SIF Monothematic Meeting

Aging Brain:

In Search for Better Neurotherapeutics

May 4th-5th, 2017

University Club

University of Calabria, Rende (CS), Italy

Programme and Book of Abstracts

(Laura Rombolà & Damiana Scuteri, Eds.)

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Department of Health Sciences

University "Magna Græcia" of Catanzaro

88100, Catanzaro, Italy

berliocchi@unicz.it

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Damiana Scuteri

Department of Pharmacy, Health and Nutritional Sciences

University of Calabria

87036 Arcavacata di Rende (CS), Italy

damianascuteri@gmail.com

SOCIETÀ ITALIANA FARMACOLOGIA

PROGRAMME

May 4th, 2017

14:00 - 15:15 Registration

15:15 – 15:25 Welcome address and meeting rationale

Gino Mirocle Crisci, Rector, Unical (Cosenza)

Sebastiano Andò, Head of Department of Pharmacy, Health Science and Nutrition, Unical (Cosenza)

Giacinto Bagetta, Chairman of Pharmacology, Unical (Cosenza)

15:25 – 15:30 Introduction

Laura Berliocchi (Catanzaro)

15:30 – 16:15 Opening lecture

“Age-related neurodegeneration”

Pierluigi Nicotera (Bonn)

16:15 – 16:30 Coffee break

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16:30 – 16:55 Tribute to Norman George Bowery

Giacinto Bagetta (Cosenza) & Doug A. Richards (Birmingham)

In memoriam of Professor Norman G. Bowery

Stefano Tacconi (Verona)

SIF Medal to Barbara Joyce Bowery

Alessandro Mugelli (Florence), SIF (Elected) President

16:55 – 17:00 Introduction

Giuseppe Passarino (Cosenza)

17.00-17.30

“Genetics in dementia"

Amalia Bruni (Lamezia Terme, Catanzaro)

17:30 – 19:30

FIRST ORAL COMMUNICATIONS SESSION

Chairpersons: Maria Pia Abbracchio (Milan) & Luigi A. Morrone (Cosenza)

Critical Role Of Kv3.4 Potassium Channel And Nav1.6 Sodium Channel In Aβ Oligomer Effects On Neuron Excitability And Cognitive Functions In Alzheimer's Disease

Ciccone R, Franco C, Secondo A, Boscia F, Piccialli I, Anzilotti S, Vinciguerra A, Di Renzo GF, Annunziato L, Pannaccione A.

The modulation of intracellular Ca^{2+} homeostasis is involved in SOD1 and ApoSOD1-induced neuroprotection of motor neurons exposed to beta-methylamino-L-alanine

Petrozziello T, Tedeschi V, Pannaccione A, Boscia F, Di Renzo GF, Annunziato L, Secondo A.

Altered protein O-GlcNAcylation profile revealed by proteomics in AD: Novel insights on protein signalling mechanisms and potential therapeutic targets

Di Domenico F, Tramutola A, Sharma N, Barone E, Cassano T, Perluigi M.

Disturbance Of Ubiquitome Profile In Down Syndrome And Alzheimer's Brain

Perluigi M, Tramutola A, Arena A, Barone E, Butterfield DA, Di Domenico F.

Effect Of Antidepressant Treatment On Neuronal Autophagic Flux

Cavaliere F, Adornetto A, Fornarelli A, Bertan F, Russo R, Bagetta G, Bano D and Nicotera P.

Anti-Inflammatory And Neuroprotective Effects Of Co-Ultrapealut In A Mouse Model Of Vascular Dementia

Casili G, Siracusa R, Impellizzeri D, Cordaro M, Crupi R, Esposito E, Cuzzocrea S.

D-Aspartic Acid Ameliorates Cognitive Impairment In A Mouse Model Of Spared Nerve Injury And Reduces B-Amyloid Aβ1-40 And Aβ1-42 Peptides

Iannotta M, Belardo C, D'Aniello A, Romano R, Luongo L, de Novellis V, Maione S.

Palmitoylethanolamide, Via PPAR-Alpha Receptor, Restores The Altered Synaptic Plasticity And Ameliorates The Compromised Pain-Related Behaviours In The Hippocampus In Neuropathic Mice.

Boccella S, Luongo L, Guida F, Belardo C, Iannotta M, Romano R, Marabese I, Palazzo E, de Novellis V, Maione S.

Influence Of Aging On Nociception In C57BL/6 Mice

Scuteri D, Berliocchi L, Morrone LA, Corasaniti MT, Sakurada T, Sakurada S, Bagetta G.

20:30 Social Dinner

May 5th, 2017

8:40 – 8:45 Introduction

Ferdinando Nicoletti (Rome)

8:45 – 9:15

“Aging and the Stressed Brain: From Mechanisms to Molecules"

Marco Riva (Milan)

9:15 – 10:45

SECOND ORAL COMMUNICATIONS SESSION

Chairpersons: Fabio Blandini (Pavia) & Diana Amantea (Cosenza)

Bioactivity Of Secondary Metabolites From Allium Species

Simin N, Četojević-Simin D, Mitić-Ćulafić D, Orčić D, Lesjak M, Nemeš I, Mimica-Dukić N.

N-Palmitoylethanolamide Prevents Parkinsonian Phenotypes In Aged Mice

Campolo M, Crupi R, Paterniti I, Filippone A, Lanza M, Cuzzocrea S, Esposito E.

CD4+ T Regulatory Cell In Peripheral Blood Of Parkinson's Disease Patients

Kustrimovic N, Rasini E, Legnaro M, Aleksic I, Blandini F, Comi C, Marino F, Cosentino M.

Neuroprotective effects of temsirolimus in animal models of Parkinson's disease

Paterniti I, Casili G, Siracusa R, Cordaro M, Crupi R, Bruschetta G, Campolo M, Cuzzocrea S, Esposito E.

Modulation Of Autophagy To Achieve Retinal Neuroprotection

Varano GP, Adornetto A, Nazio F, Morrone LA, Corasaniti MT, Cecconi F, Nucci C, Bagetta G, Russo R.

Polymorphisms Of Dopamine Receptor Genes Are Associated To Risk Of Dementia In Patients With Parkinson's Disease

Ferrari M, Comi C, Marino F, Magistrelli L, Riboldazzi G, Bono G, Cosentino M.

Progressive Supranuclear Palsy-Like Phenotype Secondary To Brain Vascular Lesions

Mancini M, Garcea T, Morelli M, Mastroianni G, Arabia G, Lupo A, Manfredini LI, Nicoletti G, Salsone M, Novellino F, Bono F, Mazza M, Ferrigno G, Rocca F, Chiriaco C, Cascini GL, Quattrone A.

10:45 – 10:55

Coffee Break

10:55 – 11:00 Introduction of the first Keynote lecture

Rossella Russo (Cosenza)

11:00 – 11:45 Keynote lecture

"Protein S-Nitrosylation, Synapse Loss, and NMDA Receptor Drug Development for Alzheimer's Disease"

Stuart A Lipton (San Diego)

11:45 – 13:15

THIRD ORAL COMMUNICATIONS

SESSION

Chairpersons: Marco Cosentino (Varese) & Anna Pittaluga (Genoa)

Biliverdin Reductase-A Mediates The Beneficial Effects Of Intranasal Insulin Administration On Alzheimer Disease Pathology In The Brain Of 3xTg-AD Mice

Barone E, Triani F, Tramutola A, Cassano T, Butterfield DA and Perluigi M.

Dimethyl Fumarate, NRF2 Dependent, As A New Therapeutic Approach For Amyloid Beta Induced Oxidative Stress

Lanza M, Campolo M, Casili G, Filippone A, Paterniti I, Cuzzocrea S, Esposito E.

Vildagliptin Improves Animal Behavior And Cognitive Impairment In A Rat Model Of Streptozotocin Induced Diabetes

Russo E, Palleria C, De Sarro C, Leo A, De Caro C, Citraro R, De Sarro G.

Impact Of Legumes And Plant Proteins Consumption On Cognitive Performances In The Elderly

Ferro Y, Mazza E, Fava A, Moraca M, Rotundo S, Provenzano F, Greco M, Foti D, Gulletta E, Bosco D, Montalcini T, Pujia A.

Immunotherapies For Alzheimer's Disease: Clinical Trials Evaluation, State Of The Art And Future Prospects

De Fina M, De Francesco A, Naturale MD, De Sarro G.

Distinct Anxiolytic Profile of Bergamot Essential Oil in Preclinical Behavioural Studies

Tridico L, Rombolà L, Corasaniti MT, Sakurada T, Sakurada S, Bagetta G, Morrone LA.

Pharmacological Basis For The Treatment Of Behavioural and Psychological Symptoms Of Dementia (BPSDs) With Aromatherapy

Scuteri D, Morrone LA, Rombolà L, Russo R, Berliocchi L, Corasaniti MT, Sakurada T, Sakurada S, Bagetta G.

13:15 – 13:20 Presentation of Closing Keynote lecture

Pierluigi Nicotera (Bonn)

13:20 – 14:05

Keynote lecture

“Modulating Innate Immunity in Alzheimer’s disease "

Michael Heneka (Bonn)

14:05 General discussion

14:15 – 15:30 Light Lunch and Posters discussion

15:30 Round Table

“Aging Brain: New Vistas in Drug Discovery Process”

Fabio Blandini (Pavia)

Ferdinando Nicoletti (Rome)

Carlo Nucci (Rome)

Anna Pittaluga (Genoa)

Laura Berliocchi (Catanzaro)

Marco Cosentino (Varese)

POSTERS

Analysis Of The Use Of Drugs For The Treatment Of Alzheimer's Dementia In Calabria Region

Garreffa MR, De Francesco AE, Labate D, Mirarchi S, Florio L.

Environmental training on clinical symptoms and synaptic defects in aged mice: a pre-clinical study to support the rehabilitative approach in clinic

Bonfiglio T, Vergassola M, Olivero G, Scimone A, Pittaluga A.

Analysis Of Adverse Reactions To Drugs In Patients With Alzheimer's Disease Or Other Dementias: Calabria Region Analysis Of ADR

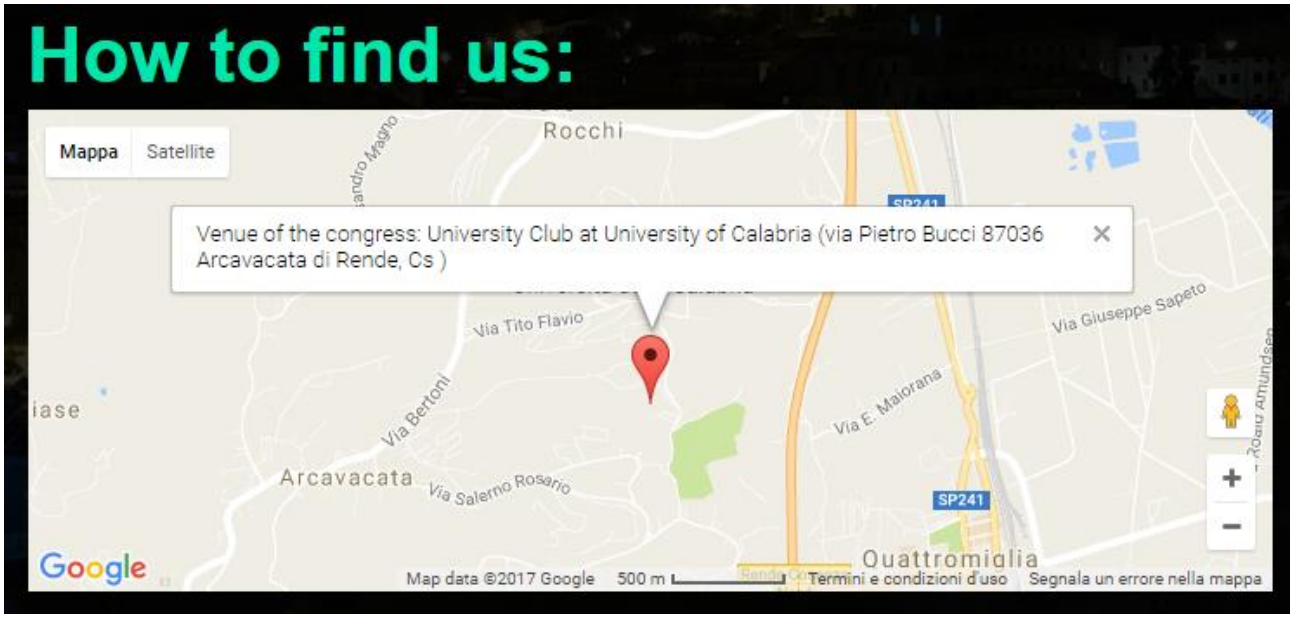
Fersini G, De Francesco AE, Maione MR, Naturale MD, Esposito S, Zito MC.

The Impact Of Natural Products On Arachidonic Acid Metabolism During Inflammation

Mimica-Dukić N and Simin N.

Prescribing Appropriateness And Analysis Of The Potential Interactions In The Population Of The Elderly Treated With Anticholinesterase Drugs Within The Health District Of Cosenza (Asp)

Altimari I, Rosselli A, Piro B, Vulnera M.



Oral presentations are organized in oral sessions as detailed in the program. Each presentation is expected to last 10-12 minutes including discussion.

Poster session: posters will be on display for all the duration of the meeting and will be discussed at lunch break.

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SOCIETÀ ITALIANA FARMACOLOGIA

ORAL COMMUNICATIONS
(C1 – C24)

POSTER COMMUNICATIONS
(P1-P5)

In memoriam of Professor Norman G. Bowery

S. Tacconi

Aptuit Center for Drug Discovery and Development, Verona, Italy

It is at the same time an honor, and a very difficult, though gratifying, task to try to outline the scientific relevance of the work of Prof. Norman Bowery, and I apologize in advance as it is impossible to recapitulate in few words the entire *corpus* of his scientific contribution.
He advanced the science, seeded and nourished at least three generations of young scientists, enjoyed the beauty of life in his professional work and his personal relationships.
Prof Bowery's scientific achievement dates back to the 70's. His first publications were immediately focused in the themes that soon became the *leitmotiv* of his interests (1). In the peripheral nervous system he studied the metabolism of GABA ranging from its depolarizing action to the transport of amino acids by neurons and glia in the superior cervical ganglia, an experimental model where he investigated many pharmacological aspects of GABAergic transmission and its modulation. The differential activities of some compounds and strange multiphasic binding parameters of others where the initial clues which started to form the idea of a second GABA receptor in his curious mind (2,3).
Consequently, in the autumn of 1979 in a very elegant paper (4), Prof Bowery demonstrated the presence of a second GABA receptor: the era of $GABA_B$ was born. The confirmation of this discovery was provided in the Nature publication in 1980 (5). From 1980 to 1984, four papers dealing with the characterization of this novel receptor with Prof Bowery as principal author were published in the ~~journal~~ scientific magazine Nature.
Inhibitory amino acid neurotransmission was not enough and soon the exciting adventure of excitatory NMDA Glutamate receptor modulation started; in 1986 (6) using the pioneering technique of receptor autoradiography, he demonstrated the presence of two glycine binding sites: a strychnine sensitive and an insensitive one. His intuition led to the confirmation of the presence of a glycine modulatory site on the NMDA receptor (7)
The technique of receptor autoradiography proved to be very important to describe and characterize the distribution of receptors (both novel and known) in central and peripheral nervous system. This was particularly true when no genetic or molecular biology tools were easily available; as an example, this technique was very useful to study the distribution of 5-HT_3 receptors and from this topographic result to infer the physiological role and potential for novel therapeutic intervention. Prof Bowery demonstrated that 5-HT_3 binding sites were present in nucleus tractus solitarius and this result was instrumental for the later development of the first 5-HT_3 drug acting as anti-emetic (Ondansetron) (8). In 1997 Bowery witnessed the cloning of $GABA_B$ receptor and with great insight described this discovery (9)
The Department of Neuropharmacology in the School of Pharmacy under his guidance was a really enjoyable place were many important scientists met and started their careers (10). In 2004, to our great but welcome surprise, Prof. Bowery joined GSK Verona as Vice President Director of Biology Department. A very academic person in the heart of "Big Pharma". Large industrial organizations needed ingenuity but not naivety and he "fitted the bill" perfectly. Collaboration between Prof. Bowery and GSK or some of the scientists who thereafter joined GSK started even before he came to Verona and we can say that they really never ended, because the seeds he dispersed in our mind are still sprouting today (11).
Prof. Norman Bowery was an important leading scientist, a rare pharmacologist, serving in Academia as well as in Industry. His contributions are multifaceted, he contributed to the advancement of knowledge in fields such as glutamatergic transmission, GABA transmission, allosteric modulation of neurotransmitter receptors and many others. Prof Bowery's attitude for science, for teaching and communicating are still with us and he is missed and remembered with great affection by the many colleagues who had the pleasure to work with him.

1. **Bowery NG**, Brown DA.(1971)"Observations on (3 H) -aminobutyric acid accumulation and efflux in isolated sympathetic ganglia." J Physiol. Oct; 218 Suppl:32P-33P
2. **Bowery NG**, Brown DA (1972) "Aminobutyric acid uptake by sympathetic ganglia "Nat New Biol. 1972 Jul 19;238 (81):89-91
3. **Bowery NG**, Brown DA. (1974)" Depolarizing actions of gamma-aminobutyric acid and related compounds on rat superior cervical ganglia in vitro" Br J Pharmacol.Feb;50 (2):205-18
4. **Bowery NG**, et al. (1979) "Baclofen: a selective agonist for a novel type of GABA receptor " Br J Pharmacol. Nov;67 (3):444P-445P
5. **Bowery NG**, et al. (1980) "(-)Baclofen decreases neurotransmitter release in the mammalian CNS by an action at a novel GABA receptor." Nature. 1980 Jan 3;283 (5742):92-4
6. Bristow DR, **Bowery NG**, Woodruff GN. (1986) "Light microscopic autoradiographic localisation of [3H]glycine and [3H]strychnine binding sites in rat brain." Eur J Pharmacol. Jul 31;126 (3):303-7
7. **Bowery NG**. (1987) "Glycine-binding sites and NMDA receptors in brain." Nature. Mar 26-Apr 1;326 (6111):338
8. Pratt GD, **Bowery NG** (1989) "The 5-HT3 receptor ligand, [3H]BRL 43694, binds to presynaptic sites in the nucleus tractus solitarius of the rat."Neuropharmacology. Dec;28 (12):1367-76.
9. **Bowery NG**, Brown DA. (1997) "The cloning of GABA(B) receptors."Nature. Mar 20;386(6622):223-4
10. Bagetta G, Nisticò G, **Bowery NG** (1991) "Characteristics of tetanus toxin and its exploitation in neurodegenerative studies". Trends Pharmacol Sci. Aug;12 (8):285-9
11. Carletti R, Ratti E, Gaviraghi G, **Bowery NG**. (1994) "Comparative receptor autoradiography of ex vivo and in vitro [3H]dizocilpine binding in mouse brain after middle cerebral artery occlusion." Neuropharmacology. Jan;33 (1):43-53

CRITICAL ROLE OF K_V3.4 POTASSIUM CHANNEL AND Na_V1.6 SODIUM CHANNEL IN Aβ OLIGOMER EFFECTS ON NEURON EXCITABILITY AND COGNITIVE FUNCTIONS IN ALZHEIMER'S DISEASE

Ciccone R., Franco C., Secondo A., Boscia F., Piccialli I., Anzilotti S., Vinciguerra A., Di Renzo GF., Annunziato L., Pannaccione A.
Division of Pharmacology, Department of Neuroscience, "Federico II" University of Naples

Alterations of neuronal excitability and ionic homeostasis are crucial events of Alzheimer's disease (AD) pathogenesis. The voltage-gated sodium (Na_V) and potassium (K_V) channels play an essential role in neuronal excitability control, suggesting their involvement in AD. "K^+-dysregulation hypothesis", due to the β-amyloid (Aβ) peptide accumulation, has been proposed for AD[1]. In 2004, Angulo et al.[2] -through a genomic analysis- show K_V3.4 up-regulation in AD brain patients. Shortly after, we demonstrated that $A\beta_{1-42}$ oligomers cause an enhancement of K_V currents in neuronal cells through the selective K_V3.4 over-expression[3]. Furthermore, we show that K_V3.4 upregulation triggers a caspase-dependent neuronal death[4] in accordance to the role of $[K^+]i$ dysregulation in neurodegeneration. On the other hand, in 2017, we demonstrated that K_V3.4 expression and activity are precociously upregulated also in astrocytes exposed to Aβ oligomers and in AD-Tg2576 astrocytes[5]. Moreover, another relevant player in neuronal excitability is represented by Na_V channels whose dysfunction has been summoned in $A\beta_{1-42}$-induced cognitive and memory deficits thus promoting neuronal hyperexcitability and epileptic seizures in AD[6,7]. In order to indentify the molecular entity underlying the dysregulation of Na^+ and K^+ homeostasis in AD, the aims of our study has been to evaluate: 1) the link between the alteration of K_V3.4 expression and activity and caspase-3-dependent cell death as well as the consequences of K_V3.4 dysfunction and the progressive cognitive decline occurring in Tg2576 mice; 2) the correlation between the degree of K_V3.4 dysfunction and AD stage in Tg2576 mice; 3) the link between the alteration of Na_V1.6, the most abundantly expressed Na_V in the central nervous system, and changes in neuronal hyperexcitability in Tg2576 mice. These objectives has been obtained by using both transgenic and neurotoxic models of AD consisting on the use of: 1) hippocampal neurons from Tg2576 mice; 2) wild type hippocampal neurons exposed to $A\beta_{1-42}$ oligomers; 3) Tg2576 mice at different stages of the disease. In these models, K_V3.4 and Na_V1.6 protein expression has been evaluated by the western blot in hippocampal cultures and total brain while their activity has been measured in neurons by patch-clamp in whole-cell configuration. Furthermore, behavioral tests have been performed in WT and Tg2576 mice at the early stage of AD to evaluate memory performance. We found that K_V3.4 overexpression, occurring together with $A\beta_{1-42}$ trimers accumulation in Tg2576 hippocampus, was linked to caspase-3 activation. Accordingly, patch-clamp experiments on Tg2576-hippocampal neurons at 12 DIV, an *in vitro* exemplification of 3-month-old Tg2576 mice, showed an upregulation of K_V3.4 activity and a caspase-3-dependent neuronal suffering. Moreover, the injection of siRNA against K_V3.4 in Tg2576 mice strongly prevented caspase-3 activation and $A\beta_{1-42}$ trimer accumulation. Furthermore, behavioral tests showed an amelioration of memory performance in Tg2576 mice when K_V3.4 was knocking down by siRNA. Furthermore, we showed that hippocampal neurons exposed to $A\beta_{1-42}$ oligomers or obtained from Tg2576 mice displayed a depolarized membranes and augmented spike frequency due to a selective upregulation of Na_V1.6 protein expression and activity. Accordingly, Na_V1.6 silencing or anisomycin treatment, reducing Na_V1.6 expression and activity, attenuated neuronal hyperexcitability in Tg2576-hippocampal neurons at 12 DIV. Collectively our results suggest that Na_V1.6 and K_V3.4 could be considered new potential pharmacological targets in AD therapy, being involved in cognitive decline and neuronal hyperactivity of Tg2576 mice.

1. Etcheberrigaray and Bhagavan, 1999
2. Angulo et al., 2004
3. Pannaccione et al., 2005;
4. Pannaccione et al., 2007
5. Ciccone at al., 2017
6. Bakker et al., 2012
7. Ren et al., 2014

HE MODULATION OF INTRACELLULAR CA^{2+} HOMEOSTASIS IS INVOLVED IN SOD1 AND APOSOD1-INDUCED NEUROPROTECTION OF MOTOR NEURONS EXPOSED TO BETA-METHYLAMINO-L-ALANINE

Petrozziello T, Tedeschi V, Pannaccione A, Boscia F, Di Renzo GF, Annunziato L, Secondo A. *Division of Pharmacology, Department of Neuroscience, Reproductive and Odontostomatological Sciences, School of Medicine, "Federico II" University of Naples, Italy*

Amyotrophic lateral sclerosis (ALS) is a human adult-onset neurodegenerative disease characterized by the loss of upper motor neurons in the cerebral cortex and lower motor neurons in the brainstem and spinal cord. As it occurs in cellular senescence, the impairment of antioxidant cellular machinery and intracellular calcium concentrations ($[Ca^{2+}]_i$) represent important mechanisms underlying cellular vulnerability in ALS. Accordingly, in about 20% of cases, familial amyotrophic lateral sclerosis as well as some sporadic cases of the disease are associated with mutations in the gene encoding Cu,Zn-superoxide dismutase (SOD1)[1,2], the enzyme that catalyzes superoxide anion dismutation. However, SOD1 mutation may cause motor neuron degeneration probably through a gain of toxic function rather than a loss of catalytic function. Indeed, mutations in metal-free apoprotein, which lacks catalytic activity, may play a more important role in ALS pathogenesis than alterations in holoprotein structure[3]. This suggests that the apo state of SOD1 (eg ApoSOD1) could exert a peculiar role in motor neuron physiology. Accordingly, in a previous study we show that the neuroprotective effect of SOD1 in motor neurons exposed to the cycad toxin L-BMAA, a model exemplifying the Guamanian form of ALS, is not dependent on its catalytic activity but rather on the activation of Ca^{2+}/Akt/ERK1/2 signaling pathway that, in turn, prevents ER stress-dependent neuronal death[4]. On the other hand, resting SOD1 release is impaired in ALS[5]. Indeed, we hypothesized that an altered $[Ca^{2+}]_i$ modulation by SOD1 -due to an improper release from motor neurons- could affect neuronal survival. To this aim, with the help of Fura2-single cell microfluorimetry, we compared the effect of SOD1, ApoSOD1 and recombinant $SOD1^{G93A}$ in the modulation of $[Ca^{2+}]_i$ in primary motor neurons. SOD1, ApoSOD1 and recombinant SOD1wt, but not $SOD1^{G93A}$, induced a rapid increase in $[Ca^{2+}]_i$ partially dependent on the release of Ca^{2+} from intracellular Ca^{2+} stores. However, this effect was partially reduced in a Ca^{2+}-free solution, thus suggesting a possible involvement of a such plasma membrane player. To this aim, we hypothesized the involvement of the Na^+/Ca^{2+} exchanger (NCX), a 10-transmembrane domain protein mainly involved in the regulation of $[Ca^{2+}]_i$ homeostasis in several neurological diseases. The isoform 1, named NCX1, was highly expressed at the plasmamembrane level of motor neurons with the highest immunosignal in the neuronal cone. However the isoform 3, NCX3, was localized intracellularly. Accordingly, Fura-2-detected NCX activity was higher in the cone than in cell body of motor neurons. Interestingly, SOD1-induced $[Ca^{2+}]_i$ increase was partially prevented by the amiloride derivative CB-DMB, a well known inhibitor of NCXs[6]. Collectively, our data indicate that SOD1 and ApoSOD1, but not $SOD1^{G93A}$, modulated $[Ca^{2+}]_i$ *via* intracellular and plasmamembrane targets, thus inducing neuroprotection in ALS.

1. Rosen et al. *Nature* 1993; **362**: 59-62.
2. Renton et al. *Nat Neurosci* 2014;17:17-23.
3. Lindberg et al. *Proc Natl Acad Sci* 2002; 99:16607-16612.
4. Petrozziello et al. *Cell Death and Diff.* 2017
5. Turner et al. *J Neurosci* 2005; 25: 108-117.
6. Secondo et al. *JPET* 2009 331:212-221

ALTERED PROTEIN *O*-GLCNACYLATION PROFILE REVEALED BY PROTEOMICS IN AD: NOVEL INSIGHTS ON PROTEIN SIGNALLING MECHANISMS AND POTENTIAL THERAPEUTIC TARGETS.

Fabio Di Domenico[1], Antonella Tramutola[1], Nidhi Sharma[1], Eugenio Barone[1], Tommaso Cassano[2], Marzia Perluigi[1].
[1] *Department of Biochemical Sciences, Sapienza University of Rome, Rome, Italy*
[2] *Department of Clinical and Experimental Medicine, University of Foggia, Foggia, Italy*

Background: PET scan analysis demonstrated the early reduction of cerebral glucose metabolism in AD patients that can make neurons vulnerable to damage *via* several mechanisms including the alteration of the hexosamine biosynthetic pathway (HBP). In turn, defective HBP lead to flawed protein *O*-GlcNAcylation coupled, by a mutual inverse relationship, with the increased protein phosphorylation on Ser/Thr residues (Hart et al., 2011). Impaired *O*-GlcNAcylation of Tau and APP have been reported in AD and are closely related with pathology onset and progression (Lefebvre et al., 2003; Kang et al., 2013; Yuzwa et al., 2014 and Förster et al., 2014). Previous studies from our laboratory (Di Domenico et al., 2010) demonstrated that aberrant O-GlcNAc of proteins occur in AD brain affecting, among others, metabolic, synaptic and a others proteins known to be involved in pathways associated with cellular insults present in neurodegeneration. As well, type 2 diabetes patients show altered GlcNAcylation/phosphorylation balance together with an increased risk to develop AD. Therefore, altered protein *O*-GlcNAcylation might represent a link between metabolic defects and AD progression.
Methods: Our study aim to decipher the status of HBP pathway, the role of total *O*-GlcNAcylation reduction and the specific protein targets of altered *O*-GlcNAcylation in brain of 12 months-old 3xTg-AD compared with age-matched wild-type mice. Hence, we analysed: 1) the Global OGlcNAc levels, as well as, the levels and activity of *O*-GlcNAc transferase (OGT) and *O*GlcNAcase (OGA), the enzymes controlling its cycling; 2) Specific O-GlcNAc levels of proteins by 2D proteomic approach coupled with ESI-MS/MS; 3) The mutual relationship between *O*GlcNAcylation and phosphorylation on our targets of interest;
Results: Our data demonstrate altered enzyme activity and expression levels of OGT and OGA, together with the decrease of total *O*-GlcNAcylation levels in 12 months-old 3xTg-AD compared to non-Tg. Data from proteomics analysis led to the identification of several proteins with differential *O*-GlcNAcylation levels, between transgenic and wild-type animals, which belong to key pathways involved in the progression of AD such as neuronal structure, degradation processes and energy metabolism. Interestingly, the majority of proteins identified by MS analysis show the concomitant alteration of phosphorylation levels suggesting that the unbalanced *O*GlcNAcylation/ phosphorylation levels may lead to altered functionality of these proteins and contribute to early cognitive defects of AD.
Conclusions: Our findings may contribute to understand the effects of altered protein *O*GlcNAcylation
profile during AD, identifying novel mechanisms of disease progression related to glucose hypometabolism. In addition, our findings may lead to the identification of novel therapeutic targets and strategies to slow or delay AD progression.

1. Hart G.W.et al., 2011 Annu. Rev. Biochem. 80, 825–858. PMID:21391816
2. Lefebvre T et al., 2003. Biochim. Biophys. Acta 1619, 167–176. PMID:12527113
3. Kang MJ et al., 2013. Exp. Mol. Med. 45. PMID:23807304
4. Yuzwa S.A.et al., 2014. J Mol Biol. 426(8):1736-52. PMID:24444746
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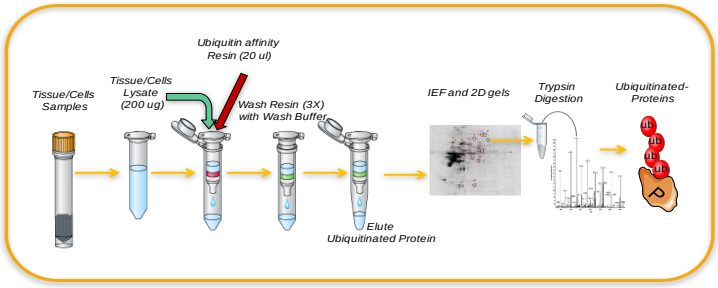
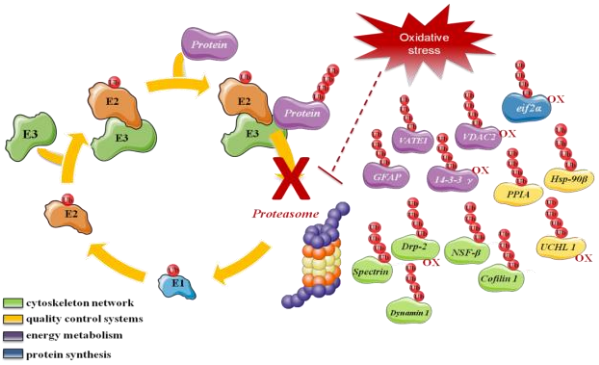
DISTURBANCE OF UBIQUITOME PROFILE IN DOWN SYNDROME AND ALZHEIMER'S BRAIN

Perluigi M[1], Tramutola A[1], A Arena[1], Barone E[1], Butterfield DA[2] and Di Domenico F[1]
[1]*Department of Biochemical Sciences "A. Rossi-Fanelli", Sapienza University of Rome, Italy;* [2]*Department of Chemistry, Markey Cancer Center, and Sanders-Brown Center on Aging, University of Kentucky, Lexington, KY 40506-0055, USA*

Background: Among the putative mechanisms proposed to be common factors in Down Syndrome (DS) and Alzheimer disease (AD) neuropathology, deficits in protein quality control has emerged as a unifying mechanism of neurodegeneration. DS offers a unique model to investigate the early molecular changes that disturb neuronal homeostasis and that with age lead slowly, but irreversibly, to neuronal death [1]. Considering that disturbance of protein degradative systems are present in DS and AD and that oxidized/misfolded proteins require poly-ubiquitination for degradation via the ubiquitin proteasome system, this study investigated if the dysregulation of protein poly-ubiquitination is associated with AD neurodegeneration in DS. **Methods:** Post-mortem brains from DS, before and after development of AD, and AD cases and their age-matched controls were analyzed. Polyubiquitinated proteins were isolated by affinity chromatography and analyzed by proteomics approach. Proteins showing altered pattern of polyubiquitination among the different groups were identified by mass spectrometry. In addition, the extent of poly-K48 and poly-K63 modifications were also analyzed.



Results: This is the first study showing alteration of the polyubiquitination profile in DS, prior and after development of AD, and AD brain compared with healthy controls. The accumulation of polyubiquitinated proteins, either K48 or K63 localized, is present in younger cases with DS without significant AD pathology, suggesting an early aging phenotype. We also found that oxidation is coupled with polyubiquitination for several identified proteins, which are mainly involved in protein quality control and energy metabolism. In the presence of reduced proteasomal activity and impaired autophagy, oxidized/misfolded proteins tagged by polyubiquitin chains may accumulate and contribute to a toxic environment for neurons in DS and AD.



Conclusions: Considering that the development of AD pathology is age dependent in DS and shows characteristic features of AD, identification of common polyubiquitinated proteins in both DS and AD can provide novel insights on the overlapping mechanisms that may lead from normal aging to the development of AD.

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EFFECT OF ANTIDEPRESSANT TREATMENT ON NEURONAL AUTOPHAGIC FLUX

F. Cavaliere[1, 2], A. Adornetto[2], A. Fornarelli[1], F. Bertan[1], R. Russo[2], G. Bagetta[2], M.T. Corasaniti[3], D. Bano[1] and P. Nicotera[1]
[1]*German Center for Neurodegenerative Disorders (DZNE), Bonn, Germany*
[2]*Department of Pharmacy, Health and Nutritional Sciences, Section of Preclinical and Translational Pharmacology, University of Calabria, 87036 Rende (CS) Italy*
[3]*Department of Health Sciences, University "Magna Graecia" of Catanzaro, 88100 Catanzaro, Italy*

Major depressive disorders are among the most frequent psychiatric illnesses and depression is the most common psychiatric complication in patients affected by aging-related neurodegenerative conditions including Alzheimer's and Parkinson's disease. Therefore, antidepressants, from tricyclic (TCAs) to selective serotonin (SSRIs) and selective serotonin/noradrenalin re-uptake inhibitors (SSNIs), are widely prescribed drugs. It is becoming clear that, besides their common principle of action influencing the monoaminergic transmission, some antidepressants exert additional effects (i.e. increase of hippocampal neurogenesis and neurotrophin release) playing a role in neuronal remodeling and synaptic plasticity. The discovery of additional targets raises important questions regarding either their actual or novel potential therapeutic applications, as well as unknown adverse effects. We have previously demonstrated that clomipramine, a widely used FDA-approved tricyclic antidepressant, affects autophagic flux and potentiates chemotherapy in tumorigenic cells (1). However, it remains under debate whether antidepressant drugs have similar effects in neurons.
Materials and Methods: Autophagosome formation and cargo degradation was investigated in primary cortical and hippocampal neurons exposed to clomipramine or fluoxetine (1 - 5 μM) for 12, 24 or 48h and mice treated with clomipramine or fluoxetine for 21 days. Assessment of GFP::LGG-1 positive puncta was performed in C. *elegans* L3 larvae exposed to clomipramine for 24h.
Results: Treatment with clomipramine and fluoxetine inhibited neuronal autophagy in primary cortical neurons. More importantly, similar findings are recapitulated in the frontal cortex of mice treated with the TCA. The latter inhibition was associated with an impairment of the lysosomal pathway degradation, leading to accumulation of aggregate-prone proteins in *C. elegans.*
Conclusion: Overall, our data suggest that clomipramine can impair autophagic flux potentially affecting neuronal function. In view of the evidence supporting a role of autophagy derangement in age-related neurodegenerative diseases (2) our observation casts some doubt in the use of these drugs for the treatment of symptoms of depression in Alzheimer and Parkinson's disease patients.

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ANTI-INFLAMMATORY AND NEUROPROTECTIVE EFFECTS OF CO-ULTRAPEALUT IN A MOUSE MODEL OF VASCULAR DEMENTIA

G. Casili[1], R. Siracusa[1], D. Impellizzeri[1], M. Cordaro[1], R. Crupi[1], E. Esposito[1], S. Cuzzocrea*[1,2]

[1] *Departement of Chemical, Biological, Pharmaceutical and Environmental Science, University of Messina, Messina, Italy*

[2] *Department of Pharmacological and Physiological Science, Saint Louis University School of Medicine, Saint Louis, USA*

Vascular dementia (VaD), the second most common cause of cognitive impairment in the population, is a disease that results from reduction in regional cerebral blood flow and involves oxidative stress and inflammation. Co-ultramicronizedPEALut (co-ultraPEALut) is a new compound with beneficial effects, which include anti-inflammatory and anti-oxidant properties. Recently, co-ultraPEALut has been shown to exhibit neuroprotective effects in models of Parkinson's disease, cerebral ischemia and Alzheimer's disease. However, its effects on VaD remain unclear. Therefore, the present study studied the potential neuroprotective actions of co-ultraPEALut containing N-Palmitoylethanolamine (PEA) and the anti-oxidant flavonoid luteolin (Lut) (10:1 by mass) in a mouse model of VaD induced by bilateral carotid arteries occlusion. At 24 hours after VaD induction, mice were orally treated with 1 mg/kg co-ultraPEALut daily for 15 days. On the 15th day, brain tissues were processed for histological, immunohistochemical, western blot and immunofluorescent analysis. Our results clearly demonstrate that co-ultraPEALut improved learning, memory ability, locomotor activity and the reciprocal social interaction. Furthermore, mice subjected to VaD and treated with co-ultraPEALut showed reduced expression of pro-inflammatory, pro-apoptotic markers and of oxidative stress. These results confirmed that the neuroprotective effects of co-ultraPEALut were associated with its anti-inflammatory and anti-oxidant propertie.

D-ASPARTIC ACID AMELIORATES COGNITIVE IMPAIRMENT IN A MOUSE MODEL OF SPARED NERVE INJURY AND REDUCES B-AMYLOID AB$_{1-40}$ AND AB$_{1-42}$ PEPTIDES

Iannotta M.[1#], Belardo C.[1#], D'Aniello A.[1,2], Romano R. [1], Luongo L.[1], de Novellis V.[1], Maione S.[1]
[1]*Department of Experimental Medicine, Università degli Studi della Campania (Luigi Vanvitelli) Naples, Italy.*
[2]*Department of Neurobiology and Comparative Physiology, Zoological Station "A. Dohrn", Naples, Italy*
[#]*These authors have contributed equally to this work*

Introduction

D-aspartate (D-Asp) is a free D-amino acid found in the mammalian brain and it is the putative precursor of endogenous N-methyl-D-aspartate (NMDA) (1) activates itself NMDA receptors (NMDARs) on the orthosteric site with a relatively high affinity on each NR2A-D receptor subunits (2,3). Furthermore, it is involved in neurological and psychiatric processes, such as cognition and affective disturbances. Depressive symptoms and other neuropsychiatric dysfunctions are common in neurodegenerative disorders, including chronic pain and dementia. A correlation between the β-amyloid protein accumulation and depression development has been suggested, however the underlying mechanisms are unknown.

Based on these evidence, our study aimed to investigate the effects of this amino acid on pain responses and pain-related affective and cognitive behaviour; the presence and the effect of D-Asp treatment on the soluble β-amyloid Aβ$_{1-40}$ and Aβ$_{1-42}$ peptides in the serum, in the medial frontal cortex (mPFC) and in the hippocampus in a long lasting model of neuropathic pain; and the levels of testosterone, progesterone and 17β-estradiol in the brain.

Material & Methods

We have validated this hypothesis using a combination of immunoenzymatic and behavioral approaches in Sham and SNI (Spared nerve injury) mice, a model of neuropathic pain (4), 1 year post-surgery. Moreover, we examined the effects of D-Asp (20 mM) in drinking solution for 1 month.

Results

In a long lasting model of neuropathic pain, SNI mice showed mechanical allodynia, anxiety and depression-like behaviour and cognitive impairments. Moreover, we observed an increase of insoluble form of Aβ$_{1-42}$ at hippocampal level and soluble form of Aβ$_{1-40}$ at serum level. D-Asp treatment improved mechanical allodynia, obsessive-compulsive and depressive-like behaviours and reduced the β-amyloid levels in the serum, mPFC, and hippocampus. Finally, DAsp chronic treatment induced a significant increase of steroid hormones synthesis in the mPFC and hippocampus.

Conclusion

We found, for the first time, that the neuropsychiatric changes and pain behaviours observed in animals with long lasting peripheral neuropathy were associated with an overall increase of Aβ1-40 and Aβ1-42 peptides. D-Asp treatment reduced abnormal behaviours and normalized the β-amyloid protein level. Our findings provide new insights into chronic pain mechanisms and suggest a possible role of β-amyloid protein in neuropathic pain-associated neurological dysfunctions.

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PALMITOYLETHANOLAMIDE, VIA PPAR-ALPHA RECEPTOR, RESTORES THE ALTERED SYNAPTIC PLASTICITY AND AMELIORATES THE COMPROMISED PAIN-RELATED BEHAVIOURS IN THE HIPPOCAMPUS IN NEUROPATHIC MICE.

S. Boccella, L. Luongo, F. Guida, C. Belardo, M. Iannotta, R. Romano, I. Marabese, E. Palazzo, V. de Novellis, S. Maione

Department of Experimental Medicine, Section of Pharmacology "L. Donatelli", Università degli studi della Campania "Luigi Vanvitelli", via S.M. di Costantinopoli, 16. 80138, Naples

Background: The hippocampus is an integral part of the Papez circuit involved in learning, memory, emotion, and motivation. Patients with chronic pain exhibit increased anxiety, depression, and deficits in learning and memory. Long-term potentiation (LTP) in the hippocampus has received attention as the biological substrate at the base of learning and memory. The activation of cannabinoid receptors, either directly by natural or synthetic agonists, or indirectly by selective inhibitors of the inactivation of endogenous cannabinoid receptor ligands (endocannabinoids), is widely supported by recent studies on neuropathic pain management (1, 2 and 3). There is evidence that palmitoylethanolamide (PEA) is able to reduce pain-related behaviors and to restore glutamatergic synapses homeostasis in the medial prefrontal cortex of neuropathic mice (4). **Aim:** In this study, to investigate the impact of chronic pain condition on the hippocampal synaptic plasticity and on the related behavioral responses, electrophysiological, behavioural and biochemical analysis were performed, in a murine model of spared nerve injury (SNI), 30 days post-surgery (5). Moreover, the possible neuroprotective effect of chronic treatment with PEA, was evaluated, in both wild-type and Ppar-α -/- SNI mice. **Results:** Our results showed, in 30 days SNI mice, a reduction of alternation in the Y-maze task, of recognition index in the Novel Object Recognition (NOR) test and of open-arm choice in the elevated plus-maze test, whereas neuropathy induced an increase of the time of immobility in the tail suspension test, as compared to the control group (Sham mice). Moreover both neuropathic wild-type and PPARα null mice showed either an altered spatial memory retention and an impairment of LTP in the granule cells of dentate gyrus induced by theta-burst stimulations (TBS) of the perforant path (PP) in the entorhinal cortex (6). In fact when the entorhinal cortex was electrically stimulated, a great potentiation of the EPSP (LTP), was observed in the ipsilateral hippocampus, in sham mice. PEA chronic treatment (14 days) increased the alternation in the Y-maze task, the recognition index in the NOR test and decreased the immobility time in the tail suspension test, suggesting that PEA was able to improve memory deficits and the depressive-like behavior but not the anxiety-like behavior associated to neuropathic pain. Finally, PEA partially restored the LTP in the dentate gyrus and ameliorated the altered spatial memory in wild-type SNI mice but not in PPARα/SNI null mice. **Conclusions:** These results suggest that neuropathic pain negatively affect the limbic and cognitive functions, which may underlie the deficiency of LTP and memory. Moreover, it opens new perspectives for the possible use of natural compounds such as PEA for the treatment of neuropathic pain and its central behavioural sequelae.

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INFLUENCE OF AGING ON NOCICEPTION IN C57BL/6 MICE

Damiana Scuteri[1], Laura Berliocchi[2], Luigi Antonio Morrone[1], Maria Tiziana Corasaniti[2], Tsukasa Sakurada[3], Shinobu Sakurada[4] and Giacinto Bagetta[1,3].
[1]Department of Pharmacy, Health and Nutritional Sciences, Section of Preclinical and Translational Pharmacology, University of Calabria, 87036 Rende, Cosenza, Italy; [2]Department of Health Sciences, University "Magna Græcia" of Catanzaro, 88100 Catanzaro, Italy; 3First Department of Pharmacology, Daiichi College of Pharmaceutical Sciences, Fukuoka, Japan; 4Department of Physiology and Anatomy, Tohoku Medical and Pharmaceutical University, 4-4-1 Komatsushima, Aoba-ku, Sendai 981-8558.

Background: It has been reported that chronic pain increases with aging and the increased life expectancy makes pain management in the elderly a hard challenge, also because of the lack of drugs effective and safe in this condition (1). The effect of aging on pain sensitivity and threshold is currently not well defined. The purpose of this research project was to study the influence of aging on pain processing at behavioural and molecular level.
Materials and methods: Assessment of basal sensitivity (Von Frey's [2], Pin-prick [3], Hargreaves' [4] and Acetone [5] tests) and of nociceptive behaviour (Formalin Test [6]) was performed in C57BL/6 mice belonging to different age groups and the molecular changes were investigated at spinal cord level through western blotting.
Results: Both mechanical and thermal sensitivity stimuli progressively increased with aging from 2 to 6 and 12 months of age, reaching a sort of *plateau* from 12 to 18 months. Moreover, the changes in mechanical sensitivity are more evident than for thermal sensitivity and cold sensitivity modifications and can be seen already at 6 months of age. Also, the nocifensive behaviour evoked by intraplantar injection of formalin resulted modified from the classical trend in aged mice with an extra peak and a varied amplitude of the response. The pharmacological effectiveness of gabapentin, one of the most currently used painkiller for chronic pain, resulted to be affected by the age of the animal, as found in the formalin test. At molecular level, the expression of some markers of autophagy, an intracellular homeostatic process implicated in aging (7) and, more recently, in chronic pain (8-9), was investigated in the L4-L5 sections of the dorsal spinal cord, highlighting a progressive decrease in Beclin-1 expression. The analysis of the L-type voltage-gated Ca^{2+}-channel subunit $\alpha_2\delta$-1 revealed an age-related "bell-shaped" trend of increase in the spinal cord expression of this biochemical marker, that could be responsible for the increased sensitivity and the different nociceptive behaviour and pharmacological response of aged mice to gabapentinoids.
Conclusions: The data gathered so far provide relevant insights on the influence of the aging process on sensitivity and nociception and, thus, for the treatment of chronic pain in the steadily increasing population of the elderly.

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BIOACTIVITY OF SECONDARY METABOLITES FROM *ALLIUM* SPECIES

N. Simin[1], D. Četojević-Simin[2], D. Mitić-Ćulafić[3], D. Orčić[1], M. Lesjak[1], I. Nemeš[1] and N. Mimica-Dukić[1]
[1]*University of Novi Sad Faculty of Sciences, Novi Sad, Serbia*
[3]*University of Novi Sad, Faculty of Medicine, Oncology Institute of Vojvodina, Sremska Kamenica, Serbia*
[2] *University of Belgrade, Faculty of Biology, Belgrade, Serbia*

Species of the genus *Allium*, especially garlic (*A. sativum*) and onion (*A. cepa*), have had a valuable place in human nutrition and medicine since ancient times. Garlic and onion are well researched species, while data on other *Allium* species are very scarce. Therefore, the aim of this study was to investigate phytochemical profile and biological activities of wild-growing *Allium* species from *Codonoprasum* section and to estimate their potential for application in medicine.
Phytochemical profile of investigated species was determined by headspace GC/MS analysis of fresh bulbs volatiles and by evaluation of presence and content of 44 phenolic compounds in methanol extracts using LC-MS/MS technique. The assessment of antioxidant activity was done by several assays (total reducing capacity, DPPH, NO, $ABTS^{\bullet+}$, $OH^{\bullet}$ assays and ability to inhibit lipid peroxidation). Anti-inflammatory activity of the extracts was evaluated by measuring their ability to inhibit COX-1 and 12-LOX enzymes. Antiproliferative activity was tested by sulforhodamine B assay in three cancer and one normal cell line. DNA damage was monitored in human fetal lung fibroblasts (MRC-5) with alkaline comet assay. Obtained results showed that dimethyl-disulphide is the main volatile sulphur compound in the bulbs, while whole plants methanol extracts are rich in phenolics, with quercetin glycosides and kaempferol 3-*O*-glucoside being the most dominant. *A. flavum* and *A. paniculatum* extracts expressed the highest antioxidant and anti-inflammatory activity. The most pronounced inhibition effect on tumor cell growth was obtained with *A. rhodopeum* and *A. paniculatum* extracts. Extracts of *A. flavum* and *A. melanantherum* reduced t-BOOH-induced DNA damage up to 70%. Obtained results strongly support the traditional use of wild-growing *Allium* species in nutrition and recommend them for further study as promising sources of therapeutic agents.

N-PALMITOYLETHANOLAMIDE PREVENTS PARKINSONIAN PHENOTYPES IN AGED MICE

Campolo M., Crupi R., Paterniti I., Filippone A., Lanza M., Cuzzocrea S., Esposito E.
a Department of Chemical, Biological, Pharmaceutical and Environmental Sciences University of Messina, Viale Ferdinando Stagno D 'Alcontres, 31-98166 Messina, Italy
b Department of Pharmacological and Physiological Science, Saint Louis University, USA

Parkinson's disease (PD) is a neurodegenerative disease characterized by degeneration of dopaminergic neurons. The canonical symptoms of this disease are: resting tremor, rigidity and hypokinesia. Aging is considered the major risk factor for generating idiopathic PD. Recently, several studies have focused the neuroprotective effects of Palmitoylethanolamide (PEA) alone or in combination with antioxidants, in an experimental model of PD after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induction. The aim of this study was to evaluate the pre-treatment effect of micronized PEA formulation (PEAm), on the neuroinflammation process and on the neuronal death on *in vivo* model of PD on aged mice. The old animals were pre-treated for 60 days with PEAm at dose of 10mg/kg. After pre-treatment, they received four injections of the dopaminergic neurotoxin MPTP and were sacrificed 7 days after induction. On the 8th days, brains were processed for histological and immunohistochemical analysis. Pretreatment with PEAm significantly ameliorated behavioral deficits, reduced the expression of specific markers of PD such as tyrosine hydroxylase (TH), dopamine transporter (DAT), as well as decreased the upregulation of α–synuclein and β3-tubulin in the substantia nigra after MPTP induction. Moreover PEAm reduced proinflammatory cytokines expression and showed a pro-neurogenic effect in hippocampus. Thus, this strategy could prevent neurodegenerative diseases associated to the old age.

CD4+ T REGULATORY CELL IN PERIPHERAL BLOOD OF PARKINSON'S DISEASE PATIENTS

Kustrimovic N[1], Rasini E[1], Legnaro M[1], Aleksic I[1], Blandini F[2], Comi C[3], Marino F[1], Cosentino M[1]
[1]*Center of Research in Medical Pharmacology, University of Insubria, Varese (I)*
[2]*Research Center for Parkinson's Disease of the Neurological Institute "C. Mondino" of Pavia (I)*
[3]*Movement Disorders Center of the University of Piemonte Orientale, Divisione di Neurologia, Ospedale Maggiore of Novara (I)*

Increasing evidence supports the involvement of the peripheral adaptive immune system in the pathogenesis of Parkinson's disease (PD), the second most common neurodegenerative disease affecting 7-10 million people worldwide (1). We previously reported a peculiar profile of CD4+ T naive and memory cells in patients in comparison to healthy subjects (HS) (2). In peripheral blood, CD4+ T cells include effector (Teff) and regulatory T lymphocytes (Treg). Treg seem to be key neuroprotective immunomodulators in animal models of PD possibly through modulation of microglial oxidative stress and inflammation (3).
We have enrolled PD patients on dopamine therapy (PD-dt, n=36, age (mean±SD): 70.0±8.1 years) and non-treated PD patients (PD-dn, n=30, 68.2±9.1 years) and HS (n=33, 65.1±11.4 years). By means of flow cytometry, we assessed the frequency of naïve (n) and activated (a) Treg cells and in vitro, examined the Treg suppression of Teff proliferation and cytokine production by usage of co-culture method (4).
Results have shown that in comparison to HS (1096.6±347.1, 10^6/L), PD-dt and PD-dn had reduced number of CD4+ T cells (799.8±278.5, P<0.001 and 850.9±277.4, P<0.002, respectively). Number of total Treg, aTreg and nTreg were significantly reduced in both groups of patients when compared to HS (Figure 1).

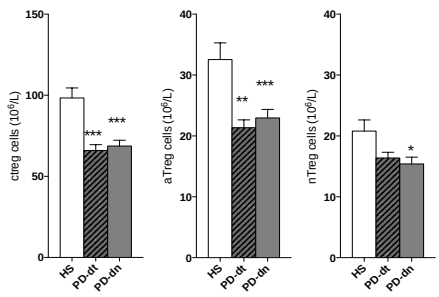

Figure 1. Absolut count of different subsets of CD4+ T regulatory cells, conventional Treg (cTreg), activated (aTreg) and naïve (nTreg) in peripheral blood of HS, PD-dt and PD-dn patients. Differences were analysed by means of two-tailed Student's t test or by Mann-Whitney test, as appropriate. *= P<0.05; ** = P<0.01 and *** = P<0.001 vs HS.

Function of Treg isolated from peripheral blood of patients was preserved regarding the capability to inhibit the proliferation of Teff. In comparison to Teff from HS (IFN-γ, 50.9±37.5 pg/mL and TNF-α 187.7±97.2 pg/mL), Teff obtained from PD-dt and PD-dn patients produced significantly higher amounts of pro-inflammatory cytokines, IFN-γ (167.1±47.7 pg/mL and 228.9±55 pg/mL, respectively, both P<0.01) and TNF-α (249.2±92.4 pg/mL and 228.2±77.9 pg/mL, respectively, both P<0.05). Treg isolated from PD patients were not able to supress production of these cytokines when cultivated together with Teff. In addition, production of anti-inflammatory cytokine IL-10 was reduced in PD-dt and PD-dn patients in comparison to HS (51.9±23.6 vs. 17.1±13.4 pg/mL, P<0.017 and 15.0±7.2 pg/mL, respectively P<0.024)
PD patients thus have complex changes in peripheral immunity, possibly suggesting the prevalence of a pro-inflammatory profile. Therapeutic strategies aimed at increasing Treg neuroprotective immunity could be beneficial in PD.

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NEUROPROTECTIVE EFFECTS OF TEMSIROLIMUS IN ANIMAL MODELS OF PARKINSON'S DISEASE.

Paterniti I, Casili G, Siracusa R, Cordaro M, Crupi R, Bruschetta G, Campolo M, Cuzzocrea S and Esposito E
University of Messina, Departement of Chemical, Biological, Pharmaceutical and Environmental Science, Messina, Italy

Parkinson's disease (PD) is a disorder caused by degeneration of dopaminergic neurons. At the moment there is no cure. Recent studies have shown that autophagy may have a protective function against the advance of a number of neurodegenerative diseases. Temsirolimus is an analogue of rapamycin that induces autophagy by inhibiting mammalian target of rapamycin complex 1. For this purpose, in the present study we investigated the neuroprotective effects of temsirolimus (5 mg/kg intraperitoneal) on 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced (MPTP) neurotoxicity in in vivo model of PD. At the end of the experiment, brain tissues were processed for histological, immunohistochemical, Western blot and immunofluorescent analysis. Treatment with temsirolimus significantly ameliorated behavioural deficits, increased the expression of specific markers of PD such as tyrosine hydroxylase, dopamine transporter, as well as decreased the upregulation of α–synuclein in the substantia nigra after MPTP induction. Furthermore, Western blot and immunohistochemistry analysis showed that temsirolimus administration significantly increased autophagy process. In fact, treatment with temsirolimus maintained high Beclin-1, p62 and microtubule-associated protein 1A/1B-light chain 3 expression and inhibited the p70S6K expression. In addition, we showed that temsirolimus has also anti-inflammatory properties as assessed by the significant inhibition of the expression of mitogen-activated protein kinases such as p-JNK, p-p38 and p-ERK, and the restored levels of neurotrophic factor expression such as BDNF and NT-3. On the basis of this evidence we clearly demonstrate that temsirolimus is able to modulate both the autophagic process and the neuroinflammatory pathway involved in PD, actions which may underlie its neuroprotective effect.

MODULATION OF AUTOPHAGY TO ACHIEVE RETINAL NEUROPROTECTION

G.P. Varano [a], A. Adornetto [a], F. Nazio [b], L.A. Morrone [a], M.T. Corasaniti [c], F. Cecconi [b], C. Nucci [d], G. Bagetta [a] , R. Russo [a].

[a]Department of Pharmacy, Health and Nutritional Sciences, Section of Preclinical and Translational Pharmacology, University of Calabria, Arcavacata di Rende, Italy; [b] Department of Biology, University of Rome Tor Vergata, Rome, Italy; [c] Department of Health Sciences, University "Magna Graecia" of Catanzaro, Catanzaro, Italy; [d] Ophthalmology Unit, Department of Experimental Medicine and Surgery, University of Rome Tor Vergata Rome, Italy.

Macroautophagy, the major subtype of autophagy, is a highly conserved catabolic pathway through which long-lived proteins and organelles are sequestered in a double membrane vesicle called autophagosome and delivered to lysosomes for degradation (1). Besides regulating the physiological turnover of cellular components, macroautophagy acts as a quality control mechanism by clearing and preventing the accumulation and toxicity of aggregate proteins and dysfunctional organelles. Glaucoma is an age-related neurodegenerative disease characterized by the progressive death of retinal ganglion cells (RGCs) (2). Alterations of the autophagic pathway have been linked to several neurodegenerative conditions, nevertheless the involvement of autophagy in the neurodegeneration occurring in RGCs exposed to glaucoma-related stressor stimuli is still debated. Here we attempted to define autophagy efficiency as a determinant for RGC survival analyzing the autophagic response and the upstream regulatory mechanisms in retinas from young and old mice exposed to an ischemic insult.

Materials and Methods: Retinal ischemia was induced in adult (3 months) and aged (24 months) wild type C57BL/6J mice by transient elevation of intraocular pressure. Expression of autophagy related proteins (ATGs) was studied by western blotting and immunofluorescence. RGCs were post-labeled after ischemia by intracollicular injection of the fluorescent tracer FluoroGold and survival was assessed in AMBRA1$^{+/-}$ mice and upon rapamycin treatment or caloric restriction.

Results: The expression of LC3II, the autophagosomal-associated form of LC3, was significantly reduced by ischemia, while the protein accumulated in the ganglion cell layer after 6 hours of reperfusion. A biphasic modulation of the autophagic substrate p62, characterized by a significant build up during the late phase of reperfusion that followed an earlier reduction, was also reported. Increased RGC death was observed in autophagy-deficient Ambra$^{+/-}$ mice subjected to retinal ischemia, while autophagy induction by rapamycin or caloric restriction improved RGC survival.

Conclusion: These results suggest that ischemic insult induces a dynamic modulation of autophagy in the adult and aged retina and identify in the catabolic pathway an important endogenous neuroprotective mechanism that can be targeted to achieve neuroprotection.

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POLYMORPHISMS OF DOPAMINE RECEPTOR GENES ARE ASSOCIATED TO RISK OF DEMENTIA IN PATIENTS WITH PARKINSON'S DISEASE

M. Ferrari[1], C. Comi[2], F. Marino[1], L. Magistrelli[2], G. Riboldazzi[3], G. Bono[3], M. Cosentino[1].
[1]Center of Research in Medical Pharmacology, University of Insubria, [2]Movement Disorders Centre, Neurology Unit, Department of Translational Medicine, University of Piemonte Orientale, Novara. [3]Departments of Biotechnology and Life Science, University of Insubria.

Introduction. Neuropsychiatric disturbances in Parkinson's Disease (PD) display variable progression: at 10 years from onset 46 % of PD patients show cognitive decline whereas 25 % of the same population have a favorable outcome (1). The dopaminergic receptor (DR) system represents an interesting target for investigating cognitive decline and dementia, indeed the role of several variants in DR genes (*DR*) have been described in Alzheimer's disease, schizophrenia, bipolar disorder and addiction (2-4). To date the only studies investigating the role of genetic variants in relation to cognitive disorders have been carried in healthy volunteers. Tsang and colleagues found, in healthy young males, that the rs5326 A allele in the promoter region of *DR*D1 gene was associated with worse cognitive performance (5) whereas Richter found that healthy subjects' carriers of the rs1800497 A allele in *DR*D2 gene showing a diminished learning-related performance enhancement (6). However, at best of our knowledge, so far, there are not data regarding the correlation between *DR* variants and cognitive decline/dementia in PD patients. The present study is aimed to determine whether genetic differences of *DR* are associated with dementia in PD patients.
Methods. In this pilot study, we enrolled retrospectively and consecutively 92 parkinsonian patients, 24 of them showed symptoms of cognitive decline/dementia, while 68 have maintained normal cognitive ability and therefore were included as controls. All subjects were genotyped for selected DR gene variants (*DR*D1 A48G and C62T, *DR*D5 T798C; *DR*D2 G2137A and C957T, *DR*D3 G25A and G712C, *DR*D4 C616G and nR VNTR 48 bp) by polymerase chain reaction and a DR score was attributed to each subject according to previously described procedures (7).
Results. Allelic frequencies did not deviate from Hardy-Weinberg equilibrium. The present preliminary results shown that patients carrying the following alleles had an increased risk of dementia, expressed as OR (95% CI, p value): allele G at *DR*D1 A48G 2.8 (1.1-7.4, p = 0.046) and allele T at *DR*D1 C62T 9.2 (3.1-26.8, p = 0.0001). No association was found between the others *DR* SNPs and cognitive decline. Moreover when genotypes were combined by assigning a score, based on published evidence regarding the effects of individual gene variants, the combination, provided a score of 3.5±1.5 for patients with cognitive decline and 2.6±1.6 for patients without cognitive decline (P = 0.008). The ROC curve of the score had an area under the curve (AUC) of 0.941 (0.885-0.996) (P<0.0001). Using the cut-off value of 1.5, dementia could be predicted with a specificity of 71% and a sensitivity 96%, with a positive likelihood ratio of 3.26. Finally, patients with *DR*D1 62TT genotype displayed shorter time to cognitive decline compared to subjects with *DR*D1 62CT and 62CC alleles.
Conclusions. PD patients with cognitive decline/dementia display higher frequency of *DR* SNPs that was associated with increased D1-like activity. Our data are in line with robust associations reported in neuropsychiatric conditions, such as nicotine and alcohol dependence and psychoses.

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PROGRESSIVE SUPRANUCLEAR PALSY-LIKE PHENOTYPE SECONDARY TO BRAIN VASCULAR LESIONS

Mancini M, Garcea T, Morelli M, Mastroianni G, Arabia G, Lupo A, Manfredini LI, Nicoletti G, Salsone M, Novellino F, Bono F, Mazza M, Ferrigno G, Rocca F, Chiriaco C, Cascini GL, Quattrone A.
Institute of Neurology, University "Magna Graecia" of Catanzaro, Catanzaro, Italy.

Introduction: Progressive supranuclear palsy (PSP) is a degenerative parkinsonism characterized by postural instability with backward falls and vertical supranuclear gaze abormalities. MR Parkinsonism Index (MRPI), calculate by multiplying the pons area-midbrain area ratio by the middle cerebellar peduncle (MCP) width - superior cerebellar peduncle (SCP) width ratio, is able to differentiate PSP from Parkinson's disease (PD), other parkinsonisms and control subjects, without overlap of individual values. PSP patients has the highest MRPI values and this index distinguished PSP from the other groups with a sensitivity, specificity, and positive predictive value of 100% when a cutoff level of 13,55 is used.[1] However, PSP-like phenotype can also results from a variety of vascular disorders characterized by the presence of white matter lesions and lacunes in the brain detected by MRI images.[2] A recent study showed that MRPI was significantly larger in PSP patients compared to Vascular parkinsonism subjects. MRPI value $\geq$ 13 distinguished the two group with a sensitivity and a specificity of 100%.[3]
Clinical case: A 75-years-old woman without familiarity for neurological disease, presented with a 5-year history of slowing of movements and progressive difficulty in walking and frequent falls with an onset in the first two years of illness. The neurological examination showed postural instability with backward falls, hypophonia, slowness of vertical saccades, symmetric bradykinesia and axial rigidity. Mini-mental State Examination score was 21. Levodopa acute test demonstrated unresponsiveness. MRI images of the brain underlined ischemic lesions of basal ganglia and midbrain, with a bilateral high mean diffusivity in putamina and caudate nuclei. Morphometric MRI analysis of brainstem structures showed the following measures: pons area, 540 mm^2; midbrain area, 104 mm^2; MCP width, 7,75 mm; SCP width, 3,69 mm. The MRPI was 10,91. Single-photon emission computed tomography with I^{123}-Iofluopane (DAT-SCAN) showed bilateral reduced uptake in basal ganglia.
Conclusions: The clinical features closely resembled idiopathic progressive supranuclear palsy (PSP). However, MRI images suggested a diagnosis of Vascular Parkinsonism and MR parkinsonism index was incompatible with a classical form of PSP. In conclusion we described a rare progressive supranuclear palsy-like phenotype secondary to vascular lesions.

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BILIVERDIN REDUCTASE-A MEDIATES THE BENEFICIAL EFFECTS OF INTRANASAL INSULIN ADMINISTRATION ON ALZHEIMER DISEASE PATHOLOGY IN THE BRAIN OF 3xTG-AD MICE

E. Barone[1], F. Triani[1], A. Tramutola[1], T. Cassano[1], D.A. Butterfield[3] and M. Perluigi[1]
[1]Department of Biochemical Sciences "A. Rossi-Fanelli", Sapienza University of Rome, Italy; [2]Department of Clinical and Experimental Medicine, University of Foggia, Via Napoli 20, 71122 Foggia, Italy; [3]Department of Chemistry, Markey Cancer Center, and Sanders-Brown Center on Aging, University of Kentucky, Lexington, KY 40506-0055, USA

Background: Biliverdin reductase-A (BVR-A) is a novel direct target of the insulin receptor, which phosphorylates BVR-A thus activating its Ser/Thr/Tyr kinase activity (1). Through this activity, BVR-A negatively regulates IRS1 activation, thus avoiding IRS1 hyper-activation and allowing the correct transduction of the insulin-mediated signalling (1,2). Our group was the first to demonstrate the impairment of BVR-A in human AD brain (3), thus hypothesising its involvement in the onset of brain insulin resistance (b.i.r.). In this picture, along the progression of AD pathology we previously identified two phases in which: (a) the early impairment of BVR-A is responsible for the hyper-activation of IRS1, which then (b) causes the stimulation of feedback mechanisms including mTOR, aimed to turn-off IRS1 hyper-activity (Fig.1), thus promoting b.i.r (4). Reduced BVR-A activity is therefore an early event triggering the onset b.i.r. (4). Intranasal insulin (I-Ins) administration is under evaluation as therapeutic strategy to alleviate b.i.r. in AD. However, the exact molecular mechanisms underlying I-Ins beneficial effects are still unclear. The goal of our project was to clarify whether the I-Ins-associated beneficial effects were mediated by the restoration of BVR-A activity. **Methods:** Changes of (a) the insulin signalling machinery (IR/IRS1/ ERK1/2/Akt/mTOR levels and activation) (b) total OS markers (PC, HNE, 3-NT) and (c) Aβ and tau levels, were evaluated in the hippocampus and cortex of 3xTg-AD and WT mice undergoing an early (4 months) or late (10 moths) I-Ins treatment (1 U/day, 3 times per week, for 2 months) (Fig.2). The morris water maze (MWM) and the novel object recognition (NOR) tasks were used to test cognitive functions. Cell-based experiments to support *in vivo* data were performed in HEK-APP$_{Swe}$ cells. **Results:** I-Ins administration prevents the impairment of BVR-A in young mice while restores BVR-A activity in old animals. Improved BVR-A activity is associated with (a) a restoration of the insulin signaling cascade, (b) reduced OS markers and (c) a reduction of Tau pathology. All these changes parallel an improved cognition. Cell-based experiments confirmed the central role of BVR-A by showing that the effects of insulin are abolished when BVR-A is knocked-down. **Conclusions:** Our data highlight that BVR-A plays a pivotal role in the regulation of the insulin signalling in the brain. Restoration of BVR-A activity first, sheds light on the molecular mechanisms underlie I-Ins-mediated beneficial effects, and then suggests the role of BVR-A as potential therapeutic target to prevent b.i.r.in AD.

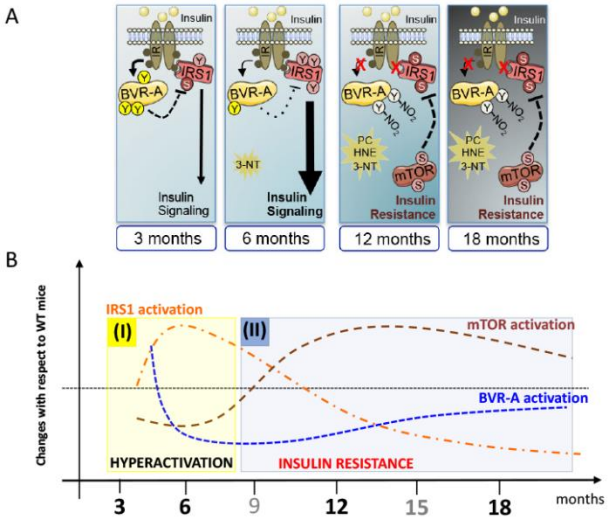

Figure 1. (A) Proposed mechanism leading to b.i.r. in AD. **(B)** Temporal profile of the events promoting b.i.r. in the hippocampus of 3×Tg-AD mice. Arrows, promotion; dotted lines, inhibition; Y, phospho-Tyr residues; S, phospho-Ser residues; Y-NO_2, 3-NT modifications.

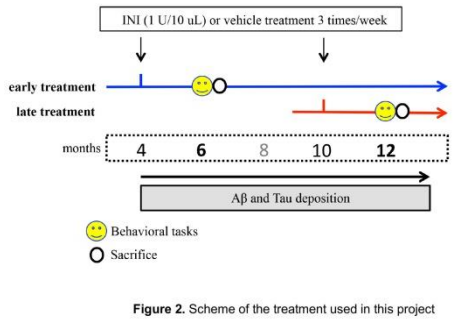

Figure 2. Scheme of the treatment used in this project

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DIMETHYL FUMARATE, NRF2 DEPENDENT, AS A NEW THERAPEUTIC APPROACH FOR AMYLOID BETA-INDUCED OXIDATIVE STRESS.

M. Lanza1, M. Campolo1, G. Casili1, A. Filippone1, I. Paterniti1, S. Cuzzocrea1,2, E. Esposito1.
1Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina, Italy
2Department of Pharmacological and Physiological Science, Saint Louis University, USA

Much of the research on Alzheimer disease has focused on the importance of oxidative processes in disease pathogenesis. Cellular changes demonstrated that oxidative stress is an event that precedes the manifestation of the hallmark pathologies of the disease such as neurofibrillary tangles and senile plaques. Nrf2 is a transcription factor that regulates the gene expression of a wide variety of cytoprotective phase II detoxification enzymes, playing a pivotal role in cellular defense system against oxidative stress. Dimethyl fumarate (DMF) is an orally bioavailable methyl ester of fumaric acid and activator of Nrf2 with potential neuroprotective and immunomodulating activities.
Therefore, the aim of the present work was to evaluate the potential beneficial effects of DMF in an in vitro Alzheimer's model such as SH-SY5Y neuroblastoma cell lines stimulated with amyloid beta (Aβ). DMF pretreatment (30 μM) preserved cellular viability from Aβ 1 μM stimulation, reducing tau phosphorylation. Moreover, DMF was able to induce an activation of manganese superoxide dismutase (Mn-SOD) and heme-oxygenase-1 (HO-1), decreasing the severity of oxidative stress. Our results showed important protective effects of DMF pretreatment from Aβ stimulation in SH-SY5Y cells, sustaining the thesis that DMF, Nrf2 dependent, could provide a valuable support to the therapies for neurodegenerative diseases today

VILDAGLIPTIN IMPROVES ANIMAL BEHAVIOR AND COGNITIVE IMPAIRMENT IN A RAT MODEL OF STREPTOZOTOCIN INDUCED DIABETES

Russo E, Palleria C, De Sarro C, Leo A, De Caro C, Citraro R, De Sarro G.
Science of Health Department, School of Medicine, University "Magna Graecia" of Catanzaro, Italy.

Increasing evidence suggests that cognitive impairment and dementia might be complications of type 2 diabetes mellitus (T2DM) (1). T2DM is a complex metabolic disease that can cause serious damage to various organs. It is a chronic disease due to an altered homeostasis of glucose and lipid metabolism characterized by insulin resistance and often followed by progressive insufficient production of insulin by the pancreatic β cells (1). GLP-1 is involved in glucose homeostasis by enhancing insulin release from pancreatic β -cells. GLP-1 receptors (GLP-1Rs) are also present in the brain mostly in the CA hippocampal regions; their activation stimulates MAPK pathway and their overexpression in the hippocampus improves learning and memory performance (1). GLP-1 has a very short half-life, as a result of its metabolism by dipeptidyl peptidase (DPP)-IV, a naturally occurring enzyme that is present in most tissues of the body and that naturally breaks down GLP-1. DPP-IV inhibitors, further than controlling blood glucose levels, possess neuroprotective effects in animal models (2, 3). Herein, we evaluated the effects of a DPP-IV inhibitor, vildagliptin, on cognitive decline associated with diabetes. Furthermore, we studied vildagliptin effects against hippocampal neurodegeneration induced by streptozotocin (STZ), a well-validated animal model of diabetes and neurodegeneration associated with cognitive decline. Diabetes and/or cognitive decline were induced in Wistar rats by intraperitoneal or intracerebroventricular injection (4) of STZ and then rats were treated with vildagliptin (3mg/kg per day os) for 4 weeks. Rats underwent behavioral tests: Morris water maze, Passive avoidance, Forced swimming test (FST), Open field test (OF). In FST, the duration of immobility was significantly reduced by vildagliptin (3 mg/kg) in comparison to control group suggesting potential antidepressant effects. In the OF anxiogenic effects were observed in all vildaglitpin-treated groups. In MWM vildagliptin improved learning and memory in STZ-treated animals. Therefore, vildagliptin has protective effects on cognitive functions in addition to its effects on blood glucose levels while it may affect mood and anxiety. Further studies are needed to disclose its clinical potential.

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IMPACT OF LEGUMES AND PLANT PROTEINS CONSUMPTION ON COGNITIVE PERFORMANCES IN THE ELDERLY

Y. Ferro[1], E. Mazza[2], A. Fava [1], M. Moraca[1], S. Rotundo[1], F. Provenzano[2], M. Greco[3], D. Foti [3], E. Gulletta[3], D. Bosco[4], T. Montalcini[2] and A. Pujia[1].
[1]*Department of Medical and Surgical Science, Nutrition Unit, University Magna Grecia, Catanzaro, Italy*
[2]*Department of Clinical and Experimental Medicine, Nutrition Unit, University Magna Grecia, Catanzaro, Italy*
[3]*Department of Health Science, Laboratory Unit, University Magna Grecia, Catanzaro, Italy*
[4]*Neurology Unit, S.Giovanni di Dio Hospital, Crotone, Italy*

Background: Numerous studies have investigated the role of the dietary factors in the prevention of cognitive decline (1) but the short-term effects of foods choice on cognitive performances in the elderly are poorly explored. Our aim was to investigate the choice of foods among elderly Italian individuals and the association with cognitive function.
Methods: In this longitudinal study, the participants were 214 individuals aged ≥ 65 years with a score > 20 at the Mini Mental State Examination. The cognitive sub-test of ADA Scale was used to detect cognitive decline progression over 12 months. Food choices was measured by a combination of a 24-hour recall and a seven-day diet record and Principal Components Analysis.
Results: The Principal Components Analysis identified four food and four nutrient patterns. MMSE and ADAS-cog score after 1 year were found to be associated with legumes pattern (Table 1).
A dietary pattern including plant proteins was independently associated with an improved ADAS-cog after 1 year (B=0.584, p=0.04; OR=1.79, CI 0.04-0.42).
Conclusions: The Principal Components Analysis is useful to investigate the choice of foods and nutrients in the elderly. We demonstrated an association between legumes pattern with cognitive performances.

Table 1
Multivariable linear regression analysis –Factors associated with MMSE and ADAS-Cog after 1 year

Dependent variable **MMSE (12 months)**	B	SE	β	p	C.I. 95% LL	C.I. 95% UL
Legumes Pattern	0.235	0.095	0.218	0.014	0.04	0.42

Note. Excluded variables: Age; Education level; SBP. CI = confidence interval; LL = lower limit. UL = upper limit.

ADAS-Cog (12 months)						
					LL	UL
Age	0.290	0.106	0.222	0.010	0.07	0.054
Education level	-0.197	0.084	0.195	0.027	-0.35	-0.022
Legumes Pattern	-0.106	0.377	0.246	0.006	-1.79	-0.304

Note. Excluded variables: Waist Circumferences; Glucose. CI = confidence interval; LL = lower limit. UL = upper limit.

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IMMUNOTHERAPIES FOR ALZHEIMER'S DISEASE: CLINICAL TRIALS EVALUATION, STATE OF THE ART AND FUTURE PROSPECTS.

M. DE FINA (1), A.E. DE FRANCESCO (2), M.D. NATURALE (1), G. DE SARRO (1).
(1) Chair of Pharmacology, Department Science of Health, University of Catanzaro, Catanzaro, Italy.
(2) Pharmacy Unit Director, Mater Domini University Hospital, Catanzaro, Italy.

BACKGROUND

Alzheimer's Disease (AD) is one of the biggest burdens of society with a dramatic and growing worldwide incidence rate of a new case every three seconds, or 9.9 million new cases of dementia every year. Worldwide in 2015 there are 46.8 million people living with dementia.
Despite the efforts made by the research, current treatments provide symptomatic fringe benefits and are not effective in preventing or modifying the disease. The progression of the disease is not well known, but toxic aggregates of misfolded Beta-amyloid peptides (ABeta) represent one of the hallmarks of AD.
One of the therapeutic approaches currently evaluated is the removal of fragments of ABeta from the brain using anti-ABeta antibodies. Immunotherapy ABeta is proving to be a potentially promising treatment strategy based on the human neuropathology and preclinical studies.
The aim of this study was to search, evaluate and analyze the recorded clinical trials studying the use of immunotherapies in patients with AD.

MATERIAL AND METHODS

The Search was performed on clinical trials registered in the last five years through the ICTRP (International Clinical Trials Registry Platform) database. Keywords were "AD" and "ABeta immunotherapy".

RESULTS

Systematic review of clinical trials identified 830 trials registered in the world, of which only 11 with ABeta immunotherapies (4 Bapineuzumab, 3 Solaneuzumab, 4 Crenezumab) in Phase III.
The results of Phase III clinical trials of monoclonal antibodies targeted to the N-terminal sequence of ABeta (bapineuzumab) and the central region of ABeta (solanezumab) gave disappointing results.
Crenezumab (humanized IgG4 monoclonal antibody) binds different forms of ABeta-oligomers, to prevent and break up their aggregation. Its clinical efficacy and safety is currently evaluated in a Phase III, randomized controlled clinical trial (CREAD) in 750 participants, with prodromal or mild AD, at dose 60mg/kg administered as an IV infusion every 4weeks for 100weeks.
Crenezumab was chosen by an international panel of experts, including the US National Institutes of Health, for use in a first-ever prevention trial in AD in a large extended family in Colombia (API-ADAD).
None of the eleven studies was conducted in Europe.

CONCLUSIONS

Clinical trials are conducted through increasingly rigorous and reproducible methodologies. From 2015 the WHO has reiterated the need to publish all the results of clinical trials. This is important to avoid risks to which future volunteers might be exposed. Nevertheless still not all databases make it all data available except ClinicalTrials.gov and the EU Clinical Trials Register. Most of the records does not provide the opportunity for sponsors to include the results in order to ensure public access.

DISTINCT ANXIOLYTIC PROFILE OF BERGAMOT ESSENTIAL OIL IN PRECLINICAL BEHAVIOURAL STUDIES

Laura Tridico[1], Laura Rombolà, Maria Tiziana Corasaniti[2], Tsukasa Sakurada[3], Shinobu Sakurada[4], Giacinto Bagetta[1] and Luigi Antonio Morrone[1].

[1]Department of Pharmacy, Health and Nutritional Sciences, Section of Preclinical and Translational Pharmacology, University of Calabria, 87036 Rende, Cosenza, Italy; [2]Department of Health Sciences, University "Magna Græcia" of Catanzaro, 88100 Catanzaro, Italy; [3]First Department of Pharmacology, Daiichi College of Pharmaceutical Sciences, Fukuoka, Japan; [4]Department of Physiology and Anatomy, Tohoku Medical and Pharmaceutical University, 4-4-1 Komatsushima, Aoba-ku, Sendai 981-8558

Background: Experimental evidences *in vitro* and *in vivo* highlight the favourable neuropharmacological profile of bergamot essential oil (BEO), a phytocomplex obtained by cold pressing of the epicarp and partly of the mesocarp of *Citrus bergamia* Risso et Poiteau (Rutaceae family, genus Citrus). In fact, BEO strengthens synaptic transmission (1), facilitates higher frequency bands of the electrocorticographic (ECoG) spectrum (2) and it shows neuroprotective (3-5) and analgesic properties (6-9). Likewise other essential oils, BEO is widely used in aromatherapy, a branch of herbal medicine, to relieve symptoms of stress-induced anxiety (10) though limited preclinical data are, actually, available. Accordingly, here we investigate the effects of the essential oil in behavioural tests in rats. The results yielded show that BEO is endowed with properties distinct from typical anxiolytic benzodiazepines, such as diazepam (DZP).

Materials and methods: The anxiolytic effect of BEO administered intraperitoneally (i.p.) was studied in rats using an open field task (OFT), an elevated plus-maze task (EPM) and a forced swimming task (FST). Jojoba oil is used as vehicle (control) of BEO.

Results: Systemic administration of BEO (250 or 500 µl/kg i.p.; n=9 and n=12 rats per group, respectively) induces in the OFT a significant (p<0.0001) decrease in the frequencies of crossing, rearing and wallrearing versus the control group (n=12 rats). Conversely, the anxiolytic dose of DZP (1.2 mg/kg i.p., n=6 rats) does not induce statistically significant changes (p>0.05) in these parameters compared to control. A statistically significant decrease is also observed for grooming behaviour in the animals treated both with BEO or DZP compared to vehicle group, while immobility is increased in the rats treated with the phytocomplex. Interestingly, at variance with the effects of BEO, the animals treated with the sedative dose of DZP (5 mg/kg, n=5) are not vigilant and active in all OFT sessions. In EPM task, the administration of BEO or the anxiolytic dose of DZP (n=9) induces a trend towards an increase of the time spent in open arms when compared to vehicle group (n=5). The rats treated with the higher dose of BEO (n=5) or the sedative dose of DZP (n=5) show a decrease in both arm entries when compared to control (n=5). Interestingly, at variance with the effects of the sedative dose of DZP, the animals treated with the higher dose of BEO are still vigilant and active. In FST task, a trend towards a decrease in swimming is observed after both treatments compared to control (n=10), whereas statistical analysis indicates a significant increase (p<0.05) in immobility behaviour in rats treated with BEO (n=6) versus the anxiolytic dose of DZP (n=5). Conversely, drowning-recovering frequency is significantly increased in DZP treated rats when compared to jojoba oil or BEO groups. No statistically significant difference (p>0.05) is observed in struggling behaviour.

Conclusions: In conclusion, BEO induces relaxant and anxiolytic effects with a behavioural pattern distinct from that of DZP. At variance with the effects of sedative dose of DZP, rats treated with BEO are vigilant throughout the duration of the tests and this deserves further investigation. Altogether, our results support the rational use of bergamot essential oil in aromatherapy in symptoms of stress-induced anxiety and are of particular interest since psychotropic drugs, like benzodiazepines, are often associated with severe side effects that adversely affect patient compliance. Benzodiazepines are also widely used to control disruptive behaviour and sleep disturbances, clustered as behavioural and psychological symptoms of dementia (BPSDs), in patients with dementia though limited evidence

exists for their clinical efficacy and safety (11-12). Incidentally, aromatherapy has recently received great interest in the complementary treatment of BPSDs and randomized clinical trials lend some support this use (10).

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PHARMACOLOGICAL BASIS FOR THE TREATMENT OF BEHAVIOURAL AND PSYCHOLOGICAL SYMPTOMS OF DEMENTIA (BPSDs) WITH AROMATHERAPY

Damiana Scuteri[1], Luigi Antonio Morrone[1], Laura Rombolà, Rossella Russo[1], Laura Berliocchi[2], Maria Tiziana Corasaniti[2] , Tsukasa Sakurada[3], Shinobu Sakurada[4] and Giacinto Bagetta[1].
[1]Department of Pharmacy, Health and Nutritional Sciences, Section of Preclinical and Translational Pharmacology, University of Calabria, 87036 Rende, Cosenza, Italy; [2]Department of Health Sciences, University "Magna Græcia" of Catanzaro, 88100 Catanzaro, Italy; [3]First Department of Pharmacology, Daiichi College of Pharmaceutical Sciences, Fukuoka, Japan; [4]Department of Physiology and Anatomy, Tohoku Medical and Pharmaceutical University, 4-4-1 Komatsushima, Aoba-ku, Sendai 981-8558

Background: Along with the cognitive decline and the memory loss, almost all the patients suffering from Alzheimer's disease (AD) develop at least one symptom belonging to the cluster of the Behavioural and Psychological Symptoms of Dementia (BPSDs). Among these symptoms, aggression and agitation remarkably reduce the individual's quality of life (QoL) and adherence to the current therapy for cognitive impairment reduces, but does not prevent, their development. Atypical antipsychotics (Risperidone, Olanzapine, Aripiprazole and Quetiapine) are the most effective and safe treatments for BPSDs if administered for no longer than 6-12 weeks, though they do not control agitation. Recent evidence strongly supports an important role for chronic pain in BPSDs development (1) and this may form the basis for lack of efficacy of atypical antipsychotics. Interestingly, it has been convincingly shown that aromatherapy with *Melissa officinalis* and *Lavandula officinalis* provides relief from all the BPSDs (2) though it does not completely control them. Due to the strong correlation between BPSDs and chronic pain, from which a large proportion of demented patients suffers, the efficacy of aromatherapy using essential oils endowed with established antinociceptive properties may provide a better control for BPSDs. Accordingly, here we report the antinociceptive profile of the essential oil of bergamot (BEO) supporting its translation into the clinic of BPSDs.
Materials and methods: The antinociceptive activity of BEO was studied in mice subjected to a neuropathic pain model such as that induced by the Spinal Nerve Ligation (SNL) (3) or by the Partial Sciatic Nerve Ligation (PSNL) (4) and, because of its defined biphasic nature, to the Formalin Test (5).
Results: Intraplantar (i.pl.) administration of BEO reduces tactile allodynia in neuropathic mice (6-7); as demonstrated in mice bearing PSNL, this antinociceptive effect is observed when BEO is given in the ipsilateral hindpaw and appears to be dose-dependent. Also, in the formalin test, BEO attenuates nociceptive response in both phases when administered ipsilaterally to the site of insult (8), suggesting a peripheral action of BEO. Quite importantly, when given in combination, the antinociceptive effect of BEO enhances the efficacy of morphine, a weak analgesic against neuropathic pain. Accordingly, the peripheral action of this essential oil involves the peripheral opioid system being this reduced by pretreatment with naloxone methiodide, known to be unable to cross the blood brain barrier.
Conclusions: The data yielded so far highlight the antinociceptive effectiveness of BEO in chronic neuropathic pain. Interestingly, deranged autophagy has been implicated in neuropathic pain development (9) and, incidentally, BEO stimulates autophagic flux (10). Collectively, our data on BEO form the rational basis for setting a randomized clinical trial for translation of this preclinical knowledge into pharmacotherapeutic management of BPSDs.

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POSTERS

ANALYSIS OF THE USE OF DRUGS FOR THE TREATMENT OF ALZHEIMER'S DEMENTIA IN CALABRIA REGION

Garreffa MR1, De Francesco AE1, Labate D2, Mirarchi S2, Florio L2
1. UO Farmacia, Azienda Ospedaliero-Universitaria "Mater Domini", Catanzaro, Italy
2. Ufficio DPC Regionale, Azienda Sanitaria Provinciale, Cosenza, Italy

Introduction

Alzheimer's dementia (AD) is the most common cause of dementia in the population above 65 years (43-64% in Italy). Acetylcholinesterase Inhibitors (AChEIs) and Memantine are the only drugs approved in Italy to treat AD. The AChEIs (Donepezil, Rivastigmine, Galantamine) are borne by the National Health Service (NHS) only with diagnosis and Treatment Plan (TP) of Alzheimer Evaluation Units (AEUs) identified by the Regions, limited to patients with mild and moderate AD; in moderate AD, Memantine is used too. Since November 2010, in Calabria the AD-drugs are supplied in private pharmacies on behalf of the Local Health Authority. Our goal is to monitor the consumption of AD-drugs to evaluate the spending trends in relation to the use.

Methods

We analyzed the prescriptions of AD-drugs (ATC N06D) supplied on behalf of the Local Health Authority in each Health District (HD) of Calabria Region (about 1.970.521 total inhabitants) in the years 2015 and 2016.

Results

Patients treated with the drugs N06D are 7091; the most of them was recorded in the Health Districts of Reggio Calabria (2620 patients; 36.95%) and Cosenza (1922 patients; 27.10%). Observing the consumption of AD-drugs in the whole Region in terms of DDD/1000inhabitants/day, comparing the two years considered, it was highlighted that the overall figure is almost the same, equal to 10.19 in 2015 and 9.93 in 2016. However a significant decrease in expenditure was registered, equivalent to 15.93% in 2016 compared to 2015. The less used molecule was Galantamine, which affects the total expenditure for a little more than 2%; the consumption of this drug decreased by 13.25% in the considered period. Also for Donepezil a significant reduction in the consumption (-17.81% in DDD/1000inh/day) was highlighted in favor of Rivastigmine (+2.08%) and especially of Memantine (+5%); indeed, the use of the latter appears in clear growth in each Health District, whereas for Rivastigmine the increase was observed only in Crotone (9.36%) and Vibo Valentia (10.25%). The spending data revealed a decreasing trend for each molecule, particularly relevant for Donepezil and Rivastigmine (with a saving of 26.03% and 19.72% respectively), but for the Memantine too (-7.44%). The HD in which a larger saving was observed are Cosenza (-20.68%) and Crotone (-16.69%); in the Vibo Valentia HD occurred a reduction in spending of 8,72%, despite the use of AD-drugs was increased by 5.28%.

Conclusions

Monitoring of use of drugs for AD is part of an integrated healthcare strategy focused on the resources optimization, also assuring the therapeutic appropriateness. Our Region intends to identify a Care Pathway having as protagonist the patient with AD since his first approach with the General Practicioner. It was adopted a Governance Pharmaceutical Plan, which includes the renegotiation of prices of drugs supplied on behalf of the Local Health Authority, including AD-drugs; as shown by our study, the decreasing of costs are obtained in favor of the patient's health, making sustainable management of the disease. We aim to deepen our analysis to verify the causes relating to fluctuations in consumption of the molecules in each Health District.

ENVIRONMENTAL TRAINING ON CLINICAL SYMPTOMS AND SYNAPTIC DEFECTS IN AGED MICE: A PRE-CLINICAL STUDY TO SUPPORT THE REHABILITATIVE APPROACH IN CLINIC

Bonfiglio T.[a], Vergassola M[a]., Olivero G[a]., Scimone A[a]., Pittaluga A[a,b].
[a]Dept. of Pharmacy, Pharmacology and Toxicology Unit, University of Genoa, Viale Cembrano 4 I-16148 Genoa, [b]Centre of Excellence for Biomedical Research (CEBR), University of Genoa, Viale Benedetto XV, 9 - 16132, Genoa, Italy

Brief introduction: Cognitive dysfunctions, depression and anxiety, poor movement control are common symptoms of central neurodegenerative diseases as well as of ageing. Life experience, sensory stimulation, cognitive activity and spontaneous physical exercise reinforce the "*brain reserve*", the synaptic plasticity which permits to cope brain damage. Rehabilitative programs reinforce "brain reserve" and are proposed as complementary to drugs for therapeutic approaches aimed to the cure of central diseases. Environmental enrichment (EE) refers to the addition of objects to the animal's environment to increase levels of novelty and complexity. It partially reproduces the physical-social activity experienced by aged patients participating to rehabilitative programs. Our study was dedicated first, to evidentiate behavioural impairments and central synaptic defects that occur in aged mice when compared to young ones and secondly, to assess whether these defects could be recovered in animals exposed to EE training

Materials and Methods: To this aim, six and twenty-old male mice were housed in enriched environment three months before sacrifice. Animals were housed in large cages containing a variety of objects such as plastic tunnels, climbing ladders, running wheels, toys in wood and plastic suspended from the ceiling, paper, cardboard boxes, and nesting material that are renewed every 2 days. Control animals (i.e. those exposed to standard environment) were housed in normal cage containing nests. The weight of animals was controlled before during and after the environmental training. The anxiety and curiosity of both trained and non-trained animals were also analysed in the "light-dark box". At the end of the training period animals were sacrificed and the spontaneous and the evoked release of preloaded [^{3}H]noradrenaline ([^{3}H]NA) as well as of endogenous glutamate and GABA monitored from isolated cortical nerve endings.

Results: Our results clearly indicate amelioration of curiosity and reduced anxiety in trained animals when compared to control. Inasmuch, [^{3}H]NA exocytosis was significantly reduced in aged mice when compared to young ones but significantly recovered in trained aged mice. GABA exocytosis also was significantly reduced in aged mice but significantly recovered to physiological level in trained old animals.

Conclusions: Our data allow to conclude that environmental training could ameliorate synaptic defects that could favour mood defects and central inflammation.

ANALYSIS OF ADVERSE REACTIONS TO DRUGS IN PATIENTS WITH ALZHEIMER'S DISEASE OR OTHER DEMENTIAS: CALABRIA REGION ANALYSIS OF ADR

G. Fersini (1), A.E. De Francesco (2), M.R. Maione (3), M.D. Naturale (4), S. Esposito (2), MC. Zito (2)
(1) *Department of Health and Environment Protection Policy Department of Health and Health Policy Sector n. 13 Drug Policy,Pharmacovigilance, Pharmacy Conventioned - Catanzaro,Italy*
(2) *Hospital pharmacy A.O.U. Mater Domini-Catanzaro, Italy*
(3) *Territorial pharmacy ASP Catanzaro-Italy*
(4) *Department of Health Sciences, University "Magna Graecia" of Catanzaro, Catanzaro, Italy.*

BACKGROUND
Dementia is the greatest challenge for the XXI century welfare systems; it is not only a serious personal health problem but it also impacts on the family, society and economy. Dementia is a clinical condition that affects 1 to 5% of the population over 65 years of age, with a prevalence which then doubles every four years, reaching a rate of around 30% at age 80. Alzheimer's type of dementia (AD) accounts for 54% of the total. In Calabria, a total of 1.970.521 inhabitants, those with AD are about 35,000. The drugs used for this disease are reversible inhibitors of acetylcholinesterase (AChE) and Memantine. Objective of the study was to verify the type and severity of suspected adverse reactions observed during the use of such drugs to deepen, in real-life, their tolerability.
MATERIAL AND METHODS
The adverse reaction reports for the period January 2015 - December 2016 for drugs used for the treatment of Alzheimer's dementia (ATC N06D) in both national and regional level were searched for through the Pharmacovigilance National Network (RNF).
RESULTS
For the period of search 440 ADRs are reported nationwide of which 360 for drugs with ATC N06DA and 80 with ATC N06DX.
The former include: donepezil, galantamine and rivastigmine while ATC N06DX find includes only memantine. Donepezil showed 30.1% very common adverse reactions (18 related to nausea, vomiting 12, 10 diarrhea), galantamine can be attributed 2.5% of ADR while 67.4% were caused by rivastigmine, which beside common adverse effects includes problems related to the replacement of the product (18 ADR) and adherence to therapy (20 ADR).
Likewise rivastigmine, the greatest number of ADRs for memantine (80) are to be referred to the replacement of the product (12 ADR).
At the regional level, 2 ADR are included in the RNF grid, e.g. 1 ADR to galantamine (skin reaction) and 1 for memantine, and these concern 7091 demented Calabrian patients.
CONCLUSIONS
Despite the many negative effects related to the use of anti-dementia drugs, examination of data obtained from the national pharmacovigilance network shows that the problem of under-reporting is still widespread. The prompt reporting of adverse reactions is essential to assess the risk profile of the drugs and the responsibility for prescribing includes a record for any damage it may cause, allowing to expand the knowledge of its risk-benefit ratio. To this end, in the face of the progressive increase in the number of dementia patients and the complexity of their clinical care situations, it is necessary to implement the reporting of adverse reactions. This could be achieved through the sharing of a regional PDTAs which would ensure not only the reproducibility of the actions taken by individual specialists in the whole region and consistency of the services provided, but also the sharing of drug safety profiles used for this scope.

THE IMPACT OF NATURAL PRODUCTS ON ARACHIDONIC ACID METABOLISM DURING INFLAMMATION

N. Mimica-Dukić and N.Simin

Department of Chemistry, Biochemistry and Environmental Sciences, Faculty of Sciences, University of Novi Sad, 21 000 Novi Sad, Serbia.

Inflammation is associated with many severe human diseases including vascular diseases, heart disease, cancer, autoimmune diseases like multiple sclerosis and rheumatoid arthritis, as well as various neurodegenerative diseases. This is why developing of new anti-inflammatory drugs is one of the primary goals for modern pharmaceutical industry. One of the most important responses in the cell attacked by some inflammatory agents is the activation of arachidonic acid (AA) metabolism and its conversion to eicosanoids: prostaglandin, prostacyclin and leucotrienes. This pathway involves activities of three different classes of enzymes: phospholipase A2 (PLA2), cyclooxygenases (COX1 and COX2) and lipooxygenases (LOX). Many of anti-inflammatory drugs interrupt AA conversions by inhibiting mentioned enzymes. Thus corticosteroids inhibit PLA2, whereas aspirin and most of nonsteroidal anti-inflammatory drugs (NSADs) express their activity by inhibiting COXs pathways. Concerning many adverse effect of corticosteroids and related drugs, and undesirable effects of non selective NSADs (significant gastrointestinal upset, gastritis, ulceration, hemorrhage etc.), scientific research was focused on COX2 selective inhibitors. However recent studies show that both COX-1 and COX-2 either alone or in concert contribute to gastric mucosal defence. Furthermore it was found that some of COX2 inhibitors (rofecoxib) could promote thrombotic cardiovascular and cerebrovascular disorder (1). Because today, a great attention is devoted to the natural compounds, such as dietary supplement and herbal remedies, which have been used for centuries to reduce pain and inflammation. In the present study the most powerful anti-inflammatory natural products (NP) and herbal remedy will be discussed. Special attention will be given to: curcumin, boswelic acid, green tea, resveratrol, pycnogenol, capsaicin, parthenolide, cucurbitacins, antocyanidins, flavonoids, and volatile terpenoids compounds. It was found that the most of them modulate various inflammatory pathways: by inhibiting COX1, COX2 and 5-LOX but also blocking the activation of NF-kB along with other inflammatory mediators. In addition some more recent study show that some natural products can act as T P2X receptors (P2XR) selective antagonists thus preventing inflammation process that is stimulated by the expression of P2XR proteins (2).

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PRESCRIBING APPROPRIATENESS AND ANALYSIS OF THE POTENTIAL INTERACTIONS IN THE POPULATION OF THE ELDERLY TREATED WITH ANTICHOLINESTERASE DRUGS WITHIN THE HEALTH DISTRICT OF COSENZA (ASP)

Ilaria Altimari, Annalisa Rosselli, Brunella Piro, Marilù Vulnera
UOC Servizio Farmaceutico Territoriale Ufficio Aziendale Farmacovigilanza

Background: Alzheimer's Disease (AD) is characterized by a slow and progressive neuronal degeneration, compromising the cognitive and functional skills of the patient. Due to aging, this disease is continuously increasing and it represents a social burden with a great economic impact. Acetylcholinesterase inhibitors (AChEI) and memantine, used in the therapy of mild (MMSE between 21 and 26) and moderate (MMSE between 10 and 20) AD (1), delay ACh metabolism. These drugs improve cognitive function and the patient's functional autonomy and contrast behavioural psychological symptoms of dementia. Comorbidity is often observed in the elderly and this is underscored by the occurrence of polytherapy. Accordingly, the scope of the study is to

- Analyze the prescribing appropriateness of AChEI (ATC N06D) in our district;
- Stratify for sex and age the population under study;
- Review the concurrent therapies for potential exposure of the patients to pharmacological interactions.

Materials and Methods: The population under study is 780.000 in the two year-period 2014-2015. All the prescriptions have been analyzed for costs, both delivered by the territorial (ASP) Pharmacy units and under convention (private pharmacies), using the DDD/1000 inhabitants/die. N06D patients have been evaluated in relation to possible pharmacokinetic interactions, scoring the RCP about those most relevant (2). Moreover, the concurrent prescriptions of FANS (ATC M01) and analgesics (ATC N02) used in the different stages of pain therapy (TDL) have been evaluated.

Results: 2.305 patients treated with AChEI. Incidence on the total population 0,30%. New patients in 2015: 487 (21,1%). The stratification of patients for sex and age shows prevalence in women: 1388 F; 917 M and increase with age: 11 patients 75 years old. Memantine is used in 34,3% of patients. The analysis of appropriateness indicates: 629 treated with donepezil (0,35 DDD/1000ab/die); 935 with rivastigmine (0,52 DDD/1000abdie); 44 with galantamine (0,03 DDD/1000ab/die); 791 with memantine (0,40 DDD/1000ab/die). The evaluation of the concurrent treatments highlights that 18 patients assume drugs interfering at metabolic level with the AChEI (2) . Among the patients treated with donezepil: four assume fluoxetine and two itraconazole, both enzymatic inhibitors of donezepil metabolism; in two cases carbamazepine is prescribed and in one rifampicin, two enzymatic inducers that interact with donepezil reducing its metabolism. The co-administration is not recommended. In the population treated with memantine, three patients assume ranitidine, that interacts at level of the transport system of the kidney, determining a potential risk of increasing drug plasma level; one patient assumes hydrochlorothiazide that reduces its plasma level; finally, in five cases the concurrent administration of memantine and L-DOPA is outlined, with possible potentiated effects. With respect to the treatment with pain therapy, in the considered two-year period, 698 patients result in co-treatment with FANS and 130 with analgesics. 20,6% of patients assuming FANS uses Ketoprofene, 19,6% Diclofenac and 15,2% Nimesulide. 48,5% of patients assuming AChEI uses Codeine as analgesic.

Conclusions: In the population of the elderly treated with AChEI, the analysis of the concurrent treatment and, in particular, of those with potential pharmacological interactions need to be furtherly deepened. The analysis should be extended to the safety and appropriateness of other drugs often co-administered by care-givers to the demented patients assisted at home or in nursing homes.

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LIST OF CONTRIBUTORS

- **Adornetto A** C6, C15
- **Aleksic I** C13
- **Altimari I** P5
- **Annunziato L** C2, C3
- **Anzilotti S** C2
- **Arabia G** C17
- **Arena A** C5
- **Bagetta G** C6, C10, C15, C23, C24
- **Bano D** C6
- **Barone E** C4, C5, C18
- **Belardo C** C8, C9
- **Berliocchi L** C10, C24
- **Bertan F** C6
- **Blandini F** C13
- **Boccella S** C9
- **Bonfiglio T** P2
- **Bono F** C17
- **Bono G** C16
- **Boscia F** C2, C3
- **Bosco D** C21
- **Bruschetta G** C14
- **Butterfield DA** C5, C18
- **Campolo M** C12, C14, C19

- Cascini GL C17
- Casili G C7, C14, C19
- Cassano T C4, C18
- Cavaliere F C6
- Cecconi F C15
- Četojević-Simin D C11
- Chiriaco C C17
- Ciccone R C2
- Citraro R C20
- Comi C C13, C16
- Corasaniti MT C10, C15, C23, C24
- Cordaro M C7, C14
- Cosentino M C13, C16
- Crupi R C7, C12, C14
- Cuzzocrea S C7, C12, C14, C19
- D'Aniello A C8
- De Caro C C20
- De Fina M C22
- De Francesco AE C22, P1, P3
- de Novellis V C8, C9
- De Sarro C C20
- De Sarro G C20, C22
- Di Domenico F C4, C5
- Di Renzo GF C2, C3
- Esposito E C7, C12, C14, C19

- Esposito S P3
- Fava A C21
- Ferrari M C16
- Ferrigno G C17
- Ferro Y C21
- Fersini G P3
- Filippone A C12, C19
- Florio L P1
- Fornarelli A C6
- Foti D C21
- Franco C C2
- Garcea T C17
- Garreffa MR P1
- Greco M C21
- Guida F C9
- Gulletta E C21
- Iannotta M C8, C9
- Impellizzeri D C7
- Kustrimovic N C13
- Labate D P1
- Lanza M C12, C19
- Legnaro M C13
- Leo A C20
- Lesjak M C11
- Luongo L C8, C9

- **Lupo A** C17
- **Magistrelli L** C16
- **Maione MR** P3
- **Maione S** C8, C9
- **Mancini M** C17
- **Manfredini LI** C17
- **Marabese I** C9
- **Marino F** C13, C16
- **Mastroianni G** C17
- **Mazza E** C17, C21
- **Mimica-Dukić N** C11, P4
- **Mirarchi S** P1
- **Mitić-Ćulafić D** C11
- **Montalcini T** C21
- **Moraca M** C21
- **Morelli M** C17
- **Morrone LA** C10, C15, C23, C24
- **Naturale MD** C22, P3
- **Nazio F** C15
- **Nemeš I** C11
- **Nicoletti G** C17
- **Nicotera P** C6
- **Novellino F** C17
- **Nucci C** C15
- **Olivero G** P2

- Orčić D C11
- Palazzo E C9
- Palleria C C20
- Pannaccione A C2, C3
- Paterniti I C12, C14, C19
- Perluigi M C4, C5, C18
- Petrozziello T C3
- Piccialli I C2
- Piro B P5
- Pittaluga A P2
- Provenzano F C21
- Pujia A C21
- Quattrone A C17
- Rasini E C13
- Riboldazzi G C16
- Rocca F C17
- Romano R C8, C9
- Rombolà L C23, C24
- Rosselli A P5
- Rotundo S C21
- Russo E C20
- Russo R C6, C15, C24
- Sakurada S C10, C23, C24
- Sakurada T C10, C23, C24
- Salsone M C17

- **Scimone A** P2
- **Scuteri D** C10, C24
- **Secondo A** C2, C3
- **Sharma N** C4
- **Simin N** C11, P4
- **Siracusa R** C7, C14
- **Tacconi S** C1
- **Tedeschi V** C3
- **Tramutola A** C4, C5, C18
- **Triani F** C18
- **Tridico L** C23
- **Varano GP** C15
- **Vergassola M** P2
- **Vinciguerra A** C2
- **Vulnera M** P5
- **Zito MC** P3