Bone marrow aluminium storage in renal failure

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SUMMARY Using the staining method for aluminium with the ammonium salt of aurine tricarboxylic acid, aluminon, 18 patients with end stage renal disease gave positive reactions in iliac crest bone biopsies and 11 of these had positive staining in the bone marrow. In one the marrow was positive and the bone negative. The marrow reaction is putatively regarded as caused by aluminium storage in unidentified cells, possibly of the macrophage system which are strongly fluorescent when examined after prior tetracycline labelling. Marrow storage should be considered when assessing the bone aluminium burden.

The application by Buchanan *et al*¹ of a specific staining technique for aluminium to bone sections from patients with renal failure has led to widespread use of this method for detection of aluminium storage in bone.^{2,3} None of these authors has noted the deposition of aluminium except in the bone itself, usually at the osteoid and mineral interface or less frequently along cement lines. While staining biopsy material for aluminium from patients with end stage renal disease we were surprised to find evidence of aluminium positive material present in the bone marrow and quite separate from the trabecular surfaces. This paper describes these findings.

Material and methods

Twenty-eight patients were studied for diagnostic purposes: nine had severe chronic renal failure and the remainder were on long term haemodialysis. Most patients were labelled with tetracycline prior to biopsy using a 2 days tetracycline—12 days off—4 days tetracycline schedule with the biopsy 2 days following the second label. Vertical trephine bone biopsies were taken from the anterior iliac crest and fixed in 1:1 10% buffered formalin phosphate and 0.5% sucrose solution for 2 h.⁴ They were then dehydrated in propanol and embedded in glycol methacrylate.⁵ The entire procedure was carried out at 4°C. Blocks prepared prior to 1981 in either Epon or methyl methacrylate were also used to examine aluminium staining. Sections ($\geq 2 \mu\text{m}$) were cut using a Porter-Blum MT2 microtome and glass

knives. Mean total section area was $2.0 \pm 0.1 \text{ cm}^2$ ($n = 10$). Sections were stained for acid phosphatase⁶ and toluidine blue, iron and Giemsa using standard techniques. Five per cent desferoxamine in either saline or phosphate buffer was applied directly to the sections for 30 min at 37°C as was 1% acetic acid or 10% EDTA, the latter at room temperature. Aluminium staining² was done on $8 \mu\text{m}$ thick sections without counterstain for recognition of bone or marrow aluminium. Fluorescence was looked for using unstained section 2-8 μm in thickness. Reactivity for non-specific esterase was examined in embedded tissue within two weeks

Fig. 1 Lack of close relation between bone and marrow staining for aluminium.

Fig. 2 Original magnification $\times 1000$, $2\ \mu\text{m}$ sections.
 (a) Aluminium stain showing positive areas in the marrow. The granular structures are stained bright red;
 (b) fluorescent picture of the same section. The patient had received tetracycline according to the protocol;
 (c) toluidine blue stain of the next section showing the coarse granules.

Effect of section pretreatment on subsequent aluminium staining in a representative patient

Pretreatment	Aluminium staining	
	Marrow	Bone
None	+++	++++
EDTA	—	—
1% acetic acid	++	—
Desferoxamine	—	+++
In phosphate buffer pH 7.2	—	++
In saline pH 4.9	+++	++++
Saline or buffer alone	+++	++++

of procurement using previously described techniques.^{5,7}

Results

The majority of biopsies with aluminium staining areas in the bone also showed positive areas in the marrow (Fig. 1). These appeared as bright red areas and corresponded to marrow cells (Fig. 2a). Of the 28 patients, in 18 the bone stained positively for aluminium and 11 of these had marrow staining. In one patient the bone was negative and the marrow positive and one patient had strongly positive marrow and only a small amount in bone. In two others the bone was strongly positive and the marrow negative. With Giemsa and toluidine blue staining these cells appeared to be macrophage in type with coarse granules in the cytoplasm (Fig. 2c). Iron stain was negative. The effect of pretreatment of the sections with different reagents is shown in the Table for a representative patient who showed strongly positive staining in both bone and marrow. Desferoxamine removed all staining from the marrow cells but had much less effect on bone, acetic acid did the reverse and EDTA abolished the staining in both areas. Examination of unstained sections for fluorescence showed that aluminium positive cells gave a strong yellow fluorescence from granules in the cytoplasm (Fig. 2b). However in patients who had not been labelled with tetracycline prior to biopsy, fluorescence was absent, although a non-specific less intense fluorescence was sporadically seen in the marrow unrelated to aluminium reactivity. Positive aluminium staining was never found in osteoblasts. Osteoclasts, whether identified by routine staining or by the more sensitive acid phosphatase reaction were also negative.

Discussion

The staining reaction for aluminium appears to be quite specific and no false positives have been reported. This is in agreement with our own experience in more than 70 biopsies where the stain has

been tested, 13 of them from normal subjects. Furthermore close correlation exists between direct chemical determination of aluminium and the extent of staining² and as well the localisation of the reaction has been confirmed as associated with aluminium using more sophisticated techniques.⁸ These considerations make it likely that the aluminium positive cells are indeed due to aluminium storage. The affinity of this material for desferoxamine in the absence of iron supports this conclusion as does the binding to EDTA. However, the clear difference between the chemical reactivity of the bone and marrow aluminium suggests that the metal is in a different form in the two sites. In the bone this would presumably be associated with mineral whereas in the cells, packaging with protein might partially protect it from dissolution with dilute acetic acid. While some relationship exists between bone and marrow storage this is not exact and some patients have positive bone staining and none in the marrow and the reverse (Fig. 1). If the bone aluminium is associated with the process of calcification then it would not be expected that the marrow storage would be closely related. The latter, if indeed involving cells of the macrophage system, would then be accumulating aluminium as part of a foreign body (? colloidal) storage similar to that described for silicon.⁹ The cell type involved in aluminium storage has not been positively identified. The negative esterase reaction would suggest these are not monocytic type cells, however their staining characteristics could be altered by the aluminium accumulation.

To our knowledge, except for a brief report by Ackrill *et al* using an unpublished technique for characterising aluminium with Solachrome,¹⁰ there have been no previous descriptions of marrow aluminium accumulation. This is probably due to the poor marrow staining with the aluminium reagent in Epon and methyl methacrylate, which has to date prevented us from examining these cells by electron microscopy. However, Maloney *et al* in Fig. 5 of their paper² show a fluorescence photograph with multiple areas in the marrow which we suspect are sites of aluminium storage.

The significance of these observations will require further study but it is suggested that in assessing aluminium storage in bone the content of both marrow and the bone itself should be assessed.

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