Proc EDTA–ERA (1985) Vol 22

IS UNRESPONSIVENESS OF ALUMINIUM-INDUCED OSTEOMALACIA DUE TO CELLULAR $1,25(OH)_2D_3$ RESISTANCE?

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Summary

Severe aluminium-induced osteomalacia is refractory to treatment with $1,25(OH)_2D_3$. This has been ascribed to diminished bone surface cell density and/or cytotoxic damage to bone cells. To explore the additional possibility of altered $1,25(OH)_2D_3$ receptor status, $1,25(OH)_2D_3$ receptors were studied in the intestinal mucosa of rats loaded with aluminium for short (3 weeks) and long periods (16 weeks). With both protocols increased binding capacity (N_{max}) with no change of equilibrium dissociation constant (K_D) was noted. It is concluded that aluminium status modulates $1,25(OH)_2D_3$ receptor expression, but this cannot explain resistance to therapy with $1,25(OH)_2D_3$.

Introduction

In some patients on maintenance haemodialysis, vitamin D-resistant osteomalacia has been described [1] which was subsequently shown to be due to aluminium toxicity [2]. When $1,25(OH)_2D_3$ is administered, such patients tend to develop hypercalcaemia. This is usually ascribed to stimulation of intestinal calcium absorption in patients who are unable to translocate calcium to the skeleton. The latter finding may be related to low rates of bone apposition [3] which result from metal-induced damage to osteoblasts [4] and bone hypocellularity. In the present study we examined the alternative or complementary hypothesis that aluminium changes bone cell $1,25(OH)_2D_3$ receptor status. Since bone cells are not readily available for binding studies, a well-defined $1,25(OH)_2D_3$ receptor-bearing target cells, i.e. intestinal mucosa, epithelial cells, were chosen for the experiment.

Material and methods

Three groups of normal male Wistar rats (initial weight 379±16g) were allowed free access to deionized distilled water and standard Altromin 1000 diet (Altromin Co,

Lage, Lippe). With the use of random numbers, animals were allocated to one of three groups: control group, sham injection group and aluminium injection group. Two different time courses were studied. In the first series, the total duration of the experiment was three weeks. In the second the duration was 16 weeks. In the three week experiment, animals received daily intra-peritoneal $AlCl_3$, the daily dose rising incrementally from 0.27 to 2.7mg, yielding a total dose of 38mg per animal. In the 16 week experiment, intra-peritoneal injections were administered on five days per week, the dose rising incrementally from 0.27 to 2.7mg, yielding a total dose of 192mg per animal.

At the end of the experiment, the intestine was removed, rinsed in four volumes of hypertonic buffer (0.3M KCl, 10mM Tris-HCl, 1.5mM EDTA, 1mM dithiothreitol; ph 7.4, 4°C) and homogenized (Polytron homogenizer; Janke Co, Stauffen). Protein was determined by the method of Lowry. Sucrose density gradient analysis and saturation analysis after Scatchard were performed as described previously [5].

Results

Sucrose density gradient analysis shows (Figure 1) binding of $1,25(OH)_2D_3$ to a 3.5S macromolecule in the nuclear fractions of intestinal mucosa of both control rats injected with solvent (left) and of experimental rats injected with aluminium for 16 weeks (middle). Svedberg constant (S) was estimated from two co-migrating [^{14}C] labelled reference proteins, bovine gammaglobulin (7.3S) and ovalbumin (3.7S). Binding of 1nM [^{3}H] $1,25(OH)_2D_3$ to the 3.5S macromolecule was specific, since it was reversed by a hundred-fold molar excess of non-labelled $1,25(OH)_2D_3$.

As shown in the middle panel, more [^{3}H] $1,25(OH)_2D_3$ was bound to the 3.5S macromolecule fraction of intestinal mucosa of rats given aluminium for 16 weeks. Such semi-quantitative assessment of binding was further substantiated by Scatchard analysis which showed higher maximal binding capacity (aluminium animals: N_{max} 30±2.7fmol/mg protein; solvent animals: 22±3.1) with no change of equilibrium dissociation constant K_D (aluminium animals 3.1± 0.4 10^{-10}M; solvent animals 2.9±0.2) in aluminium treated animals.

With sucrose density gradient analysis and Scatchard binding analysis analogous changes were found in animals with short-term (3 weeks) administration of aluminium.

Discussion

The present study clearly demonstrates modifications of $1,25(OH)_2D_3$ receptor status in the intestinal mucosa cells of animals with subchronic and chronic aluminium intoxication. The extent to which intestinal mucosa cells are representative for bone cells is unknown. The direction of change, i.e. increased binding capacity, is such that it would not explain resistance of aluminium-induced bone disease to administration of vitamin D_3 metabolites.

Further studies are required to define the mechanism by which binding

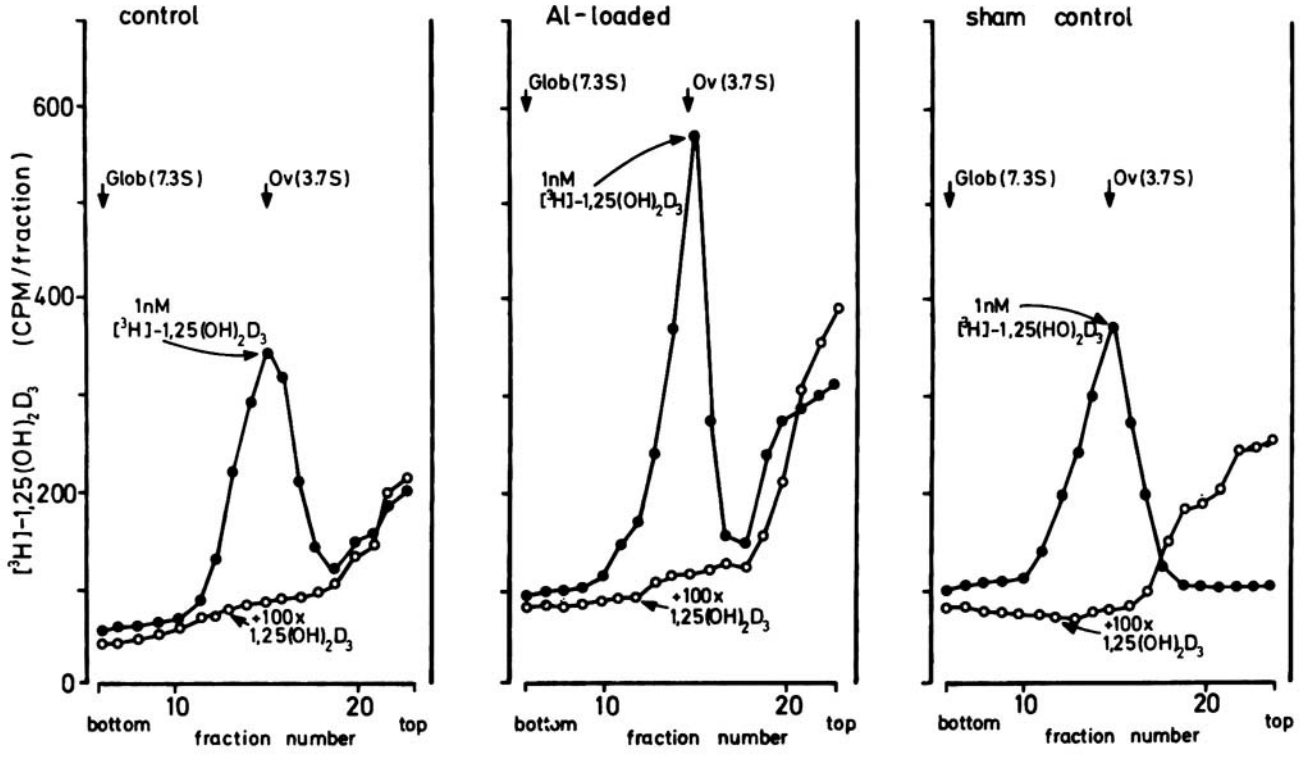

Figure 1. Sucrose density gradient analysis. Nuclear fractions of intestinal mucosa of solvent injected control rats (left panel) and $AlCl_3$ injected (16 weeks) experimental animals (middle panel). Migration of ^{14}C labelled reference proteins (bovine gammaglobulin, ovalbumin) indicated by arrows. •—• binding of [^{3}H] 1,25(OH)$_2$D$_3$ (1nM); ○—○ binding of [^{3}H] 1,25(OH)$_2$D$_3$ (1nM) in the presence of 100-fold molar excess of non-labelled 1,25(OH)$_2$D$_3$

capacity is modulated in aluminium intoxication. It appears to be of note that circulating $1,25(OH)_2D_3$ concentrations are low in patients with aluminium intoxication and in aluminium intoxicated animals [6]. It is conceivable that increased binding capacity reflects up-regulation of receptors in response to low circulating levels of the agonist.

Acknowledgment

With the support of Deutsche Forschungsgemeinschaft (Me 632/3).

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