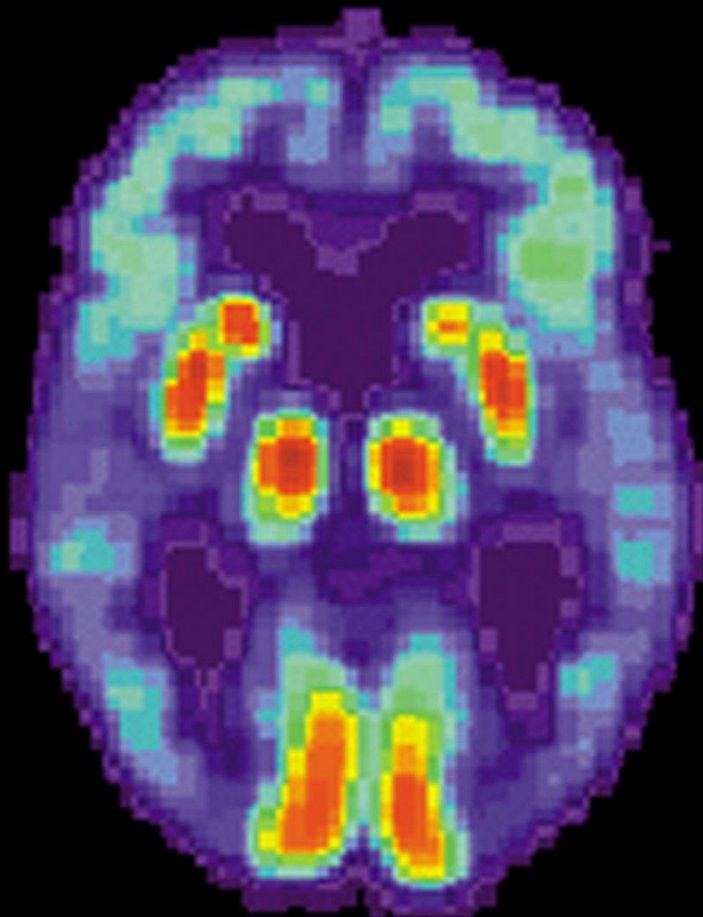


THE 2014 ALZHEIMER'S DISEASE CONGRESS

ABSTRACTS



**23rd-25th June 2014
London, UK**

EuroSciCon 

This three day event will discuss aspects of Alzheimer's Disease development and treatment in an informal academic setting. This year there are three main topics for discussion

Biomarker Discovery and Assay Development
Prevention Strategies and Vaccine Development
Drug Discovery and Development

With plenty of opportunity for networking and debate, this informal international meeting will bring you up to date with current research and thinking regarding Alzheimer's Disease.

This event has [CPD accreditation](#)

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Day 1: Alzheimer's Disease: Biomarker Discovery and Assay Development

Invited Speakers Abstracts

Why have we failed to cure AD?

Professor Amos Korczyn, Professor Emeritus, Tel-Aviv University Medical School, Israel

There is widespread recognition in the urgency to understand the causes and mechanisms of senile dementia. Attempts to find cures for Alzheimer's disease (AD) have, however, failed so far, in spite of enormous investments, intellectual and financial. We therefore have to reconsider the problem from new angles. AD is regarded as a disease because of its clinical manifestations and underlying pathology. However, this combination does not define a disease but rather a syndrome, just like hepatic cirrhosis in which liver pathology causes metabolic changes, but which can result from many different etiologies. It is unlikely that attacking a downstream phenomenon, like apoptosis or β -amyloid accumulation, can cure AD, or prevent the progression of the disease.

It is probable that senile dementia is the result of a combination of several processes, working differently in each person. Epidemiological studies have identified many risk factors for "senile dementia of the Alzheimer type", some genetic but most environmental and therefore modifiable. Therefore a concerted action to fight the dementia epidemic must be made by aggressive action against its risk factors, and this battle must begin in midlife, not in old age.

Alzheimer's disease in Down's syndrome -an ideal model for biomarker discovery?

Dr Shahid Zaman, Affiliated Lecturer & Consultant Psychiatrist, University of Cambridge & NHS, UK

People with Down's syndrome (commonly due to trisomy of chromosome 21) are living longer, into their sixties

and over. They increasingly face age-related health problems including dementia. Alzheimer's disease is much more prevalent and it develops much earlier than in the general population, and neuropathological features are evident some 10 to 15 years prior to clinical features. As the Amyloid Precursor Protein gene, located on chromosome 21 is thought to play a central role in the disease, we explored the feasibility of detecting brain amyloid using [11C]-PiB-PET and blood biomarkers as potential early diagnostic indicators of disease.

Is AD A Medical Notion of Dementia Worth Keeping in Neuroscience?

Dr Fred C. C. Peng, Honorary Consultant, Department of Neurosurgery and Neurological Institute, Taipei Veterans General Hospital, Taiwan

Researchers have refused to admit the fallacy of AD. They (1) perpetuate AD to contrast with other forms of dementia; (2) debate on the effect to seek a one-to-one cause-effect relationship (3) advocate the intervention or prevention of AD in confusion with dementia, by using animal models to mimic AD; (4) assign lesion sites to the hippocampus, unaware that Auguste had diabetes, decubitus angina, arteriosclerosis, and stupor as her brain had widespread atrophy. Dementia is neither a disease nor equivalent to AD but an illness. Plaques and tangles should be called Fischer's Disease for his dichotomy of simple dementia and presbyophrenia. MCI is a cheap reinvention of Fischer's dichotomy.

The biomarkers assessment in a Memory Clinic : is there any added value?

Professor Adrian Ivanoiu, MD, PhD, neurologist, Saint Luc University Hospital & Institute of Neuroscience, Catholic University of Louvain, Brussels, Belgium

Patients with mild cognitive impairment consulting a Memory Clinic are at risk of developing Alzheimer disease (AD), although it remains difficult to make an accurate prediction only on the basis of the cognitive examination. For a more precise diagnosis biological markers for AD have been developed and consist in a biological analysis of the cerebrospinal fluid and brain imaging by MRI and PET. They witness either an abnormally high deposition of β amyloid peptide in the brain or an early neuronal and synaptic damage related to neurodegeneration. Although many biomarkers are now available the method lacks of standardization and validation particularly in unselected clinical populations.

Amyloid hypothesis for AD: Insight from single molecule experiments and computational analyses

Professor Yuri Lyubchenko, University of Nebraska Medical Center, USA

Little progress in the treatment of Alzheimer's disease is due to a fundamental lack of knowledge of the aggregation process. Understanding mechanisms underlying self-assembly into nano-aggregates would facilitate the development of efficient therapeutic and diagnostic tools for AD. We developed AFM force spectroscopy experimental approach enabling us to characterize misfolded amyloids. These studies led to the discovery that the dimerization of proteins dramatically stabilizes their misfolded states suggesting that the formation of dimers is the key step for amyloids aggregation. The contribution of these finding to a mechanistic understanding of Alzheimer's disease early onset will be discussed.

Cerebrospinal fluid Presenilin-1: a potential new biomarker for Alzheimer's disease

Dr Javier Sáez-Valero, Professor and Group Leader, Instituto de Neurociencias de Alicante, Universidad Miguel Hernández-CSIC, & Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), Spain

Presenilin-1 (PS1) is the active component of the γ -secretase complex, and its presence in cerebrospinal fluid (CSF) has not been measured to date. Recently we have demonstrated that PS1 is present in CSF as 100–150-kDa hetero-complexes containing both the N- and C-terminal fragments of the protein, as well other γ -secretase components, but differ from active γ -secretase membrane-complexes. Particularly, the levels of the highly stable PS1 complexes are increased in CSF samples from Alzheimer's disease (AD) cases, suggesting potential value as a biomarker for AD. Estimation of PS1 CSF levels may be useful to challenge the disease-modifying effects of newer Alzheimer's therapy.

Investigation of novel functional and metabolic MRI biomarkers for the preclinical assessment of tauopathy in AD

Dr Niall Colgan, Research Associate, UCL Centre for Advanced Biomedical Imaging, London UK

A key neuropathological hallmark of Alzheimers Disease (AD) is the presence of intracellular neurofibrillary tangles(NFTs) of hyperphosphorylated Tau protein. There is a need for greater understanding of the relationship between tau burden and non-invasive imaging data for improved diagnosis and therapeutic assessment of this devastating disease. In this work, a mouse model of tauopathy was used to investigate how elevated tau expression affects the clinically implementable MRI parameters: i) cerebral blood flow (CBF) using arterial spin labelling (ASL) ii) amide proton transfer(APT) using chemical exchange saturation transfer (CEST) iii) brain glucose metabolism using glucoCEST iv) the diffusion properties of tissue water using diffusion tensor imaging (DTI) v) brain morphology using high resolution 3D structural imaging with tensor based morphometry (TBM). This is the first application of ASL, CEST and DTI to the tauopathy as well as glucoCEST to investigate neurodegenerative disease.

Oral Presentation Abstracts

WHAT DO WE ASSESS USING MEMORY TESTS? A VOLUMETRIC MRI STUDY OF THE FCSRT AND DMS48

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The aim of the present volumetric study was to explore the neuro-anatomical correlates of the two tests most widely used in France to assess verbal and visual anterograde memory. More precisely, we wanted to determine whether the two tests relied on the medial temporal lobe (MTL), and could be predictive of Alzheimer's disease (AD), which pathological changes typically starts in the MTL.

We analysed data from a cohort of 140 right-handed patients with mild cognitive impairment (MCI) participating in the longitudinal multicentric clinical research study BALTAZAR (Biomarker of Amyloid peptide and Alzheimer's disease Risk). Verbal memory was assessed using the Free and Cued Selective Reminding Tests (FCSRT; Grober *et al.*, Neurology 1988;38(6):900-3) and visual recognition memory using a computerized forced-choice task, the DMS-48 (Barbeau *et al.*, Neurology 2004;62:1317–1322), among a larger cognitive baseline. Performances in verbal and visual recognition memory using the two tests were correlated to local gray matter atrophy via structural MRI using Voxel Based Morphometry (VBM, SPM12 in Matlab 8.2).

Our results confirm the involvement of the MTL prominently on the left for the FCSRT and on the right for the DMS-48 in the whole group of MCI patients (FWE, $p < 0.05$). Interestingly, MTL remained implied only in the subgroup of patients who had deficient scores on the cued recall or recognition, but not on the free recall phase of the FCSRT. For the DMS-48, MTL remained implied only for the group of patients whose performances degraded between Set 1 and Set 2.

In conclusion, the FCSRT and the DMS-48 are able to detect specific features of memory loss due to MTL damage, under the condition that the cued recall or delayed recognition scores are deficient. These results have significant clinical implications with regards to the clinical diagnosis of the underlying etiologies of MCI, such as Alzheimer's disease.

THE KINASE PKR IS A BIOMARKER AND A THERAPEUTIC TARGET IN ALZHEIMER'S DISEASE

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In Alzheimer's disease (AD), the neuropathological lesions are senile plaques composed of A β 1-42 peptide, neurofibrillary tangles made of hyperphosphorylated tau protein and synaptic and neuronal losses. According to the "Amyloid Cascade Hypothesis" A β 1-42 monomers and oligomers could be responsible for neurodegeneration. In AD, the A β 1-42 cerebrospinal fluid (CSF) levels are reduced and CSF tau and phosphorylated tau (pTau) are increased. PKR is a pro-apoptotic kinase that is able to phosphorylate the initiation factor eIF2 α and block protein translation. The activation of PKR is triggered by bacteria, virus, cytokines, calcium, and A β 1-42. PKR can modulate inflammatory signals, the levels of BACE 1 and A β 1-42 production as well as GSK3 β activation and tau phosphorylation. PKR^{-/-} mice have an improved memory compared to wild type mice. Activated PKR is increased in degenerating neurons of AD brains. We have performed a study analyzing CSF levels of PKR and activated PKR (pPKR) in AD patients compared to neurological controls and patients with amnesic Mild Cognitive Impairment (MCI). Forty five AD patients, 11 patients with Mild Cognitive Impairment and 35 neurological controls were included and followed for a period of two to three years. All patients had initially, a lumbar puncture to determine CSF levels of A β 1-42, Tau, pTau, PKR and pPKR using ELISA or western blot procedures. Cognitive assessment was performed twice a year. All patients or care givers gave their written informed consents. The mean CSF pPKR level was increased by 300% in AD patients compared to neurological disease controls. The sensitivity was 91.1% and the specificity was 94.3%. pPKR levels were also increased in most MCI patients. In AD patients PKR and pPKR levels correlate with pTau levels. AD patients with high levels of CSF pPKR had a more severe cognitive decline compared to AD patients with lower levels of CSF pPKR

In conclusion, the analysis of CSF PKR and pPKR levels represent a new tool for AD diagnosis and prognosis and PKR is a valuable new therapeutic target to attenuate abnormal molecular signals leading to neurodegeneration in AD.

PROTEOME METASTABILITY IN NEURODEGENERATIVE DISEASE

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Maintaining protein solubility is fundamental to proteostasis, as the formation of diverse aggregated species is associated with a variety of cytotoxic events and disorders, including Alzheimer's and Parkinson's diseases. Increasing evidence indicates that protein aggregation can be widespread in living systems, as many different proteins aggregate upon cellular stress. The origins of this proteomic metastability, however, remain unclear, as do the reasons why only certain proteins aggregate in vivo. We have applied a simple model of protein supersaturation to quantify metastability at a proteome scale. We show that the proteins most vulnerable to aggregation are those whose cellular concentrations are high relative to their intrinsic solubilities. These supersaturated proteins constitute a metastable sub-proteome involved in forming pathological assemblies in stress, ageing, and neurodegenerative disease. We find that such proteins are overrepresented in the biochemical processes associated with neurodegenerative disorders, helping to rationalize their specific cellular pathologies. In Alzheimer's, the core pathological processes are almost all supersaturated, whereas only select classes of more distal processes — in particular those proteins associated with oxidative phosphorylation — are supersaturated. A meta-analysis of autopsy-derived transcriptional data from Alzheimer's patients shows a marked downregulation of genes and pathways that correspond to supersaturated proteins. We propose that the machinery of protein homeostasis typically enables some proteins to be soluble at supersaturated levels, but that a decline in proteostasis puts these proteins and the entire cellular network at proteotoxic risk. Hence, there is a systematic repression of the expression of these proteins when cellular stress is high, such as it is in the brains of Alzheimer's patients. We anticipate that the concept of supersaturation will provide a generally applicable basis for tracking the instability of proteomes — as well as the mechanisms that manage it — in aging, stress, and disease.

Poster Presentation Abstracts

RELATIONSHIP BETWEEN FLORBETAPIR-PET AND STRUCTURAL MRI IN CLASSIFYING MILD COGNITIVE IMPAIRMENT FROM HEALTHY CONTROLS AND AD DEMENTIA IN ADNI

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OBJECTIVE: To compare regions of interest (ROIs) between florbetapir (FBP) PET and volumetric MRI (vMRI) as biomarkers to differentiate stages of Alzheimer's disease (AD).

METHODS: Baseline imaging and cognitive scores of 170 healthy controls (HC), 252 early mild cognitive impairment (EMCI), 136 late MCI (LMCI), and 75 dementia subjects from ADNI-2 were used. We compared FBP-PET standard uptake value ratios (SUVr) for 6 ROIs (anterior cingulate, posterior cingulate, precuneus, frontal medial-orbital, parietal, and temporal) comprising the composite, plus hippocampus and occipital ROIs, to vMRI hippocampus, anterior cingulate, posterior cingulate, precuneus, and lateral ventricle (after intracranial volume correction). Other measures were age, education, animal fluency, Functional Assessment Questionnaire (FAQ), and Montreal Cognitive Assessment. Univariate analyses were done using five logistic regression models: 1.) HC versus MCI (all); 2.) HC versus EMCI; 3.) HC versus LMCI; 4.) EMCI versus LMCI; and 5.) MCI versus AD dementia. Classification performance was compared to random variables.

RESULTS: In all five models, FBP-PET composite SUVr and all ROIs except hippocampus and occipital cortex classified MCI better than chance. Hippocampus did not differentiate in any models and occipital cortex only significantly differentiated groups in Models 1, 3 and 5. For vMRI, hippocampus classified MCI and dementia (Models 1, 3, 4, and 5), but did not distinguish HC from EMCI (Model 2). vMRI posterior cingulate, precuneus, and lateral ventricle classified MCI and dementia (Model 5). Additionally, precuneus distinguished LMCI from HC or EMCI (Models 3 and 4). FAQ had the best classification performance among clinical variables compared with imaging variables in all models.

CONCLUSIONS: FBP-PET distinguished early disease stages better than vMRI (HC from EMCI or LMCI), while vMRI performed better as a later stage biomarker (LMCI and dementia). Occipital FBP-PET performed more like vMRI as a late-stage biomarker, while FAQ reflected functional decline across stages.

EARLY DIAGNOSIS OF ALZHEIMER'S DISEASE BY COMBINATION OF CEREBROSPINAL FLUID CORE BIOMARKERS AND VISININ-LIKE PROTEIN-1 (VILIP-1)

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Mild cognitive impairment (MCI) is a syndrome characterized by cognitive impairment without dementia, which primarily affects episodic memory. Patients with MCI often have an initial stage of Alzheimer's disease (AD). In this study we compared the effectiveness of 6 CSF biomarkers ($A\beta_{1-42}$, total tau, p-tau 181, p-tau 199, p-tau 231 and VILIP-1) in differentiation of AD patients (n = 96) from healthy controls (HC, n = 22). Biomarker levels among AD, MCI (n = 46), and HC, were compared using the Kruskal-Wallis test ($A\beta_{1-42}$: ($\chi^2=10.763$; $df=2$; $p=0.005$); **total tau**: ($\chi^2=34.182$; $df=2$; $p<0.001$); **p-tau 181**: ($\chi^2=28.329$; $df=2$; $p<0.001$); **p-tau 231**: ($\chi^2=28.215$; $df=2$; $p<0.001$); **p-tau 199**: ($\chi^2=24.101$; $df=2$; $p<0.001$); **VILIP-1**: ($\chi^2=15.588$; $df=2$; $p<0.001$)), followed by the Mann-Whitney U test for pairwise comparisons ($A\beta_{1-42}$: AD vs MCI (U=1032; Z=-3.366; $p=0.001$); AD vs HC (U=823.5; Z=-0.860; $p=0.390$); MCI vs HC (U=347.5; Z=-1.224; $p=0.221$); **total tau**: AD vs MCI (U=1110; Z=-4.786; $p<0.001$); AD vs HC (U=428.5; Z=-4.336; $p<0.001$); MCI vs HC (U=391; Z=-1.508; $p=0.132$); **p-tau 231**: AD vs MCI (U=183; Z=-3.712; $p<0.001$); AD vs HC (U=48; Z=-4.816; $p<0.001$); MCI vs HC (U=141.5; Z=-1.820; $p=0.069$); **p-tau**

181: AD vs MCI (U=878; Z=-2.892; p=0.004); AD vs HC (U=200; Z=-4.793; p<0.001); MCI vs HC (U=152; Z=-3.363; p=0.001); **p-tau 199:** AD vs MCI (U=197.5; Z=-3.861; p<0.001); AD vs HC (U=69; Z=-4.160; p<0.001); MCI vs HC (U=158.5; Z=-0.990; p=0.322); **VILIP-1:** AD vs MCI (U=831; Z=-3.295; p=0.001); AD vs HC (U=300.5; Z=-2.931; p=0.003); MCI vs HC (U=279.5; Z=-0.467; p=0.641). Phospho-tau biomarkers, especially p-tau 199, reached the highest specificity, sensitivity and area under curve (AUC), as revealed by ROC analysis. MCI patients with a high risk of AD development were detected based on cut-off levels for p-tau biomarkers. Five MCI patients (21,8%) had increased both p-tau 199 and p-tau 181, while 8 MCI patients (34,8 %) had increased p-tau 199 only. Additionally, 5 MCI patients also had increased both p-tau 199 and p-tau 231. These 5 MCI will be further monitored as they are at risk of AD development. VILIP-1 is not as reliable as phospho-tau biomarkers, but adds to the overall specificity, sensitivity and AUC in differentiating AD from MCI and HC.

RAPID IDENTIFICATION OF A PLATELET-DERIVED ALZHEIMER'S DISEASE-SPECIFIC PHENOTYPE BY BIOCHIP ARRAY TECHNOLOGY

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Background: Alzheimer's disease (AD) is a chronic, neurodegenerative disorder and one of the major health care challenges of the 21st century. Due to still unknown aetiology, 'definite' AD diagnosis relies on post mortem brain examination, while ante mortem only a diagnosis of 'probable' AD is possible; this is achieved either by neuropsychological assessment or brain scans. On the molecular level, AD-specific cerebrospinal fluid biomarkers are promising, however, the invasive lumbar puncture renders their routine application impossible. Consequently, alternative sample materials and minimally invasive diagnostic procedures are urgently needed. Based on the shared biological similarities of platelets and neurons, these peripheral cell fragments were used to characterize AD-specific biomarkers.

Methods: The study was approved by the local ethics committee and blood was collected from 62 patients with clinically suspected AD and 63 age/sex-matched controls. Their platelet proteomes were resolved in two independent discovery and verification phases using fluorescence 2D-DIGE; AD-regulated proteins were tryptically digested and MS-identified. *APOE ε4* and *GSTO1*A140* genotyping was performed. For high-throughput analysis with the novel multiplex protein biochip, platelet-rich plasma (PRP) was prepared from whole blood (20 min, 80 x g) and stored at -80 °C. After thawing, platelets were isolated and lysed with SDS before addition of 2% BSA/PBS to bind excess SDS. Lysates were applied to the protein biochip (immunoassay sandwich principle) and protein biochips subsequently imaged on the Evidence Investigator analyser.

Results: Four proteins were highly significantly up-regulated in LOAD samples: monoamine-oxidase-B (MaoB), tropomyosin-1 (Tm1), apolipoprotein E4 (ApoE4), and glutathione S-transferase omega1 isoform A140 (*GSTO1*A140*). While both isoforms result from single nucleotide polymorphisms, the latter was highly predominant in *APOE ε4*-negative patients: genotyping revealed significantly more *APOE ε4* carriers in the AD (66%) than in the control (11%) group and presence of exclusively two *GSTO1*A140* alleles in non-*APOE ε4* AD patients (n=20) relative to 38% in controls (30% in non-*APOE ε4* controls) and 32% in *APOE ε4*-positive AD patients. Biochip analysis correctly identified 98% of all samples of the *GSTO1*A140* and 100% of the *APOE ε4* genotype by normalisation with either ERK2 or panApoE concentrations. Biochip quantification of Tm1 and MaoB (ERK2-normalised) also replicated the higher expression of these two proteins in AD patients relative to controls. An algorithm utilising these four biomarkers yielded the highest separation power for AD and control samples with a ROC AUC of 0.969 (95% CI=0.944-0.994).

Discussion: The combination of four AD-regulated platelet proteins, including the most powerful genetic risk factor of late-onset AD, *APOE ε4*, enabled identification of AD patients with a sensitivity of 94% and a specificity of 89%. This demonstrates the utility of this innovative multiplex device as reliable peripheral diagnostic tool to aid ante mortem AD diagnosis in a routine blood-based clinical screening.

POTENTIAL EFFECT OF AMYLOID IMAGING ON DIAGNOSIS AND INTENDED MANAGEMENT OF PATIENTS WITH COGNITIVE DECLINE: IMPACT OF APPROPRIATE USE CRITERION.

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Background

Published appropriate use criteria (AUC) by Johnson et al, 2013, provide guidelines for selecting patients for whom amyloid PET could be useful.

Objectives

To evaluate the impact of amyloid PET on diagnosis and intended management in a set of patients likely to meet AUC.

Methods

We examined 229 cases from a completed study of Florbetapir amyloid PET in patients either undergoing or having recently completed an evaluation for cognitive decline. In these cases, Alzheimer's Disease (AD) was suspected and there was uncertainty (<85% confidence) in the clinical diagnosis. All cases received a provisional diagnosis, and an intended treatment/management plan prior to PET scan, and a final diagnosis and management plan post PET scan. Based on the retrospective review of prescan diagnosis and demographics, cases were classified as likely meeting AUC (AUC-like) or not.

Results

125/229 (55%) subjects were classified as AUC-like. The nonAUC cases included typical AD, Mild Cognitive Impairment due to AD (MCI due to AD), Cognitive Decline without objective evidence of impairment (CD) and dementia or cognitive impairment with specific nonAD diagnosis (for example Fronto Temporal Dementia - FTD). 59/125 (47%) AUC-like cases were amyloid positive (A β +). Within the nonAUC group, the proportion of A β + cases ranged from 6/21 (29%) in CD patients, to 49% in subjects clinically diagnosed as MCI due to AD, to 53% in patients with a clinical diagnosis of non AD and to 22/30 (73%) in typical AD. Diagnosis changed after PET scan for 62% of AUC cases vs 45% of nonAUC cases. The proportion of patients with change in management plan was high (>85%) regardless of AUC category.

Conclusions

PET amyloid imaging altered diagnosis and management in patients selected according to AUC. Additionally, appropriate use criteria exclude patients with a relatively high (typical AD) or low (CD) probability of a positive A β scan in most cases. However, in two groups of patients classified as nonAUC by the strictest interpretation of the criteria (provisional diagnoses of MCI due to AD and nonAD dementia) the proportion of A β + scans was close to chance (49 and 53%).

MICRO-RNA MODULATION MEDIATED BY ELECTROMAGNETIC FIELDS: A CYTOPROTECTIVE EFFECT

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INTRODUCTION

Epidemiological studies have shown an impact of low frequency electromagnetic field (LF-EMF, ranging from 1 to 300 Hz) exposure on development of pathological conditions of cells and tissues. However, some studies have suggested a therapeutical role of LF-EMF in fighting neurodegenerative diseases, considering their capability *in vitro* to modulate gene expression, acting on transcriptional and post transcriptional processes (Nikolova et al., 2005; Remondini et al., 2006; Zhou et al., 2011). These mechanisms include a class of small non-coding RNA molecules, called microRNAs (miRNA), able to regulate gene expression (Krützfeldt and Stoffel, 2006). Noteworthy, miRNAs regulate the expression of key proteins involved in AD pathogenesis and the expression of certain miRNAs is altered in AD patients (Nelson et al., 2010- 2011; Smith et al., 2011; Junn and Mouradian, 2012; Kuman et al., 2013; Leidinger et al., 2013), thus suggesting that a dysfunctional miRNAs-based regulatory system may represent a new etiologic factor for AD. In this scenario, a deeper understanding of the relation between AD, EMFs and miRNAs may help to shed more light on the molecular bases of this pathology. Moreover, a better comprehension of miRNA role in AD could represent a good start point to identify novel circulating biomarkers and, hopefully, effective therapeutic approaches. In this study we focused our attention on miRNA-30a, which mediates a repressive effect on Beclin1 expression, that regulates autophagy and plays an important role in development, tumorigenesis, and neurodegeneration (Zhong et al., 2009). Beclin-1 and its binding partner class III phosphoinositide 3-kinase (PI3K) are required for the initiation of the formation of the autophagosome in autophagy.

MATERIALS AND METHODS

Modulation of autophagy was investigated in human neuroblastoma SH-SY5Y cells, which were also subsequently exposed to A β peptides.

Autophagy-related markers (ATG7 and LC3B-II) and expression of miRNA-30a were analyzed after treatment with LF-EMF. To produce a pulsed electromagnetic fields was used a magnetic bioreactor with the following characteristics: intensity 2 mT, amplitude 5 mV, frequency 75 Hz , impulse duration 1.3 ms.

RESULTS

The results primarily point that LF-EMF induce a significant reduction of microRNA 30a (miR-30a), with consequent increase of Beclin1 transcript. Our results show also an increase of the expression of autophagy-marker (ATG7 and LC3B-II). As well known, autophagy is encharged of enhancing the clearance of proteins typically associated with AD. These data suggest that specific LF-EMF treatments can modulate *in vitro* the expression of a microRNA sequence, affecting autophagy Beclin1-mediated. Considering the crucial role of autophagy in neurodegeneration, we could exploit the capability of LF-EMF to modulate the destruction of protein aggregates to induce a cytoprotective effect, triggered by autophagy.

A POSSIBLE ROLE OF ELECTROMAGNETIC FIELDS ON MODULATION OF miRNA-335 AND mi-RNA-107 IN PBMC FROM AD PATIENTS

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INTRODUCTION

Alzheimer's disease (AD) is the most frequent form of dementia worldwide. Histopathologically speaking, β -Amyloid plaques and neurofibrillary degeneration represent the pathognomonic elements of AD (Bergamasco & Mutani 2006). Involvement of hippocampus and amygdala in early phases cause synaptic dysfunctions, such as the block of long term potentiation (LTP), with consequent damage of processes of learning and memory (Robbins & Coltran 2008). Effects of PEMFs are still controversial, however it has been demonstrated that transcranial electromagnetic stimulation of brain through PEMFs in AD patients could modulate the neurophysiologic activity of pathological circuits, resulting in clinical benefits (Laxton et al. 2010). miRNAs constitute a new class of circulating molecules involved in epigenetic regulation. Recently it has been shown that miRNAs, besides their autocrine action, play also a role in down regulation of mRNA of a distant target cell, far from its original site (Vickers KC, et al., 2011). The aim of this work is to evaluate the expression of specific miRNA, following exposure of mononuclear cells in peripheral blood (PBMC) of AD patients to LF-EMF, so to discover the molecular pathways involved in the process. We have selected two miRNAs: miRNA hsa-mir-107, whose target is BACE 1 (Beta-site Amyloid precursor protein Cleaving Enzyme1). Overexpression of BACE1 will therefore lead to β -amyloid plaques reduction (Nelson et al., 2010). The second miRNA that we analyzed in our work is miRNA hsa-mir-335-5p, whose targets are MAPK1 and GRIA1 genes, which encode, respectively, for protein ERK, which is one of the mitogenically activated proteins, involved in cellular growth, and receptor AMPA, which plays a major role in triggering the LTP process. Consequently, a low expression of these miRNAs could enhance both cellular regeneration and memory and learning capacities.

MATERIALS AND METHODS

PBMC have been obtained from peripheral blood samples of AD patients. Analysis in PBMC coltures were done in 4 different experimental conditions: CTR (incubation without electromagnetic field exposure); T1 (15 minutes exposure to EMF); T2 (30 minutes exposure to EMF); T3 (60 minutes exposure to EMF). Following RNA extraction, fluorometric analysis was performed (Qubit®, Invitrogen). cDNA obtained from retro transcription was used in order to evaluate miRNA expression, via qRT-PCR (quantitative Real-Time Polymerase Chain Reaction).

RESULTS

These initial data show that both miRNAs analyzed have been modulated following exposure to LF-EMF. miRNA-107 and miRNA-335 expression appeared to be significantly reduced at T1, their expression increases progressively with T2 and T3, always being inferior to controls. Since miRNAs negatively regulate the target mRNA, a reduced expression of miRNA 107 and miRNA 335 lead to increased expression of their own targets. Reduction of miRNA 107 implies a greater expression of ERK and AMPA receptors, involved in cellular regeneration and LTP processes. Taken together these data suggest that further studies should be performed in order to find the optimal exposure time able to induce clinical benefits.

BIOLOGICAL EFFECTS OF ELECTROMAGNETIC STIMULATION IN ALZHEIMER'S-LIKE SYSTEM

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INTRODUCTION

Alzheimer's disease (AD) is the most common and diffuse neurodegenerative disease. Pathological mechanisms leading to the disease are not yet completely understood, however two characteristic lesions have been identified: extracellular A β plaques deposition and neurofibrillar tangles of hyperphosphorylated Tau protein. In addition, AD is characterized by a massive inflammatory component and innate immune response. The inflammatory reaction takes place in vulnerable sites of the brain, such as microglia, neurons, astrocytes. These cells, once activated, produce pro-inflammatory cytokines, chemokines, prostaglandins, leukotrienes, thromboxanes, coagulation factors and ROS, that play a role in the reduction of protein debris accumulation. It has been shown, that activated microglia contribute to decrease A β accumulation, by increasing its clearance, degradation and phagocytosis.

It is well known that not only chemical molecules but also physical stimuli can influence the physiological functions and pathological conditions of cells. At a molecular level, it has been postulated that EMFs can affect the redox status within cells, thus evoking a general stress response (Goodman and Blank, 2002) and increasing the expression of stress-related proteins (Osera et al., 2011). As shown on AD-model mice, high-frequency EMFs treatment induced an improvement of cognitive functions, ascribed by the authors to an enhanced clearance of amyloid plaques (Arendash et al., 2010). Conversely, in an *in vitro* cellular AD-model over-expressing APP, a prolonged EMFs caused a significant increased secretion of A β ₁₋₄₂ (Del Giudice et al., 2007), one of the most prone-to-aggregation fragment derived by APP (Zhang et al., 2011). In particular we focused on the effects of electromagnetic fields (EMF) exposure on the production of IL-1 β , TNF- α , ROS.

MATERIALS AND METHODS

Biological systems involved in the study:

1. Murine microglia cells (BV2)
2. Hippocampal neuron murine cells (HN)
3. Peripheral system PBMCs from AD patients

To reproduce deposition of insoluble A β plaques (AD-like condition) *in vitro* cellular models were treated with A β oligomers mixture.

A magnetic bioreactor (polymethylmetacrylate, parallel planes) was used to produce a pulsed electromagnetic field, with the following characteristics: intensity 2 mT, amplitude 5 mV, frequency 75 Hz, impulse duration 1.3 ms.

RESULTS

Following LF-EMF exposure it was observed a statistically-relevant increase in the production of ROS, IL-1 β and TNF- α , compared to controls. These results suggest that LF-EMF could act as a preconditioning agent to induce an activation of the clearance process of A β deposits, operated by glial cells. Taken together we could hypothesize that LF-EMF might represent a new therapeutical approach in the treatment of preclinical (MCI) and early-stage AD patients.

CROSSTALK BETWEEN GENETICS, GENE EXPRESSION AND BIOCHEMICAL MARKERS SUPPORTS SYSTEMIC IRON HOMEOSTASIS DYSREGULATION IN ALZHEIMER'S DISEASE

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INTRODUCTION: The distinction between normal aging and Alzheimer's disease (AD) is a relevant step to combat this disease efficiently. Thus, the identification of biomarkers and genetic factors underlying AD pathology is extremely important. Oxidative injury in the brain, mediated by the imbalance of redox-active metals as iron (Fe) has been recognized to contribute to the pathology of AD.

OBJECTIVES: To test this hypothesis we: (i) screened a set of SNPs in Fe metabolism-related genes and *APOE* in a sample of 116 AD patients and 98 healthy controls; (ii) compared serum biomarkers of Fe metabolism in the same samples and (iii) analyzed the expression level of several Fe metabolism genes in the peripheral blood mononuclear cells (PBMCs).

METHODS: Genetic analysis was performed through high density SNP genotyping of the candidate genes *CYBRD1*, *HAMP*, *HFE*, *ACO1*, *IREB2*, *SLC11A2*, *SLC40A1*, *TF*, *TFR2*, and *APOE*. Biochemical analysis was assessed for: serum Fe, transferrin (Tf), ferritin (Ft) and Tf saturation. The expression of *TFR1*, *TFR2*, *SLC40A1*, *HAMP* and *SLC11A2* genes were determined by quantitative Real-Time PCR in PBMCs.

RESULTS: Several significant SNP associations with AD were found in this study. Besides the previously reported association with *APOE* ($P=0.0007$), we also showed association with three SNPs in *TF* ($0.0147 < P < 0.0537$) and one SNP in *TFR2* ($P=0.0055$), *ACO1* ($P=0.0258$) and *SLC40A1* ($P=0.0210$) genes. Also, significant differences have been found in the biochemical markers of Fe metabolism ($P=0.003$, overall MANCOVA). These are mainly driven by the significant decrease of serum Fe concentration measured in AD patients compared to controls. Finally, the mRNA levels of *TFR1*, *TFR2* and *SLC40A1* were significantly decreased in PBMCs of AD patients ($P < 0.001$), while no significant differences have been found in the *HAMP* and *SLC11A2* gene expression. We are currently evaluating the expression of other eight iron metabolism-related genes and the *APP* gene in this dataset.

CONCLUSIONS: We hypothesize that the low systemic Fe status profile observed in AD patients could be due to impaired regulation of cellular Fe efflux. The intracellular accumulation of Fe, particularly in the brain would lead to a rise in oxidative damage, contributing to the AD pathophysiology.

RESPONSE TO REDOX STRESS IN LYMPHOCYTES FROM PATIENTS WITH ALZHEIMER'S DISEASE AND MILD COGNITIVE IMPAIRMENT

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Despite intensive research, the pathogenesis of Alzheimer's disease (AD) is poorly understood. therefore an effective cure and reliable early markers are missing. It is well known that AD is characterized by complex alterations in cellular processes that occur not only in neurons, but also in peripheral cells such as lymphocytes. Recently, we have demonstrated that sporadic AD lymphocytes show G1 phase arrest and increased levels of p21 protein, the key regulator of G1/S cell cycle checkpoint and apoptosis (1). Hence we aimed to elucidate the effects of these changes on the apoptotic response of AD lymphocytes to oxidative stress.

In the first step we compared apoptotic response to oxidative stress evoked by treatment with 2-deoxy-D-ribose (2dRib) in EBV-immortalized B-lymphocytes from 8 patients with familial AD (FAD) bearing 8 different mutations in PS1: P117R, M139V, L153V, H163R, S170F, F177L, I213F, E318G, and 16 patients with the sporadic form of AD (SAD). Control lymphocytes came from two groups of healthy individuals aged correspondingly to FAD and SAD, respectively. We found that 24 hours after 40mM 2dRib treatment, the percentage of surviving lymphocytes in the MTT assay was significantly decreased in SAD comparing to the age-matched controls ($p < 0,05$) and FAD ($p < 0,005$). In agreement with these data, early apoptosis measured by AnnexinV staining was higher in SAD lymphocytes than in control ($p < 0,05$) and FAD ($p < 0,005$) cells. Also measurements of mitochondrial membrane potential (MMP) using cationic dye JC-1 showed differences in the response to 2dRib between SAD and FAD: MMP of SAD lymphocytes was significantly decreased comparing to control ($p < 0,05$) and FAD ($p < 0,005$) cells. Accordingly, SAD lymphocytes showed an increased percentage of cells with fragmented DNA in SubG1-phase, when

compared to control and FAD lymphocytes. Comparing two control groups, age-matching SAD and FAD, respectively, we found that the differences in the apoptotic response between SAD and FAD cells are not due to aging. Thus, FAD lymphocytes seem to be significantly more resistant to pro-oxidative apoptotic stimuli than SAD cells. Moreover, higher apoptotic response in SAD lymphocytes correlated with the increased level of p21 protein. Next we analyzed whether changes in apoptotic response can be detected at the early stage of AD. With this aim, we measured selected parameters of apoptosis in lymphocytes from Mild Cognitive Impairment (MCI) patients. Our preliminary results have shown no statistically significant changes in late apoptotic response, as evaluated by SubG1 measurements. However, mitochondrial potential of MCI cells after treatment with 2dRib clearly tended to be decreased, similarly to SAD cells. These results indicate that changes in the apoptotic response may appear early in the AD pathogenesis and gradually increase with the development of the disease. Altogether, our results showed that SAD lymphocytes are more vulnerable to 2dRib than age-matched control and FAD cells and thus the mechanism of apoptosis distinguishes cells from SAD and FAD patients. Our data indicate that lymphocytes may be useful for the development of new early AD diagnostic methods based on analysis of the apoptotic response.

¹ Bialopiotrowicz E, Kuzniewska B, Kachamakova-Trojanowska N, Barcikowska M, Kuznicki J, Wojda U. Cell cycle regulation distinguishes lymphocytes from sporadic and familial AD patients. *Neurobiol Aging* 2011 32(12):2319.e13-26.

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Day 2: Alzheimer's Disease: Prevention Strategies and Vaccine Development

Invited Speakers Abstracts

SemiAlloGeneic Vaccines for Alzheimer's Disease

Professor Mark S. Kindy, Medical University of South Carolina, USA

Alzheimer's disease (AD) is a progressive neurodegenerative disorder. Amyloid β ($A\beta$) peptide-containing plaques and neurofibrillary tangles are neuropathological hallmarks of AD. Recent studies suggest that antibody-mediated immunotherapy could reduce AD pathology and enhance cognition in AD patients, but this approach carried the risk of severe neuronal inflammatory side effects. Semiallogenic (SAG) vaccines are derived from both self and non-self components that functionally mimic antigen producing cells by concomitantly stimulating alloantigen-specific T helper cells via allogeneic MHC and antigen-specific cytotoxic T lymphocyte precursors via antigen presentation. We have shown that SAG vaccines can prevent and reduce AD pathogenesis and improve cognition.

What did we learn from the first clinical trial of $A\beta$ immunotherapy?

Dr Delphine Boche, Senior lecturer (Associate Professor), University of Southampton, UK

Alzheimer's disease (AD) is characterised by abnormal aggregation in the brain of amyloid- β (A) peptide and hyperphosphorylated tau, associated with inflammation and neuronal loss. We have performed a clinical and neuropathological follow-up of AD patients who were actively immunised with A β 42 (AN1792, Elan Pharmaceuticals). Post-mortem neuropathology has identified changes in the AD process in immunised patients including a lower A β load and hyperphosphorylated tau. However, all the immunised AD patients progressed to severe dementia prior to death. The findings show that A β immunisation can modify AD pathology but this does not seem to prevent progression of the cognitive decline.

Multiscale computational approach illuminating novel common pathways between diabetes and AD

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Backgrounds Diabetes may increase the risk of Alzheimer's disease (AD). Many epidemiology studies indicate that people with diabetes are at higher risk of eventually developing AD or other dementias. It is estimated that hyperinsulinemia in elderly people could account for 39% of cases of AD. However, how

these two diseases are connected remains unknown. Possible mechanisms include shared genetic or microbial risk factors, which will be systematically investigated in this study.

Methods We accessed large genetic association studies (e.g., genome wide association studies, GWAS) in dbGap (The database of Genotypes and Phenotypes) databases with endpoints of diabetes type 2 (D2T), AD and related phenotypes. We applied various p value thresholds and examined the overlap between diabetes and AD GWAS hits. A significant overlap indicates shared genetic components underlying the two diseases. Further, by leveraging ENCODE (Encyclopedia of DNA Elements) and gene co-expression networks of relevant tissues, the studies were designed to inform the functionality of the shared genetic signals. We are currently focusing on 75 subjects meeting AD and T2D criteria and 75 T2D but AD-free subjects. We are currently re-sequencing genetic loci found in shared pathways/gene-networks identified in GWAS and search for polymorphisms showing different frequencies in the two groups. Gut microbiome of the two groups and search for fecal bacteria responsible to AD pathogenesis among T2D subjects will reveal additional associations.

Results We surveyed GWAS reports, and found 195 genetics loci for AD traits and 364 genetic loci for T2D traits which reached genome-wide significance. We identified several inflammatory genes, e.g., CLU, CR1, CD33, EPHA1, and MS4A4A/MS4A/6A, among others. This evidence strongly support the hypothesis inflammation is one of the genetic risk factors shared by AD and diabetes.

Conclusions By leveraging GWAS, ENCODE, gene co-expression networks, genome- and fecal bacteria-sequencing data, using multiscale biology approaches, data will be presented to reveal common genetic and microbiome pathways between diabetes and AD. The study will clarify a potential common etiology of the two diseases, information that will help identify T2D cases that are likely to develop AD. The study will also inform about mechanisms for novel therapeutic approaches.

Regulatory T cells as new targets for immunotherapy in Alzheimer's disease?

Dr Guillaume Dorothee, Senior Investigator, INSERM, France

Severe complications in the first clinical trial of A β vaccination, together with the recent failure of subsequent passive immunotherapy approaches underline the need for better understanding immune responses to A β . Conflicting clinical and preclinical data highlight a complex implication of T cells in response to A β vaccination. Recent reports also suggest the involvement of T cells in the pathophysiology of AD, but the nature and role of implicated populations remain poorly defined. Our studies in animal models suggest that regulatory T cells critically control vaccine-induced T cell responses to A β , and play a beneficial role in the pathophysiology of AD, highlighting the therapeutic potential of Treg-based immunotherapy strategies.

Moving dna immunization toward an alzheimer's disease clinical trial

Professor David H. Cribbs, Professor and Associate Director, Institute for Memory Impairment and Neurological Disorders, University of California, Irvine, USA

Immunotherapy targeted at clearing amyloid from the brain has been successful in rodent models and humans. However, adverse events associated with both active and passive immunotherapy have hampered the development of vaccines for Alzheimer disease (AD). We have developed a series of DNA and recombinant protein-based epitope vaccines composed of 3 copies of immunogenic A β 42 B cell epitope fused with a platform of multiple universal foreign Th epitopes (MultiTEP) that are widely recognized by human MHC class II molecules. We have now shown that these greatly improved epitope vaccines can induce therapeutically potent anti-amyloid antibodies in mice, rabbits and monkeys.

ACE I/D POLYMORPHISM SHOWS CONTRARY RESPONSE TO ANGIOTENSIN CONVERTING ENZYME INHIBITOR IN REGULATION OF ACE PROMOTER ACTIVITY IN NEURON.

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BACKGROUND: In addition to being a popular prescription used in cardiovascular and kidney diseases, angiotensin converting enzyme (ACE) inhibitors have also been indicated to have potential as therapeutic agents in dementia. Likewise, ACE inhibitor-induced ACE signal leading changes in gene expression of ACE itself has been proved. Due to the associated role of ACE I/D polymorphism in Alzheimer's disease (AD), the pharmacogenetic effect of ACEi on ACE I/D genotype in AD has been also investigated. However, the mechanism remains unclear.

OBJECTIVE: Based on our previous report [1], the Alu element of ACE gene shows functional regulation in activity of ACE promoter. The present study aimed to identify whether the regulatory function of ACE promoter affected by ACE I/D polymorphism could be further modulated by ACEi.

METHODS: SH-Sy5y cells were transfected with the pACEpro(f)-SEAP-Bas, p-I-ACEpro-SEAP or p-D-ACEpro-SEAP, respectively. After 24 hours of treating 100uM of Lisinopril (an ACE inhibitor), SEAP assay and pGL4 (transfection efficiency control) were examined to value the transcriptional activity of ACE promoter in each reporter vector.

RESULTS: We found that Lisinopril indeed increased ACE promoter activity by approximate 16.8% as compared to the control group (without Lisinopril). Furthermore, the transcriptional activity of p-I-ACEpro-SEAP vector (with Alu element, I allele) was upregulated by 17.2% ($p < 0.001$), whereas the activity of p-D-ACEpro-SEAP (without Alu element) was suppressed by 18.7% ($p < 0.01$) after the cells were stimulated with Lisinopril in comparison to the cells without stimulation.

CONCLUSION: Our results first indicate that the pharmacogenetic function of ACEi on ACE I/D polymorphism in neurons. This encouraging finding may bridge the decade elusive gap between the protective role of ACEi found in cognitive decline in patients with dementia and the pharmacogenetic association study of ACEi in ACE I/D of AD.

Keywords: angiotensin converting enzyme (ACE) inhibitors, ACE I/D polymorphism, pharmacogenetics, Alzheimer's disease, enzyme-mechanism.

[1] Wu SJ, Hsieh TJ, Kuo MC, Tsai ML, Tsai KL, Chen CH, Yang YH. *Neurobiol Aging*. 2013 Jul;34(7):1921.e1-7

SUPERSATURATION : A RISK FACTOR FOR PROTEIN AGGREGATION

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HEPARAN SULFATE SULFOTRANSFERASES AND PATHOLOGIC PHOSPHORYLATION OF TAU IN ALZHEIMER'S DISEASE-RELATED TAU PATHOLOGY

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The accumulation of abnormally phosphorylated tau is a central event in Alzheimer's disease. Although *in vivo* the critical pathologic phosphorylation of tau is assumed to be mediated by the same kinases that phosphorylate tau in normal brain, *in vitro*, these kinases abnormally phosphorylate tau at disease

specific sites only if the enzymatic reaction takes place in the presence of certain polyanions such as heparin, a highly sulfated analogue of the complex heparan sulfates family of glycosaminoglycans. Currently, it is unknown whether this complex family of molecules, which structural and functional diversity in brain results from the action of several heparan sulfate sulfotransferases, is involved in the cellular mechanism leading to tau pathology. Recently, we have observed that the expression of two specific heparan sulfate biosynthesizing enzymes are significantly increased in Alzheimer's disease brain and that the products of these enzymes are critically involved in the biochemical and cellular mechanisms leading to the pathologic phosphorylation of tau. Inhibiting the expression of one of these enzymes strongly attenuates the pathologic phosphorylation of tau induced in cells by oxidative stress or by expression of hTau^{P301L}, indicating an essential role of specific heparan sulfates domains in the mechanism leading to the abnormal phosphorylation of tau at Alzheimer's disease characteristic sites. We propose a novel hypothesis for the better comprehension of the pathophysiological mechanisms leading to Alzheimer's disease related tauopathy, with groundbreaking therapeutic outcomes.

DEVELOPMENT OF MIXED MUSCARINIC RECEPTOR LIGANDS AND SIGMA-1 RECEPTOR AGONISTS AS NEUROPROTECTANTS IN ALZHEIMER DISEASE: PRECLINICAL DATA

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Selective muscarinic, particularly M1, cholinergic receptor ligands have shown some efficacy in Alzheimer's disease (AD) rodent models. Activation of the M1 receptor triggers a neuroprotective pathway, involving PLC/PKC activation. It eventually leads to inactivation of GSK-3 β , the main kinase involved in Tau hyperphosphorylation, and activation of α -secretase, decreasing amyloid- β (A β) protein accumulation. The sigma-1 receptor (S1R) is a ligand-operated molecular chaperone expressed in the brain and localized on endoplasmic reticulum (ER), mitochondria and plasma membranes. Its activation modulates IP₃ receptor-dependent Ca²⁺ mobilizations, facilitates the activation of sensors of the ER stress and kinase pathways. Under chronic activation, it is also involved in recomposition of lipid domains on the plasma membrane, which are highly functionalized domains. Interestingly, the chaperone can be directly activated (or inactivated) by several classes of natural and synthetic ligands, explaining its historic misunderstanding as a classical membrane receptor. Ligands modulating the S1R are potent neuromodulatory and protective drugs. We analyze the potency of mixed muscarinic/S1R ligands, the aminotetrahydrofuran derivatives ANAVEX1-41 and ANAVEX2-73, for their neuroprotective potential in AD. Two main AD models are used, a nontransgenic model by direct icv injection of an oligomeric A β protein fragment [25-35] (oA β) in mice and transgenic animals overexpressing hAPP_{Swe}. The pathology is analyzed in terms of ER and oxidative stress, inflammation, mitochondrial damage, cell loss, memory deficits, increased APP processing and Tau hyperphosphorylation. The drugs were injected acutely or chronically. Biochemical, morphological and behavioral studies were conducted between 1 to 3 weeks after oA β , or after 2 month treatments in Tg mice. In the oA β model, the selective S1R agonist PRE-084, ANAVEX1-41 or ANAVEX2-73 prevented cell loss, astrocytic reaction, oxidative stress and induction of proapoptotic caspases in the hippocampus and cortex. oA β -induced learning deficits were also alleviated. ANAVEX2-73 prevented oA β -induced inhibition of AKT activity, activation of GSK-3 β activity and the resulting Tau phosphorylation, showing that both amyloido- and Tauopathies were alleviated. In Tg mice, a 2-month per os treatment with ANAVEX2-73 prevented the appearance of memory deficits and alterations in synaptic markers. The mixed compounds showed a synergistic efficacy between their cholinergic and S1R activities, being active at low doses in the 10-100 μ g/kg range. Moreover, the combination of ANAVEX2-73 and donepezil appeared synergic, leading to the design of ANAVEX PLUS (ANAVEX2-73 + donepezil) as a drug candidate for clinical development. In conclusion, we identified S1R agonists as neuroprotective agents and outlined the interest in targeting both muscarinic receptors and S1R chaperones in AD related neurodegenerative pathologies.

MIGHT A COMPARABLE RADICAL PROCESS CONTRIBUTE TO CREUTZFELDT JAKOB, ALZHEIMER'S AND LEWY BODY DISEASES ?

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In Creutzfeldt Jakob, Alzheimer and Lewy body diseases, similar pathological hallmarks have been described, such as brain deposition of abnormal protease-resistant proteins in beta sheet conformation. It has been shown that in several of these pathologies, copper bound to proteins is able to protect against free radicals by copper reduction from cupric valence Cu^{++} to cupreous Cu^+ . We have previously demonstrated in bovine brain homogenate that free radicals produce proteinase K-resistant prion after manganese is substituted for copper. Since low brain copper levels have been described in bovine brain spongiform encephalopathies and in various brain regions in Creutzfeldt Jakob, Alzheimer's and Lewy body diseases, we propose a mechanism possibly underlying the neurodegenerative processes that ensue when copper protection against free radicals is impaired. In peptide sequences the alpha acid hydrogen near the peptide bond is highly mobile and can be pulled out by free radicals present in situ. This process will produce a trivalent α -carbon radical and induce a radical chain process. Following epimerization, it will be in a position to generate a D-amino acid configuration in the peptide sequence and to produce larger peptide structures by condensation between free radical peptides. Since only L-amino acids are physiologically present in mammalian and human proteins, it may be supposed that physiological enzymes such as proteases can only recycle physiological L-peptides. If a D-amino acid is found in the peptide sequence subsequent to deficient copper protection against free radicals, it will not be recognized and might alter the recycling of L-amino acid from brain peptides. In the brain, there will ensue an accumulation of abnormal protease-resistant proteins such as those observed in Creutzfeldt-Jakob, Alzheimer and Lewy body diseases.

Poster Presentation Abstracts

RGH-235, A NEW POTENT AND SELECTIVE HISTAMINE H_3 RECEPTOR INVERSE AGONIST

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Targeting the histamine H_3 receptor alone or in combination with AChE inhibitors may be a viable approach for the symptomatic treatment of cognitive deficits.

RGH-235 is a new potent, selective and orally active histamine H_3 receptor inverse agonist developed by Gedeon Richter Plc. It displays high affinity for either human ($K_i = 3.0$ nM) or rat ($K_i = 9.2$ nM) receptors, without showing significant affinity for other (H_1 , H_2 , H_4) histamine receptors or more than 100 other targets (receptors, ion channels, transporters and enzymes). In the *in vitro* functional tests, RGH-235 antagonized the imetit-induced ERK1/2 phosphorylation ($\text{IC}_{50} = 6.5$ nM) and behaved as an inverse agonist ($\text{IC}_{50} = 0.6$ nM) in the [^{35}S]GTP γ S binding assay.

RGH-235 showed high oral bioavailability and appropriate PK profile. Rodent models of learning and memory revealed pro-cognitive potential of the compound. Oral treatment of rats (in the Novel Object Recognition paradigm) or mice (in the Place Recognition paradigm) with RGH-235 resulted in significant improvement of natural forgetting vs. scopolamine-induced amnesia, respectively, in the 0.3–1 mg/kg dose range.

Based on the above findings the compound is a promising histamine H_3 inverse agonist with procognitive potential.

ALZMED: A PROPOSAL FOR SURVIVAL ON ALZHEIMER'S DISEASE IN THE MEDITERRANEAN AREA

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INTRODUCTION: Alzheimer's Disease, the most common cause of Dementia, is a progressive neurodegenerative brain pathology that affects the elderly. Symptoms like memory loss, cognitive deficiency, communication difficulties and mood changes worsen over the time until the victims are no longer able to perform the most basic daily life activities.

Dementia is one of the biggest challenges of our generation's public health. Actually more than 35 million people all over the world live this situation, and this number is expected to double within 2030 and probably triple reaching 115 million people within 2050. That means that will become increasingly

necessary to have long-term care forms, which may include home-nursing care, communities and home assistance and long hospital stays.

The European population is getting older, and particularly is increasing the number of seniors coming from the south Mediterranean and African countries.

On December 2013 was organized the G8 Summit on Dementia in UK, recognizing dementia as one of the most significant public health crises of the 21st century. In this way it is required a collaborative action in order to face one of the most expensive public health problems. In their joint report, the ADI (Alzheimer's disease international) and WHO call upon all governments to develop a common strategy against dementia, implementing awareness-raising initiatives and improving health-care and social assistance systems.

TARGET: on the basis of these issues, it is clear how widespread is the question of having a diagnosis and an evidence of dementia and Alzheimer's patients. The purpose of our project is to underline the problems related to dementia diffusion in the Mediterranean area. Especially in developing countries, where still there is not a solid dementia diagnostic system, this project could represent the engine for the development of new positive integrated actions. The guiding principles are that of "Living well with dementia" that could be a reachable goal, in order to maintain a good quality of life and achieve it as a final goal.

PROPOSAL: in conclusion, our project was born on the basis of epidemiological data and problems of our territorial, local, regional, and international-world reality and it is aimed at obtaining a general evaluation of issues of dementia in the Mediterranean area. This project involves the collection of information on existing realities in order to develop general hypotheses to improve the care pathway, research and the quality of life for people with cognitive deficits and suffering from Alzheimer's disease and their families. In order to reach a more powerful and effective assistance of these patients, we propose to tackle the disease by acting on the deficiencies of the entire system, introducing important indicators like:

- .verifying the existence and the levels of ambulatory care procedures of AD patients;
- .proposing support services for relatives and care-givers by coordinating the service and supporting network
- .strengthen the research upon the cognitive degeneration;
- .verifying the possibilities to act by early diagnosis interventions by biomolecular and cognitive markers;
- .proposing international cooperation projects in order to organize information and awareness activities on dementia question in developing countries.

SARCOPENIA IMPROVEMENT IN ALZHEIMER DISEASE, FOLLOWING SUPPLEMENTATION WITH A MIXTURE OF EAAs, FOLATE AND VITAMIN D

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INTRODUCTION

Sarcopenia is an aging-associated condition, which is currently characterized by the loss of muscle mass and muscle strength. The first aim of the study is to assess the effect of an orally administered special mixture of amino acids (EAAs), folate and vitamin D.

MATERIAL AND METHODS

An unbalanced, double blind, placebo-controlled clinical trial was performed in 130 elderly subject (females/males 86/44; age $81,9 \pm 6.02$). Data from demographic variables, body composition (DXA) and nutritional indexes, have been collected at baseline (t_0) and after the 2-months treatment (t_1). Subjects received either EAAs supplementation twice a day (t_0 : n=48, sex=34/14, age= $81.8 \pm 6.3y$, BMI= 24.1 ± 5.3 kg/m², RSMM= 6.2 ± 1.1 kg/m²), aminoacids and folate/vitamin D compound (gr.4 / mg.15 or U 10000) supplementation (t_0 : 18, 11/7, $81.8 \pm 7.3y$, 24.1 ± 4.4 kg/m², 6.6 ± 1.3 kg/m²) or placebo (t_0 : 64, 41/23, $82.1 \pm 5.5y$, 24.4 ± 4.5 kg/m², 6.5 ± 1.2 kg/m²). Statistical analysis was performed using linear mixed-effects regression models for repeated measures.

RESULTS

In account of RSMM (t_1) the average changes occurred from RSMM (t_0) were $+0.27$ Kg/m² (P=0.001) in EAAs group and $+0.32$ Kg/m² (P=0.002) in mixed supplementation group, compared with placebo. The two supplementations are statistically equivalent. In account of lean mass (t_1), comparing with placebo, the average changes occurred from baseline values was $+1418.5$ g (P=0.002<0.05) in EAAs group. Mixed

supplementation did not return significant performance ($P=0.60>0.05$) on lean mass. All results were adjusted for sex, age and lean mass regression model was adjusted for fat mass (t_0) also. These results indicate that a mixture of EAAs, vitamin D and folic acid could play a more efficient role in the management of sarcopenia in comparison to alone administration of EAAs.

NRF2 REGULATES NGF MRNA EXPRESSION IN HUMAN GLIOMA CELLS AND NORMAL HUMAN ASTROCYTES

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ABSTRACT

Nerve growth factor (NGF) is a neurotrophic factor that plays an important role in neuronal cell development and survival. Especially, NGF is a potent trophic factor for brain cholinergic neurons including those in the basal forebrain that are frequently affected in Alzheimer's disease (AD). Carnosic acid (CA), a major constituent of the herb rosemary, induces NGF production in human T98G glioblastoma cells, but the mechanism through which it works remains unknown. We found a redox-sensitive transcription factor, Nrf2, that coordinately regulate the expression of cytoprotective phase 2 genes, also participates in CA-inducible *NGF* expression. In T98G cells, CA caused *NGF* gene induction in a dose- and time-dependent manner without affecting *NGF* mRNA stability. Simultaneously, CA increased Nrf2 nuclear accumulation and activated expression of Nrf2 target genes such as heme oxygenase 1 (*HO-1*) and thioredoxin reductase 1 (*TXNRD1*). Nrf2 knockdown by Nrf2-specific siRNA significantly reduced constitutive and CA-inducible *NGF* gene expression. In addition, knockdown of Keap1, a negative regulator of Nrf2, enhanced *NGF* gene expression. Furthermore, CA induced *NGF* expression in normal human astrocytes in an Nrf2-dependent manner. These results highlight a role of Nrf2 in *NGF* gene expression in astroglial cells.

Edaravone (MCI-186, 3-methyl-1-phenyl-2-pyrazolin-5-one) is a free radical scavenger and has been shown to ameliorate post-ischemic neuronal dysfunction in patients with acute ischemic stroke. It is clinically used only in Japan for the patients. We found that edaravone (~1 mM) enhances CA (~50 μ M)-inducible *NGF* expression as well as *HO-1* expression in U373MG human astrocytoma cells. Nrf2 knockdown by siRNA suppressed the synergistic effects of edaravone and CA on *NGF* expression. However, the co-treatment of edaravone did not alter the level of CA-induced Nrf2 nuclear accumulation, indicating that some other transcription factors may cooperate with Nrf2 in the induction of *NGF*. Elucidation of such combinations of transcription factors may be useful in the prevention of early phase of mild cognitive impairment (MCI) due to AD.

Day 3: Alzheimer's Drug Discovery and Development

Invited Speakers Abstracts

Going beyond Preclinical Animal Models : Quantitative Systems Pharmacology to support Alzheimer's Disease Research & Development

Dr Hugo Geerts, Chief Scientific Officer, In Silico Biosciences, USA

Despite increasing sophistication in transgene animal models and the large investment by pharma industry, the clinical success rate of AD drugs is disappointingly low. We propose a humanized CNS Quantitative Systems Pharmacology as a novel out-of-the-box platform for supporting R&D. This approach, similar to formal Computer Aided Design used in other industries, uses computer-based mechanistic modeling integrating the neurophysiology and neuropathology of relevant brain networks, functional imaging of genetics, the pharmacology of drug-receptor interactions and parametrization with clinical data. We will show examples of blinded clinical predictions, clinical trial failures, the prediction of genotype and comedication effect on clinical outcome.

New era in AD drug design: intracellular and exosomal targets

Dr Botond Penke, Professor, University of Szeged, Department of Medical Chemistry, Hungary

AD is a multifactorial disease, caused by a combination of different risk factors. The newest genetic (Jonsson T. 2012) and integrative genomic studies (Rhinn H. 2013) very recently provided solid proof of the amyloid hypothesis. Conventional AD drug development failed for many reasons (the therapeutic targets, e.g. extracellular amyloid, have been incorrect; drugs have been used too late in the disease process; proof of concept problems, etc.). Amyloid pathology correlates with progression at earlier stages

of the disease. In the new era of AD drug design we use new targets: intracellular Abeta formation, early stages of Abeta aggregation and direct or exosomal cell-to-cell transmission of prionoids (Abeta and pTau).

The enemies within: the role of herpes simplex virus type 1 (HSV1) and APOE-e4 in Alzheimer's disease

Professor Ruth Itzhaki, Researcher, University of Manchester, UK

We showed that HSV1 is present and active in a high proportion of elderly brains and that in APOE-e4 carriers, it confers a strong risk of AD. In direct linkage to AD, we found that HSV1-infected human neural cells in culture strikingly increase beta amyloid (Abeta) and AD-like-tau (P-tau) levels, and Abeta deposits form in brains of HSV1-infected mice. Further, in AD brains, HSV1 DNA is located specifically within amyloid plaques. Antiviral agents reduce greatly A β and P-tau levels in HSV1-infected cells, suggesting the usage of antivirals to reduce progression of AD. Epidemiological, immunological and virological studies by others substantiate the role of HSV1 in AD.

Modulators of γ -secretase activity can facilitate the toxic side-effects and pathogenesis of Alzheimer's disease

Dr Željko M. Svedružić, Assistant Professor, Faculty of Medicine, and Department of Biotechnology, University of Rijeka, Croatia

We describe modulation of A β production in cells, by studying how different drug-candidates affect changes in γ -secretase activity caused by gradual increase in A β metabolism. A β secretion in presence of γ -secretase modulators shows biphasic activation-inhibition dose-response curves. These synergistic activation-inhibition effects can drastically reduce γ -secretase's capacity to process its physiological substrates, and thus facilitate the toxic side-effects and the pathogenic events. The presented mechanism can explain why moderate inhibition of γ -secretase did not lead to effective therapies, and how quantitative analysis of the catalytic capacity of γ -secretase can be used to prepare novel drug-candidates and early diagnostic strategies.

Combining drug-like fragments in multitarget new chemical entities for Alzheimer's disease

Professor Andrea Cavalli, University of Bologna and Italian Institute of Technology, Italy

Fragment-based drug discovery has played a relevant role in the identification of drug-like multi-target compounds. Indeed, several different strategies can be utilized to combine in a single multi-target compound two (or more) fragments carrying the pharmacophoric functions responsible for the interaction with two (or more) different targets [1]. Here, we designed, synthesized, and tested a series of dual-target compounds based on drug-like fragments. Using galantamine, a drug in the market for the treatment of Alzheimer's disease (AD) as anti-cholinesterase, and memantine, the only available non-competitive NMDA receptor antagonist for the treatment of AD, we obtained a new series of dual-inhibitors (AChE/NMDA) potentially useful for the treatment of AD [2]. Among the new compounds, we identified potent inhibitors of AChE, while others were promising NMDA receptor antagonists. Surprisingly, some of them were also antagonists of the NR2B-containing NMDA receptor. A few of these compounds displayed balanced activities against the two selected targets, showing a quite promising dual-target profile. Pharmacokinetics and pharmacodynamics data were also generated for a small selection of these new molecules. In this lecture, major results and drawbacks will be discussed from a neurodegenerative diseases drug discovery standpoint.

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How to Prevent Dementia and Alzheimer's

Dr Allen J. Orehek, Innovator/Physician, Dementia Prevention Center, USA

Dr Orehek's unique talent is the prevention of dementia. New scientific concepts will open the door to your personal dementia prevention. The brain does not age, however suffers damage over time. The DPC will inform you on how best to identify your baseline or your current degree of damage. Identification of the causes of damage (micron strokes), allows for the mitigation of additional damage. In addition to explaining these new concepts in a medical and scientific manner, Dr Orehek will also provide a mathematical explanation that can be measured, tested, evaluated and judged. Enjoy!

Activities of daily living: a new approach to discovering Alzheimer therapies

Dr Robert Deacon, University of Oxford, UK

Activities of daily living (ADL) become impaired in Alzheimer's (AD). Hitherto, the search for new AD therapies has relied heavily on animal models of learning and memory. At Oxford we have developed simple tests for quantifying ADL of mice. Not only are these tests cheap and sensitive, they provide a novel approach to the search for AD therapies. In terms of mouse welfare, they involve virtually no stress, as deprivation of food/water and aversive motivation is unnecessary, minimising the possibly

confounding effects of stress. The aetiological and face validity of these ADL models is enhanced by their dependence on the hippocampus, one of the first brain areas to be affected in AD.

Galanin-like peptide (GALP) have anti-obesity effect via the activation of hepatic lipid metabolism

Dr Satoshi Hirako, Post-doctoral fellow, Dept of Anatomy, Showa University School of Medicine, Tokyo, Japan

Galanin-like peptide (GALP) is produced in neurons in the hypothalamic arcuate nucleus and is well known as a neuropeptide regulating feeding behavior and energy metabolism. In this study anti-obesity effect was obtained by the 7-day intranasal administration of GALP in obese mice. The respiratory exchange ratio (RER) of GALP group was lower than the saline group. In addition, fatty acid oxidation-related gene mRNA levels were increased in liver by administration of GALP. The present study indicates that anti-obese effect of GALP may be caused by anorexigenic effect and improvement of lipid metabolism in the liver.

Antipsychotics induced obesity: Direct actions on the adipocytes

Professor Nira Ben-Jonathan, Professor of Cancer and Cell Biology, University of Cincinnati, United States
Atypical antipsychotics (AAP) are prescribed to millions of patients with mental diseases. Although AAP ameliorate mental dysfunctions, they have serious metabolic side-effects such as weight gain, diabetes, and cardiovascular disease. We discovered expression of functional dopamine and serotonin receptors in human adipocytes and found that AAP altered many of their functions. We propose that direct actions of AAP on adipose tissue contribute to weight gain and the metabolic syndrome. Human adipocytes could be integrated into the screening paradigm of candidate new drugs for the identification of undesirable metabolic activities prior to costly animal studies and clinical trials.

Oral Presentation Abstracts

THE AGED BEAGLE MODEL OF ALZHEIMER'S DISEASE PROGRESSION

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Several therapeutic candidates for Alzheimer's disease (AD) showed improvements in standard mouse models of the disease, but failed in the clinic, suggesting that improved animal models with better translational ability are required for the development of Alzheimer therapeutics. Aged Beagle dogs are a natural AD model with spontaneous onset of the disease, as per most AD patients. Aged dogs demonstrate domain-specific cognitive decline and progress to typical AD neuropathology, modelling the entire spectrum of AD progression, without any need for rare amyloid or tau mutations. Specifically, the observed neuropathology includes amyloid deposition, hyperphosphorylation of tau, cerebral atrophy and neuronal loss. This physiological demise is accompanied by increasing deficits in spatial working memory, executive function, spatial learning ability, and decreasing selective attention, which has been established in standardized cognitive assays at InterVivo Solutions. Multiple biomarker changes, reported to predict conversion to AD in patients, are seen in the aging dogs: reduced content of A β ₄₂ in CSF, reduced fluorodeoxyglucose uptake in several brain regions and longitudinal atrophy of the frontal cortex and hippocampus. Therefore the dog appears ideally suited to assess both symptomatic and disease modifying therapeutics.

In a symptomatic therapy study, 1.5 mg/kg of p.o. donepezil improved spatial working memory in aged dogs between 3 and 5 hours, but not 1 hour, after administration. Plasma levels of donepezil were within the established human therapeutic range between 2 to 5.5 hours, but not after 1 hour, following administration. Consequently, the aged Beagle dog model accurately predicts the cognitive improvements at the same therapeutic plasma levels established in humans.

In a disease modifying therapeutic study, an active amyloid vaccine, which was targeting fibrillar A β , was tested in dogs over 2.4 years, examining cognitive function. Although amyloid plaque load was significantly reduced in immunized dogs, no improvement in learning, spatial attention or spatial memory was found, which is consistent with the findings observed in multiple phase III human clinical trials employing various A β antibodies.

In summary, these results suggest that the aged Beagle dog model of Alzheimer's disease progression can be employed to predict the effectiveness of symptomatic, as well as perceived disease modifying therapies for the treatment of AD. Current data suggest this natural and spontaneous AD model has improved translational ability over all established AD models, still used for screening. Consequently, preclinical evaluation of novel therapeutics in standardized cognitive screens and biomarker studies in aged Beagle dogs is expected to reduce the very expensive attrition of clinical AD candidates, ultimately reducing the excessive cost associated with advancing AD drug candidates.

COGNITIVE EFFECTS, NEUROPROTECTIVE PROPERTIES, SELECTIVITY AND CO-CRYSTAL STRUCTURES OF LEUCETTINES, A FAMILY OF DYRK/CLK INHIBITORS

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There is growing evidence implicating DYRKs (dual specificity, tyrosine phosphorylation regulated kinases) and CLKs (cdc-like kinases) in the onset and development of Alzheimer's disease and Down syndrome. A random screen of natural products has allowed us to identify the marine sponge Leucettamine B as an inhibitor of DYRKs and CLKs (Debdab et al., 2011). Synthesis of over 500 analogues (collectively referred to as Leucettines) led to an optimized product, Leucettine L41. Leucettines were co-crystallized with DYRK1A, DYRK2, CLK3, PIM1 and GSK-3 β . Key interactions were identified which will help in the synthesis of further derivatives. The selectivity of L41 was extensively studied (Tahtouh et al., 2012.) (i) by its effects on the catalytic activity of two panels of recombinant kinases, (ii) in an interaction assay with 402 kinases, (iii) by an affinity chromatography approach (L41 immobilized on sepharose beads) (Burgy et al., 2013.) and (iv) by a competition affinity assay. Proteomic and transcriptomic studies shed light on the range of molecular actions of L41 in cells. L41 displayed dose- and time-dependent neuroprotective effects on glutamate-induced cell death in immortalized mouse hippocampal cell line HT22. L41 enhanced autophagy without inducing cell death, most probably as a result of CLK inhibition (Fant et al., 2014). L41 also reduced amyloid precursor protein (APP)- induced cell death in cultured rat brain slices. L41 prevented cognitive deficits triggered by icv injection of amyloid peptide A β 25-35 in mice. L41 is currently being tested in tgBACDyrk1a mice, a model which expresses one extra copy of the DYRK1A gene, and in Ts65Dn mice, a partial trisomy model. Both models overexpress DYRK1A (about 1.5 fold increase in mRNA and protein levels and in catalytic activity). L41 treatment of mice leads to normalization of the DYRK1A activity and fully corrects the novel object recognition deficit displayed by these two Down syndrome mouse models. The unusual multi-target selectivity of Leucettines may account for their neuroprotective effects. This family of kinase inhibitors deserves further optimization as potential therapeutics against neurodegenerative diseases such as Alzheimer's disease and Down syndrome.

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HEPARAN SULFATE FRAGMENTATION LOWERS AMYLOID BURDEN IN ALZHEIMER'S DISEASE TRANSGENIC MICE

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FUNCTIONAL INTERACTION BETWEEN PRION AND A β IN NEURONAL MEMBRANES.

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Alzheimer's disease (AD) is a progressive neurodegenerative pathology that affects the human brain and causes cognitive and behavioral disorders. A major characteristic of AD is the presence of β -amyloid peptide (A β) oligomers in the brain. We have previously shown that A β oligomers (A β o) associate with the neuronal membrane and induce the formation of perforations, causing an influx of calcium ions and increasing the release of synaptic vesicles that leads to a delayed synaptic failure produced by vesicle depletion.

Recent studies suggested that A β interacted with several proteins of the plasma membrane, such as the amyloid precursor protein (APP) and cellular Prion (PrPc). Therefore, these proteins could be regulating some of the toxic effects of A β in brain neurons. With the aim of determining if the levels of these two proteins affect the association and the toxic effects of A β on hippocampal neurons, we performed a series of experiments enhancing and reducing the membrane levels of APP and PrPc.

Our results show that A β readily associated to the plasma membrane of HEK cells and hippocampal neurons after 1 h of incubation. The use of phospholipase C (PLC) decreased the levels of membrane PrPc in hippocampal neurons and also reduced A β o association to these neurons. An anti-PrP antibody (6D11) decreased the association of A β to the hippocampal neurons and diminished the co-localization between A β and PrPc. Furthermore, this antibody was able to block the increase of calcium induced by A β . The latter suggests that the state of A β aggregation is important for its interaction with other membrane proteins.

We conclude that A β interacts with membrane proteins like Prion. The results support the idea that the interaction of A β with PrPc could be important in some of the toxic effects induced by A β . Future studies blocking A β interaction with Prion and other membrane proteins could be important for the discovery of new therapeutic strategies for AD.

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LATE DANTROLENE TREATMENT REDUCED AMYLOID BURDEN IN ALZHEIMER TRIPLE TRANSGENIC MICE

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Increasing studies suggest that intracellular Ca²⁺ dysregulation via over activation of ryanodine receptors (RyR) play important roles on neuropathology and cognitive dysfunction in Alzheimer's disease (AD). Our recent study demonstrated that early treatment of dantrolene, a RyR antagonist and a clinically used drug to treat malignant hyperthermia and others, significantly decreased amyloid burden and nearly abolished the memory and learning loss in Alzheimer's triple transgenic (3xTg-AD). However the possible therapeutic effects of late dantrolene treatment are still not clear and were then investigated in the current study. 15 months old 3xTG-AD or wide type (WT) control mice were treated with dantrolene 5 mg/kg orally for consecutive 6 months. Corn syrup in PBS was used as vehicle control. Learning and memory were measured with the Morris Water Maze (MWM) at 21 months. Mice brain volumes were measured by MRI immediately after MWM tests. Brains were then removed and the amyloid burdens in the different brain regions were examined using immunohistochemistry. Dantrolene treatment significantly reduced amyloid burden and 6E10 positive cells in hippocampus in 21-month-old 3xTG-AD mice. There were no significant difference on hippocampus volume measured by MRI and cognitive function measured by MWM between the WT control and 3xTG-AD mice at 21 months old. Our results suggested that late treatment of dantrolene long after initiation of amyloid aggregation could still significantly decrease amyloid burden in hippocampus of 3xTG-AD mice. However, the possible beneficial effects of dantrolene-mediated amyloid burden reduction on improvement of cognitive function could not be evaluated in this study due to lack of memory and learning loss in these very aged 3xTG-AD mice compared to WT controls.

INTRACELLULAR A β ALTERS THE TIGHT JUNCTION OF RETINAL PIGMENT EPITHELIUM IN ALZHEIMER'S DISEASE ANIMAL MODEL OF 5XFAD MICE

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Extracellular deposit of amyloid beta (A β) is a common pathologic feature in both age-related macular degeneration (AMD) and Alzheimer's disease (AD), but the role of intracellular A β on the tight junction of the retinal pigment epithelium (RPE) is unknown. In this study, we investigated the intracellular A β expression and its role on the outer blood retinal barrier (BRB) in the retina of 5XFAD mice, a mouse model of AD. The retina of 5XFAD mice showed the pathologic features of AMD with intracellular A β in the RPE. With increasing intracellular A β accumulation, zonular occludens-1 (ZO-1) and occludin were markedly attenuated and lost their integrity as tight junctions in the RPE of 5XFAD mice. A β ₄₂ uptake by ARPE-19 cells induced the tight junction breakdown of ZO-1 and occludin without cell death. These results implicate that intracellular A β ₄₂ could play a role in the breakdown of outer BRB in 5XFAD mice. 5XFAD mice could be a mouse model of dry AMD with regard to the A β ₄₂ related pathology.

MULTI-TARGET-DIRECTED LIGANDS (MTDLS) AS POTENTIAL DRUGS IN ALZHEIMER DISEASE - HIGHLY SELECTIVE INHIBITION OF BUTYRYLCHOLINESTERASE BY NOVEL MELATONIN-TACRINE HETERODIMERS.

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Alzheimer Disease (AD) has multifactorial origin and it's not surprising that the current drug design paradigm of one-drug-one-target may not be a sufficient model to develop treatment regimens for AD. Drugs hitting a single target are insufficient for the treatment of diseases like neurodegenerative disorders. That's why the doctors apply their patients multiple-medication therapy (MMT) also known as "cocktail" or "combination of drugs". MMT is composed of different drugs that combine different therapeutic mechanisms. In order to facilitate taking medications second approach might be used - multiple-compound medication (MCM) also called "single-pill combination". Medicinal research is constantly seeking to improve the efficiency of drugs. The third strategy is to combine in one molecule pharmacophores of different drugs in the same structure, to afford hybrid molecules - MTLDS (multi-target-directed ligands), with the intention to hit several targets at once. [1].

Already synthesized hybrids show that such coupling results in higher biological activity. This single compound may be able to hit multiple targets. Molecule may contain an inhibitor of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) and, for example, calcium channel blocker, radical scavenger, that may protect neurotransmitting system. After the coupling the molecule acquires better properties. For example, the inhibitor's activity increases, it can connect with different parts of the enzyme (peripheral and active binding sites) [2].

To meet today's expectations, we strive to create molecules, that are new hybrid cholinesterase inhibitors, which combine today's pharmaceuticals in AD, cholinesterase inhibitors and serotonin (5-hydroxytryptamine 5-HTA, an important neurotransmitter) which should act in the hybrid molecule as a regulator of neurotransmission, antioxidant and antidepressant, linked by carbamate bond. The obtained derivative may thus serve multiple functions, not only limited to the inhibition of progression of the disease [3]. The next step is to evaluate in vitro the biological activity of the novel heterodimers toward cholinesterase inhibition using Ellman's method [4]. Some compounds obtained by our group turned to be highly active and selective inhibitors of cholinesterases. Those compounds are covered by a pending patent and constitute an invention reported to the Patent number WO 2012165981 A1 entitiled "Novel hybrid cholinesterase inhibitors" [5].

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