Degenerative changes in the white matter appear to be more prominent than those in the cortex. Based on our recent observation of iron accumulation and ferritin immunoreactivity in the aged dog brain, we suspected that iron-induced oxidative injury may be related to pathogenesis of the severe age-related morphological changes in the cerebral white matter. In this study, we performed immunohistochemical analysis using antibodies against laminin and fibrinogen to determine whether age-related morphological changes of blood-brain-barrier (BBB) occur in the white matter, resulting in an exudation of serum materials (*ie.* iron) which may cause oxidative injury. In addition, we examined iron deposition and astrocytic reaction for oxidative stress with the use of Prussian blue DAB and post DAB enhancement (PBD-PDE) method and superoxide dismutase (SOD) immunohistochemistry (IHC). Brains from 9 young dogs, ranging in age from 6 months to 5 years, and 22 old dogs from 10 to 18 years, were used. In young dogs, there were no significant findings in histological and immunohistochemical examinations. In contrast, a decrease of laminin immunostains was shown in the basement membranes of some capillaries and venules in the white matter of aged dogs. Fibrinogen IHC showed positive immunoreactions in some macrophages accumulating in perivascular spaces and some astrocytes. PBD-PDE method technique for iron demonstrated positive stains in some macrophages and astrocytes. There were some astrocytic processes positive for SOD in the white matter. These results suggest a possibility that a collapse of vascular basement membranes may occur during aging, leading to a disturbance of BBB function and thereby to perivascular exudation of serum materials including an iron. The vascular changes may be related to severe degenerative changes in the white matter.

108 HEAVY METALS AND PCBS PROMOTE BETA-AMYLOID AGGREGATION AND ITS CYTOTOXCITY IN PC12 CELLS.

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Alzheimer disease (AD) is a gradual and irreversible, progressive neurodegenerative disorder which is characterized with the accumulation of amyloid fibrils. The major constituents of amyloid fibrils in the AD brain are 39-43 amino acid amyloid β (Aβ) peptides, which are protein fragments snipped from a larger protein called amyloid precursor protein (APP). The amyloid hypothesis states that Aβ deposition is a major causative factor for the onset of AD. This is further supported by other studies, which showed that Aβ is toxic only when it is aggregated. The factors that cause the aggregation of Aβ are not clearly known, however it has been proposed that exposure to environmental toxic agents such as heavy metals and persistent organic pollutants may exaggerate the aggregation of this peptide. The present study is aimed to screen the role of various environmental agents, which could influence Aβ aggregation. Synthetic Ab peptide (1-40) was dissolved in urea, diluted with PBS (pH 7.4) and incubated in the presence of 0, 0.1 μM, 1 μM and 10 μM of Ba/Hg/Pb/Zn/PCBs. Peptide aggregation was studied by fluorometric determination with bis-ANS as well as measuring the sedimentation of the aggregated peptide by a protein assay. The protein assay showed that Pb, PCBs and Zn significantly promoted the aggregation of Aβ, whereas Ba and Hg had minimal effects. Similar results were obtained with the bis-ANS studies. Control studies conducted using both techniques and another protein, bovine serum albumen (BSA), showed non-significant effects on aggregation. Furthermore, the cytotoxicity of the Abeta peptide in the presence of these toxicants was investigated in PC12 cells. It was observed that toxicants that promoted peptide aggregation also enhanced the cytotoxicity of Aβ. These findings suggests that environmental agents are potential risk factors for the development of AD by promoting the aggregation of Abeta and/or enhancing its cytotoxicity.

109 ALUMINUM MALTOLATE-INDUCED CYTOTOXICITY IN NEURO-2a CELLS INVOLVES APOPTOSIS AND NECROSIS.

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Aluminum maltolate (Al-malt) has been shown to cause neurodegeneration following *in vivo* exposure and apoptosis plays a prominent role. The objective of this study was to define the mode of cell death induced by Al-malt. Neuro-2a cells were treated with Al-malt for 24 h in the presence or absence of pretreatment with a variety of pharmacological agents. Al-malt concentration-dependently increased cell death as indicated by lactate dehydrogenase (LDH) release and the appearance of cells with hypodiploid DNA content. Flow cytometry using acridine orange (high-live, low-apoptotic) and ethidium bromide (necrotic) dual staining showed that the mode of cell death was a combination of apoptosis and necrosis. Treatment with Al-malt resulted in caspase 3 activation and the externalization of phosphatidyl serine, both indicative of apoptosis. In addition, nuclear condensation and fragmentation were evident in Hoechst 33258 stained nuclei from Al-malt-treated cultures. Interestingly, pretreatment with cyclohexamide (CHX) markedly reduced Al-malt-induced apoptosis indicating that this mode of death is dependent on *de novo* protein synthesis in the current culture model. Necrotic cell death was not prevented by CHX suggesting that only apoptosis was dependent on gene expression. Pretreatment with antioxidants and kinase inhibitors (mitogen activated protein kinases and PKC) did not reduce Al-malt toxicity suggesting independence from oxidative stress and major kinase signaling pathways. The results provide insight into the mechanisms of Al-malt neurotoxicity and support the involvement of this metal in neurodegeneration.

110 ALUMINUM-INDUCED NEURODEGENERATION INVOLVES DIFFERENTIAL REGULATION OF PROINFLAMMATORY CYTOKINE AND NEUROTROPHIN GENE EXPRESSION.

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The etiology of human neurodegenerative diseases including Alzheimer's disease (AD) is exceedingly complex and our understanding of the mechanisms involved is far from complete. The experimental neurotoxicology of aluminum has been shown to recapitulate virtually every pathophysiological feature of AD and therefore represents a useful model to study the mechanisms involved in neurodegeneration. The present study investigated the effects of aluminum maltolate (Al-malt) on the delicate balance that exists between pro-inflammatory cytokines and neurotrophins using primary brain rotation-mediated aggregate cultures. Aggregates were treated with Al-malt (5-150 μM) on day 15 *in vitro* for 72 h. Cell death increased in a time- and concentration-dependent manner reaching significance in aggregates treated with 150 μM Al-malt in 48 h and 50 μM by 72 h. Analysis of gene expression at 72 h revealed a concentration-dependent increase in tumor necrosis factor α and macrophage inflammatory protein-1α suggestive of a state of inflammation. In contrast, a dramatic concentration-dependent decrease in the expression of nerve growth factor (NGF) and brain derived neurotrophic factor was observed. In fact, NGF expression could not be detected in aggregates treated with 50 and 150 μM Al-malt. These changes in gene expression correlated with a decrease in aggregate size and an increase in neurodegeneration as indicated by Fluoro-Jade B staining. The results indicated a differential regulation of pro-inflammatory cytokines and neurotrophins in brain tissue following treatment with Al-malt. Such findings provide insight into the possible involvement of deregulation of the cytokine/neurotrophin balance in the etiology of neurodegeneration.

111 ALUMINUM IN DRINKING WATER PROMOTES NEURO-INFLAMMATORY INDICES *IN VIVO*.

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Several epidemiological studies indicate a positive relationship between the incidence of Alzheimer's Disease (AD) and aluminum (Al) concentration in drinking water. However, others have not found such an association and the controversy regarding the role of aluminum in AD is still ongoing and unresolved. Existing evidence suggests that Al is capable of causing inflammation systemically and in the central nervous system. In order to determine if a biologically relevant amount of Al is capable of producing neuroinflammation *in vivo*, we exposed mice for 10 weeks to Al lactate (0.01, 0.1, 1 mM) in drinking water. The pro-inflammatory cytokine TNF-α was increased in the brain of mice treated with the highest dose of Al lactate. The inflammation related transcription factor NF-κB was increased in the mice brains dosed with the lowest concentration of Al. NF-κB activation was not observed at the highest Al dose. This may be due to homeostatic processes which down regulate gene expression of pro-inflammatory elements by negative feedback. No effect on these inflammatory markers was seen in the liver or serum. Therefore, the effects of Al observed were not due to a systemic response, but rather, suggest a selective neurological reaction.

112 EFFECTS OF ALUMINUM ON MEMBRANE PROPERTIES AND BIOGENIC AMINE METABOLISM IN RESTING PC-12 CELLS.

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Effects of aluminum (Al) on membrane properties of excitable cells are not fully understood. Evidence suggests that oxidative stress and membrane alterations play important roles in Al neurotoxicity. The present study was conducted to investigate