Possible role of deficiency of selenium and vitamin E in atherosclerosis

Ellis et al\(^1\) draw attention to the enigma of the association of selenium and vitamin E deficiency with coronary artery disease. One theoretical explanation is provided by the hypothesis that monocyte-derived macrophages in the early plaque are causing damage by oxidising the lipids they contain.\(^2\) This springs from the observation that macrophage like cells in even the earliest human plaques contain ceroid, the production of which might well be preceded by release of diffusible oxidised lipids that are cytotoxic.\(^3\) Furthermore, the insoluble ceroid appears to be laid down around the periphery of soluble lipid droplets in membrane bound vesicles and persists in the necrotic base of advanced plaques.\(^4\)** The central, soluble lipid may be difficult to disperse in vivo because of this skin of ceroid. This activity of the macrophage’s microbicidal oxidative systems might prove an important, or even essential, stage in the development of the plaque. Deficiency of antioxidants or components of antioxidants would therefore accelerate the disease.

Further work to test this hypothesis is in progress in this laboratory.

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Localisation of aluminium and iron by histochemical and laser microprobe mass analytical techniques in bone marrow cells of chronic haemodialysis patients

We wish to corroborate the observations of Dr Kaye,\(^7\) who noticed a positive aluminium staining\(^8\) in the bone marrow cells of some patients with end stage renal disease. The positive staining in the bone marrow cells is putatively regarded as caused by aluminium storage in unidentified cells, possibly of the macrophage system. Iron stain, however, was negative. This is surprising, since by Prussian blue staining for iron heavy iron loading is often found in bone marrow macrophages of chronic haemodialysis patients.\(^9\)

We studied transilic bone biopsies (obtained with a 7 mm diameter trephine)\(^10\) of three chronic haemodialysis patients (A, B, and C), who showed aluminium induced osteomalacia.\(^11\) Patients A and B showed considerable bone marrow iron storage, in contrast to patient C. Bone aluminium and iron concentrations (microgram of the element per gram of bone, wet weight) were determined using electrophoretic atomic absorption spectrometry, as described elsewhere.\(^12\) Respective bone aluminium concentrations were 25.2 µg/g, 63.1 µg/g, and 83.6 µg/g, and the corresponding iron concentrations were 736 µg/g, 386 µg/g, and 64 µg/g. For histological examination the bone specimens were fixed in Burkhardts’ solution for 24 h\(^13\) and subsequently transferred to absolute methanol. The specimens were embedded in methyl methacrylate and sections were cut from the undecalcified biopsies with a Jung K sledge microtome. Eight micrometer thick sections were stained by means of Goldner’s method\(^14\) for qualitative histology, and 2 µm sections were alumnum stained.\(^15\)

Patient A showed very few bone marrow cells with positive aluminium staining, while in the bone marrow of patient C numerous bright red cells were seen. The number of aluminium stained cells in patient B was intermediate between those of patients A and C. Histologically, these cells appeared to be macrophage in type, as were those reported by Dr Kaye.\(^7\)

In order to verify the results of the aluminium staining, laser microprobe mass analysis (LAMMA)* was performed on the alumnum stained sections. With LAMMA, which provides multi-element mass spectra at the ultrastructural level,\(^*\) not only iron but also aluminun was detected in many of the histochemically aluminon negative bone marrow cells of patients A and B (Figure). On the other hand the mass spectra of the aluminon positive bone marrow cells of patient C showed aluminon and no appreciable iron signal. According to the LAMMA spectra, the amounts of aluminon in the histochemically aluminon negative cells of patients A and B were of the same order of magnitude as those in the aluminon positive cells of patient C.

Our findings are in conflict with the supposition of Dr Kaye\(^7\) that aluminon staining is unable to demonstrate aluminon in bone marrow cells in methyl methacrylate embedded sections. That aluminon positive marrow cells had not been previously reported might have been due to the different fixation procedures used by other authors.

The aluminon staining of undecalcified bone sections shows the presence of aluminon with high specificity. Positive histochemical staining with aluminon at the osteoid/calcified bone boundary has been confirmed with several microanalytical methods.*\(^16\) Nevertheless, LAMMA appears to demonstrate aluminon in bone marrow cells, which contain much iron and are histochemically aluminon negative. The LAMMA results presented here indicate that aluminon and iron may be stored concomitantly in bone marrow cells of chronic haemodialysis patients. This observation provides additional support to the assumption of Dr Kaye that the aluminon storing bone marrow cells are indeed macrophages. The concurrent storage of iron and aluminon in bone marrow macrophages is a striking analogy to the accumulation of both elements in hepatocyte and Kupffer cell lysosomes of patients with iron and aluminon overload\(^*\) and to their concomitant occurrence at the osteoid/calcified bone boundary.\(^*\)

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References


Laser microprobe mass spectrum originating from one of the aluminum negative bone marrow macrophages of patient A. The brown colour of these cells suggested considerable iron storage.


Dr Kaye comments as follows: The confirmation by Dr Van de Vyver and co-workers that aluminium can be demonstrated in marrow cells using the aluminium stain and verified using a microprobe analytical technique is gratifying. The combined storage of iron and aluminium is not unexpected, although it was not seen in our material. This is probably because our patients tend to be kept mildly iron deficient and marrow iron stores are usually meagre. One of our patients with clinically severe aluminium bone disease was treated with desferoxamine for 8 months, after which a repeat bone biopsy showed disappearance of trabecular aluminium staining but persistence in the marrow, implying different chemical reactivity of aluminium in the two sites.

I have not systematically looked at the reason(s) for the apparently less satisfactory staining in methyl methacrylate or epon embedded material and this could be due to fixation or other factors. Whatever the explanation it is further evidence of a difference between the reactivity of the marrow and mineral aluminium deposits. The authors observations are very interesting and amplify the results previously reported.

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Acute myeloid leukaemia in a patient with congenital antithrombin III deficiency

Congenital antithrombin III deficiency is well recognised as a rare cause of venous thrombosis. The occurrence of acute myeloid leukaemia in a patient with congenital antithrombin III deficiency has not previously been reported. A 45 year old woman presented in July 1982 with a five week history of tiredness, spontaneous bruising, and gingival swelling. She had a history of postoperative venous thrombosis on two occasions and a striking family history of thrombotic disease: her father and paternal uncle had had major thrombotic episodes while in their twenties. The maternal side of the family was unaffected.

At presentation fever (39°C), pallor, and widespread bruising were noted, but no lymphadenopathy, hepatosplenomegaly, or sternal tenderness was found. Haemoglobin concentration was 10-0 g/dl, white cell count 5.5 × 10⁹/l, and platelet count 290.0 × 10⁹/l. Blood film showed 95% blast cells. Histology and cytochemistry of a marrow aspirate led to a diagnosis of acute myeloid leukaemia (M1).

Chemotherapy, comprising daunorubicin, cytosine arabinoside, and thioguanine (DAT) was started. After the first pulse of chemotherapy, blast cells were no longer apparent in the peripheral blood and progression was uneventful. Eighteen days after admission sudden severe pleuritic chest pain developed, and there was pain in the right calf. Electrocardiogram was normal, but chest x ray examination showed opacification of left upper and right lower zones, an elevated right hemidiaphragm, and a small right sided pleural effusion. Pulmonary embolism was diagnosed, although the patient was unfit for isotope scan. There was no evidence of disseminated intravascular coagulation on baseline coagulation screening. Heparin by continuous intravenous infusion was cautiously instituted in a dosage of 10 000 IU every 12 h in view of the pronounced thrombocytopenia, and monitored according to the partial thromboplastin time with kaolin. Platelet concentrates were given every 12 h throughout this period.

Despite these measures the patient developed recurrent chest pain and haemoptysis with subsequent hypotension and acute renal failure, and she died 23 days after admission. Necropsy revealed multiple small and medium sized pulmonary emboli, bilateral pulmonary infarcts, and multiple thrombi in the calf areas of the right leg.

An amidolytic antithrombin III assay performed on a blood sample taken before heparinisation using a commercially available chromogenic substrate (Kabi S 2238) showed a substantial reduction in antithrombin III at 43% (normal range 80-115%). Samples taken later from the patient's mother showed normal antithrombin III levels (90%). No other relatives were available for study.

Acquired antithrombin III deficiency can occur as a result of disseminated intravascular coagulation in a number of conditions.²³ The absence of disseminated intravascular coagulation in this case together with the striking past and family history of thrombosis strongly support a diagnosis of congenital antithrombin III
Localisation of aluminium and iron by histochemical and laser microprobe mass analytical techniques in bone marrow cells of chronic hemodialysis patients.

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