALS AND METHODS: we explored the efficacy of nanoparticle-mediated delivery of TEMNAP by investigating the molecular and the morphological properties of LPS-stimulated BV2 microglia. In addition, we explored the potential neuroprotective effects TSPO modulation in transgenic mice Tg2576, a well-known model of Alzheimer.
RESULTS: our results demonstrated that the exposure of BV2 microglia to TEMNAP significantly reduced the LPS-induced microglia proliferation and strongly prevented the expression of the pro-inflammatory marker iNOS, as well as the production of nitric oxide.
In addition, we found that nanoparticle-mediated delivery of TEMNAP was more effective in preventing the LPS-induced proliferation of hyperactivated microglia and in reducing the upregulation of the M1 pro-inflammatory markers iNOS and CD86.
In vivo experiments carried out in transgenic mice Tg2576 confirmed the neuroprotective effects of TEMNAP systemically administered.
CONCLUSIONS: collectively, our results revealed a strong neuroprotective effect of the new compound TEMNAP and confirm that TSPO may represent a useful theranostic target in dementia.

IMPAIRMENT OF REDOX HOMEOSTASIS AND ENERGY METABOLISM IN THE BRAIN OF DOWN SYNDROME: A SYNERGISTIC PATH TO ALZHEIMER DISEASE

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OBJECTIVE: Down Syndrome (DS) is the most common genetic disorder due to the abnormal triplication of chromosome 21 resulting in a variety of pathological phenotypes. Among these, individuals affected by DS show with ageing the accumulation of oxidative damage associated with defects of the proteostasis network. DS is currently considered a human genetic model of early onset Alzheimer disease (AD). We hypothesize that redox dysregulation is closely linked to metabolic defects, including reduced glucose metabolism, energy production and aberrant insulin signaling. Further, loss of protein quality control, including proteasome and autophagy, contributed to the accumulation of oxidative damage and AD neuropathological hallmarks.
MATERIALS AND METHODS: frontal cortex from post-mortem brains from a cohort of DS individuals, prior to and after development of AD neuropathology (DS > 40 years, DSAD), *vs.* respective age-matched controls, and brain (cortex and hippocampus) isolated from Ts65Dn mice (a mouse model of DS) were analyzed to evaluate: oxidative modifications of proteins by redox proteomics, mTOR/insulin signaling, markers of autophagy and mitochondrial activity.
RESULTS: collected data demonstrated the early accumulation of oxidative damage in the brain of DS individuals before the onset of AD neuropathology. We identified oxidized proteins in DS and DSAD cases involved in neuronal trafficking; proteostasis network; energy metabolism and mitochondrial function. In addition, we showed that aberrant mTOR/insulin signaling contributes to disturbance of protein quality control and energy production.
CONCLUSIONS: the picture that emerges from these studies suggests that aberrant redox homeostasis represents an early event in DS brain and likely contributes to dysfunction of several brain functions ultimately leading to cognitive decline. It is likely that perturbation of redox signaling accelerates the onset of AD neuropathology in DS population.

NCX3-INDUCED MITOCHONDRIAL DYSFUNCTION IN MIDBRAIN LEADS TO NEUROINFLAMMATION IN STRIATUM OF A53T-α-SYNUCLEIN TRANSGENIC OLD MICE

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OBJECTIVE: the molecular mechanisms involved in the pathogenesis of selective dopaminergic neurodegeneration are complex and not completely understood. The increase of alpha-synuclein aggregates, oxidative stress, altered intracellular Ca^{2+}-homeostasis, mitochondrial dysfunction and disruption of mitochondrial integrity are considered among the pathogenic factors leading to dopaminergic neuronal loss. In this regard, it is noteworthy that mitochondria critically regulate inflammatory processes, and that neuroinflammation also emerged as a potential causative mechanism of neuronal demise, as suggested by preclinical and clinical studies. A positive correlation between the serum levels of inflammatory mediators and Parkinson's Disease (PD) onset, as well as a key role for mitochondria in this process have been recently hypothesized.
This study aims to investigate the molecular mechanisms leading to mitochondrial dysfunction and their relationship with the activation of the neuroinflammatory process occurring in PD.
MATERIALS AND METHODS: to address these issues, experiments were performed *in vivo,* in a familial model of PD represented by mice carrying the human mutation of α-synuclein A53T under the prion murine promoter, and *in vitro*, in primary culture of neuronal and glial cells obtained from midbrain and striatum isolated from WT and α-synuclein A53T transgenic mice. In these models, the expression and activity of NCX isoforms, a family of important transporters regulating ionic homeostasis in mammalian cells working in a bidirectional way, were evaluated. Mitochondrial function was monitored by confocal microscopy and fluorescent dyes to measure mitochondrial calcium content, mitochondrial membrane potential and Free radical production in neuronal and glial cells.
Parallel experiments were performed in 4 and 16 months old A53T-α-synuclein transgenic mice to correlate the functional data obtained *in vitro* with mitochondrial dysfunction and neuroinflammation through biochemical analysis.
RESULTS: the results obtained demonstrated: 1. in α-synuclein A53T-transgenic mice mitochondrial dysfunction occurs early in midbrain and later in striatum, 2. mitochondrial dysfunction occurring in the midbrain is mediated by the impairment of NCX3 protein expression in neurons and astrocytes, 3. mitochondrial dysfunction occurring early in midbrain triggers neuroinflammation later into striatum, thus contributing to PD progression during mice aging.
CONCLUSIONS: the results reported in the present study demonstrate that mitochondrial dysfunction might exert a detrimental role in PD progression. Specifically, mitochondrial dysfunction occurring in mesencephalic neurons from α-synuclein A53T-transgenic mice at the early stage of the disease promotes neuronal degeneration and activates microglial cells in the striatum. The activated microglial cells can in turn promote proinflammatory factors release in the striatum of these mice with consequent glial activation and progressive impairment of dopaminergic neuronal plasticity in the midbrain at the late stage of the disease.

NOVEL ENHANCEMENT MECHANISMS OF THE NOCICEPTIVE RESPONSE BY SERUM EXOSOMES IN A MOUSE MODEL OF NEUROPATHIC PAIN

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OBJECTIVE: exosomes are small (50-150 nm) membrane vesicles of endocytic origin that are found in body fluids and support their role in intercellular communication. Although recent studies have demonstrated that various biomarkers are involved in the extent of pain from the serum exosomes, the effects of exosomes on pain have not been elucidated. Our preliminary experiments showed that the administration of serum exosomes isolated from mice with partial sciatic nerve ligation (PSNL), a mouse model of neuropathic pain, to other PSNL mice did not affect the intensity of allodynia. We attributed this to the very short half-life of exosomes in the blood (2-30 min). Therefore, we focused on formalin-induced nociceptive behavior, an established model of acute pain, and investigated the effects of serum-derived exosomes from PSNL mice on formalin-induced nociceptive behavior and its molecular mechanisms.
MATERIALS AND METHODS: PSNL model mice were constructed by partially ligating the sciatic nerve in 5-week-old *ddY* male mice (20-22 g). Blood was collected on the 7th day after PSNL operation, and the serum was crudely purified by adding an exosome enrichment reagent (ExoQuick®) and then purified using a size exclusion chromatography column (EVSecond L70®). Immediately after intrathecal (i.t.) administration of serum-derived exosomes to normal mice, 0.5% formalin (20 μL) was administered within the right hind paw plantar. Nociceptive behavior was assessed by calculating the number of seconds the mouse was licking or biting the administration site every 30 seconds for up to 5 minutes after administration.
RESULTS: we found that 0.5% formalin-induced nociceptive response was significantly enhanced by i.t. pre-treatment with serum exosomes isolated from PSNL but not from sham-operated mice. In addition, exosomes isolated from PSNL were digested with trypsin, and the surface protein ''shaved" exosomes or samples in which the exosome structure was disrupted by detergent treatment were ineffective on formalin-induced response. Proteome analysis identified 736 proteins from whole exosomes derived from mouse serum, and 38 of them showed a 1.5-fold or greater increase in expression in the PSNL compared to the sham-operated. Among them, complement C5 performed that the majority in the sham-operated group was intrinsic to the exosomes, whereas the wild-type PSNL group increased the amount expressed on the membrane. Furthermore, at doses in which formalin-induced nociceptive behavior was enhanced in exosomes derived from serum from wild-type PSNL mice, nociceptive behavior was not exacerbated in exosomes derived from complement C5 deficient mice serum. Finally, pre-treatment of normal mice with the antagonist of complement C5a receptor (PMX53) antagonized the aggravation of nociceptive stimulation by exosomes derived from the serum of wild-type PSNL mice.
CONCLUSIONS: complement C5, located on the exosomal membrane derived from PSNL mouse serum cleaves to C5a in the spinal subarachnoid space and plays an important role in potentiating formalin-induced nociceptive behavior via the C5a receptor.

PEA-OXA AMELIORATES ALLODYNIA, NEUROPSYCHIATRIC AND ADIPOSE TISSUE REMODELLING INDUCED BY SOCIAL ISOLATION

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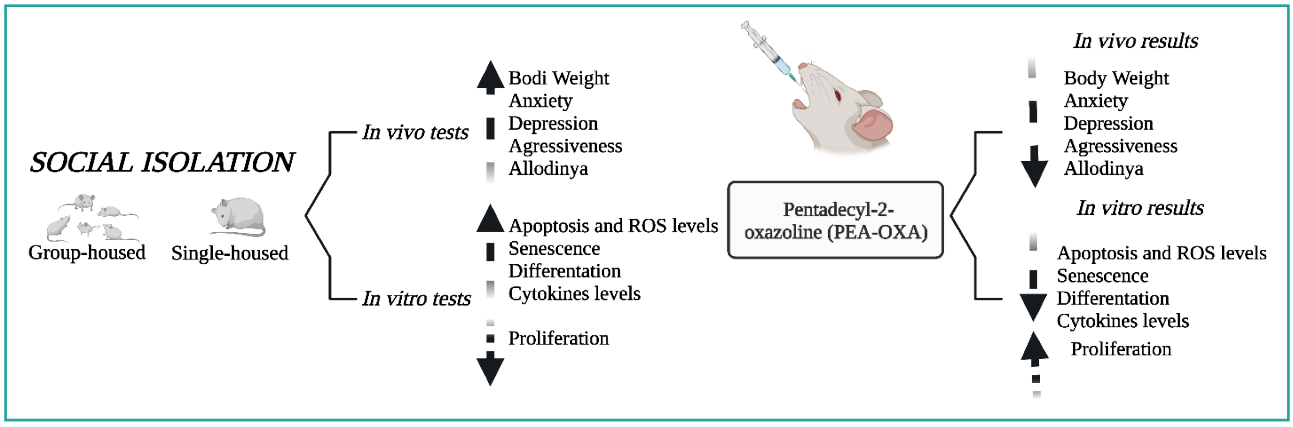
OBJECTIVE: chronic social isolation generates a persistent state of stress associated with obesity along with some neuro-endocrine disorders and central behavioural sequelae (*e.g.*, anxiety, depression, aggression, and allodynia). In this study, we evaluated the effect of social isolation on body weight, depressive- and anxious-aggressive-like behavior, as well as on phenotypic changes of adipocytes from visceral adipose tissue of control (group-housed) or socially isolated (single-housed) male mice. The effect of treatment with pentadecyl-2-oxazoline (PEA-OXA), a natural alpha2 antagonist and histamine H3 protean partial agonist, on these alterations was also evaluated.
MATERIALS AND METHODS: single or group-housed mice treated with vehicle or PEA-OXA underwent body weight, mechanical allodynia, social, anxious-, depressive- and aggressive-like behavior measurements. Proliferation rate, apoptosis, senescence, expression of fat lineage genes, lipid droplets and proinflammatory cytokines were measured on white adipose tissue adipocytes from group- or single-housed mice.
Results: single housed mice developed weight gain, mechanical allodynia at the von Frey test, aggressiveness in the resident intruder test, depression- and anxiety-like behavior in the tail suspension and hole drop tests, respectively. Social recognition memory was also impaired, suggesting cognitive impairments. Single housed mice receiving PEA-OXA showed a general resolution of both, physical-metabolic and behavioural alterations associated with social isolation. Furthermore, adipocytes from the adipose tissue of socially isolated mice showed an evident inflamed phenotype (*i.e.*, a reduced rate of proliferation, apoptosis, senescence, and ROS hyper-production together with an increased expression of IL-1β, IL-10, IL-17, and TNF-α and a decrease of IL-6). The treatment with PEA-OXA on adipocytes from single housed mice produced a protective/anti-inflammatory phenotype with an increased expression of brown adipose tissue biomarker.
CONCLUSIONS: this study confirms that persistent stress caused by social isolation predisposes to obesity and neuropsychiatric disorders. PEA-OXA, through its multi-target activity on alpha2 adrenoceptor and histamine H3 receptors, which have recently aroused great interest in the neuropsychiatric field, reduces weight gain, systemic pro-inflammatory state, allodynia, and affective disorders associated with social isolation.

Graphical abstract



NEW TECHNOLOGIES UNVEIL FUTURE RESOURCES FROM THE GLORIOUS PAST OF THE NATURAL SUBSTANCES

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Nature, the master of craftsman of molecules created almost an inexhaustible array of molecular entities. It stands as an infinite resource for drug development, novel chemotypes and pharmacophores, and scaffolds for amplification into efficacious drugs for a multitude of disease indications and other valuable bioactive agents. Although the use of bioactive natural products as herbal drug preparations dates back hundreds, even thousands, of years ago, their application as isolated and characterized compounds to modern drug discovery and development started only in the 19th century. It has been well documented that natural products played critical roles in modern drug development, especially for antibacterial and antitumor agents. A huge number of natural product-derived compounds in various stages of clinical development highlighted the existing viability and significance of the use of natural products as sources of new drug candidates.

Natural products discovered so far have played a vital role in improving the human health and have been the drugs of choice despite facing a tough competition from compounds derived from computational and combinatorial chemistry, due to their safety and efficacy. The most striking feature of natural products in connection to their long-lasting importance in drug discovery is their structural diversity that is still largely untapped.
Revitalization of the natural products is bringing newer challenges with respect to quality control and standardization along with cost effectiveness. The renewed interest in the development of natural products requires the confluence of the modern techniques and harmonization of regulations related to their research and development between various fields of science.

THE AMYLOID PRECURSOR PROTEIN PHOSPHORYLATION AT THE TYROSINE-682 RESIDUE AS DRUGGABLE SIGNATURE IN ALZHEIMER DISEASE

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OBJECTIVE: Alzheimer's disease (AD) is ranked the sixth leading cause of death among elderly in Italy and accounts for 4.3% of total deaths. Concerningly, there are currently no biomarkers for the early detection nor accurate diagnosis for the preclinical stages of AD. As such, the identification of new therapeutic targets that can be pharmacologically targetable is crucial for the development of new potentially effective therapy options for AD.
We previously demonstrated that the excessive phosphorylation of the amyloid precursor protein (APP) Tyr682 residue on the 682YENPTY687 motif precedes amyloid β accumulation and leads to neuronal degeneration. Consistently, APP pTyr682 increases in neurons or fibroblasts from AD patients. In addition, Fyn tyrosine kinase (TK) elicits APP phosphorylation at the Tyr682 residue (pTyr682) and Fyn TK Inhibitors prevent APP pTyr682 and neuronal degeneration in human neurons from AD patients.
We here investigated whether APP pTyr682 status changes in monocytes from patients with AD using a tandem mass spectrometry procedure.
MATERIALS AND METHODS: peripheral blood was collected from 130 subjects: 33 healthy volunteers and 90 patients, 30 with a diagnosis of subjective cognitive impairment, 30 mild cognitive impairment and 37 AD. Aβ42/40 ratio as well as TNFα levels were assessed in the plasma of these subjects. APP pTyr682/Tyr682 levels were quantified in monocytes from 10 subjects using tandem mass spectrometry procedure.
RESULTS: APP pTyr682/Tyr682 ratio increases in the peripheral blood monocytes of 5 out of 7 AD patients analyzed, but not in the 3 healthy volunteers. Higher TNFα and Aβ42/40 levels were found in the plasma from 35 AD patients when compared to 33 healthy subjects.
CONCLUSIONS: changes in the levels of APP Tyr682 phosphorylation might become a new peripheral blood biomarker in AD patients.

THE COMPLEX MODE OF ACTION OF ANTI-NMDA AND ANTI-AMPA ANTIBODIES IN THE CNS: TRANSLATIONS TO CLINICAL DISORDERS

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OBJECTIVE: in recent years evidence emerged that a variety of patients with diverse neuropsychiatric phenotypes carry autoantibodies directed against receptors in the central nervous system. Among the possible candidates, NMDA receptor and AMPA receptor subunits emerged as preferential immunogenic proteins able to induce the production of antibodies that impact differently the insertion of the cognate receptors in plasmamembranes, also altering their functions in controlling the glutamatergic pathways in the central nervous system (CNS).
First evidence proved the main role of autoantibodies recognizing the Glu1 and the GluN2 subunits in the development of encephalitis and convulsive symptoms. Soon later autoantibodies recognising the GLUA2 and the GLUA3 subunits of the AMPA receptors came to the attention of researchers for their participation to the course of frontal cortical dementia and in general to loss of cognitive and memory ability.
As to the impact of the autoantibodies recognizing the NMDA receptor subunits on central transmission, incubation of cultured hippocampal neurons with either commercial antibodies recognizing the GLUN1 and the GLUN2A and B subunits or sera from patients suffering from immune-mediated encephalitis causes a significant internalization of the NMDA receptors, subtracting them to synaptic connectivity. Differently, antiGluA2 and anti-GluA3 autoantibodies stabilize the AMPA receptors in plasma membranes then prolonging their activity, but the sera of patients affected by fronto-temporal dementia having a high titre of circulating anti-GluA autoantibodies reduced the central AMPA-mediated signalling.
MATERIALS AND METHODS: by using purified isolated nerve endings (the synaptosomes) we investigated the impact of commercially available antibodies raised against the external sequences of the GluN subunits (namely the GluN1, the GluN2a and B, the Glun3 subunits) and of the GluA subunits (*i.e.*, the GluA1 to 4 subunits) on the NMDA and the AMPA receptor-mediated control of glutamate exocytosis.
RESULTS: our results confirmed that anti-GluN antibodies silence the NMDA-mediated facilitation of glutamate release by increasing the internalization of the receptor proteins within the synaptosomes particles. Differently, incubation of synaptosomes with anti-Glua2 and anti-GluA3 antibodies significantly reinforced the AMPA evoked release of glutamate from cortical synaptosomes, while the anti-Glua1 and 4 antibodies were inactive. Antibody-evoked facilitation of the AMPA-evoked glutamate release was dependent on a reduced internalization of the AMPA receptors, due to its stabilization in plasmamembranes but also to an increased insertion of the GluA3 subunits. Lastly, the exposure of synaptosomes to the sera of patients suffering from fronto-temporal dementia that were titred for their content in anti-GluA3 antibodies caused a significant reduction, instead of a potentiation, of the AMPA-evoked glutamate exocytosis. The possible mechanism underlying the apparent discrepancy between the impact of anti-GluA antibodies and that elicited by sera enriched in anti-GluA3 subunits still is matter of debate.
CONCLUSIONS: the data obtained with synaptosomes suggests that synaptosomes could provide a useful experimental approach to verify the impact of autoantibodies on the insertion and the efficiency of NMDA and the AMPA receptors in synaptic boutons. Furthermore, the antibody-mediated control of the receptor-mediated transmitter exocytosis from these particles is predictive of the participation of the targeted subunit in the subunit assembly involved in the receptor expression, indirectly allowing the pharmacological characterization of the subunit composition of these receptors. This "immunopharmacological approach" would improve our knowledge of the role of AMPA and NMDA receptors in central autoim-

mune disease as well as of their pharmacological characterization bypassing the problem due to the lack of selective subunit ligands.

NEURAL-DERIVED EXTRACELLULAR VESICLES (nEVs): A NOVEL APPROACH TO IDENTIFY BRAIN ALTERATIONS LEADING TO ALZHEIMER DISEASE

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OBJECTIVE: intellectual disability, accelerated aging and early-onset Alzheimer's disease (AD) are pathological features of Down syndrome (DS). Brain insulin resistance is a key AD neuropathological hallmark, and our group reported the accumulation of brain insulin resistance markers in post-mortem brain from adult DS individuals even before AD development. However, no reliable diagnostic tools for identifying such alterations in living subjects are available. Plasma-resident neuronal-derived extracellular vesicles (nEVs) show great potential in that regard. Indeed, increased markers of brain insulin resistance in nEVs predict the development of AD in elderly individuals. While growing research aims at the identification of molecular pathways underlying the aging trajectory and AD development in DS population, data young DS individuals are missing.
MATERIALS AND METHODS: Neuronal-derived extracellular vesicles (nEVs) were isolated form healthy donors (HD, n = 17) and DS children (n = 18) aging from 2 to 17 years, who underwent medical history collection, clinical work-up and fasting blood sampling for routine biochemistry and experimental evaluations. nEVs content was interrogated for markers of insulin/mTOR pathways. Moreover, levels of proteins regulating synaptic plasticity mechanisms (syntaxin-1A, PSD95 and CamKIIα) were evaluated. The whole set of measured mediator signals was used to build a multivariate classification model, with the aim of evaluating whether they could provide a differentiation between controls and participants with DS.
RESULTS: nEVs isolated from DS children were characterized by a significant increase of pIRS1Ser636, a marker of insulin resistance, and the hyperactivation of the Akt/mTOR/p70S6K axis downstream from IRS1, likely driven by the higher inhibition of PTEN. High levels of pGSK3βSer9 were also found. No significant differences were observed for synaptic proteins levels between DS and controls. Remarkably, a negative association between pCamK IIα^{Thr286} and both pAkt^{Ser243} and pmTOR^{Ser2448} in HD but not in DS individuals was observed, suggesting that the crosstalk between the insulin/mTOR pathway and CaMK IIα may be disrupted in DS, thus contributing to intellectual disability.
CONCLUSIONS: the alteration of the insulin signalling/mTOR pathways represents an early event in DS brain and likely contributes to the cerebral dysfunctions and intellectual disability observed in this unique population. Persistence of brain insulin resistance throughout the life triggers early AD development in DS.

NEUROPHARMACOLOGY OF BERGAMOT ESSENTIAL OIL FOR CLINICAL APPLICATION

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OBJECTIVE: neuropharmacological studies have recently demonstrated that bergamot essential oil (BEO) produces remarkable neurobiological activities in animals and humans. The purpose of the present work was to examine the efficacy of BEO against nociception and allodynia in animal models of acute and chronic pain.
MATERIALS AND METHODS: to investigate pain-relieving activity of BEO, capsaicin and formalin were used to evoke acute- and acute/persistent-nociception in mice, respectively. The mouse was injected capsaicin (1.6 μg) or formalin (2.0%) into the plantar surface of right hindpaw. Licking/biting behaviour induced by intraplantar (i.plant.) injection of capsaicin and formalin was measured for a period of 5 min and 30 min, respectively. Antiallodynic effect of BEO was examined in the mouse partial sciatic nerve ligation (PSNL) model. The spinal activity of extracellular signal-regulated protein kinases (ERKs, ERK-1 and ERK-2) was analyzed by western blotting.
RESULTS: the nociceptive behavioural response evoked by capsaicin and formalin was inhibited significantly by i.plant. injection of BEO. The antinociceptive effects were antagonized by pretreatment with naloxone hydrochloride and naloxone methiodide. The mechanical allodynia was observed within 3 days after the surgery, peaked at day 7 and lasted for 3 weeks. The PSNL produced a significant increase in the spinal expression of ERKs at day 7. Pretreatment with β-funaltrexiamine (β-FNA), a selective μ-opioid receptor antagonist, reversed antiallodynic effect of BEO. In western blotting analysis, BEO reduced the increase of phosphorylated ERKs in mice with PSNL, which was antagonized by β-FNA. The opioid receptor antagonists for δ and κ receptors, were ineffective on BEO-induced antiallodynic activity. In addition, BEO-induced antiallodynia was reversed by pretreatment with antisera against β-endorphin.
CONCLUSIONS: preclinical pharmacological data demonstrates that BEO-induced analgesia is mediated by peripheral opioid-receptors and associated with a reduction of spinal ERK activation. The injection of BEO may stimulate a release of β-endorphin from keratinocyte in the epidermis of hindpaw, which act at local neuronal μ-opioid receptors to inhibit nociception. Peripherally acting BEO may represent a promising therapeutic approach for the treatment of neuropathic pain conditions.

2-PENTADECYL-2-OXAZOLINE PREVENTS COGNITIVE AND SOCIAL BEHAVIOUR IMPAIRMENTS IN THE AMYLOID β-INDUCED ALZHEIMER-LIKE MICE MODEL: BRING THE α2 ADRENERGIC RECEPTOR BACK INTO PLAY

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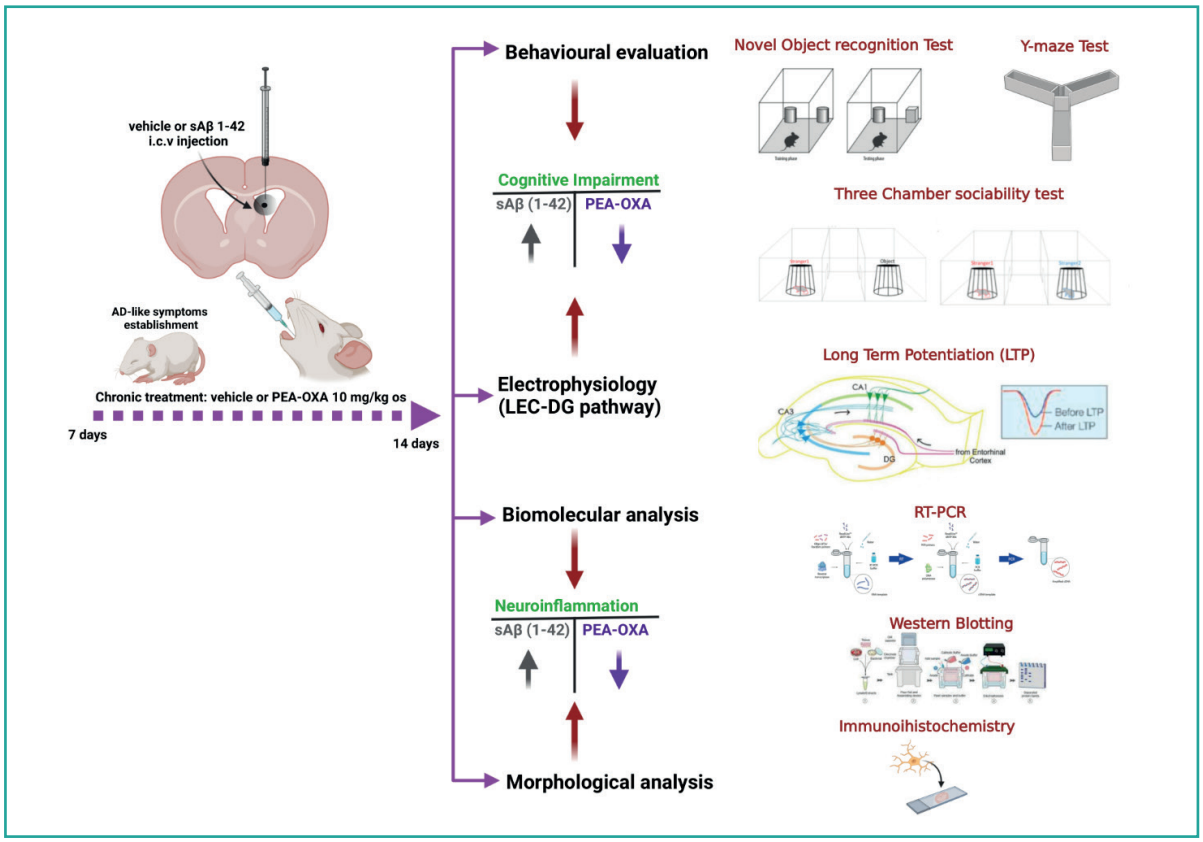
OBJECTIVES: the 2-pentadecyl-2-oxazoline (PEA-OXA) is a natural compound with protective action in neuro-inflammation. We have previously shown that PEA-OXA behaves as an α2 adrenergic receptor (α2AR) antagonist and a putative protean agonist on histamine H3 receptors. Recently, neuroinflammation and monoaminergic neurotransmission dysfunction has drawn particular attention in Alzheimer Disease (AD) pathophysiology. In this context, the objective of this study was to investigate the effects of the dual-acting PEA-OXA in an AD-like model in mice.
MATERIALS AND METHODS: a combined computational and experimental approach was used to evaluate the ability of PEA-OXA to bind α2A-AR subtype, and to investigate the effects of PEA-OXA treatment on neuropathological (behavioural and functional) effects induced by soluble Amyloid β 1-42 (sAβ 1–42) intracerebroventricular injection.
RESULTS: computational analysis revealed the PEA-OXA ability to bind the α2A-AR, a pharmacological target for AD, in two alternative poses, one overlapping the Na^+ binding site. *In vivo* studies indicated that chronic treatment with PEA-OXA (10 mg/kg, os) restored the cognitive (discriminative and spatial memory) deficits and social impairments induced by sAβ injection. Consistently, electrophysiological analysis showed a recovery of the long-term potentiation in the hippocampus (Lateral Entorhinal Cortex-Dentate Gyrus pathway), while neuroinflammation, *i.e.*, increased pro-inflammatory cytokines levels and microglia cells density were reduced.
CONCLUSIONS: these data provide the basis for further investigation of the pro-cognitive aptitude of PEA-OXA by proposing it as an adjuvant in the treatment in AD, for which the available pharmacological approaches remain unsatisfactory. Moreover, this study offers new future direction in research investigating the role of α2AR in neuropsychiatric illness and therapies.

Graphical abstract



EFFECTS OF CHRONIC TREATMENT WITH A PHYTOSOMAL PREPARATIONS CONTAINING *CENTELLA ASIATICA L.* AND *CURCUMA LONGA L.* ON COGNITIVE PERFORMANCE IN RATS: INVOLVEMENT OF NEUROPLASTIC MECHANISMS

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BACKGROUND: it has been demonstrated that different drugs and phytochemicals lead to beneficial effects at molecular levels and improve memory and cognitive functions by targeting Bdnf machinery. Indeed, the Brain-derived neurotrophic factor (Bdnf) is the most diffuse neurotrophin, involved in several positive functions both during the development and at adulthood. Moreover, local protein synthesis is a very complex and fine-regulated mechanism, associated with synaptic plasticity and memory.
MATERIALS AND METHODS: a phytosomal preparation containing *Centella asiatica L.* (Gotu kola, Asiatic pennywort) and *Curcuma longa L.* (Turmeric) (50 mg/kg or 250 mg/kg) was administered for 10 days to naïve rats and Bdnf expression and its high-affinity receptor TRKB were evaluated. Furthermore, we assessed whether the increase in Bdnf signaling was paralleled by an upregulation of *de novo* protein synthesis by focusing on the prefrontal cortex.
RESULTS: we provide evidence that the chronic administration of the phytosomal preparation containing *C. asiatica* and *C. longa* positively affects neuroplastic mechanisms by increasing the expression of Bdnf and the activation of the downstream pathways via TRKB receptor. Moreover, we observed that the increase of the neurotrophin is paralleled by changes in the translation machinery with an enhanced activation of proteins involved in cognitive and memory processes.
CONCLUSIONS: our data support the use of phytosome preparation in ameliorating brain plasticity in the PFC and despite the necessity of more detailed analyses and clinical trials, the use of this phytosomal preparation could be used as supportive therapy in BDNF-impaired subjects characterized by memory and cognitive dysfunctions such as elderlies and patients with Alzheimer's disease.

EFFECT OF CANNABIDIOL ON HUMAN POLYMORPHONUCLEAR LEUKOCYTES: RELEVANCE FOR NEUROINFLAMMATION AND NEUROPATHIC PAIN

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OBJECTIVE: cannabidiol (CBD), the main non-psychoactive component of cannabis plants (*Cannabis sativa L.*), is increasingly considered as an effective treatment for inflammatory disorders of the nervous system, including multiple sclerosis, Alzheimer disease and more others. CBD is effective on several neuroinflammation-associated symptoms such as pain and spasticity, possibly due to its anti-inflammatory and immunomodulating properties. In depth characterization of the relevant targets acted upon by CBD will greatly help to fully exploit its therapeutic potential.
Neuroinflammation is due to a complex interplay between peripheral immunity and central nervous system-resident immune cells, ultimately leading to neuronal damage. Among peripheral immune cells, human polymorphonuclear leukocyte (PMN) are rapidly recruited to the sites of injury in peripheral and central nervous system damage. In previous studies, we have extensively characterized the ability of CBD to inhibit functional responses of PMN to proinflammatory stimuli.
In animal models of neuropathic nerve injury circulating PMN exhibit a primed profile, resulting in a significant increase in their response to activating stimuli. Primed PMN are critical for PMN-mediated tissue injury in many different diseases.
AIMS: to investigate, in isolated human PMN, the ability of CBD to counteract priming-enhanced responses. To this end, we measured apoptosis, cell migration, reactive oxygen species (ROS) generation, and adhesion molecules expression, all steps critically involved in tissue invasion and inflammatory cascade activation.
MATERIALS AND METHODS: experiments were performed in human PMN isolated from buffy coats of healthy donors as previously described. PMN priming was induced by LPS (100 or 1000 ng/ml, as appropriate for each model). Cells were cultured under resting or primed conditions and finally ROS generation was measured by spectrofluorimetric analysis, cell migration through a microscopic evaluation and apoptosis and adhesion molecule expression by cytofluorimetric assay.
RESULTS: CBD (0.1-1 μM) was unable to affect all the cell functions tested under resting conditions. In all the tested conditions, CBD (0.1-1 μM) was unable to affect apoptosis in primed PMN, while on the contrary priming-induced ROS generation was significantly prevented by CBD, although the statistical significance was obtained only with CBD 1 μM. Primed induced adhesion molecules expression was not affected by CBD in the whole concentration range tested. Finally, fMLP-induced cell migration was significantly enhanced by LPS priming and CBD concentration-dependently reduced the effect of priming (0.1-1 μM), reaching the statistical significance at 0.1 μM.
CONCLUSIONS: the present study shows that CBD extensively affects human PMN functions in a safe range of concentrations. We have extended our previous observations showing that CBD not only affect some PMN key functions, but also that CBD is able to prevents priming-induced effects on these functions (migration and ROS generation), although without affecting priming-induced adhesion molecule expression.
It will be of extreme interest to investigate if the *in vitro* observed effects will be maintained *in vivo* in diseases in which PMN priming play a pivotal role (*e.g.*, neurodegenerative diseases, peripheral inflammatory diseases). Studies in patients can provide data to achieve additional information's about the possibility to use of this safe product in different pathological conditions including inflammation-related pain or other diseases both CNS and in the periphery in which pain and inflammation play a key role.

DEMENTIA THERAPEUTICS: BIOTECH DRUGS

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OBJECTIVE: Alzheimer's disease (AD) is the most prevalent type of age-associated dementia in the world. The main pathological

features consist of amyloid-β (Aβ) plaque deposits and neurofibrillary tangles constituted of hyperphosphorylated tau protein. Up to now, only a few AD treatments approved have been approved by regulatory agencies, but the majority are limited for partial symptomatic relief to patients and are not able to modify the disease progression.
The main focus of this review is to summarize recent evidence on the mechanism, therapeutic effects and clinical trial results of immunotherapy against amyloid-beta (Aβ) compounds in patients with AD, mainly focusing on the impact of aducanumab and lecanemab, as well as other antibodies targeting Aβ, on AD pathology and clinical manifestations.
MATERIALS AND METHODS: a literature search of MEDLINE/PubMed and a search of ClinicalTrials.gov were conducted through last years.
RESULTS: all compounds analysed are safe and passed the Phase 1. In Phase 2, the biological effect has been proven, *i.e.*, decreased brain Aβ load at Amy-PET. In Phase III trials, clinical outcomes have been evaluated, leading to many trial failures. Reasons for this are the late treatment and the lack of biomarkers for patient inclusion. In light of the above, more recent clinical trial design has been modified including patients with prodromal AD, leading to promising results in terms of safety, positive effects on decreasing brain Aβ levels and improving cognitive impairment.
CONCLUSIONS: the novel highly specific antibodies targeting Aβ as disease modifying therapies for AD have been developed and tested in several clinical trials. In particular aducanumab and lecanemab showed beneficial effects. However, the efficacy of these compound in patients is still not certain and there are many open questions to be answered.
Lastly, disease modifying interventions with antibodies for AD must be performed early, otherwise treatment will be likely not effective due to massive neuronal loss.

TRANSCRIPTION FACTORS GENE EXPRESSION IN CD4+ T-CELL FROM ALZHEIMER'S DISEASE PATIENTS: A PILOT STUDY

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OBJECTIVE: in recent years neuroinflammation has been hypothesized as an important contributing factor of Alzheimer's Disease (AD) pathogenesis. Several evidences suggest that adaptive immune system plays a key role into neuroinflammation in neurodegenerative disorders, such Parkinson's Disease. Based on these assumptions, we have designed a case-control pilot-study, with the aim of identifying biomarkers of immune dysregulation in AD, by evaluating the expression of CD4+Tcells transcriptional-factors and pro/anti-inflammatory cytokines profile, in patients with Mild-Cognitive-Impairment (MCI) and AD, compared with No-Cognitive-Impairment (NCI) subjects.
MATERIALS AND METHODS: between December 2019 and October 2021, we recruited subjects, selected from Centre-for-Cognitive-Disorders of Neurology-Department, ASST-Settelaghi-Hospital, enrolled in MCI-group with a MMSE > 24 and CDR: 0.5 and AD-group with a MMSE: 20-24 and CDR: 1. The NCI group were healthy subjects with MMSE > 27 and CDR: 0, matched by age, sex, and schooling. During the enrolment visit, data concerning demographic (age, sex, schooling, BMI), clinical-anamnestic (hypertension, diabetes, oncological disorder, heart disease, dyslipidemia) and instrumental (brain CT/brain MRI/brain FDG-PET/amyloid PET only for MCI and AD group) variables were recorded and blood samples were collected for CD4+Tcells isolation and for blood cytokines (TNF-α, IL-10, IFN-γ, IL-6) determination. CD4+Tcells were processed for identification of transcriptional-factors-mRNA-levels (Nurr1/RORγC/GATA3/Tbet1/FoxP3/STAT1/STAT3/STAT4/STAT6). Demographic, clinical, instrumental and laboratory data were analysed. Statistical significance of the difference among groups was analysed by means of Student's t-test and the chi-q test for continuous and categorical variables.
RESULTS: we have enrolled 50 subjects: 25-NCI-group, 9-MCI-group and 16-AD-group, homogeneously distributed for anamnestic and demographic variables. In terms of "gene-pattern" of CD4+Tcells, Tbet1 transcriptional-factor, related to Th1 differentiation, specifically differentiated MCI-group from AD and NCI-groups ($p = 0.02$). No differences were found in TNF-α, IFN-γ, IL-10 and IL-6 plasma levels among the three groups. Examining the correlation between the presence of microvascular encephalopathy, known to be a predisposing neuroinflammatory condition, in MCI and AD group (by brain CT/MRI) and the specific "gene-pattern" of CD4+Tcells, in MCI with microvascular encephalopathy Nurr1, related to Treg cells-lineage, was less expressed ($p = 0.02$), while STAT4, related to Th1-lineage, was more expressed ($p = 0.03$). No differences emerged in Tbet1 expression ($p = 0.12$). A final sub analysis was conducted on brain FDG-PET pattern and peripheral lymphocytes profile of MCI and AD group. In MCI-group Nurr1 was less expressed in "bilateral frontal-parietal-temporal-occipital-hypometabolism" ($p = 0.02$). In the AD-group "bilateral-temporo-parietal-profile" was related with an increased Nurr1,GATA3,STAT1,STAT4 expression ($p < 0.05$). In "bilateral-frontal-parietal-temporal-occipital-hypometabolism" there was a reduced RORγC and STAT-4 expression ($p < 0.,05$). In "bilateral-frontal-parietal-temporal-hypometabolism" there was a STAT-1 reduction ($p < 0.05$).
CONCLUSIONS: MCI shows an inflammatory state, that is no longer detectable in NCI and AD. The higher expression of Tbet1 in MCI-group is not related to higher incidence of associated vascular-encephalopathy, but to the presence of AD itself. The most-diffuse-brain-AD-hypometabolism showed a reduced expression of transcriptional-factors, implied in pro-inflammatory immune response. A less-advanced-AD-hypometabolism showed an increased expression of transcriptional-factors, implied in anti-inflammatory immune response. These data confirm the hypothesis that a pro-inflammatory process could be exist in an early Alzheimer's Disease stage and gradually it could be decrease in advanced stages.

DEMENTIA THERAPEUTICS AND COGNITIVE REHABILITATION

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OBJECTIVE: it is well known that patients with Alzheimer's disease (AD) have an increased risk of spontaneous recurrent seizures (SRSs) and epilepsy. Similarly, people with epilepsy (PWE)

display an augmented risk of cognitive impairment. To date, the correlation between epilepsy and cognitive deficit still needs to be fully clarified, solving substantial doubts about if epilepsy causes dementia or *vice versa* and if shared mechanisms underlie both pathologies.
MATERIALS AND METHODS: here, we review evidence indicating that patients with AD could present subclinical epileptiform discharges, which in turn, can hasten cognitive decline and worsen the quality of life in these patients. Targeted pharmacological intervention to decrease anomalous network hyperexcitability could represent a valid therapeutic strategy to delay the onset of later neurodegenerative alterations and consequential cognitive deficits by several years in patients. A prompt diagnosis and management of seizures in patients with AD should be practiced. In this context, no guidelines are available for epileptic activity treatment.
CONCLUSIONS: randomized clinical studies are needed not only to update clinicians about the symptomatic treatment of seizures in AD patients but also to recognize if treatment with antiseizure medications (ASMs) might have disease-modifying properties. Furthermore, it will be crucial to increase the use of experimental models of AD to comorbid conditions, including epilepsy, both to uncover the processes underlying seizure onset and to describe their role in cognitive impairment. These models might also be suitable for discovering pharmacological compounds that are therapeutically effective and reliable early biomarkers for SRSs in AD.

IMAGING OF DEMENTIA AND ARTIFICIAL INTELLIGENCE

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Dementia is one of the major causes of disability and dependency in older people worldwide, affecting an estimated 50 million people, according to the World Health Organization. As the global population ages, this number is expected to rise to 150 million by 2050 as life expectancy increases.
Early recognition of preclinical and prodromal stages of dementia (Mild Cognitive Impairment, MCI) is of crucial importance to take preventive actions and appropriate treatments that may delay or improve symptoms or the transition to dementia.
Unfortunately, early diagnosis of dementia is a rather complicated process, relying mainly on the patient's clinical data, while traditional imaging plays a secondary role, lacking in the early stages of obvious degenerative changes that only manifest in advanced disease. The main changes consist primary of selective atrophies and focal perfusion/metabolic deficits.
The latest imaging techniques such as fMRI/PET/SPECT can not only identify degenerative changes, secondary to hypometabolism, amyloid plaques, and atrophy, but also provide quantitative information on certain biomarkers, which may suggest the diagnosis and prognosis of a particular disease.
In recent years, artificial intelligence (AI) is taking on an increasingly prominent role in the field of neuroimaging, aimed at researching models and algorithms that support the radiologist's decision making in clinical and research settings.
One of the areas that has been the immediate and most frequent target of AI applications is dementia, with a focus on Alzheimer's disease, consequently leading to a significant acceleration of research in this field.
Indeed, the high prevalence of neurodegenerative diseases has allowed the creation of large publicly accessible neuroimaging databases, the largest of which is the Alzheimer's Disease Neuroimaging Initiative (ADNI).
The existence of these freely accessible databases has attracted great interest in the scientific community dealing with artificial intelligence.
AI techniques have found special application in the field of dementia whose diagnosis consists of looking for subtle anatomical or functional changes that are often difficult to detect and combining multiple biomarkers, both clinical and imaging. Of particular interest is the application of AI in predictive diagnostics of dementia.
Several Machine Learning (ML) and deep learning (DL) techniques, using Artificial Neuronal Networks (ANNs), have been proposed as useful aids in the diagnosis of dementia. These techniques showed good potential in enabling classification of various dementias, recognition of underlying causes of various neurological disorders with cognitive impairment, and thus valuable support for early diagnosis.

IMAGING BIOMARKERS FOR ALZHEIMER'S DISEASE AND GLAUCOMA: CURRENT AND FUTURE PRACTICES

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OBJECTIVE: glaucoma is the most important cause of global blindness. Intraocular pressure (IOP) is the major risk factor for the disease. However, other unrelated factors have been correlated with the risk of development and progression of the disease. Glaucoma, in fact, is a complex disease, and it has been linked with other neurodegenerative disorders such as Alzheimer's disease (AD). Nowadays, the diagnosis of glaucoma only relies on clinical aspects of the optic nerve, visual field test, and optical coherence tomography. Though the multidisciplinary aspect of the disease suggests that other biomarkers may be useful for the diagnosis, thus underling the importance of novel imaging techniques supporting clinicians.
MATERIAL AND METHODS: we evaluated the frequency of glaucoma-like alterations in a group of patients with AD using diagnostic criteria based on Frequency Doubling Technology perimetry and Heidelberg Retinal Tomography-3. Then we evaluated glaucomatous patients using advanced neuroimaging techniques searching for degenerative changes in the visual pathway. In particular, we employed Diffusion Kurtosis Imaging (DKI) to evaluate normal-appearing white matter (NAWM) changes in patients with primary open-angle glaucoma (POAG) as compared with healthy controls in a regionally unbiased, voxel-wise manner. Then, POAG patients were examined using resting-state functional magnetic resonance imaging (rs-fMRI) which is commonly employed to study changes in functional brain connectivity, by combining multi-shell diffusion-weighted imaging, multi-shell, multi-tissue probabilistic tractography, graph theoretical measures and a recently designed 'disruption index', which evaluates the global reorganization of brain networks.
RESULTS: the evidence of a potential connection between AD and glaucoma emerged because patients with AD, despite lower mean IOP, showed a fivefold increase in glaucoma compared to controls. Remarkably, the neuroimaging studies evaluating the modifications of the visual pathways and brain functions in glau-

comatous patients reported abnormalities in structural, ultrastructural, and functional brain networks compared to controls. In this regard, after estimating both DTI indices (mean diffusivity and fractional anisotropy), whole-brain, voxel-wise statistical comparisons were performed in white matter using Tract-Based Spatial Statistics (TBSS). We found widespread differences in several white matter tracts in patients with glaucoma compared to controls in several metrics (mean kurtosis, kurtosis anisotropy, radial kurtosis, and fractional anisotropy) which involved localization well beyond the visual pathways, and involved cognitive, motor, face recognition, and orientation functions amongst others. In addition, POAG patients showed statistically significant group-wise differences in subject-wise disruption indexes in all local metrics. Two brain regions serving as hubs in healthy controls were not present in the POAG group. Instead, three hub regions were present in POAG but not in controls. We found both global and local structural connectivity differences between POAG patients and controls, which extended well beyond the primary visual pathway and were localized in the left calcarine gyrus, left lateral occipital cortex, right lingual gyrus, and right paracentral lobule. Group-wise and subject-wise disruption indices also differed between POAG patients and controls.
CONCLUSIONS: overall, we found, in patients with POAG, a whole-brain structural reorganization that spans a variety of brain regions involved in visual processing, motor control, and emotional/cognitive functions. Interestingly, several imaging alterations found in glaucoma patients are like those found in disconnection syndromes, such as AD. This evidence supports the hypothesis of the existence of common pathological pathways bringing to the development of the two diseases. Many pathogenetic mechanisms underlie both these diseases, leading to many common biomarkers that neuroimaging techniques can detect and follow up. This also implies several potential benefits for the diagnosis and treatment of both diseases.

COGNITIVE REHABILITATION: A PRACTICAL FRAMEWORK OF ENABLEMENT FOR PEOPLE LIVING WITH DEMENTIA

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OBJECTIVE: this talk describes the development of, and evidence for, cognitive rehabilitation (CR) for people with mild-to-moderate dementia. CR is a goal-oriented personalised behavioural intervention addressing the impact of cognitive impairment on everyday functioning. CR does not set out to train cognition or improve performance on cognitive tests; rather, it uses a goal-oriented, problem-solving approach to facilitate improved management of functional disability. CR is conducted on an individual basis in the home setting, with carers fully involved and supported wherever possible. CR focuses on what is important to each person and is tailored to the person's needs through collaborative goal-setting. The main outcome is the extent to which personal goals are attained.
MATERIALS AND METHODS: we gathered initial evidence for efficacy through a series of single-case designs which provided proof of concept, and we conducted two single-site trials, one with people diagnosed with Alzheimer's disease and the other with people who had Parkinsonian dementias. We then recruited nearly 500 people living with mild-to-moderate Alzheimer's, vascular or mixed dementia to a large multi-site trial, the GREAT trial. Following this we conducted an implementation study to examine the effectiveness of CR when provided as part of routine health services rather than under trial conditions.
RESULTS: people who engaged in CR improved their functioning in relation to the personal goals targeted in the intervention. The two single-site trials demonstrated superiority of CR to both active control and treatment as usual conditions at post-intervention follow up. The GREAT trial demonstrated superiority of CR to treatment as usual at post-intervention and 6 month follow up. The same or stronger effects were demonstrated when CR was provided as part of routine health services. Based on these findings we produced an e-learning course for practitioners and co-produced a self-management resource based on CR principles for people with dementia.
CONCLUSIONS: CR can enable people with dementia to improve their functioning in relation to activities that are important and meaningful for them. Supporting functional ability can form a helpful element in post-diagnostic support and may also have worthwhile longer-term benefits.

MANAGEMENT OF NEURODEGENERATIVE LANGUAGE DISORDERS

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OBJECTIVE: there is increasing evidence to support the effectiveness of targeted speech rehabilitation therapy in primary progressive aphasia (PPA), with or without the addition of non-invasive brain stimulation. For example, in the nonfluent variant (nfvPPA), motor speech disorder and agrammatism have shown positive effects to structured oral reading task training and VISTA (video-implemented script training for aphasia) therapy. In addition, when coupled with speech therapies, transcranial direct current stimulation (tDCS) has demonstrated improvements in various speech and language performances in all variants of PPA.
MATERIALS AND METHODS: review of the available evidences about PPA management; meta-analysis of effectiveness of language training and non-invasive brain stimulation on oral and written naming performance in PPA.
RESULTS: the available evidence supports the effectiveness of neuromodulation techniques associated with speech and language rehabilitation (SLR) in PPA patients. The results are however heterogeneous and prediction of outcome at the individual subject level is not presently possible.
CONCLUSIONS: management options need to be offered to PPA patients through the organization of a network of specialized centers providing neuromodulation, SLR and telemedicine support. At the research level, an increased understanding of the mechanisms responsible for the effects of non-invasive neuromodulation techniques in Primary Progressive Aphasia (PPA) on the large-scale language networks of the human brain is a necessary step for the prediction of individual response to neurostimulation and for the development of a precision-medicine approach based on personalized neuromodulation protocols.

TARGETING PAIN TO CONTROL NEUROPSYCHIATRIC SYMPTOMS OF DEMENTIA: THE NEED FOR TAILORED CLINICAL TRIALS

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OBJECTIVE: in the last decades improved social conditions increased life expectancy and, consequently, the impact of age-related neurodegenerative diseases such as dementia. In fact, it is estimated that some 55 million people are affected by dementia and this number is going to triple by 2050. Despite recent developments in biotechnology, we still do not have any disease-modifying drugs. Aside cognitive impairment, after the diagnosis or even before the onset some 97% of patients develop neuropsychiatric symptoms (NPS) remarkably reducing quality of life. Among NPS depression is tightly linked to dementia through a phenomenon of reverse causation and agitation is on of the symptoms most resistant to treatment. The therapy of NPS consists in the use of atypical antipsychotics, often off-label and increasing of 1.6-1.7-fold the risk of death for cardiocerebrovascular accidents. One of the most important triggers of agitation during severe dementia is unrelieved pain because of lack of self-reporting.
MATERIALS AND METHODS: over the years various drugs with the most disparate mechanisms of action were tested for the therapy of NPS, from classic and atypical antipsychotics to antidepressants such as citalopram and escitalopram, up to anticonvulsants and mood stabilizers such as carbamazepine, gabapentin and valproic acid. However, a safe and effective treatment is not available. Here we propose the multi-step process that we implement to obtain this purpose.
RESULTS: the investigation of the treatment of pain and NPS in community-dwelling patients suffering from dementia was evaluated for the first time on a wide real-world sample. The results demonstrate poor treatment of chronic and, particularly, neuropathic pain, with underdiagnosis of dementia and off-label elevated use of antipsychotics and antidepressants. Since pain assessment is the main barrier to pain relief in severe dementia, the validation trial (protocol No. 31/2017 approved by Calabria Region Ethical Committee) provided, for the first time, the Italian setting with I-MOBID2, a pain scale unique since it detects even concealed musculoskeletal and visceral pain, that proved to be valid and reliable and unravel pain in over a half of the cohort recruited suffering from severe dementia. 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. Moreover, in order to provide patients with a safe and effective needs-oriented and patient-centered treatment, the essential oil of bergamot (BEO) that demonstrated sound preclinical evidence of analgesic activity, was engineered in a nanotechnology delivery system to allow the study of its activity in a double-blind clinical trial. In fact, the Bergamot rehabilitation AgaINst agitation in dementia (BRAINAID) randomized, double-blind, placebo-controlled trial to assess the efficacy of furocoumarin-free bergamot loaded in a nanotechnology-based delivery system of the essential oil in the treatment of agitation in severe dementia patients (Mini-Mental State Examination ≤ 12) is registered on FDA repository (NCT04321889) and it is actually recruiting.
CONCLUSIONS: NanoBEO confirms the analgesic activity of BEO and it guarantees the titration and stability of the active principles of the phytocomplex and the consequent reproducibility of the effects. In addition, NanoBEO traps odor making double-blind study possible. The NCT04321889 BRAINAID clinical trial might provide the rationale for safer treatment of agitation and pain, and it can confirm the analgesic properties of BEO in the clinic, offering a significant improvement in the quality of life of patients with dementia. Moreover, these studies shed light on the step-by-step preclinical-to-clinical pathway followed for BEO to overcome typical biases of research in the field of essential oils to provide high certainty of the body of evidence.

IMPORTANCE OF THE AUTONOMIC AND BEHAVIORAL RESPONSE IN THE ASSESSMENT OF PAIN IN PATIENTS WITH DISORDERS OF CONSCIOUSNESS

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OBJECTIVE: the pain was defined as "an unpleasant sensory and emotional experience arising from actual or potential tissue damage or described in terms of such damage" (International Association for the Study of the Pain). However, its nature is subjective and implies that pain may only be detected when a patient reports its presence. The pain assessment in patients with disorders of consciousness is important for its possible prognostic value. However, assessing pain in these patients is challenging, and the only behavioral assessment could be no exhaustive. We aim to investigate the behavioral and autonomic response to the nociceptive stimulus to define a more effective approach to pain assessment.
MATERIALS AND METHODS: they were considered peer-reviewed publications regarding the behavioral assessment or/and autonomic nervous system (ANS) analysis in DOC patients.
RESULTS: the only behavioral assessment may produce a misinterpretation of the results. Analysis of the (ANS) response could help more effectively assess pain in these patients.
CONCLUSIONS: the combined behavioral and ANS assessment improves the detection of pain in patients with disorders of consciousness.

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AGE AND SEX-DEPENDENT DIFFERENCES IN GABA TRANSMISSION IN MICE HIPPOCAMPAL NERVE TERMINALS

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OBJECTIVE: major depressive disorder (MDD) is a psychiatric disorder characterized by depressed mood, diminished interests, impaired cognitive functions, and vegetative symptoms, such as disturbed sleep or appetite. An episode of major depression may occur only once in a person's lifetime, but it is more likely to recur throughout a person's life. The prevalence of adults with a major depressive episode was highest among individuals aged 18-25 and it occurs about twice as often in women than in men. For years, the monoaminergic system (serotonin, norepinephrine, and dopamine) has received the most attention in the neurobiology of MDD, and most therapeutic drugs have been developed to target these systems. Although the neurobiological basis remains elusive, the hypothesis emerged that altered glutamatergic and GABAergic transmissions are pivotal to the disease. While the role of glutamate transmission has been investigated, the implication of GABAergic network largely remains unknown. In this context we aim at investigating the efficiency of GABA release from nerve endings (synaptosomes) isolated from the hippocampus of 20 day postnatal, (P20), adolescent (40 day postnatal, P40), young adult (3 months old), adult (6 months old), and aged (16 months) male and female mice. In addition, biochemical analyses were performed to compare the density of several proteins involved in the production, degradation, and activity of GABA in synaptosomes isolated from the same experimental groups.
MATERIALS AND METHODS: synaptosomes were isolated from the hippocampus of mice of different ages and from both sexes. They were preloaded with [^{3}H]GABA (30 nM) and the release of the neurotransmitter was investigated with the "up-down superfusion" approach in different depolarizing conditions (12 or 20 mM KCl-enriched solution). In parallel, Western blot analysis was carried out to compare the density of the vesicular GABA transporter (VGAT) and the glutamate decarboxylase 65/67 (GAD 65/67).
RESULTS: the efficiency of [^{3}H]GABA exocytosis from hippocampal synaptosomes increases starting from 20 days postnatal and reaches the maximal level at 6 months in either male and female mice, then decreasing in older animals. Comparing the two sexes, the functional studies demonstrated that [^{3}H]GABA exocytosis in significantly higher in hippocampal synaptosomes from P40 male mice when compared to female ones but it is rapidly recovered in synaptosomes from 3 months old female mice. Western blot analysis unveiled age and sex-dependent differences in the density of VGAT and GAD 65/67 protein expression in hippocampal synaptosomes.
CONCLUSIONS: our study demonstrates the existence of sex and age-dependent changes in the hippocampal GABAergic transmission that might have a role in dictating synaptic plasticity especially in the early developmental ages.

THE INVOLVEMENT OF TRPV2 CHANNELS IN THE NEUROPROTECTIVE EFFECTS OF CANNABIDIOL IN A MODEL OF *IN VITRO* ISCHEMIA

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OBJECTIVE: in the last few years a number of scientific studies have demonstrated a key role for cannabinoids in numerous pathological mechanisms of the central nervous system (CNS), including brain injury following cerebral ischemia. Cerebral ischemia is the second cause of death in industrialized countries with a high incidence and mortality rate. It results from a transient or permanent reduction in cerebral blood flow and restricts the delivery of substrates causing delayed neuronal loss and neurodegeneration. There are two types of ischemia: focal ischemia, which is confined to a specific region of the brain; and global ischemia, which encompasses wide areas of brain tissue. However, the role of cannabinoids in the mechanisms leading to neurodegeneration following cerebral ischemia is yet unclear. Many studies have produced conflicting results on cannabinoids putative protective and/or toxic effects.
MATERIALS AND METHODS: we investigated the effects of the selected cannabinoids, Δ^9-tetrahydrocannabinol (THC), cannabidiol (CBD), and cannabigerol (CBG) in rat organotypic hippocampal slices exposed to oxygen and glucose deprivation (OGD), an *in vitro* model of forebrain global ischemia. Cell death in the CA1 subregion of slices was quantified by propidium iodide fluorescence and morphological analysis and tissue organization were examined by immunohistochemistry and confocal microscopy.
RESULTS: incubation with THC exacerbated, whereas incubation with CBD attenuated CA1 injury induced by OGD. THC toxicity was prevented by CB1 receptor antagonists while the neuroprotective effect of CBD was blocked by TRPV2, 5-HT1A and PPARγ antagonists. Confocal microscopy confirmed that CBD, but not THC, had a significant protective effects toward neuronal damage, tissue disorganization and glia activation caused by OGD in organotypic hippocampal slices mediated, at least in part, by TRPV2 channels, since the TRPV2 antagonist tranilast blocked them. The TRPV2 expression decreased after OGD in CA1 pyramidal neurons, but it increased in activated, phagocytic microglia. CBD increased TRPV2 expression, decreased microglia phagocytosis, and increased rod microglia after OGD.
CONCLUSIONS: our results show that cannabinoids have different effects in ischemia and play different roles in the mechanisms of post-ischemic neuronal death. CBD showed neuroprotective effects while THC worsened the neurodegeneration caused by ischemia. The effect of CBD was related to its agonistic activity on the TRPV2 channel.

PRESYNAPTIC COMPLEMENT, A SYNAPTIC ORGANIZER IN THE CNS: RELEVANCE TO SYNAPTOPATHY

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OBJECTIVE: complement recently emerged as a main player in white and grey matter lesions in experimental autoimmune encephalomyelitis (EAE) mice, an animal model of multiple sclerosis. To improve the knowledge of its activity, we investigated the impact of C1q and C3 proteins in the cortex of EAE mice at the acute stage of disease as promoters of synaptic pruning and modulators of glutamate release from cortical nerve endings (synaptosomes) and astrocytic processes (gliosomes).
MATERIALS AND METHODS: synaptosomes and gliosomes from the cortex of EAE mice at 21 ± 1 day post immunization (d.p.i.) were analysed for C1q and C3 protein content, glutamate release efficiency, viability and ongoing apoptosis.
RESULTS: in EAE mice at 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 control animals, 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: our results unveil new mechanisms of actions of the complement in the CNS of EAE mice that could be relevant to disease progression and suggest new therapeutic targets for the management of MS.

HEMOKININ-1 ACTIVATES TRANSIENT RECEPTOR POTENTIAL VANILLOID 1 CHANNELS IN THE SPINAL CORD TO INDUCE NOCICEPTIVE BEHAVIORS

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OBJECTIVE: hemokinin-1, which is a homologue of substance P, mainly acts on the neurokinin-1 (NK1) receptors and is involved in pain transmission in the spinal cord. The transient receptor potential vanilloid 1 (TRPV1) channel plays a key role in pain transmission in the spinal cord. Details of pain transmission by hemokinin-1, including the involvement of TRPV1 channel, have not been explored. Here, we investigated the mechanisms that underlie hemokinin-1 induced nociceptive behaviors.
MATERIALS AND METHODS: hemokinin-1 was administered intrathecally into the spinal cord of mice, and nociceptive behaviors initiated immediately after. A combination of directed biting, licking, and scratching in mice was recorded for the total response time of these nociceptive behaviors in different pharmacological conditions. We used western blotting to confirm that hemokinin-1 activated the TRPV1 channel by measuring the amount of phosphorylated TRPV1 channel.
RESULTS: intrathecal administration of hemokinin-1 evoked nociceptive behaviors consisting of licking and scratching with biting of the hind limbs in mice. Hemokinin-1-induced nociceptive behaviors were decreased by capsazepine, a TRPV1 channel antagonist. Pre-treatment with anti-TRPV1 channel antibody eliminated the hemokinin-1 induced nociceptive behaviors. Moreover, the phosphorylation of the TRPV1 channel in the spinal dorsal horn was increased by intrathecal administration of hemokinin-1.
CONCLUSIONS: hemokinin-1 elicits nociceptive behaviors through the TRPV1 channels as well as the NK1 receptors in the spinal dorsal horn. Hemokinin-1 may regulate pain transmission via TRPV1 channels on primary afferents.

ROLE OF SPINAL CHOLECYSTOKININ-8, NOCICEPTIN, AND HEMOKININ-1 IN DIABETIC ALLODYNIA

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OBJECTIVE: lack of effective drugs for allodynia, a painful neuropathy caused by diabetes mellitus, decreases the quality of life of patients. It is assumed that various neurotransmitters are involved in the spinal cord during the development of allodynia caused by hyperglycemia-induced neuropathy, but studies have not yet been conducted on the details of this phenomenon. Therefore, neurotransmitters of the spinal cord in streptozotocin-induced diabetic allodynia were examined.
MATERIALS AND METHODS: allodynia was strongly developed from day 3 to day 14 after streptozotocin administration. In this study, the von Frey filament technique was performed using a mouse model of diabetic neuropathic pain 7 days after intravenously administration of streptozotocin. Antibodies of representative neurotransmitter peptides were intrathecally administered to allodynia-induced mice.

RESULTS: streptozotocin-induced allodynia was reduced by intrathecally administered antibodies of cholecystokinin-8, nociceptin, and hemokinin-1. In contrast, intrathecally administered antibodies of substance P, somatostatin, and angiotensin-2 did not affect streptozotocin-induced diabetic allodynia. Diabetic allodynia was decreased by intrathecally administered CI-988, a cholecystokinin CCK-B receptor antagonist, and JTC-801, a nociceptin receptor antagonist. The amount of mRNA of CCK-B receptors increased in the spinal cord, but not dorsal root ganglion, of streptozotocin-induced diabetic allodynia mice.
CONCLUSIONS: these results suggest that diabetic allodynia is expressed by transmitting it to the spinal dorsal hone by cholecystokinin-8, nociceptin, and hemokinin-1 released from primary afferent neurons of the spinal cord.

NUTRACEUTICAL APPROACHES FOR THE MANAGEMENT OF AGING: PROMISING ANTIAGING ACTIVITY OF A NEW FORMULATION OF ELLAGIC ACID

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OBJECTIVE: aging is a progressive physio-pathological process that causes both central and peripheral complications. Ellagic acid (EA) is a nutraceutical product, contained in pomegranate fruit, that has been shown to be beneficial in peripheral disorders, but also in central neuroinflammatory and neurodegenerative disorders. In this study, we analyzed the impact of the oral administration of an EA micro-dispersion (EAm), a new formulation that increase the EA solubility and therefore the bioavailability, in young and old mice.
MATERIALS AND METHODS: young (3 months) and old (20 months) mice were chronically (14 days) administrated with vehicle (mineral water) or with the EAm suspension, dissolved in the drinking water. Weight, beverage, and behavioural skills of both young and old mice were monitored. Cortical nerve endings (synaptosomes) were incubated with [^{3}H]NA and release experiments were performed to evaluate changes in noradrenaline exoxytosis. PCR and western blot analysis were performed to support by a biochemical point of view the functional observations.
RESULTS: oral EAm did not modify animal weight and motor and anxiety behavioral skills in young and old mice, but significantly recovered changes in *ex-vivo, in vitro* parameters in old animals. The exocytosis of noradrenaline, which has an important role in the control of mood and anxiety, but also in contrasting inflammation, is decreased in the cortex of old mice. EAm administration did not modify noradrenaline release in young animals but recovered it in old mice. Furthermore, the content of GFAP (astrocytosis marker) was increased in the cortex of the old mice, while IBA-1 (marker of microglia activation) and CD45 (marker of lymphocytes) immunopositivities were unchanged when compared to young ones. EAm treatment significantly reduced CD45 signal in both young and old mice cortical lysates, it diminished GFAP immunopositivity in young mice, but failed to affect IBA-1 expression in both young and old animals. Finally, EAm treatment significantly reduced IL1b expression in old mice.
CONCLUSIONS: these results suggest that EAm is beneficial to aging and could represent a promising nutraceutical ingredient for elders.

2-PENTADECYL-2-OXAZOLINE (PEA-OXA) TREATMENT REDUCES THE COGNITIVE DAMAGE AND IMPAIRED NEURAL PLASTICITY IN VITAMIN D DEFICIENCY

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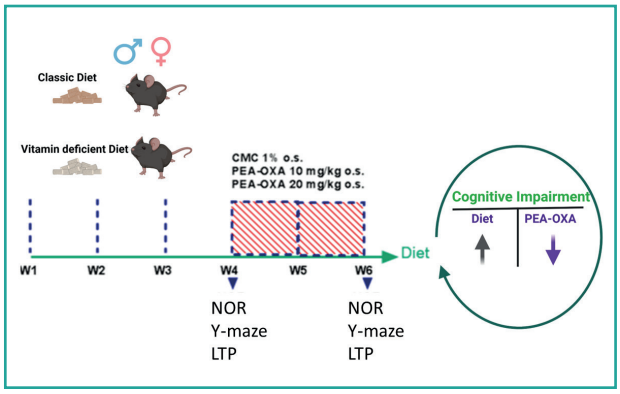
OBJECTIVE: 2-pentadecyl-2-oxazoline (PEA-OXA) is a natural compound, that is a secondary metabolite, found in green and roasted coffee beans, with anti-inflammatory and neuroinflammatory properties. We have previously shown that PEA-OXA is a α2 adrenergic receptor (α2AR) antagonist and a putative protean agonist on histamine H3 receptors. Vitamin D deficiency is considered a risk factor for the development of several immune-mediated pathological condition, including aging and dementia.
MATERIALS AND METHODS: in this study, we used a vitamin D deficiency condition induced by diet in male and female mice and evaluated the possible protective effects of PEA-OXA on the cognitive damage associated with low vitamin D levels.
RESULTS: we found that vitamin D deficiency induced a deficit of discriminative and spatial memory deficits, as revealed by the Novel Object Recognition (NOR) test and Y Maze Forced Alternation test. Consistently, electrophysiological studies revealed an impaired neural plasticity (Long-term potentiation LTP) in the hippocampus (Lateral Entorhinal Cortex-Dentate Gyrus pathway). Chronic treatment with PEA-OXA (10 mg/kg, os) restored cognitive damage and electrophysiological changes in both male and female mice.
CONCLUSIONS: Our findings may suggest PEA-OXA as a novel compound for the treatment of cognitive decline in different neurodegenerative diseases.

Graphical abstract



ANXIOLYTIC-LIKE/RELAXANT EFFECTS OF BEO ARE USEFUL TO MANAGE ANXIETY AND AGITATION SYMPTOMS OBSERVED IN PATIENTS WITH DEMENTIA

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OBJECTIVE: preclinical results indicate that essential oil obtained by *Citrus Bergamia* (Risso et Poiteau) (BEO) shown interesting anxiolytic-relaxant effects useful to treat behavioral and psychological symptoms of dementia (BPSDs), including agitation, depression and anxiety.
MATERIALS AND METHODS: to study the involvement of GABAergic and 5-HTergic transmissions in BEO behavioral effects, male Wistar rats (250-300 g) (n = 3-8) were systemically pretreated with diazepam (1.2 and 5 mg/kg, i.p.) and 8-hydroxy-2-(di-n-propylamino)tetralin ((±)8-OH-DPAT) (1 mg/kg, i.p.), selective agonists of GABA-A and 5-HT1A receptors, respectively, or with the selective antagonists flumazenil (3 mg/kg, i.p.) and WAY-100635 (N-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-N-(2-pyridinyl)cyclohexane-carboxamide) (1 mg/kg, i.p.),. The effects of BEO (250 and 500 µl/kg, i.p.) were analyzed in open-field, elevate plus-maze and forced swimming tasks.
RESULTS: the data yielded show that anxiolytic-relaxant effects of BEO are not superimposable to that observed with the benzodiazepine and that 5-HT1A receptor is indirectly involved.
CONCLUSIONS: further experiments are needed to deeply elucidate the mechanism of action of the essential oil that will allow a rational use in management of early BPSDs observed in patients with dementia.

ROLE OF mGlu5 AND mGlu2/3 RECEPTORS IN BEHAVIOURAL EFFECTS OF BERGAMOT ESSENTIAL OIL

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OBJECTIVE: bergamot essential oil (BEO) shows an interesting anxiolytic/relaxant action useful in the management of agitation, aggression and anxiety, typical behavioural and psychological symptoms of dementia (BPSD). Pharmacological results indicate that the behavioural effects of BEO do not completely overlap with those of benzodiazepines and are indirectly modulated by the 5-HT1A receptor. It is known that serotonin modulates glutamatergic neurotransmission particularly by metabotropic receptors. Among glutamatergic receptors, the mGluR5 and mGluR2/3 subtypes seems to play a key role in the control of anxiety.
MATERIALS AND METHODS: Male Wistar rats (250-300 g) (n = 3-8) were systemically pretreated with MPEP (3 and 10 mg/kg, i.p.), selective antagonist of the mGlu5 receptor, and with LY-341495 (3 and 10 mg/kg, i.p.), selective antagonist of mGlu2/3 receptor. Behavioral effects of BEO (500 µl/kg, i.p.) were analyzed in open-field, elevate plus-maze and rotarod tasks.
RESULTS: the results show that the antagonists differently modulate BEO effect. Particularly, the effects observed in the EPM seem to suggest that the mGlu5 receptor is more involved in the anxiolytic action of the phytocomplex.
COCLUSIONS: these data indicate that complex and fine mechanisms underlying behavioral effects of BEO that however need to be further elucidated.

PAIN ASSESSMENT FOR BETTER PHARMACOLOGICAL TREATMENT OF PAIN AND AGITATION IN SEVERE DEMENTIA: I-MOBID2 VALIDATION

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OBJECTIVE: it is estimated that some 55 million people are affected by dementia and 97% develops neuropsychiatric symptoms (NPS), among which the most resistant is agitation. Its treatment consists in the use of atypical antipsychotics, often off-label and increasing of 1.6-1.7-fold the risk of death for cardiocerebrovascular accidents. Agitation is, at least in part, caused by unrelieved pain because of lack of self-reporting, with consequent assessment difficulties. Thus, the purpose of the present clinical trial is to validate for the clinical use in the Italian setting the Mobilization-Observation-Behaviour-Intensity-Dementia (MOBID2) tool that unravels concealed musculoskeletal inflammatory and neuropathic pain, through active guided movements, and also pain from head, skin and internal organs.
MATERIALS AND METHODS: according to the current international guidelines the validation trial, approved by Calabria Region Ethics Committee protocol No. 31/2017, consisted in three phases: translation, cross-cultural adaptation and validation has been conducted in a cohort of 11 non-verbal, severe demented patients aged ≥65. The psychometric properties of the validat-

ed Italian I-MOBID2 were measured through content validity index, Spearman's rank order correlation and intraclass correlation coefficient.
RESULTS: the I-MOBID2 allowed to disclose pain in 63.6% patients. The psychometric analysis demonstrated good scale content validity index (0.89), high construct validity (Spearman rank order correlation Rho = 0.748) and good-to-excellent inter-rater (Intraclass correlation coefficient, ICC = 0.778) and test-retest (ICC = 0.902) reliability with 5.8 min average execution time.
CONCLUSIONS: this validation trial provided, for the first time, the Italian setting with I-MOBID2 a valid and reliable tool, that proved to unravel pain in over a half of patients suffering from severe dementia. This pain assessment scale is suitable also for future development in community setting. The effect of establishment of appropriate pain treatment on reduction of the Cohen-Mansfield Agitation Inventory score and on as-need agitation rescue medications deserves investigation.

NEED FOR A CLINICAL TRIAL INVESTIGATING EFFICACY AND SAFETY OF ORAL SPRAY NAbiximols FOR PAIN AND AGITATION TREATMENT IN SEVERE DEMENTIA PATIENTS

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OBJECTIVE: some 55 million people are affected by dementia and up to 80% of residents in long-term care facilities suffer from chronic pain, contributing to the development of fluctuant neuropsychiatric symptoms, and agitation in particular. Evidence is accumulating in favor of the involvement of the endocannabinoid system in pain and behavior processing. Therefore, the purpose of the present study is to propose a double-blind, placebo-controlled, randomized trial to investigate efficacy and safety of oral spray nabiximols, containing Δ9-tetrahydrocannabinol and cannabidiol (Sativex®), for pain and agitation treatment in severe dementia patients (Mini-Mental State Examination ≤12) over 65.
MATERIALS AND METHODS: the trial NACTOPAISD (NAbiximols Clinical Translation To the treatment of Pain and Agitation In Severe Dementia) is approved by Calabria Region Ethical Committee (protocol n. 118 of April 21st, 2022). It is planned to be conducted in agreement with the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) and the Consolidated Standards of Reporting Trials (CONSORT) statements.
RESULTS: the trial has two coprimary endpoints, consisting in efficacy on pain and agitation, assessed through the recently validated Italian Mobilization-Observation-Behavior-Intensity-Dementia and the Cohen-Mansfield Agitation Inventory. The secondary endpoint is the evaluation of efficacy duration after wash-out and the assessment of quality of life through the Dementia Quality of Life measure DEMQOL. The results obtained will be subjected to a statistical analysis plan. The assessors are going to note down the occurrence of any adverse events.
CONCLUSIONS: NACTOPAISD, following the way paved by studies assessing the different effects of cannabinoids on neuropsychiatric symptoms, might provide sound rational evidence supporting the efficacy and safety of nabiximols in the treatment of agitation and pain during severe dementia. Since the therapy of nuropsychiatric symptoms of dementia consists in potentially harmful, often off-label, antipsychotics, nabiximols may represent a safer treatment able to improve the quality of life of these fragile patients.

AUTOMATED DIAGNOSIS OF ALZHEIMER'S DISEASE WITH NEUROPSYCHOLOGICAL MEASURES: A META-ANALYTICAL REVIEW

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OBJECTIVE: today it is still difficult to predict patients at risks of Alzheimer's disease (AD) and whether and when individuals at risk (with Mild Cognitive Impairment, MCI) will progress to AD-type dementia and how much time will lapse for progression. Thus, the current challenge is to identify markers that capture MCI and discriminate between patients with MCI who will be convert (MCI converters, MCIc) and who will not convert (MCI not converters, MCInc) to AD-type dementia. In this study we performed a meta-analytic evaluation of the contribution of machine learning (ML) and neuropsychological measures for the automated classification of AD and MCI patients and the prediction of MCIs' conversion to AD-type dementia
MATERIALS AND METHODS: this systematic review was conducted on papers published on the use of ML applied to neuropsychological assessment for the automatic classification of AD, MCI and prediction of conversion of MCI to Alzheimer's type dementia, in accordance with the PRISMA statement. Meta-analytic accuracy, sensitivity and specificity and heterogeneity index were calculated using the MADA package.
RESULTS: evaluating data from 59 published studies, the majority (70%) with low risks of bias, we demonstrated that neuropsychological measures alone can lead to a successful automatic classification of prodromal AD phenotypes regardless of the employment of different ML algorithms. The contrasts MCI *vs* HC, MCIc *vs* MCInc and AD *vs*. HC were automatically recognized with a pooled accuracy of 0.9, 0.759 and 0.914, respectively. Moreover, ML algorithms are able to extract relevant categories of neuropsychological tests that maximize the classification accuracy. In particular: a)

MMSE, for evaluating the global cognitive status; b) AVLT, for evaluating the long-term memory performance; c) Category Fluency Test, for evaluating the language ability; and d) Digit Span Forward and Backward, for evaluating verbal short-term memory, sustained attention and working memory capacities (**figure 1**).

CONCLUSIONS: this meta-analytic review demonstrates that ML applied on neuropsychological measures can be useful to automatically classify AD patients, even at an early stage of the disease, and to identify a combination of optimal neuropsychological predictors.

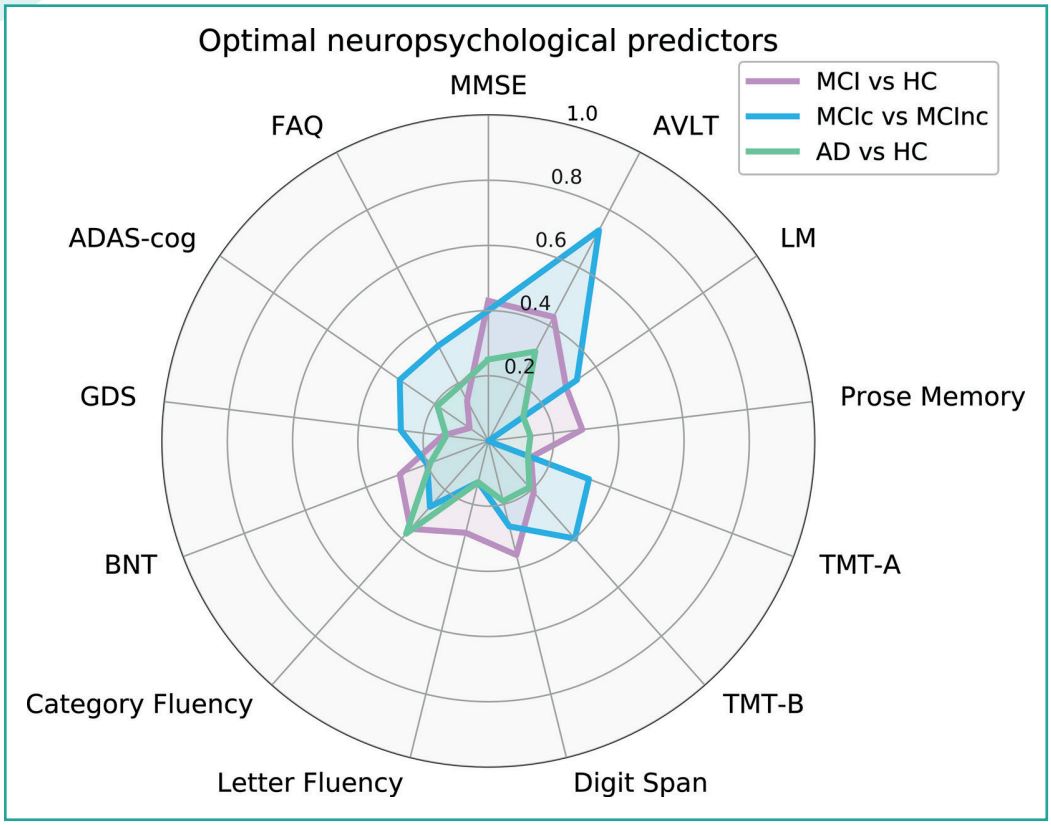

Figure 1. Radar plot of the most frequent optimal predictors (≥25 % frequency), for the different comparisons, MCI *vs*. HC (violet), MCIc *vs*. MCInc (blue), and AD *vs*. HC (green).

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