

Aging brain: in search for better neurotherapeutics

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Abstract. During the past 25 years, international meetings organized every second year by the pharmacologists of the University of Calabria have represented an awaited appointment largely participated by young scientists to discuss basic and clinical research concerned with hot topics in biomedicine. This year, high caliber international scientists met on May 4th-5th, 2017 with young researchers at the Club of the University of Calabria (Cosenza, Italy) in order to make the point on neurodegenerative diseases, affecting mainly the aging brain and also to share emerging strategies for a better therapeutic management of these diseases. Participants from USA, Germany, Russia, Japan, England, Serbia and Italy created a friendly atmosphere that allowed a lively and open discussion making the meeting a success. The beauty of the Norman-Swabian Castle of Byzantine origin (a.d. 937) of Cosenza, the Civic Museum of the Byzantine Icons and Traditions of Frascineto (CS) and the hospitality of Altomonte (CS) made the rest.

Key words: aging, brain, Alzheimer's disease, Parkinson's disease, aromatherapy, bergamot essential oil, pain, BPSDs

INVECCHIAMENTO CEREBRALE: ALLA RICERCA DI MIGLIORI TERAPIE

Riassunto. Durante i passati 25 anni, i congressi internazionali organizzati con cadenza biennale dai farmacologi dell'Università della Calabria hanno rappresentato un appuntamento atteso e largamente partecipato da giovani ricercatori per discutere di ricerca di base e clinica inerente argomenti caldi di biomedicina. Quest'anno, scienziati di calibro internazionale hanno incontrato nei giorni 4 e 5 maggio 2017 giovani ricercatori presso il Club dell'Università della Calabria (Cosenza, Italia) per fare il punto sulle malattie neurodegenerative che prevalentemente si osservano nel corso dell'invecchiamento cerebrale ed anche per condividere le strategie emergenti per un miglior controllo terapeutico di queste malattie. Partecipanti provenienti dagli USA, Germania, Russia, Giappone, Gran Bretagna, Serbia ed Italia hanno creato un'atmosfera amichevole che ha consentito una discussione vivace ed aperta facendo del meeting un vero successo. La bellezza del Castello Normanno-Svevo di origini Bizantine (a.d. 937) di Cosenza, il Museo Civico delle Icone e Tradizioni Bizantine di Frascineto (CS) e l'ospitalità della città rinascimentale di Altomonte (CS) ha fatto il resto.

Parole chiave: invecchiamento, cerebrale, malattia di Alzheimer, malattia di Parkinson, aromaterapia, olio essenziale di bergamotto, dolore, BPSD

ENVEJECIMIENTO CEREBRAL: BUSCANDO MEJORES TERAPIAS

Resumen. Durante los últimos 25 años, congresos internacionales organizados con una cadencia bienal por los farmacólogos de la Universidad de Calabria representaban una cita esperada y ampliamente asistido por jóvenes investigadores para discutir la investigación básica y clínica sobre temas candentes de la biomedicina. Este año, científicos de calibre internacional han se reunieron los días 4 y 5 de mayo de 2017 jóvenes

investigadores en el Club de la Universidad de Calabria (Cosenza, Italia) hacer un punto sobre las enfermedades neurodegenerativas que se observan principalmente durante el envejecimiento cerebral y también para compartir estrategias emergentes para un mejor control terapéutico de estas enfermedades. Participantes de Estados Unidos, Alemania, Rusia, Japón, Gran Bretaña, Serbia y Italia han creado un ambiente agradable que ha permitido una discusión animada y abierta haciendo de la reunión un verdadero éxito. La belleza del castillo normando-swabian de los orígenes bizantinos (a.d. 937) de Cosenza, el Museo Cívico de los Iconos y las Tradiciones Bizantinas de Frascineto (CS) y la hospitalidad de la ciudad renacentista de Altomonte (CS) hizo el resto.

Palabras clave: envejecimiento, cerebro, enfermedad de Alzheimer, enfermedad de Parkinson, aromaterapia, bergamot aceite esencial, dolor, BPSDs

Introduction and meeting rationale

The steadily increasing epidemiological prevalence of age-related neurodegenerative diseases and the deepening of knowledge on basic mechanisms underlying physiologic and pathologic aging processes of the brain account for the need of constant up to date of data emerging from fundamental as well as translational and clinical research. "Aging Brain: In Search for Better Neurotherapeutics", a monothematic meeting sponsored by the Italian Society of Pharmacology (SIF), provided a very high quality setting to host established scientists from internationally recognized laboratories and young researchers from several countries in order to fulfil the above need and to highlight emerging molecular pharmacological mechanisms upon which to build innovative therapeutic strategies and to prompt the development of new research lines for PhDs and post-docs. The attendees were welcomed by Prof. Gino Mirocle Crisci, Rector of the University of Calabria, and by the Head of Department of Pharmacy, Health Science and Nutrition, Prof. Sebastiano Andò. The rationale of the meeting was illustrated by Prof. Giacinto Bagetta, Chairman of Pharmacology at the University of Calabria, who also took the opportunity to pay a tribute to Prof. Norman George Bowery, who hosted him at the School of Pharmacy (London) for a very productive (see Bagetta et al., 1991) (1) three year post-doctoral research stage working on the underlying mechanisms of neurodegeneration caused by the unopposed glutamate excitation emerging from tetanus toxin impaired GABA mediated inhibition in rodent hippocampus. Prof. Bowery died on

the 25th October 2016; he loved Italy in such a special way that during periods of the 80's and 90's the majority in his laboratory were young Italian pharmacologists and the British a minority. Prof. Bowery is recognized worldwide for the fundamental discovery of the second GABA receptor, the GABA_B receptor. Dr. Stefano Tacconi (Aptuit Center for Drug Discovery and Development, Verona, Italy), one of the Bowery's Italian pupils, together with Dr. Doug A. Richards, member of Bowery's research group at the Department of Pharmacology, University of Birmingham (UK), was asked for providing the audience with a detailed note of his scientific profile as well as hints of private life, respectively. Lady Barbara Joyce Bowery was awarded a SIF Medal by the SIF (Elected) President Prof. Alessandro Mugelli (Florence, Italy), who attended the ceremony of the Laurea Honoris Causa given to Norman George Bowery by the University of Florence. For the generosity of SIF Prof Giacinto Bagetta is personally indebted with the President, Prof. Giorgio Cantelli Forti.

Main lectures report

During aging several pathological changes affect the nervous system fostering neurodegeneration. The main age-related neurodegenerative diseases are dementia and Parkinson's Disease (PD). Alzheimer's Disease (AD) is the most common cause of dementia and data gathered by the World Health Organization (WHO) in 2012 state that 54% of all the cases of dementia are linked to AD (2). Dr. Amalia Bruni, neu-

rologist and Head of the Regional Neurogenetic Centre at Lamezia Terme (Catanzaro, Italy), introduced by Giuseppe Passarino, professor of Genetics at the University of Calabria (Cosenza, Italy), illustrated the historical changes since dementia was misdiagnosed and confused with psychotic disorders. Furthermore, a very interesting study of genetics in Calabrian families affected by dementia accounted for the social burden of this condition that involves us and is nearer than generally believed. The unsolved etiological issue of neurodegenerative diseases is at the root of the lack of disease modifying drugs able to counteract neurodegeneration. The central topic of age-related neurodegeneration was masterfully discussed by Prof. Pierluigi Nicotera, Scientific Director of the German Center for Neurodegenerative Diseases (DZNE, Bonn, Germany), whose enlightening keynote lecture provided an overview of the possible causes and mechanisms and a wide landscape on the stage of the therapy for neurodegenerative diseases and on the recent advances in another very important component that is neuroinflammation. Prof. Nicotera reasoned that, as the population gets older, the prevalence of these diseases remarkably increases and their complexity is at the root of the failure of a lot of pharmacological approaches. Therefore, to delay the progression of neurodegenerative diseases and of AD, in particular, it seems mandatory to address more than one target through drugs exerting a synergistic effect. Moreover, he explained that this increased longevity predisposes to an augmented possibility to accumulate cell damages; at this point epigenetics comes into play. Epigenetics is actually the programming of how our DNA is expressed. In dividing cells an overexpression of histone H3-H4 prevents genomic instability from occurring, thus delaying processes linked to aging. Accordingly, detection of the chromatin structures causally linked to aging becomes necessary, as the latter may represent therapeutic targets in age-related human diseases (3). Activation of innate immunity and the assembly of microglial cells at brain sites of Alzheimer disease pathology has long been regarded as bystander phenomenon, which does not actively contribute to disease pathogenesis and progression. At variance with this old view, Prof. Michael Heneka (DZNE, Bonn, Germany), introduced by Prof. Pierluigi Nicotera, illustrated the importance of modulating innate immunity in AD. In

fact, data emerging from genetics, clinical imaging and animal experimentation point to an intimate and mutual interaction of innate immune mechanisms and neurodegenerative processes. Inhibition of inflammasome activation just begins to prove beneficial and protective effects against cognitive deficits and neuronal death in animal models of AD and cell cultures, respectively, thereby opening a new venue for therapeutic intervention. Moreover, Prof. Heneka demonstrated that innate immunity influences amyloid oligomerization, fiberization and deposition and, therefore, the progression of the disease. The inflammatory reaction initiates the amyloid deposition process and it is fundamental for the progression of the disease; this is a true disease-specific mechanism. However, the different microglial populations present depending on the stage of disease and the high variability among the brains of different individuals do not make microglia an ideal target for AD treatment. Accordingly, microglia can be used as a target not to cure but to prevent AD and to predict the progression of the pathology, though, a better understanding of immune cell functioning, including the role of receptors for neurotransmitters on these cells, is compulsory for a drugable target.

Along this line, Prof. Marco Riva (Milan, Italy) explained that, when brain is dysregulated - and immune mechanisms are now recognized to have an important role in these conditions - stress is a key element for neuropsychiatric disorders. There may be a sort of common mechanism between aging and stress that can alter synaptic functions and neuronal plasticity. Depression is the most common psychiatric disorder in patients suffering from AD and PD and often a challenge for caregivers and doctors.

The lack of established mechanisms responsible for the onset of neurodegenerative diseases, such as AD and PD, makes the road to the discovery of disease modifying drugs still very long. However, during the past two decades important findings have been made in the field of neurodegeneration. During his brilliant keynote lecture, introduced by Prof. Rossella Russo (Cosenza, Italy), Prof. Stuart A. Lipton (San Diego, USA) made the audience focus on an aspect of the utmost importance: in AD the hallmarks known for years are A β plaques and hyperphosphorylated Tau protein tangles, but the best pathological correlate with clinical dementia is the

loss of the synapses. Therefore, the best available index of disease is the number of synapses and the amount of preserved or increased synapses needs to be the main goal of drugs. Hence, drugs must increase the number and the function of synapses. How does this synaptic loss occur? Oxidative stress and S-nitrosylation (transfer of a NO group to a critical cysteine -SH group) of mitochondria induces damage. Mitochondrial fission and fusion are fundamental for neuronal and synaptic function and these are affected in neurodegenerative diseases, as in AD. Finally, mitochondrial damage induces apoptosis and therefore synaptic loss. In particular, the mitochondrial fission dynamin-related protein 1 (Drp1), a GTPase, is S-nitrosylated in AD brains, as well as Parkin in early-onset PD. It was demonstrated that Drp1 is correlated with A β and with a reduction of synaptic density. Thus, A β in AD and toxins as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in PD may induce S-nitrosylation of proteins importantly involved in synaptic loss. For this reason it is necessary to use in a "safe" manner drugs acting on brain receptors responsible for both neuroprotection and damage, like the N-methyl-D-aspartate (NMDA) receptors. These receptors play a pivotal role in neuronal death and memantine, a low affinity non-competitive NMDA-receptor antagonist, preferentially blocks extrasynaptic channels when excessively open, sparing normal synaptic activity. Therefore, memantine offers neuroprotection in AD via an uncompetitive/fast-off rate (UFO) action. NMDA receptors can have either excitatory or inhibitory function, depending on the type of neuron on which they are located. Moreover, physiological synaptic NMDA receptor activity promotes survival pathways, through cAMP response element-binding protein (CREB), Brain-Derived Neurotrophic Factor (BDNF) expression etc., while extrasynaptic NMDA receptors inhibit BDNF expression and prompt excessive NO generation, toxicity pathways, protein misfolding and synaptic damage. A β induces aberrant extrasynaptic NMDA receptors (eNMDARs) activity and this induces further production of A β oligomers. Likely eNMDARs aberrant activity induces Tau protein hyperphosphorylation, activation of caspase 3 and S-nitrosylation of proteins like Drp1, with consequent synaptic loss. This accounts for the efficacy of memantine which blocks aberrant activity, acting on eNMDARs

predominantly. A newer drug, nitromemantine, provides a dual site antagonism of eNMDAR (4). Indeed, nitromemantine provides voltage-dependent blockade of excessively open eNMDARs, like memantine, but in addition it delivers a nitro group to NMDARs. Since the blocker spends a lot of time near the channel mouth, in particular during hypoxia, NO has a higher probability to interact with allosteric cysteine residues of the NMDAR, thus reducing its excessive, aberrant activity. Therefore, nitromemantine reduces the excessive activity of eNMDARs through the classic mechanism of action of memantine inside of the channel, but also through a novel mechanism S-nitrosylation outside of the channel (4).

Young researchers communications report

A series of original data were lively discussed in oral sessions, making possible the interaction of young PhDs and Post-Docs with senior scientists. Apart from the two hallmarks of AD amyloid β -peptide (A β) plaques and neurofibrillary tangles (NFTs), an upregulation of the voltage-gated KV3.4 potassium channel was found in AD cerebral samples. During the first oral communications session, chaired by Prof. Maria Pia Abbracchio (Milan, Italy) and Prof. Luigi A. Morrone (Cosenza, Italy), Dr. Roselia Ciccone (Naples, Italy) outlined the possibility that KV3.4 and also voltage-gated sodium channels NaV1.6, responsible for neuronal excitability, may turn out to be new potential pharmacological targets in AD, according to the findings obtained in Tg2576 mice. Early reduction of cerebral glucose metabolism, detected through positron emission tomography (PET), is another feature of AD brains likely linked to an aberrant O-GlcNAcylation of Tau and correlated with the disease onset and progression. The contribution of Dr. Antonella Tramutola (Rome, Italy) focused on these processes of alteration and hypometabolism of glucose in AD. Evidence supporting the possibility that that cognitive impairment and dementia arise as complications of type 2 diabetes mellitus has emerged. Prof. Emilio Russo (Catanzaro, Italy) highlighted the effects of vidagliptin, a dipeptidyl peptidase (DPP)-IV inhibitor, on cognitive impairment and hippocampal neurodegeneration in a streptozocin-induced animal model of diabetes and

neurodegeneration associated with cognitive decline. Furthermore, down syndrome (DS) and AD seem to be correlated in their neurodegenerative processes, since disorders of the protein degradation were identified in both conditions. Prof. Marzia Perluigi (Rome, Italy) presented the first study showing alteration of the polyubiquitination profile in DS, both before and after the development of AD. The second most common cause of cognitive impairment is Vascular Dementia (VaD). Mechanisms at the root of VaD involve reduced cerebral blood flow and oxidative stress. Hence, a new compound made up of N-Palmitoylethanolamine (PEA) and luteolin (Lut) (10:1 by mass), of which the name is co-ultraPEALut, was demonstrated to be effective in a mouse model of VaD by Dr. Giovanna Casili (Messina). The efficacy of the pre-treatment with micronized PEA formulation on neuro-inflammation and neuronal death was also studied in an *in vivo* model of PD aged mice by Dr. Michela Campolo (Messina). Indeed, the peripheral immune system is involved in PD. The peculiar profile of CD4+ T naive and memory cells with likely consequent pro-inflammatory changes of immune system in PD patients was demonstrated by Dr. Natasa Kustrimovic (Varese, Italy) during the second oral communications session, chaired by Dr. Fabio Blandini (Pavia, Italy) and Prof. Diana Amantea (Cosenza, Italy). Another unfortunate characteristic of patients affected by PD is that they are likely to present cognitive decline or dementia with higher frequency if single nucleotide polymorphism (SNP) of the dopaminergic receptor DR, that was associated with increased D1-like activity, occurs and this was highlighted by Dr. Marco Ferrari (Varese, Italy). Recently, autophagy and, in particular, the highly conserved catabolic pathway of long-lived proteins and organelles known as macroautophagy (5), has turned out to be heavily involved in aging and neurodegeneration. Clomipramine, a tricyclic antidepressant of common use, exerts some effects also on autophagic flux and potentiates chemotherapy (6). In her oral communication, Dr. Annagrazia Adornetto (Cosenza, Italy) presented new findings according to which mice treated with clomipramine displayed inhibition of cortical neurons autophagy. Dr. Irene Paterniti (Messina, Italy) outlined the neuroprotection provided by temsirolimus, an analogue of rapamycin, that inhibits the mammalian target of rapamycin complex 1 (mTOR1), thus inducing autophagy. Glaucoma is an age-related

neurodegenerative disease characterized by the progressive death of retinal ganglion cells (RGCs). Dr. Giuseppe Varano (Cosenza, Italy) showed that ischemic insult caused by transient elevation of intraocular pressure (IOP) induces a dynamic modulation of autophagy in the adult and aged retina of wild type C57BL/6J mice. Increased death of RGCs was shown in autophagy-deficient *Ambra+/-* mice that underwent retinal ischemia and the use of rapamycin or caloric restriction, to induce autophagy, increased RGCs survival. Another very serious neurodegenerative disease typical of adult age is amyotrophic lateral sclerosis (ALS), which consists of the loss of cerebral cortex upper motor neurons and brainstem and spinal cord lower motor neurons. In this field of expertise Dr. Tiziana Petrozziello (Naples, Italy) showed that the Cu/Zn superoxide dismutase (SOD1), often subjected to mutation in ALS, and ApoSOD1 can induce neuroprotection in ALS *via* modulation of intracellular calcium concentrations ($[Ca^{2+}]_i$).

The increased longevity predisposes also to chronic pain conditions, often misunderstood and resistant to the common use painkillers and, thus, remarkably reducing the patient's quality of life (QoL). Dr. Monica Iannotta (Naples, Italy) underlined that mice subjected to a long-lasting spared nerve injury (SNI) (7) develop mechanical allodynia, anxiety, depression-like behaviour, cognitive impairment and increased levels of hippocampal insoluble A β 1-42 level and of serum soluble A β 1-40. Both molecular and behavioural alterations were improved by treatment with D-aspartate. Neuropathic pain-linked memory impairment and depressive-like but not anxiety-like behaviour could be improved by PEA, as demonstrated by Dr. Serena Boccella (Naples, Italy). However, mechanisms through which aging can affect basal sensitivity and pain threshold are currently not well understood. Dr. Damiana Scuteri (Cosenza, Italy) demonstrated the pivotal influence of the aging process in C57BL/6 mice, belonging to different age groups, subjected to the assessment of mechanical (Von Frey's and Pin-prick tests) and thermal sensitivity (Hargreaves' and Acetone tests) and of nociceptive behaviour (Formalin Test) (8). In the spinal cord, molecular changes of the main chronic pain marker, the L-type voltage-gated Ca^{2+} -channel $\alpha 2\delta$ -1 subunit, and of Beclin 1 may account for the observed variations in pain sensitivity and in the response to gabapentin, one of the most used drugs against neuropathic pain.

A summary of the clinical trials on AD immunotherapy was reported by Dr. Mariarosanna De Fina (Catanzaro) during the third oral communications session, chaired by Prof. Marco Cosentino (Varese, Italy) and Prof. Anna Pittaluga (Genoa, Italy). The possible future role of dimethyl fumarate (DMF), an orally bioavailable methyl ester of fumaric acid and activator of the transcription factor Nrf2, which regulates the expression of genes encoding for detoxification enzymes, was shown by Dr. Marika Lanza (Messina, Italy) in an *in vitro* model of AD, consisting in the stimulation of SH-SY5Y neuroblastoma cell lines with A β . Furtherly, the possible involvement of biliverdin reductase-A in the activity of the intranasal insulin administration, under evaluation as therapeutic strategy for brain insulin resistance in AD, was highlighted by Dr. Eugenio Barone (Rome, Italy). An unsolved clinical syndrome in demented people is represented by the behavioural and psychological symptoms of dementia (BPSDs). Management of BPSDs is still attributed to atypical antipsychotics, whose use for more than 6-12 weeks (9), (10) increases the risk of death and provides poor control of agitation. Actually, aromatherapy with *Melissa officinalis* and *Lavandula officinalis* (11) proved to be efficacious in randomized clinical trials, even though it does not definitely control BPSDs. Association of BPSDs with undertreated chronic pain makes the essential oil bergamot (BEO) a good candidate with which to handle this clinical condition, being this phytochemical complex endowed with antinociceptive properties as demonstrated by Dr. Damiana Scuteri (Cosenza, Italy). Indeed, BEO antinociceptive effectiveness has been established both in the capsaicin test inflammatory model (12), (13) and in the spinal nerve ligation (SNL) (14) and partial sciatic nerve ligation (PSNL) neuropathic pain models (15), as well as in the formalin test (16). These effects may stem from BEO modulation of hippocampal synaptic levels of amino acid neurotransmitters (17) and enhancement of basal and induced autophagy in human SH-SY5Y neuroblastoma cells (18): indeed, autophagy is involved in aging (19) and in chronic pain, since it is differentially altered in neuropathic pain models (20), and abnormalities of the autophagy process have been implicated in AD (21). Naloxone hydrochloride and methiodide (not able to cross blood brain barrier) attenuated BEO-induced antinociception, only if administered ipsilaterally, thus supporting an involvement of the pe-

ripheral opioid system in BEO activity (16). Therefore, aromatherapy with BEO may reduce the use of opioids, endowed with serious side effects and still of common use in spite of the lack of strong evidence for effectiveness in chronic non-cancer pain (22). Moreover, Dr. Laura Tridico (Cosenza, Italy) showed that BEO produces anxiolytic-like/relaxant behaviour devoid of sedation, unwanted side effect of benzodiazepines in the elderly (23). The latter data are in agreement with previous results demonstrating that systemic BEO was associated to augmented alpha electroencephalographic (EEG) frequency relaxation and beta brainwave activity, pointing to relaxation and alert (23-25). Hence, randomized clinical trials (RCTs) in order to translate aromatherapy with BEO for BPSDs management into clinical practice are needed. Moreover, the possible association between legumes consumption and cognitive capabilities was highlighted by Dr. Yvelise Ferro (Catanzaro, Italy). The phytochemical profile and biological activities of *Allium* species and the effects of natural compounds on inflammation were reported by Dr. Natasa Simin and by Prof. Neda Mimica-Duki (Novi Sad, Serbia). The importance of rehabilitative programs in the treatment of neurodegenerative diseases was underlined through the study of Dr. Tommaso Bonfiglio (Genoa, Italy) focusing on environmental training: environmental enrichment (EE) consists in the addition of objects to the animal's environment in order to strengthen the synaptic plasticity. Dr. Manuela Mancini (Catanzaro, Italy) illustrated a rare case report of progressive supranuclear palsy (PSP)-like phenotype, characterized by postural instability with backward falls, linked to ischemic lesions of basal ganglia and midbrain. Finally, a pharmacoepidemiologic analysis of AD drugs and their prescribing appropriateness with the adverse reactions and possible interactions was reported by Dr. Maria Roberta Garreffa (Catanzaro, Italy), Dr. Adele De Francesco (Catanzaro, Italy) and Dr. Ilaria Altimari (Cosenza, Italy).

Conclusions

Altogether, the meeting offered the perfect setting to face very interesting topics and to share studies and knowledge, thus widening the view of younger participants and giving rise to novel research lines.

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