Effects of thyroparathyroidectomy, phosphate depletion and diphosphonate therapy on acute uraemic extra-osseous calcification in the rat

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Summary
1. The effects of acute uraemia on arterial and visceral calcium concentrations were studied in acutely uraemic rats. The influences of thyroparathyroidectomy, phosphate depletion and diphosphonate therapy on extra-osseous calcium concentrations were assessed in this model.

2. Aortic and visceral calcium concentrations were greater in acutely uraemic rats than in non-uraemic rats. Both prior thyroparathyroidectomy and prior phosphate depletion resulted in lower aortic and visceral calcium concentrations in non-uraemic rats and prevented the increase in aortic and visceral calcium concentrations with acute uraemia. Diphosphonate given for 5 days before and for 2 days after the induction of acute uraemia resulted in lower tissue calcium concentrations than in non-diphosphonate-treated acutely uraemic rats. In contrast, diphosphonate given only immediately before or only after induction of acute uraemia did not prevent the increase in extra-osseous calcium concentrations with acute uraemia.

3. It is concluded that acute uraemia results in an increase in arterial and visceral calcium concentrations. Both thyroparathyroidectomy and phosphate depletion are effective in preventing the increase in extra-osseous calcium concentrations in acute uraemia. Diphosphonates may have a future role in preventing such calcification.

Key words: acute uraemia, arterial calcification, diphosphonate, phosphate depletion, thyroparathyroidectomy, visceral calcification.

Abbreviation: EHDP, ethane-1-hydroxy-1,1-diphosphonate.

Introduction
Arterial and visceral calcification is a well recognized complication of chronic renal failure both in man [1-4] and in experimental animals [5-7]. In contrast, extra-osseous calcification has been less extensively studied in acute uraemia. Diffuse soft-tissue calcification can, however, occur in patients with acute renal failure [8, 9] and in animals with experimentally induced acute uraemia [10]. Extraskeletal calcification in uraemia is clearly of importance; in animals [10], as well as in man [1, 8, 10], once renal function is re-established either by resolution of the acute renal failure [8] or by transplantation [1, 11], the extraskeletal soft-tissue calcium deposits may not dissolve and may intensify in the non-uraemic state [10]. Since these calcium deposits can severely affect renal [12, 13], myocardial [14], arterial [3] and pulmonary [8, 15] function, it would appear desirable to prevent their occurrence if possible.

The present study was undertaken to determine the effects of acute uraemia on arterial and visceral calcium concentrations and to determine if correction of some of the metabolic alterations (hyperphosphataemia and hyperparathyroidism) associated with acute renal failure would prevent the development of extraskeletal calcification. In addition, since diphosphonates have prevented soft-tissue calcification in other conditions, their effect on the prevention of extraskeletal calcification in acute renal failure was studied.
Methods

Experimental design

Four groups of male Sprague-Dawley rats (Sorson Labs., CA, U.S.A.) weighing 200–300 g were studied.

Control group. This group comprised 78 rats, 40 of which were killed 2 days after ureteral ligation and the rest (38) 2 days after laparotomy without ureteral ligation.

Thyroparathyroidectomy group. Thyroparathyroidectomy was performed by cautery and was considered successful if the serum calcium 9 days after surgery was less than 1.75 mmol/l. Laparotomies were performed 14 days after thyroparathyroidectomy. Eight rats were killed 2 days after bilateral ureteral ligation and eight 2 days after laparotomy alone.

Phosphate-depleted group. Phosphate depletion was produced in 15 rats by feeding a low-phosphate diet (0.02%, w/w) (Teklad Test Diets, Madison, WI, U.S.A.) and Al(OH)$_3$ (5:1, w/w) for 42 days before surgery. Nine rats were killed 2 days after laparotomy and bilateral ureteral ligation and six 2 days after laparotomy alone.

Diphosphonate group. Forty-eight rats were given ethane-1-hydroxy-1,1-diphosphonate (EHDP) intraperitoneally before and/or after laparotomy. Five treatment protocols were studied. (1) Non-uraemic group. Twelve rats were given EHDP (10 mg day$^{-1}$ kg$^{-1}$ body weight) for 7 days. Laparotomies were performed on day 5 and the animals killed on day 7. (2) Pre- and uraemic-treated group. Eighteen rats were given EHDP (10 mg day$^{-1}$ kg$^{-1}$) for 7 days. Bilateral ureteral ligations were performed on day 5 and the animals killed on day 7. (3) Pre-uraemic-treated group. Six rats were given EHDP (10 mg day$^{-1}$ kg$^{-1}$) for 5 days before bilateral ureteral ligation and none during the 2 days of acute uraemia. (4) Uraemic-treated group. Six rats were given 10 mg of EHDP/kg at the time of bilateral ureteral ligation and on the day after surgery. These rats were killed 2 days after ureteral ligation. (5) High-dose uraemic-treated group. Six rats were given 40 mg of EHDP/kg at the time of ureteral ligation, 10 mg of EHDP/kg on the day after surgery and were killed 2 days after ureteral ligation.

Laboratory methods

Serum samples were analysed in a Technicon II auto-analyser for urea nitrogen [16] and creatinine [17]. Phosphorus was determined by the American Monitor (American Monitor Corporation, Indianapolis, IN, U.S.A.) method, an automated modification from Fiske & Subbarow [18]. Calcium concentrations were measured with an atomic absorption spectrophotometer (Perkin-Elmer 370, Perkin-Elmer Instruments Div., Norwalk, CT, U.S.A.). Specimens of aorta, kidney, lung and heart were dried for 16 h at 130°C. The tissue was then ground to a fine powder and defatted by rinsing four times with 2 ml of ether/petroleum ether (1:1, v/v). The tissue was then redried at 130°C for 1 h and weighed. Ten to 100 mg of the dry defatted tissue was digested by standing in 1 ml of nitric acid for 16 h and then heating. The resulting clear liquid was diluted to either 5 or 25 ml, depending on the weight of tissue used, with deionized water. Calcium concentrations were measured as above. The results were expressed as millimoles of calcium per kilogram of fat-free dry solid.

Statistical comparisons were made by analysis of variance.

Results

Before induction of acute uraemia thyroparathyroidectomized rats had lower serum calcium and calcium–phosphorus products than did controls and phosphate-depleted rats had lower serum phosphorus, higher serum calcium and lower serum calcium–phosphorus products than did controls ($P < 0.001$) (Table 1). EHDP treatment for 5 days resulted in no significant changes in serum biochemical values from the control rats (Table 1). Serum biochemical values 2 days after bilateral ureteral ligation are shown in Table 1.

In non-uraemic animals tissue calcium concentrations were less in the thyroparathyroidectomized rats than those in the control animals in the aorta ($P < 0.01$), kidney and heart ($P < 0.001$). Phosphate-depleted non-uraemic rats had lower aortic and heart ($P < 0.001$) calcium concentrations than did non-uraemic control rats. Non-uraemic rats that had received EHDP for 7 days had lower aortic ($P < 0.001$), kidney ($P < 0.001$), lung ($P < 0.01$) and heart ($P < 0.001$) calcium concentrations than did non-uraemic control rats (Table 2).

Control rats killed after 2 days of acute uraemia had greater aortic, kidney and heart calcium concentrations than did non-uraemic control rats (Table 2).

Acute uraemic thyroparathyroidectomized rats had lower aortic, kidney, lung and heart calcium concentrations than the acutely uraemic control rats. Aortic, kidney and lung calcium concentrations were not significantly different in
Acute uraemic extra-osseous calcification

thyroidectomized rats was greater than in the acutely uraemic thyroparathyroidectomized rats. The mean heart calcium concentration in the acutely uraemic thyroparathyroidectomized rats was greater than in the non-uraemic thyroparathyroidectomized rats, but was less than in the non-uraemic control rats (P < 0.005) (Table 2).

Phosphate-depleted acutely uraemic rats had lower aortic, kidney and heart calcium concentrations than the acutely uraemic control rats. Aortic and visceral calcium concentrations were not significantly different in acutely uraemic and in non-uraemic phosphate-depleted rats (Table 2).

Aortic and visceral calcium concentrations were significantly less in acutely uraemic animals which received EHDP for 5 days before and for 2 days after the induction of acute uraemia than in acutely uraemic control animals. Tissue calcium concentrations were greater in acutely uraemic EHDP-treated animals than in non-uraemic EHDP-treated animals, but were greater than in non-uraemic control animals only in the kidney (P < 0.005) (Table 2).

Treatment with EHDP for 5 days before induction of acute uraemia alone did not prevent extraskeletal calcium deposition nor did EHDP given only during the 2 day acute uraemic period in a total dose of 20 or 50 mg/kg (Table 2).

Discussion

The present study has demonstrated that increases in arterial and visceral calcium concentrations are common in acutely uraemic rats. There are few previous studies of experimental acute uraemic extra-osseous calcification. These have shown that arterial and myocardial calcification is uncommon in dogs after bilateral

Table 1. Serum biochemical values (mmol/l) in 40 control, eight thyroparathyroidectomised (TPTX), nine phosphate-depleted (PO,D) and 18 EHDP-treated (10 mg day⁻¹ kg⁻¹ body weight for 7 days) rats before and 2 days after bilateral ureteral ligation

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>TPTX</th>
<th>PO,D</th>
<th>EHDP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea nitrogen</td>
<td>7.9 ± 0.4</td>
<td>9.6 ± 0.4</td>
<td>8.9 ± 0.4</td>
<td>9.3 ± 0.4</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.04 ± 0.001</td>
<td>0.05 ± 0.002</td>
<td>0.07 ± 0.004</td>
<td>0.04 ± 0.002</td>
</tr>
<tr>
<td>Calcium</td>
<td>2.38 ± 0.03</td>
<td>1.80 ± 0.05*</td>
<td>2.88 ± 0.05*</td>
<td>2.53 ± 0.03</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>2.36 ± 0.06</td>
<td>2.58 ± 0.06</td>
<td>0.71 ± 0.03*</td>
<td>2.58 ± 0.03</td>
</tr>
<tr>
<td>Calcium–phosphorus product</td>
<td>5.62 ± 0.12</td>
<td>4.64 ± 0.12*</td>
<td>2.04 ± 0.13*</td>
<td>6.53 ± 0.07</td>
</tr>
</tbody>
</table>

Results are means ± SEM. Significance of differences between treated groups and control group: *P < 0.001, **P < 0.001 vs preligation.

Table 2. Tissue calcium concentrations in control, thyroparathyroidectomised (TPTX), phosphate-depleted and EHDP-treated rats

<table>
<thead>
<tr>
<th></th>
<th>Aorta</th>
<th>Kidney</th>
<th>Lung</th>
<th>Heart</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-uraemic (38)</td>
<td>24 ± 2</td>
<td>10 ± 1</td>
<td>15 ± 1</td>
<td>8 ± 1</td>
</tr>
<tr>
<td>Acute uraemic (40)</td>
<td>173 ± 31*</td>
<td>31 ± 3*</td>
<td>28 ± 7</td>
<td>19 ± 3*</td>
</tr>
<tr>
<td>TPTX</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-uraemic (8)</td>
<td>12 ± 4</td>
<td>7 ± 1</td>
<td>11 ± 1</td>
<td>3 ± 1</td>
</tr>
<tr>
<td>Acute uraemic (8)</td>
<td>12 ± 1†</td>
<td>7 ± 1†</td>
<td>11 ± 1†</td>
<td>5 ± 1‡</td>
</tr>
<tr>
<td>Phosphate-depleted</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-uraemic (6)</td>
<td>13 ± 2</td>
<td>10 ± 1</td>
<td>17 ± 2</td>
<td>5 ± 1</td>
</tr>
<tr>
<td>Acute uraemic (9)</td>
<td>22 ± 10†</td>
<td>10 ± 1†</td>
<td>16 ± 3</td>
<td>4 ± 1†</td>
</tr>
<tr>
<td>EHDP-treated groups</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a) Non-uraemic (12)</td>
<td>11 ± 1</td>
<td>7 ± 1</td>
<td>12 ± 1</td>
<td>5 ± 1</td>
</tr>
<tr>
<td>(b) Pre- and uraemic (18)</td>
<td>27 ± 5*†</td>
<td>20 ± 3*‡</td>
<td>16 ± 2*</td>
<td>8 ± 1†‡</td>
</tr>
<tr>
<td>(c) Pre-uraemic (6)</td>
<td>57 ± 28*</td>
<td>56 ± 12*</td>
<td>64 ± 25*</td>
<td>40 ± 28*</td>
</tr>
<tr>
<td>(d) Uraemic (6)</td>
<td>92 ± 36*</td>
<td>42 ± 5*</td>
<td>59 ± 22*</td>
<td>13 ± 3*</td>
</tr>
<tr>
<td>(e) High-dose uraemic (6)</td>
<td>151 ± 48*</td>
<td>57 ± 4*</td>
<td>180 ± 20*</td>
<td>11 ± 1*</td>
</tr>
</tbody>
</table>
nephrectomy [19], although brain calcium concentration does increase after ureteral ligation [20, 21]. In man extensive extra-osseous calcification does occur with acute renal failure [8, 9] although the prevalence of this complication has yet to be defined.

Both parathyroidectomy and phosphate depletion were shown in this study to be effective in preventing increases in extra-osseous calcium concentrations in acute uraemia. Similar beneficial results have been obtained in experimental chronic uraemia. Parathyroidectomy is associated with lower tissue calcium concentrations in chronic uraemic animals [7, 22] and phosphate depletion prevents nephrocalcinosis and progressive deterioration of renal function, both in the remnant kidney model of chronic renal failure [12] and in experimental glomerulonephritis [23].

Prevention of increases in uraemic extra-osseous calcium concentrations by both parathyroidectomy and phosphate depletion could be due either to a lack of parathyroid hormone or to a lower calcium–phosphorus product. In this, as in previous studies [24–26], phosphate-depleted rats had moderate hypercalcaemia which would tend to suppress endogenous parathyroid hormone production. In phosphate-depleted non-uraemic rats hypoplasia and decreased activity of the parathyroid glands occur [24, 26] and serum parathyroid hormone levels fall with phosphate depletion in man [27]. Thus phosphate depletion, in addition to preventing secondary hyperparathyroidism in chronic uraemia [28], may be equally as effective in preventing the hyperparathyroidism which occurs within 6 h of the onset of acute uraemia [29, 30].

The calcium–phosphorus product with acute uraemia was lower in both the phosphate-depleted and thyroparathyroidectomized animals as compared with that of the control rats. In the thyroparathyroidectomy group the product fell as a result of the severe hypocalcaemia which occurred in the fasted uraemic state. Although attempts were made to eliminate this factor by treating an additional group of acutely uraemic thyroparathyroidectomized rats with large pharmacological dosages of 1,25-dihydroxycholecalciferol, it was found that it was not possible to normalize the serum calcium level. Thus it cannot be stated at this time which is of prime importance in the pathogenesis of extra-osseous calcification in acute uraemia; a high circulating parathyroid hormone concentration or a raised calcium–phosphorus product.

Thyroparathyroidectomized non-uraemic rats in the present study had lower tissue calcium concentrations than did non-uraemic control rats. This reduction in soft-tissue calcium concentration cannot be explained by reduced serum calcium levels in thyroparathyroidectomized animals, since phosphate-depleted animals had comparable reductions in tissue calcium concentrations in association with elevated serum calcium levels. Parathyroid hormone stimulates the uptake of calcium by cells in tissue culture [31] and parathyroidectomy results in a decrease in skin and soft-tissue calcification in uraemic patients [20]. A similar reduction in tissue calcium concentration in association with hypercalcaemia was found in non-uraemic phosphate-depleted rats. It is thus possible that a lack of parathyroid hormone in both parathyroidectomized and phosphate-depleted rats could be responsible for the reduction in tissue calcium concentrations in non-uraemic rats.

The present study has also demonstrated that EHDP is effective in preventing the increases in extraskeletal calcium concentrations that occur in acute uraemia. However, the period of administration of EHDP appears to be a critical factor for it to have this effect. Whereas EHDP prevented the increases in calcium concentrations when it was administered both before and during the uraemic phase, it was ineffective when given only in the period immediately before the induction of uraemia or when given only during the uraemic period.

One possible explanation for this finding is that circulating levels of diphosphonates may be important in preventing calcium deposition. The 5 day treatment period with EHDP before induction of uraemia might saturate the binding sites on bone for diphosphonates. The diphosphonate given only during the uraemic phase, when the ureters were ligated, would they stay in the extracellular compartment, since it would be neither extracted by bone nor excreted in the urine. This would explain why diphosphonate given only during the 5 day period before induction of uraemia was ineffective. Similarly, diphosphonate loading in the uraemic phase alone would be equally as ineffective if bone binding sites were available to bind all of the diphosphonate given. In an attempt to determine if this was the reason for the ineffectiveness of EHDP given during acute uraemia, a group of rats was given large dosages of EHDP on the first day of uraemia in an attempt to load bone binding sites followed by a smaller injection on day 2. However, this proved to be equally as ineffective as the smaller dosage schedules.

Other studies have demonstrated the effectiveness of diphosphonates in the prevention of
extraskeletal calcification in chronic uraemia. Diphosphonate therapy prevents kidney and muscle calcification in chronically uraemic rats [32] and may be of benefit in the treatment of arterial and soft-tissue calcification in patients with chronic renal failure [33].

It is concluded that, besides the beneficial effect of phosphate control on the prevention of bone disease [34], parathyroid hyperplasia [28] and extraskeletal calcification [12, 23] in chronic uraemia, control of serum phosphorus is also of importance in the prevention of increases in arterial and visceral calcium concentrations in the acutely uraemic state. Increases in acute uraemic extraskeletal calcification may also be prevented by parathyroidectomy or EHDP treatment. However, the latter may have limited clinical applicability, since it would appear that for diphosphonates to be effective they must be given both before and after the induction of acute uraemia. Additional studies are necessary to determine what accounts for the effectiveness of diphosphonates in this dosage schedule as opposed to its ineffectiveness in other dosage schedules.

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