The Effect of High Parathyroid Hormone Levels on the Development of Aluminum-Induced Osteomalacia in the Rat

Arnold J. Felsenfeld, M.D., Lorraine Machado, B.A., and Mariano Rodriguez, M.D.

A.J. Felsenfeld, L. Machado, M. Rodriguez.
Department of Medicine, Wadsworth Veterans Administration and University of California, Los Angeles, Medical Centers, Los Angeles, CA
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ABSTRACT
A relative deficiency of parathyroid hormone (PTH) is generally observed in dialysis patients with aluminum-associated osteomalacia or aplastic bone disease. It has been suggested that high PTH levels may protect against the development of aluminum-associated bone disease. Through the use of a previously established model of aluminum-induced osteomalacia in the rat, the protective effect of PTH was evaluated. Aluminum was administered intraperitoneally at doses of 0, 5, 10, and 20 mg during a 2-day period, and rats were sacrificed 5 and 12 days after aluminum administration. PTH (bovine 1-34) was administered via a subcutaneously implanted Alzet pump at 2 U/h starting 4 days before aluminum administration and continuing until sacrifice. As the aluminum dose was increased to 20 mg, the osteoblast surface and the bone formation rate decreased. PTH supplementation increased the osteoblast surface at all doses of aluminum and increased the bone formation rate at 0 and 5 mg of aluminum. However, even with PTH supplementation, osteoblast surface decreased as the aluminum dose increased. In the absence of PTH supplementation, osteoblast surface was markedly reduced when the serum aluminum concentration was greater than 400 μg/liter or stainable trabecular aluminum surface exceeded 15%. When the stainable trabecular aluminum surface was greater than 12%, the bone formation rate was zero even during supplemental PTH administration. A significant correlation was observed between serum aluminum and stainable trabecular aluminum surface (r = 0.80 at 5 days and r = 0.86 at 12 days; P < 0.001). However, after PTH administration, less stainable trabecular aluminum was present for the same serum aluminum concentration. Both with and without PTH, the slope of the correlation between serum aluminum and stainable trabecular aluminum surface was steeper at 5 days after aluminum administration than at 12 days. In conclusion, for an equivalent aluminum exposure, high PTH levels protected against the development of low-turnover aluminum bone disease in the rat.

Key Words: Alzet miniosmotic pump, osteoblast surface, bone formation rate, osteoid, aluminum

A relative deficiency of parathyroid hormone (PTH) is a frequent finding in dialysis patients with aluminum-associated osteomalacia or aluminum-associated aplastic bone disease (1–4). The PTH response to hypocalcemia is also reduced in these patients (5,6). In addition, in patients with osteitis fibrosa, a marked reduction of PTH levels as a result of parathyroidectomy may predispose them to the development of aluminum-associated osteomalacia (7) or aluminum accumulation on the trabecular bone surface (8,9). Thus, it has been suggested that high levels of PTH may protect against the development of low-turnover aluminum-associated bone disease (both osteomalacia and aplastic bone disease) in dialysis patients.

The authors of this report and others have shown that aluminum administration in the rat with renal failure produces osteomalacia (10–12). The development of aluminum-induced osteomalacia in the rat is associated with a decreased osteoblast surface (11). At the same time, we have shown that during aluminum administration, the simultaneous administration of PTH markedly increased the osteoblast surface but did not correct the mineralization defect (13). However, at lower aluminum doses, sufficient to produce osteomalacia, PTH administration may protect against the aluminum-induced impairment of mineralization. Thus, in this study, the purpose was to evaluate if PTH protects against the development of aluminum-induced osteomalacia. To accom-
to accomplish this goal, PTH was infused in rats receiving a range of aluminum doses.

METHODS

Pair-fed male Wistar rats weighing 300 to 340 g were studied. In all rats, renal failure was surgically induced by a % nephrectomy in two stages. First, ligation of two main arteries in the left kidney was performed, followed 1 week later by a total right nephrectomy. Rats were randomly assigned to groups receiving 0, 5, 10, or 20 mg of aluminum or the same dose of aluminum plus PTH. Aluminum was administered intraperitoneally in divided doses during a 2-day period and was begun 9 days after the right nephrectomy. In a previous study, we showed that aluminum administered intraperitoneally in divided doses during a 2-day period produced osteomalacia (14). Bovine 1-34 PTH (Peninsula Laboratories Inc., Belmont, CA) was administered at 2 U/h via a subcutaneously implanted Alzet pump. The PTH administration was started 4 days before the aluminum administration and continued until sacrifice. Rats were sacrificed at either 5 or 12 days after aluminum administration. Rats sacrificed at 12 days after aluminum administration received double tetracycline labeling. Tetracycline (20 mg) was administered intraperitoneally on days 2 and 8 before sacrifice. On the day of sacrifice, rats were anesthetized with 50 mg of Nembutal given intraperitoneally and sacrifice was then accomplished with intracardiac puncture and exsanguination. Blood obtained at sacrifice was used to measure serum calcium, phosphorus, creatinine, and aluminum.

After exsanguination, the ilium was detached from the skeleton, placed in 70% ethanol, and processed as described previously (11). Five-micron Goldner-stained sections were examined for osteoblasts, osteoclasts, osteoid, and fibrosis; five-micron sections were stained for aluminum by the method of Maloney et al. (15). Fifteen-micron unstained sections of cancellous bone were analyzed for tetracycline labels. Quantification was performed at a magnification of \( \times 500 \) with a Merz-Schenk reticle (11).

The categories listed below for the measurements of cancellous bone conform with the nomenclature recommended by the American Society for Bone and Mineral Research (16): (1) osteoblast surface, the fraction of trabecular surface covered by osteoblasts, (2) osteoid surface, the fraction of trabecular surface covered by osteoid, (3) osteoid volume, the fraction of trabecular bone volume occupied by osteoid, (4) osteoid thickness as measured directly with an eyepiece reticle, (5) osteoclast surface, the fraction of trabecular surface covered by osteoclasts, (6) osteoclast number, the number of osteoclasts per square millimeter of cancellous bone, (7) fibrosis volume, the fraction of cancellous bone volume occupied by endosteal fibrosis, (8) aluminum surface, the fraction of trabecular bone surface covered by aluminum, (9) the bone formation rate as obtained by multiplying the fraction of double-labeled tetracycline surface times the mineral apposition rate. (The latter is obtained by measuring the distance between tetracycline labels and dividing by the number of days between doses of tetracycline.), and (10) the adjusted apposition rate as obtained by the following formula: fraction of double tetracycline-labeled trabecular surface times the mineral apposition rate divided by the fraction of osteoid surface.

Serum calcium was measured by atomic absorption (model 5300, The Perkin-Elmer Corp., Norwalk, CT). Serum creatinine was measured by using a specific analyzer for creatinine (Creatinine Analyzer 2; Beckman Instruments, Inc., Fullerton, CA). Aluminum was measured by flameless atomic absorption with a graphite furnace (17). Serum phosphorus was measured by a specific kit (Fast Phosphorus Test Set; Stanbio, San Antonio, TX).

Statistical Analysis

For comparisons between experimental and control groups, the unpaired \( t \) test was used. For comparisons of more than two groups, ANOVA and the Duncan's range test were used. Correlations between two parameters were analyzed by regression analysis. Significance was achieved when the \( P \) value was less than 0.05. Values are expressed as the mean ± SE.

RESULTS

The biochemical data are shown in Table 1. At 5 and 12 days, the rats receiving PTH had higher serum calcium levels and a tendency toward lower serum phosphorus levels. The serum creatinine was not different between groups at 5 days; at 12 days, the serum creatinine was minimally increased in the chronic renal failure (CRF) + PTH group. Weight gain, not shown, was not significantly different among the four groups at 5 days and at 12 days.

The bone histology data are shown in Table 2. Aluminum administration decreased the osteoblast surface, and PTH supplementation increased the osteoblast surface. However, even during PTH administration, aluminum reduced the osteoblast surface (CRF + PTH versus CRF + PTH + AL). Aluminum administration increased the osteoid surface at 12 days (CRF versus CRF + AL), and PTH supplementation increased the osteoid surface at both 5 and 12 days (CRF versus CRF + PTH). Osteoid volume was increased by aluminum administration at 12 days (CRF versus CRF + AL). The osteoid thickness, which depends on the rate of matrix deposition (osteoblasts), versus the rate of matrix removal (bone for-
mation) was increased by both aluminum and PTH administration (CRF versus CRF + AL and CRF versus CRF + PTH) at both 5 and 12 days. However, at 12 days, aluminum administration did not significantly increase the osteoid thickness in the PTH-supplemented rats (CRF + PTH versus CRF + PTH + AL) because the combination of PTH and aluminum decreased the osteoblast surface and bone formation rate proportionally. Aluminum administration decreased the osteoclast surface and osteoclast number at 12 days (CRF versus CRF + AL). Fibrosis was observed only in groups receiving supplemental PTH.

As shown in Figure 1, when the groups were stratified for the dose of aluminum, PTH supplementation increased the osteoblast surface at all doses. In the absence of PTH supplementation, a multiple comparison analysis was performed by the Duncan's range test and a significant decrease in the osteoblast surface was observed at both 5 and 12 days as the dose of aluminum was increased from 0 to 20 mg (5 days) and from 0 to 10 mg (12 days). Although PTH supplementation increased the osteoblast surface, a significant decrease in osteoblast surface was observed in PTH-supplemented rats at 5 and 12 days as the dose of aluminum was increased from 0 to 10 mg (5 days) and from 0 to 20 mg (12 days).

When the groups were stratified for the dose of aluminum, the osteoid thickness increased during PTH supplementation at 0 and 20 mg of aluminum at both 5 and 12 days and at 10 mg of aluminum at 12 days (Figure 2). At 5 mg of aluminum, no differences were observed.

The bone formation rate and the adjusted apposition rate for each dose of aluminum administered are shown in Figure 3. Since time-spaced tetracycline labels could only be given to rats sacrificed at 12 days, bone formation rates and adjusted apposition rates are only available at 12 days. Supplemental PTH increased the bone formation rate when no aluminum or 5 mg of aluminum was given. However, no differences were observed in the adjusted apposition rate. When the bone formation rate was plotted

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**TABLE 1. Biochemical data**

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<thead>
<tr>
<th>Groups</th>
<th>Concentrations in Serum (mg/dL)</th>
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<tr>
<td></td>
<td>Calcium</td>
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<tr>
<td>5 days</td>
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<tr>
<td>CRF (N = 7)</td>
<td>10.5 ± 0.2</td>
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<tr>
<td>CRF + AL (N = 23)</td>
<td>10.5 ± 0.1</td>
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<tr>
<td>CRF + PTH (N = 5)</td>
<td>12.9 ± 0.5&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>CRF + PTH + AL (N = 21)</td>
<td>11.9 ± 0.3&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>12 Days</td>
<td></td>
</tr>
<tr>
<td>CRF (N = 7)</td>
<td>10.4 ± 0.1</td>
</tr>
<tr>
<td>CRF + AL (N = 23)</td>
<td>10.5 ± 0.2</td>
</tr>
<tr>
<td>CRF + PTH (N = 7)</td>
<td>11.3 ± 0.5</td>
</tr>
<tr>
<td>CRF + PTH + AL (N = 26)</td>
<td>11.2 ± 0.2&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Values are mean ± SE.
<sup>b</sup> p < 0.05 versus CRF.
<sup>c</sup> p < 0.05 versus CRF + AL.
<sup>d</sup> p < 0.05 versus CRF + PTH.

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**TABLE 2. Bone histology**

<table>
<thead>
<tr>
<th>Group</th>
<th>Osteoblast Surface (%)</th>
<th>Osteoblast Surface (%)</th>
<th>Osteoid Volume (%)</th>
<th>Osteoid Thickness (micron)</th>
<th>Osteoclast Surface (%)</th>
<th>No. of Osteoclasts</th>
<th>Fibrosis Volume (%)</th>
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<td>5 Days</td>
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<td></td>
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<tr>
<td>CRF (N = 7)</td>
<td>23 ± 2</td>
<td>27 ± 2</td>
<td>3.7 ± 0.6</td>
<td>3.9 ± 0.3</td>
<td>10 ± 1</td>
<td>5.0 ± 0.5</td>
<td>0</td>
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<tr>
<td>CRF + AL (N = 23)</td>
<td>10 ± 2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20 ± 3</td>
<td>3.7 ± 0.5</td>
<td>5.3 ± 0.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10 ± 1</td>
<td>4.6 ± 0.5</td>
<td>0</td>
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<tr>
<td>CRF + PTH (N = 5)</td>
<td>53 ± 5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>55 ± 5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14 ± 3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.8 ± 0.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15 ± 2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.4 ± 0.7</td>
<td>3 ± 1</td>
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<tr>
<td>CRF + PTH + AL (N = 21)</td>
<td>36 ± 3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>44 ± 4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>14 ± 3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.7 ± 0.9&lt;sup&gt;d&lt;/sup&gt;</td>
<td>10 ± 1&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.1 ± 0.3</td>
<td>4 ± 2</td>
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<td>12 Days</td>
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<tr>
<td>CRF (N = 7)</td>
<td>21 ± 2</td>
<td>24 ± 2</td>
<td>3.7 ± 0.7</td>
<td>4.4 ± 0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11 ± 1</td>
<td>4.1 ± 0.3</td>
<td>0</td>
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<td>CRF + AL (N = 20)</td>
<td>10 ± 2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>37 ± 5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14 ± 2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.8 ± 0.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6 ± 1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.0 ± 0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0</td>
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<tr>
<td>CRF + PTH (N = 7)</td>
<td>48 ± 7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>62 ± 7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>27 ± 7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.4 ± 4.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8 ± 2</td>
<td>3.2 ± 0.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4 ± 2</td>
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<tr>
<td>CRF + PTH + AL (N = 26)</td>
<td>29 ± 4&lt;sup&gt;c,d&lt;/sup&gt;</td>
<td>54 ± 4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>27 ± 3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>18 ± 1.8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8 ± 1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.5 ± 0.4</td>
<td>2 ± 1</td>
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</table>

<sup>a</sup> Values are mean ± SE.
<sup>b</sup> p < 0.05 versus CRF.
<sup>c</sup> p < 0.05 versus CRF + AL.
<sup>d</sup> p < 0.05 versus CRF + PTH.
against stainable trabecular aluminum surface (Figure 4), no bone formation was observed if the stainable trabecular aluminum surface exceeded 12%. The exogenous infusion of PTH during aluminum administration did not alter this finding.

In Figure 5, the relationship between osteoblast surface and both the serum aluminum and stainable trabecular aluminum surface at 5 days is presented. Once the serum aluminum (Figure 5A) exceeded 400 μg/liter or the stainable trabecular aluminum surface (Figure 5B) exceeded 10%, osteoblast surface fell to very low values unless PTH was given. The infusion of PTH during aluminum administration maintained the osteoblast surface at higher levels despite the serum aluminum exceeding 400 μg/liter and the stainable trabecular aluminum surface exceeding 10%. At 12 days (Figure 6), when the serum aluminum was greater than 300 μg/liter (Figure 6A) or when the stainable trabecular aluminum surface was greater than 15% (Figure 6B), osteoblast surface was less than 10%. The infusion of PTH during aluminum administration tended to maintain osteoblast surface despite increasing serum aluminum and stainable trabecular aluminum surface.

PTH supplementation during aluminum administration changed the relationship between serum aluminum and stainable trabecular aluminum surface at both 5 and 12 days (Figure 7). At 5 days (Figure 7A), without PTH supplementation, the correlation between serum aluminum and stainable trabecular
Figure 3. The bone formation rate (A) and adjusted apposition rate (B) are shown at four different doses of aluminum (0, 5, 10, and 20 mg) with and without PTH supplementation. These results were obtained 12 days after aluminum administration. P values shown are the comparison at each dose of aluminum with and without PTH supplementation.

aluminum surface was significant ($r = 0.86, P < 0.001$); similarly, during PTH supplementation, serum aluminum correlated with stainable trabecular aluminum surface ($r = 0.60, P < 0.01$). However, PTH supplementation shifted the relationship; for the same serum aluminum level, stainable trabecular aluminum surface was less during PTH supplementation. At 12 days (Figure 7B), with PTH ($r = 0.94, P < 0.001$) and without PTH ($r = 0.80, P < 0.001$) supplementation, serum aluminum correlated with stainable trabecular aluminum surface. At 12 days, both with and without PTH supplementation, the slope was steeper than that observed at 5 days. Also at 12 days, PTH supplementation shifted the relationship between serum aluminum and stainable trabecular aluminum surface downward. Thus, for any serum aluminum value, stainable trabecular aluminum surface was less with PTH supplementation.

In Table 3, serum and stainable trabecular aluminum surface values are shown at 5 and 12 days for the two groups receiving aluminum (CRF + AL and CRF + PTH + AL). At 5 days, serum aluminum levels were greater in the PTH-supplemented group (CRF + PTH + AL) but, despite higher serum aluminum levels, stainable trabecular aluminum surface was less in the CRF + PTH + AL group. At 12 days, serum aluminum levels were greater in the PTH-supplemented group (CRF + PTH + AL). However, despite higher serum aluminum levels, the stainable trabecular aluminum surface was similar.

**DISCUSSION**

The study in the rat presented here evaluated the effect of increasing the dose of aluminum on the development of osteomalacia and its modification by PTH supplementation. As the administered aluminum dose was increased from 0 to 20 mg, the osteoblast surface and the bone formation rate decreased. PTH supplementation increased the osteoblast surface and increased the bone formation rate both in the absence of aluminum and at low doses of aluminum. However, even with PTH supplementation, the osteoblast surface decreased as the aluminum dose increased. In the absence of PTH supplementation, the osteoblast surface was markedly reduced when the serum aluminum exceeded 400 μg/liter or when the stainable trabecular aluminum surface exceeded 15%. Supplementation with PTH increased the osteoblast surface for comparable levels of serum aluminum and stainable trabecular aluminum surface. A stainable trabecular aluminum surface greater
Figure 5. (A) The relationship between osteoblast surface and serum aluminum is shown 5 days after aluminum administration. When serum aluminum exceeded 400 µg/L, the osteoblast surface fell to low values unless supplemental PTH was administered. PTH administration in the absence of aluminum increased the osteoblast surface. (B) The relationship between osteoblast surface and stainable trabecular bone aluminum surface is shown 5 days after aluminum administration. When stainable trabecular bone aluminum surface exceeded 10%, the osteoblast surface fell to low values unless supplemental PTH was administered. In the absence of aluminum, supplemental PTH administration increased the osteoblast surface.

Figure 6. (A) The relationship between osteoblast surface and serum aluminum is shown 12 days after aluminum administration. When the serum aluminum was greater than 300 µg/L, osteoblast surface was less than 10% unless supplemental PTH was administered. (B) The relationship between osteoblast surface and stainable trabecular bone aluminum surface is shown 12 days after aluminum administration. In the absence of supplemental PTH administration, the osteoblast surface was less than 10% if stainable trabecular bone aluminum surface was greater than 15%.

than 12% markedly reduced the bone formation rate even during PTH supplementation. Finally, a significant correlation was observed between serum aluminum and stainable trabecular aluminum surface,
Figure 7. The correlation between serum aluminum and stainable trabecular bone aluminum surface is shown 5 days (A) and 12 days (B) days after aluminum administration. Both with and without supplemental PTH administration, the correlation between serum aluminum and stainable trabecular bone aluminum surface was significant. Supplemental PTH administration shifted the correlation to the right. Thus, with PTH administration, stainable trabecular bone aluminum surface was less for the same serum aluminum concentration. At 12 days, both with and without PTH supplementation, the slope was steeper than that at 5 days. The dotted and solid lines represent 95% confidence levels.

and, for the same serum aluminum concentration, less stainable trabecular aluminum surface was observed after PTH supplementation.

While we evaluated the effect of PTH supplementation on aluminum toxicity, several animal studies have evaluated the effect of the removal of PTH. In one study, with dogs, parathyroidectomy retarded bone aluminum accumulation; however, in those dogs, both renal function and calcitriol levels were normal (18). Contrary to those results, we found that parathyroidectomy in azotemic rats increased bone aluminum accumulation (19). In another study, cal-
TABLE 3. Serum and bone aluminum

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<th>Aluminum in:</th>
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<tr>
<td></td>
<td>Serum (µg/L)</td>
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<td>Trabecular</td>
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<tr>
<td></td>
<td></td>
<td>Bone (%)</td>
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<td>5 Days</td>
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<td>CRF + AL (N = 23)</td>
<td>417 ± 57</td>
<td>13 ± 2</td>
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<td>CRF + PTH + AL (N = 21)</td>
<td>610 ± 74*</td>
<td>6 ± 2</td>
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<td>12 Days</td>
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<tr>
<td>CRF + AL (N = 20)</td>
<td>258 ± 39</td>
<td>24 ± 4</td>
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<td>CRF + PTH + AL (N = 26)</td>
<td>572 ± 76*</td>
<td>23 ± 4</td>
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* Values are mean ± SE.

P < 0.05 versus CRF + AL (5 days).

P < 0.05 versus CRF + AL (12 days).

citriol supplementation in parathyroidectomized, azotemic dogs reduced bone aluminum accumulation (20). Supplementation with PTH did not decrease the aluminum accumulation; however, only physiologic doses of PTH were used and, as a result, neither hypercalcemia nor increased cellular activity of bone was produced. Thus, these studies do not contradict our findings that high PTH levels decreased bone manifestations produced by aluminum.

Maintenance dialysis patients with aluminum-associated osteomalacia or aluminum-associated aplastic bone disease have a marked decrease in osteoblast surface in addition to a relative deficiency of PTH (1-4). In the study reported here, increasing the dose of aluminum decreased the osteoblast surface. The simultaneous infusion of PTH increased the osteoblast surface at the same aluminum dose; but, at the same time, a progressive decrease in the osteoblast surface was observed in the PTH-supplemented group as the dose of aluminum increased. As has been previously suggested by both in vitro and in vivo studies, aluminum may be directly toxic to the osteoblast (21-24). Because both clinical and in vitro studies have suggested that aluminum may decrease PTH secretion (4,5,14,25,26), it is possible that the effect of aluminum could have been the result of reduced PTH levels. However, the finding of a similar reduction in osteoblast surface during supplemental PTH administration indicated that aluminum was directly toxic to the osteoblast. The other notable finding was that PTH supplementation increased the osteoblast surface at all doses of aluminum (Figure 1). Thus, at both 5 and 12 days, the osteoblast surface in the 20-mg aluminum, PTH-supplemented group was similar to the osteoblast surface in the CRF group receiving no aluminum.

As expected, the groups receiving supplemental PTH had higher serum calcium levels. Despite a similar degree of renal failure, the serum aluminum was higher during PTH supplementation at both 5 and 12 days. Because, for comparable levels of serum aluminum, stainable trabecular aluminum surface was less with PTH supplementation, it is possible that PTH: (1) removed aluminum from the bone surface, (2) accelerated aluminum incorporation into bone, or (3) prevented the deposition of aluminum.

The osteoid thickness was increased at 5 and 12 days as a result of aluminum administration. The osteoid thickness reflects the balance between matrix deposition (osteoblasts) and removal (bone formation). Although the rate of matrix deposition was not measured, the decrease in osteoblast surface during aluminum administration would suggest that aluminum decreased the rate of matrix deposition. In addition, if mineralization stopped at the time of aluminum administration and matrix deposition, which should be the same as the mineral apposition rate of 1.4 micron/day, remained normal, the osteoid thickness should increase by approximately 17 microns by day 12. From Figure 2, it can be ascertained that in the absence of PTH supplementation, the administration of 10 and 20 mg of aluminum resulted in an increase in osteoid thickness of 5.7 and 5.6 microns, respectively. This is less than expected if the rate of matrix deposition remained normal. Similarly, in rats receiving 10 and 20 mg of aluminum plus PTH supplementation, the osteoid thickness increased by 8.6 and 3.7 microns, respectively, which is also less than would be expected with a normal rate of matrix deposition. Thus, assuming that mineralization stopped at day 0, these results suggest that the rate of matrix deposition decreased as a consequence of aluminum deposition.

Another finding was that aluminum administration reduced the osteoclast surface and osteoclast number at 12 days. This may have been because of the reduced number of osteoblasts at 5 days. Previous studies have suggested that the presence of osteoblasts are important for osteoclast recruitment (27,28). However, it is also possible that aluminum administration reduced PTH levels (14,29) and this resulted in the decreased osteoclast surface and number.

When the serum aluminum exceeded 400 µg/liter or the stainable trabecular aluminum surface exceeded 15%, osteoblast surface was markedly reduced. Because serum aluminum correlated with stainable trabecular aluminum surface, it is difficult to know if circulating aluminum or deposition of aluminum on the bone surface was responsible for the decreased osteoblast surface. Aluminum in cell
culture has been shown to reduce osteoblast-like cells (30,31). The supplementation of PTH maintained or increased osteoblast surface even when the serum aluminum exceeded 400 μg/liter or when the stainable trabecular aluminum surface exceeded 15%.

As the aluminum dose was progressively increased to 20 mg, the bone formation rate decreased. PTH supplementation increased the bone formation rate at the tissue level when 0 or 5 mg of aluminum was administered. Thus, in this model of acute aluminum toxicity, high PTH levels provided some protection against the development of aluminum bone disease. Once stainable trabecular aluminum surface exceeded 12% the bone formation rate fell to zero and PTH supplementation did not improve the bone formation rate. In contrast, PTH supplementation maintained or increased the osteoblast surface despite high percentages of stainable trabecular aluminum surface. Thus, from these data, it would appear that aluminum may separately affect the osteoblast and bone mineralization.

At both 5 and 12 days after aluminum administration, serum aluminum correlated with stainable trabecular aluminum surface. However, as compared with that at 5 days, the slope at 12 days was steeper. Thus, for the same serum aluminum concentration, the stainable trabecular aluminum surface was greater at 12 days. As a result of PTH supplementation, the relationship between serum aluminum and stainable trabecular aluminum surface was shifted to the right. Thus, for the same serum aluminum concentration, the stainable trabecular aluminum surface was less. This effect may have been secondary to enhanced movement of aluminum into bone or decreased deposition of aluminum. Whether the decreased stainable trabecular aluminum surface was important in the protection against bone toxicity from aluminum remains to be determined.

In summary, progressive increments in the dose of aluminum decreased osteoblast surface and the bone formation rate. Supplementation with PTH increased osteoblast surface at each dose of aluminum and the bone formation rate at lower doses of aluminum. However, even with PTH supplementation, aluminum decreased the osteoblast surface. Supplementation with PTH increased the osteoblast surface despite high serum aluminum levels or increased the stainable trabecular aluminum surface. PTH supplementation improved the bone formation rate when the stainable trabecular aluminum surface was less than 12%. Supplementation with PTH changed the relationship between serum aluminum and stainable trabecular surface. Thus, for the same serum aluminum concentration, stainable trabecular aluminum surface was less during PTH supplementation. In conclusion, our results in this model of acute aluminum toxicity indicate that for an equivalent aluminum exposure, high levels of PTH protected against the development of aluminum-induced bone disease.

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REFERENCES


