64 Aluminosilicate particulate and β-amyloid in vitro interactions: a model of Alzheimer plaque formation

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Alzheimer’s disease is characterised pathologically by the accumulation of extracellular insoluble fibrillar β-amyloid (Aβ) protein aggregates within the senile plaques of the dementia brain. The identification of aluminosilicate deposits within the plaque cores has prompted studies into the potential role of toxic metals, particularly aluminium, in the aetiopathogenesis of the neurodegenerative disorder [1]. Evidence for the possible adverse effect of environmental exposure to aluminium and its compounds has been obtained from a number and variety of studies: epidemiological investigations into human occupational and community populations, comparative trace element analysis of dementia brain tissue samples, experimental animal toxicological tests, toxic effects on cultured brain cells, and modification/aggregation of Aβ peptides treated in vitro with various aluminium compounds [2]. However, whilst the influence of genetic factors in the pathogenesis of Alzheimer’s disease is widely accepted, the role of putative environmental agents such as aluminium, remains contradictory, uncertain, and controversial [3]. The proposed pathological role of reactive fibrillar plaque components and associated microglial chronic inflammatory interactions in the neurodegenerative process in Alzheimer’s disease [4], is consistent with subsequent in vitro studies. These have shown the stimulatory effect of analogous model natural and synthetic aluminosilicate particles [5] and Aβ [6] in promoting the generation of neurotoxic reactive oxyradicals and nitric oxide by activated microglial cells in culture.

In the present in vitro study, the capacity of various aluminium compounds and model natural mineral and synthetic aluminosilicate particles to interact with Aβ peptide has been studied using an immunochemical assay. Synthetic Aβ(1-42) residues peptide (50 µg/ml) was incubated with the sample aluminium materials (1mg/ml) in pH7.4 phosphate buffered saline at 4°C in slowly rotating vials for 16hr. Assays were performed in quadruplicate. Following incubation, the mixtures were centrifuged at 13,000g for 5min, and the Aβ estimated in the resultant supernatant and pellet. The soluble Aβ in the supernatant was assayed directly, whilst the aggregated Aβ in the pellet was resuspended in diluent equal to the original incubation volume prior to assay. The Aβ was adsorbed onto PVC wells and detected by synthetic Aβ(1-42) and labels senile plaques in Alzheimer meningeal pamyloid. This antibody recognises Aβ(1-42) and labels senile plaques in Alzheimer brain tissue.

<table>
<thead>
<tr>
<th>Alumino sample</th>
<th>Ratio Aβ aggregated/soluble</th>
</tr>
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<tbody>
<tr>
<td>ECC China clay</td>
<td>6.69</td>
</tr>
<tr>
<td>ECC kaolin BP</td>
<td>4.88</td>
</tr>
<tr>
<td>Alusil N precipitate</td>
<td>0.35</td>
</tr>
<tr>
<td>Alusil ET precipitate</td>
<td>0.11</td>
</tr>
<tr>
<td>Commercial aluminium sulphate</td>
<td>0.30</td>
</tr>
<tr>
<td>Control</td>
<td>0.12</td>
</tr>
</tbody>
</table>

The results indicate that the ECC China clay and ECC kaolin BP mineral aluminosilicates were the most active in promoting Aβ protein aggregation, with the two synthetic alusil aluminosilicate precipitate samples ranking along with the commercial aluminium sulphate sample in exhibiting similar to marginally elevated reactivity compared to the low levels of the buffer control. Confirmation of the formation of the fibrillar nature of the Aβ protein aggregates was provided by electronmicrography. Supplementary studies were also undertaken to examine the effect of modifying the incubation conditions. Namely: dose response to aluminosilicate particles, changes in temperature and time, variable aluminium concentrations and Aβ 'aged' state.

The capacity of minerals to act as nucleation sites for the adsorption of proteins [7] and hence promote the aggregation and deposition of β-amyloid, provides a possible pathogenic mechanism for the enhanced formation of neurotoxic Aβ fibrils. It is suggested that the mineral surface adsorptive process diminishes the activation energy barrier and thus catalyses the interconversion of the non-amyloidogenic to the amyloidogenic Aβ peptide conformer [8]. Comparable nucleation phenomenon may also be of particular relevance to the seeding of prion proteins present in s-rapie, BSE and CJD [9]. The findings suggest that comparable in vivo deposition of fibrillar β-amyloid aggregates within the Alzheimer plaques may be exacerbated by interactions with environmental aluminosilicate particulates, so-called cephaloconiosis [10]. Access of environmental toxins directly to the brain via the olfactory tract has been hypothesised [11].

Hence it is proposed that by the particulate aluminosilicate-catalysed and free radical-mediated [12] promotion of fibrillar Aβ formation, the associated enhanced stimulation of microglia-generated free radicals causes a chronic inflammatory cascade process resulting in neurodegeneration and Alzheimer’s dementia.

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