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ALZHEIMER'S DISEASE: A PATHOGENIC ROLE FOR ALUMINOSILICATE-INDUCED PHAGOCYTIC FREE RADICALS

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The occurrence of aluminosilicate deposits within the cerebral plaques in Alzheimer's senile dementia sufferers has prompted further consideration of the possible role of such materials in the aetiology and pathogenesis of the disease. We have monitored the ability of various natural and synthetic model aluminosilicate particulates of differing morphological and chemical composition to stimulate the generation of phagocyte-derived free radical reactive oxygen metabolites (ROM) using an *in vitro* chemiluminescent technique on purified human blood-derived polymorphonuclear leukocytes (PMN). The results indicate that an enhanced chemiluminescent response is produced by calcium-bearing fibriform particulates. It is proposed that an analogous *in vivo* particle-induced and phagocyte-mediated oxidative stress could provide a potential pathogenic mechanism in the development of Alzheimer's disease.

KEY WORDS: Alzheimer's disease, aluminosilicates, free radicals, phagocytes

INTRODUCTION

The detection by x-ray microprobe analysis and magic-angle-spinning nuclear magnetic resonance of aluminosilicate deposits within the plaques found in brain tissue from Alzheimer sufferers[1] invites questions as to their role in the pathogenesis of the disease.[2] Aluminosilicates are widely distributed throughout the Earth's crust, comprising a large number of clay and zeolite minerals. Zeolites are open crystalline structures built from corner-sharing SiO_4^{4-} and AlO_4^{5-} tetrahedra and including intracrystalline cavities and channels of molecular dimensions. The negative charge of the aluminosilicate framework is balanced by exchangeable cations, such as sodium, calcium and iron, located in the channels.[3,4] Crystal structure on the atomic level is often reflected in the morphology of the particles if they are allowed to crystallise for a sufficiently long time, as in the case of geological deposits. For example, the neutral zeolites offretite and erionite, which contain one-dimensional channel systems, adopt a fibrous morphology. Epidemiological[5] and experimental[6,7] *in vivo* and *in vitro* reports that erionite, a zeolite mineral found, for example, in Cappadocia, Turkey, and Oregon, U.S.A., is a potent pulmonary carcinogen are of particular interest.

Studies of cellular mechanisms involved in dust-related pulmonary diseases such as asbestosis and cancer, suggest that particle-induced stimulation of the respiratory burst of phagocytic cells,[8,9] i.e. PMN and macrophages, which migrate into the lung[10] and the consequent production of potentially injurious free radical and related reactive oxygen metabolites (ROM), such as superoxide and hydroxyl radicals and

hydrogen peroxide,[11] play a significant role. It is striking that erionite, an even more potent carcinogen than asbestos, also exhibits the greatest stimulatory activity of PMN ROM production as monitored by luminol-enhanced chemiluminescence.[8] The damaging effect of ROM has been implicated in a variety of pathological processes including inflammation, fibrosis, emphysema and cancer.[12] The oncogenic activity of fibriform particulates is related to fibre dimensions, with fibres longer than ca. 8 μm being the most carcinogenic.[13] Similarly, *in vitro* chemiluminescent studies of dust-stimulated PMN have revealed that comminution of asbestos and erionite fibres by ball-milling also causes a decrease in free radical production, despite the increase in the number of particles.[14] This evidence is consistent with the hypothesis[15] that the enhanced pathogenicity of fibriform particulates is dependent on the phenomenon of "frustrated phagocytosis", i.e. the partial cellular endocytosis of large elongated particles and the consequent increased extracellular release of damaging ROM. The precise role of fibre morphology and composition has not, however, been established.

We have examined how various natural and synthetic model particulate zeolites of different structure, morphology, crystal size and cationic form stimulate the production of PMN-derived ROM as monitored by luminol-enhanced chemiluminescence.[16] The sodium and calcium cationic forms of Turkish and Californian erionite, and of the synthetic zeolites omega and offretite were studied. All are structurally related and contain a one-dimensional channel system. Scanning electron microscopy shows that the Turkish erionite samples contain a greater number of fibres approximating to the proposed pathogenic fibrous dimension of ca. 8 μm compared to the Californian erionite. The synthetic zeolites were in the form of spherulites ca. 1 μm in diameter.

METHODS

Na^+-exchanged forms were prepared by treating the samples with a large excess of 1M aqueous NaCl at 80°C for 25 hours, and Ca^{2+}-exchanged forms by a similar treatment with 1M $CaCl_2$. PMN were purified from heparinized human blood by sedimentation in 3% dextran, followed by centrifugation with Ficoll-Paque (Pharmacia). Residual contaminating erythrocytes were lysed with NH_4Cl (155 mM)/$NaHO_3$(10 mM)/EDTA (0.1 mM) solution for 5 minutes. The resultant purified (> 95%) PMN were twice washed in calcium-free buffer before being resuspended in Krebs-Ringer Hepes (25 mM) buffer at pH 7.35. Comparative chemiluminescent assays were carried out in the presence of the luminescent enhancing agent luminol (10 μM) at a standardised dust and PMN concentration of 400 μg/ml and 10^6 cells/ml respectively, using an automatic multichannel Berthold luminescence analyser at 37°C. All experiments were performed in triplicate.

RESULTS

Chemiluminescent-stimulated PMN responses monitored over a 20 minute reaction period are given in Figure 1. Both cationic forms of zeolite omega produce a monotonic and virtually linear increase in chemiluminescence, unlike the other zeolites which give a more rapid initial increase in activity, with a peak occurring within 8 minutes. Both cationic forms of Turkish erionite are highly active, much more so than the other samples. Calcium forms of Californian erionite and synthetic offretite have

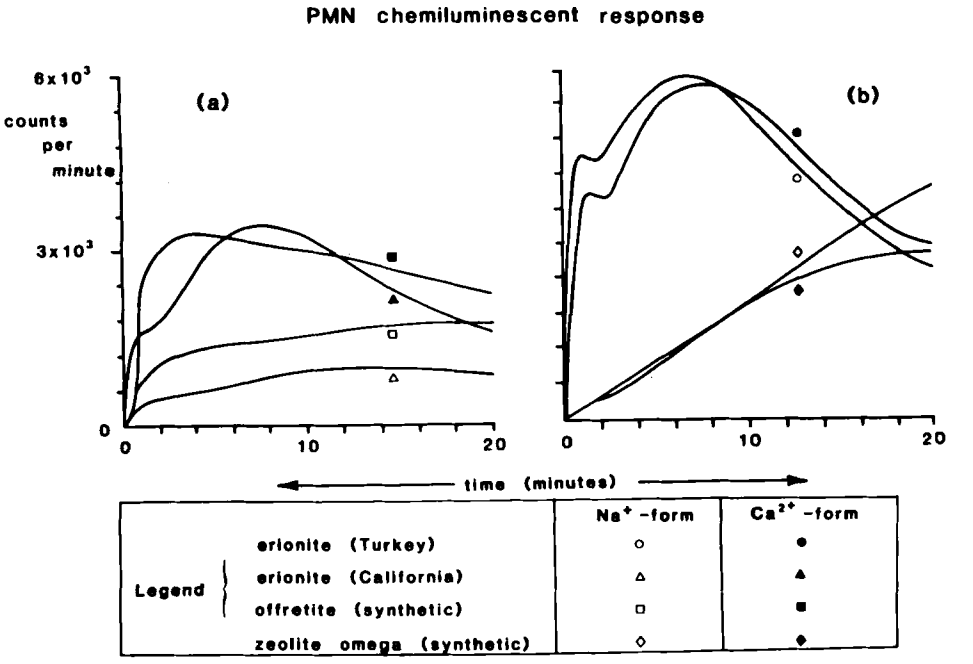

FIGURE 1 PMN-stimulated luminol-enhanced chemiluminescent activity of zeolite samples. (a) Californian erionite (triangles) and synthetic offretite (squares); (b) Turkish erionite (circles) and zeolite omega (diamonds).

very similar activity, in each case greater than the corresponding sodium forms. The rank order of activity of the samples which produce a peak in chemiluminescent response is

Turkish erionite (Ca, Na) $\gg$ Californian erionite (Ca) = synthetic offretite (ca) $\gg$ synthetic offretie (Na) > Californian erionite (Na).

DISCUSSION

The result that the largest chemiluminescent response is produced by natural erionites from Turkey and California confirms the importance of fibre morphology and size in stimulating PMN ROM production.[14] Also, while Na^+ - and Ca^{2+} - forms of Turkish erionite have similar activities, in both Californian erionite and the synthetic offretite the *calcium* form gives a much higher response than the *sodium* form. It is interesting to note in this context that montmorillonite aluminosilicate clay with Ca^{2+} as the exchange cation produces a large PMN cellular chemiluminescent response.[17] The specific contributions of the crystal structure, large internal surface and catalytic activity of zeolites to the chemiluminescent response remain to be established.

While the mechanisms by which aluminosilicates deposit in the brain are unknown, the involvement of the olfactory area of the brain and the uptake of aluminium by the brain via the nasal-olfactory neural pathway has been demonstrated,[18] and a similar

mechanism has been suggested for the transport of inhaled aluminosilicates.[19] The disruption in the blood-brain barrier which may occur as a result of traumatic head injury, a known risk factor in Alzheimer's disease,[20] indicates a possible involvement in the pathogenic process of blood-derived leucocytes, which have been detected in the brains of Alzheimer's subjects.[21] The presence of microglial macrophages in the brain adjacent to plaque amyloid fibrils,[22] and their ability to generate ROM when stimulated by particulate, immunological and soluble agents, e.g. tumour promoter phorbol myristate acetate,[23] indicate that endogenous brain cells are also capable of contributing to phagocyte-dependent cerebral oxidant stress.

Free radicals and ROM are implicated in mediating various biochemical processes of direct relevance to neural functioning in senile dementia, including modification of vascular permeability,[24] synaptic neurotransmitter function[25] and indeed as a primary mechanism of the aging process.[26] The brain itself is peculiarly prone to the injurious effects of oxidative stress because of its high content of unsaturated fatty acids and low levels of the antioxidant enzyme glutathione peroxidase,[27] and accumulation of lipofuscin in the periphery of Alzheimer plaque is additional evidence of lipid peroxidative changes.[28] An increased level of lipid peroxides and a decreased activity of the antioxidant enzyme superoxide dismutase have been found in brain tissue following ingestion of aluminium,[29] a suspected environmental toxin in Alzheimer's disease. Interestingly, decreased levels of antioxidants such as vitamin E[30] and zinc[31] have also been detected in the serum and brains, respectively, of Alzheimer's subjects. The capacity of zinc to protect against the free radical generating action of "decompartmentalised" iron[32] may be of particular relevance to the finding of iron within senile plaque cores[33] and the capacity of aluminium ions to enhance iron-mediated membrane lipid peroxidative alterations.[34]

The role of calcium in aging neural tissue[35] and in the regulatory control of cellular functions including cytoskeletal changes and PMN activation has also been extensively investigated.[36] Of special relevance are the increased cellular calcium levels found in cells from Alzheimer's donors[37] and the action of calcium in catalysing the transglutaminase-dependent crosslinking of rigid and insoluble neurofilament polymers.[38] The enhanced activation of PMN by the calcium forms of zeolites shows that aluminosilicates act as a special kind of calcium ionophore, comparable with the antibiotic ionomycin which primes PMN stimulatory activity.[39]

We propose therefore that aluminosilicates deposit in the brain plaques as insoluble fibrillar mineral-type particles of such chemical composition, morphology and dimensions as to mimic the toxic effect as demonstrated by the model fibrous zeolite particulates examined. The consequent cellular and catalytic production of injurious phagocyte-derived free radical oxidant species may provide a significant aetiological mechanism in the pathogenesis of Alzheimer's disease.

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