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Background: Mitochondrial aldehyde dehydrogenase 2 (ALDH2) is believed to play a major role in acetaldehyde detoxification in the ethanol metabolism. The alcohol sensitivity is associated with a mutant allele of the ALDH2 gene (ALDH2*2), which results in a substitution of Glu 487 to Lys, acting in a dominant negative fashion. Our molecular epidemiologic analysis has shown the association of Alzheimer’s disease (AD) with ALDH2*2 allele. The concentration of lipid peroxides (LPO) was significantly higher in defective ALDH2 females than nondefective ones, suggesting that ALDH2 functions as a protector against oxidative stress. Indeed, ALDH2 detoxifies reactive aldehydes, such as 4-hydroxyl-2-nonenal (4-HNE), that are derived from LPO.

Methods: We constructed transgenic mice by introducing the mouse-version of the ALDH2*2 gene, and named the resultant mice DAL (Dominant negative of ALDH2) mice. Results: Female DAL mice exhibited no particular abnormality on physical examinations compared with control C57BL/6 mice when observed until 18 months after birth. However, primary cultured neocortical cells from DAL mouse embryos exhibited increase in vulnerability to 4-HNE. Therefore, autopsy of the brain was performed in 6-month-old mice, but no difference compared with the brain of control C57BL/6 mice was noted, whereas in 18-month-old DAL mice, signs of neurodegeneration such as atrophy of the hippocampus and associated loss of pyramidal cells and activation of glial cells were observed. These changes were sporadically observed from 12-month-old and increased with aging. The old-aged DAL mice also developed prominent deficits in special memory in the Morris water-maze test, whereas young DAL mice (6-month) looked normal. Accumulation of oxidative stress in the brain of DAL mice will be further discussed.

Conclusions: Brain degeneration and decline in spatial cognitive ability in DAL mice are considered to be due to decrease in resistance to oxidative stress. Analysis of these mice will clarify the relationship between lesions characteristic of AD and oxidative stress.

THE COMPLEXATION OF AMYLOID-β WITH HEME FORMS A PEROXIDASE: MECHANISM OF NEUROTOXICITY IN ALZHEIMER’S DISEASE

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Background: The molecular mechanism by which Amyloid-β (Aβ) peptide causes the neurodegeneration in Alzheimer’s disease (AD) is not known. We previously proposed that excessive Aβ binds to regulatory heme, triggering functional heme deficiency (HD), causing the key cytopathologies of AD including mitochondrial dysfunction, loss of complex IV, iron accumulation, and oxidative stress (Atamna et al. 2004, PNAS).

Objectives: In the current study we searched for additional evidence that Aβ binding to regulatory heme is the mechanism by which Aβ causes HD.

Methods: The experiments included techniques of tissue culture, biochemistry, radiobiology, and HPLC.

Results: We found that heme binds to Aβ forming an Aβ-heme complex. We also found that Aβ induces heme synthesis and iron uptake in human neuroblastoma cells, which provides strong support that Aβ triggers HD. Interestingly, we also found that the Aβ-heme complex is a peroxidase, which catalyzes the oxidation of serotonin and DOPA by H2O2. Curcumin, which lowers oxidative damage in the brain in a mouse model for AD, inhibits the Aβ-heme peroxidase.

Conclusion: The binding of Aβ to heme supports a unifying mechanism by which elevated levels of Aβ induces HD, causes oxidative damage to macromolecules, and depletes specific neurotransmitters. The relevance of the depletion of regulatory heme by excessive Aβ and Aβ-heme peroxidase to mitochondrial dysfunction, oxidative stress, neurotoxicity and other cytopathologies of AD will be discussed.

P3-366

P3-367 ASSESSMENT OF ALTERED BLOOD/BRAIN IRON METABOLISM AS A RISK FACTOR FOR THE DEVELOPMENT OF ALZHEIMER’S DISEASE

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Background: Iron regulatory protein-2 (IRP-2), a post-transcriptional regulator of proteins responsible for iron uptake and storage, has shown abnormal localization in Alzheimer’s Disease (AD) brain. Moreover, IRP-2 knockout mice accumulate higher iron levels in certain brain regions followed by axonal degeneration. Objectives: To address the role of brain/blood iron perturbation in the pathogenesis of AD two groups of elderly participants, one cognitively intact and the other mildly cognitively impaired (MCI) have been studied longitudinally for changes in level and function of IRP-2 in peripheral blood.

Methods: Over the past 21 months whole blood RNA and serum samples were obtained from 28 control and 76 amnestic multiple-domain MCI participants. Quantitative Realtime PCR (QPCR) was used to investigate expression levels of IRP-2. To assess the functionality of IRP-2, the expression of three alternative splice forms of the divalent metal transporter 1 (DMT1), one of which contains an iron response element by which IRP-2 controls its translation and two of which do not, is being evaluated.

Results: Through nested PCR and DNA sequencing we were able to identify several alternative splicing variants of IRP-2 whose function and possible correlation to AD is currently being assessed in relation to participant’s clinical course. QPCR done on RNA samples of nine participants has shown a differential expression of IRP-2 between MCI and either control or progressive MCI (PMCI) patients. A larger scale study involving 20 control, 20 MCI and 13 PMCI age and gender matched participants is currently under way to confirm that finding.

Conclusions: To our knowledge, this is the first report on the identification of IRP-2 splice variants. The significance of these in the face of AD is under current investigation. Furthermore, we were able to show expression differences of IRP-2 between MCI and either control or PMCI patients. This finding will be confirmed by a larger study to permit final conclusions as to its significance. Regarding the function of IRP-2, final experiments are currently being done to compare the ratios of DMT1-IRE to DMT1-nonIRE between different stages of cognitive impairment. This research was funded by NIH Grant #AG20948.

P3-368 ENDOGENOUS HUMAN APP POSSESSSES OXIDASE ACTIVITY

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Background: Amyloid precursor protein (APP) is a type-1 transmembrane metal-binding protein with oxidase activity. Candidate substrates include ferrous iron and cholesterol. We have recently identified a region of APP with homology to the ferritin H subunit in residues 250-273 in APP-695 that has significant ferroxidase activity. Since APP also possesses a copper-binding domain at the amino terminus whose structure has recently been solved, it is possible that the ferroxidase activity of APP might be copper dependent, like ceruloplasmin. Objective: To characterize the oxidase activity of APP purified from normal human plasma and various cell lines. The impact of urea denaturation, as well as copper supplementation and deprivation on APP oxidase activity were also investigated. Methods: APP and ceruloplasmin were purified from human plasma, neuronal cell lines (M17 and SY5Y), fibroblasts and hepatocytes (HepG2) using anion-ex-
change column chromatography, SDS-PAGE and electrophoresis. Activities from these partially purified preparations were compared with purified recombinant APP751 expressed in yeast, and ceruloplasmin obtained from commercial sources. Oxidative activity was characterized using various substrates (including PPD; p-Phenylenediamine dihydrochloride) and Fe²⁺ oxidation, in the presence and absence of Cu²⁺ and chelators. **Results and Conclusion:** Plasma APP and ceruloplasmin separated by electrophoresis both readily oxidized PPD. Cellular APP derived from neuronal (M17 and SY5Y) and hepatic (HepG2) cell lines also oxidized PPD, indicating a ubiquitous activity. Moreover, the oxidase activity of APP is not apparently dependent upon delivery of copper by the copper transporter APT7A (Menkes protein) as activity was still detected with APP derived from APT7A-null fibroblast. As the PPD oxidase activity of APP does not require Cu²⁺, it is mechanistically more similar to that of ferritin than that of ceruloplasmin.

**P-3-369**  
**INTRANEURONAL AMYLOID-β42 MAY SILENCE OXIDATIVE STRESS IN ALZHEIMER’S DISEASE**  
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**Background:** A growing body of evidence suggests an initial role of intraneuronal accumulation of amyloid-β (Aβ) in the pathological cascade of Alzheimer’s disease (AD), but on the other hand, we have reported that oxidative stress is one of the earliest events in vulnerable neurons of AD. Indeed, both intraneuronal Aβ accumulation and oxidative stress precede extraneuronal deposition of Aβ plaque in patients with Down syndrome and in transgenic mice models of AD, which suggests a possible connection between the two events. **Objective(s):** To investigate the relationship between the levels of intraneuronal Aβ accumulation and oxidative damage to RNA in AD brains, the latter of which was previously shown to parallel oxidative damage to protein. **Methods:** Immunocytochemically, Aβ40, Aβ42 and the oxidized RNA nucleoside, 8-hydroxyguanosine (8OHG) were identified in postmortem tissue sections of the hippocampal region from 16 cases of AD (age 65-93 years). **Results:** Intraneuronal Aβ42 and 8OHG immunoreactions were observed in the hippocampal pyramidal neurons in all the subjects, while 8OHG-positive neurons were more widely distributed compared with Aβ42-positive neurons. Intraneuronal Aβ40 immunoreaction was faint in most of the subjects. The QCB42 antibody (Biosource) showed the same pattern and level of Aβ42 immunoreaction in the neuronal cytoplasm that the MBC42 antibody (H.Y.) showed, while the former recognizes both monomeric and oligomeric Aβ42 species and the latter recognizes mainly monomeric Aβ42. However, the antibody specific to Aβ oligomers (provided by Dr. C. Glabe) hardly immunostained the neuronal cytoplasm, suggesting that the neuronal cytoplasmic Aβ42 observed in this study might be mainly monomeric. Relative density measurements revealed an inverse relationship between intraneuronal Aβ42 and 8OHG immunoreactivities (r = -0.61, p < 0.02), while there was no significant relationship between intraneuronal Aβ40 and 8OHG immunoreactivities. **Conclusions:** Together with recent evidence that Aβ possibly acts as an antioxidant through its capability to capture redox metal ions, these results suggest that intraneuronal accumulation of Aβ42, especially the monomeric form, is related to a compensatory response to neuronal oxidative stress in AD. We suggest that excessive removal of Aβ may lead to increased, rather than decreased, neuronal oxidative damage in AD.

**P-3-370**  
**APOE GENOTYPE HAS NO INFLUENCE IN PLATELET AND ERYTHROCYTE OXIDATIVE STATE**  
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**Background:** In a previous study, we showed increased platelet and erythrocyte oxidative stress in aging and Alzheimer’s Disease (AD) (Kawamoto et al, Neurobiol Aging, 26:857-64, 2005). ApoE is an antioxidant glycoprotein, and ApoE4 has lower antioxidant capacity and is a susceptibility factor for sporadic AD. **Objective(s):** To evaluate the influence of APOE genotype in oxidative stress production in aging and AD. **Methods:** We evaluated 32 elderly subjects without dementia (19 women, 70.1±6.3 y) and 49 patients with probable AD (33 women, 73.2±7.3 y). TBARs and cGMP content and nitric oxide synthase (NOS), superoxide dismutase (SOD) and Na,K-ATPase activities were compared between individuals with and without APOE ε4 allele, using unpaired Student “t” test. **Results:** The following results were found: 1) 62% of AD patients and 29% of controls were carriers of at least one ε4 allele. 2) Significant statistical difference between controls and AD was observed in TBARS content (p<0.0001) and in NOS (p<0.005) and Na,K-ATPase activities (p<0.0005). 3) We did not find any difference (p>0.05) between individuals with and without ε4 allele in any of the variables studied in AD patients. In the control group, non-ε4 allele carriers showed higher SOD activity than carriers (p<0.05). 4) When the analysis were performed using only APOE genotype, independently of group, ε4 allele carriers showed higher NOS activity than non-carriers (p<0.05). **Conclusions:** APOE ε4 allele carriers did not show important increase in platelet and erythrocyte oxidative stress markers than do non-carriers. Therefore, APOE genotype was not directly related with the oxidative state in this sample.  
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**P-3-371**  
**MITOCHONDRIAL GSH DEPLETION SENSITIZES HUMAN NEUROBLASTOMA CELLS TO BETA-AMYLOID PEPTIDE-INDUCED OXIDATIVE STRESS AND APOTOTIC CELL DEATH**  
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**Background:** The pathogenesis of Alzheimer disease is not completely understood at present, although the generation of toxic beta-amyloid peptide (Aβ) is thought to play a prominent role. One of the cytotoxic effects of the Aβ is reactive oxygen species (ROS) overgeneration. Although, the exact mechanisms of this process are not well defined, emerging evidence points to mitochondria as a source for Aβ-induced ROS generation. Moreover, disturbances in cholesterol homeostasis promote the formation and deposition of Aβ and the progression of AD. Recent findings from our laboratory reveal that cholesterol enrichment of mitochondria depletes selectively mitochondrial glutathione (mGSH), a major defense against ROS generated from mitochondria. **Objectives:** The aim of this work was to study how mGSH regulates the oxidative stress and the apoptotic pathways triggered by Aβ and to analyze its regulation by cholesterol in rat brain mitochondria and in the human neuroblastoma cell line SH-SYSY. **Results:** Rat brain mitochondria were exposed to the Aβ (1-42) peptide, determining H₂O₂ generation and lipid peroxidation spectrophotometrically. Aβ incubation elicited a burst of peroxide production within 2h. However, mGSH depletion prior to Aβ exposure potentiated the H₂O₂ generation (2-3 fold) and lipid peroxidation (5-10 fold) by Aβ, which correlated inversely with mGSH content. Furthermore, pre-treatment of SH-SYSY cells with (R,S)-3-hydroxy-4-pentenoate, which is biotransformed into a Michael electrophile in the mitochondrial matrix, caused selective mGSH depletion (50-70%), sparing cytosol GSH. Exposure of mGSH-depleted SH-SYSY cells to Aβ enhanced ROS generation and cell death (40-70%), compared to mGSH-repleted cells (20-30%) that was prevented by the caspase inhibitor qVD-OPH. To ascertain the role of cholesterol in modulating the susceptibility to Aβ, we isolated brain mi-