BIOLICAL ACTIVITY OF ENDOGENOUS AND EXOGENOUS CALCITONIN IN PATIENTS WITH OSTEITIS FIBROSA AND CHRONIC RENAL FAILURE

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Summary

Successful treatment of osteitis fibrosa with 1α-hydroxycholecalciferol (1α-OHD₃) in 9 patients with end-stage chronic renal failure was associated with a significant increase in plasma levels of immunoreactive calcitonin (iCT) independently of changes in plasma calcium, and a decrease in levels of parathyroid hormone (iPTH). In 9 further patients whose plasma alkaline phosphatase activity failed to suppress with 1α-OHD₃, changes in iPTH were associated with proportionate changes in iCT. This suggests that a rise in endogenous calcitonin (CT) secretion contributes to the success of treatment with 1α-OHD₃. In 13 further patients, injections of salmon CT induced a fall in plasma calcium and phosphate which was proportional to the prevailing level of plasma alkaline phosphatase. These data provide further evidence that bone resorption can be effectively inhibited when CT levels are raised either by exogenous CT or its endogenous stimulation.

Introduction

In experimental animals calcitonin (CT) is a potent inhibitor of bone resorption, and this property has led to its use in Paget’s disease and other disorders of mineral metabolism. Despite considerable knowledge of the pharmacology of CT, the role of endogenous CT in skeletal physiology and in the pathogenesis of human bone disease is uncertain. Previous observations have led us to postulate that osteitis fibrosa in chronic renal failure may be due not only to raised levels of parathyroid hormone (PTH) but also to defective secretion of CT [1,2,3]. This investigation examines whether or not effective treatment of osteitis fibrosa might be accompanied by the stimulation of endogenous CT or achieved by the administration of exogenous CT.
Patients and Methods

Eighteen patients with symptomatic bone disease and chronic renal failure were treated for 1 year with 1α-hydroxycholecalciferol (1α-OHD₃). All patients had marked impairment of renal function (GFR <20ml/min) and 15 were established on intermittent haemodialysis (Kii1 multipoint dialyser, 12-18h/wk; dialysate calcium 1.53-1.85 mmol/L). The selection of patients was based on a consistently increased pretreatment level of plasma alkaline phosphatase (>130 i.u./L) and the presence of osteitis fibrosa on histological examination of the iliac bone biopsies [3]. The initial dose of 1α-OHD₃ (2µg daily by mouth) was continued until hypercalcaemia occurred, when the dose was reduced to maintain plasma calcium below hypercalcaemic levels but above pretreatment values. Clinical details of these patients and a fuller account of their responses to treatment have been described previously [4,5].

The acute responses to exogenous synthetic salmon calcitonin (20-40 MRCu i.v. by a single injection or infused over 6 hours) were assessed in 13 additional dialysis-treated patients with a wide range of plasma alkaline phosphatase levels. Patients were fasted and not given dialysis treatment on the day of the test.

With the exception of hormone assays, biochemical measurements were performed on the Vickers multiple channel analyser. Plasma levels of iCT [6] and iPTH [7] were measured using antisera previously shown to give values that correlate well with indices of bone cell activity in chronic renal failure [1-5].

Results

Effects of 1α-OHD₃

Plasma alkaline phosphatase activity, which was above normal (120 i.u./L) in all 18 patients before treatment, fell to normal in 9 patients but remained unchanged in the other 9 (Table I). These patients were termed treatment ‘successes’ and ‘failures’ respectively. During treatment with 1α-OHD₃, plasma calcium increased significantly in both groups of patients though the increment was less in treatment failures since their pre-treatment levels of calcium (and iPTH) were greater (Table I). Hormonal responses also differed. Plasma levels of iPTH fell and iCT rose markedly in successful treatment (reciprocal change), whereas in treatment failures changes in plasma iCT were inconsistent. Though iPTH levels decreased significantly in this group, they did not suppress to normal.

Examination of individual responses (Figure 1) showed that one of the patients, apparently a treatment failure, showed changes in hormone measurements typical of a good responder. Plasma alkaline phosphatase levels had not yet fallen to constant values in this patient. Indeed, when treatment was continued, plasma alkaline phosphatase fell to normal suggesting that this patient had been prematurely and therefore inappropriately classified.
TABLE I. Mean (with SEM) biochemical measurements in plasma before and after 1 year of treatment with 1α-hydroxycholecalciferol. Patients have been divided into treatment successes and failures (see text for details). The significance of differences between mean values before and after treatment (P¹) and of good and poor responders before treatment (P²) are indicated. N.S. = not significant.

<table>
<thead>
<tr>
<th></th>
<th>Treatment successes n=9</th>
<th>Treatment failures n=9</th>
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<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>Alkaline phosphatase (i.u./l)</td>
<td>381 (58)</td>
<td>67 (10)</td>
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<tr>
<td></td>
<td>(0.07)</td>
<td>(0.06)</td>
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<tr>
<td>Immuno-reactive calcitonin (µg/l)</td>
<td>0.20</td>
<td>0.38</td>
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<td></td>
<td>(0.07)</td>
<td>(0.06)</td>
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<tr>
<td>Immuno-reactive parathyroid hormone (µg/l)</td>
<td>2.31</td>
<td>0.62</td>
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<tr>
<td></td>
<td>(0.29)</td>
<td>(0.11)</td>
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<tr>
<td>Calcium (mmol/l)</td>
<td>2.21</td>
<td>2.59</td>
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<tr>
<td></td>
<td>(0.10)</td>
<td>(0.06)</td>
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<tr>
<td>Phosphate (mmol/l)</td>
<td>1.60</td>
<td>1.68</td>
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<td>(0.20)</td>
<td>(0.15)</td>
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**Effects of Salmon Calcitonin**

Acute responses to salmon CT were assessed on hourly blood samples as the change induced from pretreatment levels of plasma calcium and phosphate. Hypocalcaemia and hypophosphataemia were less marked or absent in patients with normal levels of alkaline phosphatase compared with those with high levels. Indeed the degree of response was proportional to the initial alkaline phosphatase value (Figure 2). The route of drug administration did not affect the degree or duration of response.

**Discussion**

Plasma levels of iCT are increased in a proportion of patients with end-stage chronic renal failure [1-3, 8]. Since the kidney is an important site for its degradation [9], this may be due in part to a prolonged metabolic clearance rate of fragments of iCT with doubtful biological activity. Although this makes interpretation of radioimmunoassay difficult in end-stage chronic renal failure, there are good correlations between levels of iCT (iPTH) and direct and indirect indices of bone cell activity [1-5]. The assays used appear to have biological relevance, at least in patients on dialysis.
Figure 1. Plasma levels of immunoreactive parathyroid hormone (iPTH) and calcitonin (iCT) in 18 patients with osteitis fibrosa treated with 1α-OHD₃. Lines connect values measured before treatment (●) and at 1 year (arrow). The solid diagonal lines enclose the values for iPTH and iCT found in normal subjects and patients without bone disease (see Figure 3). Patients have been sub-divided into treatment successes and failures. Good responders (left) show a fall in plasma iPTH in addition to an increase in plasma iCT. In contrast, plasma levels of iPTH and iCT in poor responders (right), showed less consistent changes.

Figure 2. Acute effects of salmon CT in patients with end-stage chronic renal failure. The change induced in plasma calcium and phosphate (value at 6 hours minus initial value) was inversely proportional to the initial activity of alkaline phosphatase.

Further evidence for abnormalities of CT secretion in chronic renal disease is suggested by the finding that patients with the higher levels of iCT respond more vigorously to provocative tests of CT secretion [10]. Increased secretion of endogenous CT may serve to protect the skeleton from osteitis fibrosa since
patients with renal bone disease (osteitis fibrosa) have significantly lower plasma levels of iCT than patients without bone disease [1-3]. In patients without bone disease as well as in health, plasma levels of iCT are directly proportional to levels of iPTH whereas, in the presence of skeletal disease, levels of iCT are ‘inappropriately’ low for the prevailing secretion rate for iPTH [1,2] (see Figure 3). The relationship between iPTH and iCT in patients with raised levels of alkaline phosphatase differs from those with normal levels in that the former have the lower levels of iCT and the higher levels of iPTH. Because of the direct relationship between plasma iCT and iPTH in normal subjects, the ratio iPTH/iCT discriminates more effectively between patients with and without bone disease than the use of either assay alone.

A causal relationship between osteitis fibrosa and CT is difficult to establish in cross-sectional studies but longitudinal studies argue in favour of this. Thus after bilateral nephrectomy in dialysis-treated patients, transient falls in plasma alkaline phosphatase and bone cell counts are associated with transient increase in plasma iCT [3]. Since plasma levels of iPTH do not change, the rise in iCT may contribute to the decrease in bone turnover after bilateral nephrectomy. In the present longitudinal study, successful treatment of hyperparathyroid bone disease with 1α-OHD₃ was also associated with an increase in plasma iCT.

Figure 3. The relationships between immunoreactive parathyroid hormone (iPTH) and calcitonin (iCT) observed in healthy subjects (+) and in dialysis-treated patients with normal (○) or increased activities of alkaline phosphatase (●), from reference 1, with permission. The lines show the common regression (dashed) with 95% confidence limits (continuous) of values from normal subjects and patients without bone disease.
(and a decrease in IPTH) such that the relationship between IPTH and ICT became normal (Figure 1). A return to normal of the IPTH/ICT relationship was not seen in patients with poor responses to 1α-OHD₃, save in the one patient who was probably classified inappropriately as a treatment failure. It seems possible, therefore, that stimulation of CT secretion is a significant component of a successful outcome to the treatment of osteitis fibrosa with 1α-OHD₃.

The variability of the response to 1α-OHD₃ and the factors which modify responses have been discussed elsewhere [4,5] but of particular interest is the observation that treatment failures had the higher pretreatment levels of plasma calcium (Table 1). Although pretreatment plasma levels of ICT did not differ between successful and unsuccessful treatment, it is possible that the smaller increment in plasma calcium attainable in treatment failures (to avoid hypercalcaemia) resulted in a smaller, perhaps sub-threshold stimulus to the secretion of CT. Not surprisingly there was a relationship between the percentage change in calcium with that of ICT but detailed studies in individual patients (unpublished) indicate that rises in ICT may be delayed for several months whereas increases in plasma calcium are more rapid. This suggests that factors other than plasma calcium contribute to the rise in CT.

Although the detailed mechanisms by which CT is stimulated are unknown, the responses observed are consistent with the hypothesis that endogenous CT is an important regulator of skeletal metabolism in chronic renal disease and that changes in its endogenous secretion rate may modify renal bone disease.

The question arises whether or not the administration of exogenous calcitonin would decrease bone cell activity as seen for example, in Paget’s disease. The induction of hypocalcaemia and hypophosphataemia in the absence of significant renal function [11, Figure 2] suggest that osteoclasts are sensitive to calcitonin, albeit in pharmacological amounts. Whether these acute responses are maintained during long-term treatment is not yet clear.

Acknowledgments

We are grateful to Leo Laboratories Ltd and the Armour Pharmaceutical Company for supplies of 1α-OHD₃ and synthetic CT. These studies were generously supported by the National Kidney Research Fund, the Wellcome Trust and the Belgian FRSM (no: 20305).

References

2 Heynen, G, Kanis, JA, Oliver, D, ledingham, JGG and Russell, RGG (1976) Lancet, ii, 1322
Open Discussion

MADSEN (Copenhagen) These are interesting data and now we know that the aetiology of renal osteodystrophy may involve some defect in vitamin D metabolism which is not solely a lack of 1 alpha hydroxylase, and moreover some of these patients may also lack calcitonin, I would like to ask Dr Kanis if he has done any experiments on the combined effect of 1 alpha and exogenous calcitonin supplements.

KANIS I think that is a very interesting question because one of the factors which seems to predispose to failure to respond to 1 alpha hydroxycholecalciferol is a high initial level of plasma calcium possibly due in part to the so-called tertiary hyperparathyroidism. If one could perhaps give larger doses of 1 alpha and allow patients to tolerate that because they are on calcitonin, this may have beneficial effects. The other approach may be for you to stick with the low dialysate calcium which might allow your patient to tolerate more 1 alpha hydroxycholecalciferol.

KOKOT (Katowice) Have you some observations about calcitonin secretion in patients treated by chronic haemodialysis but without supplemental administration of 1 alpha cholecalciferol? I am just wondering whether this calcitonin secretion is increasing also in patients not treated by 1 alpha cholecalciferol.

KANIS You mean is this just a time dependent effect? Are we sure that this is associated with treatment or might it just have occurred if we had not done anything to the patient? I think it is highly unlikely that it would have occurred. We have looked at calcitonin levels in individual patients separated by a period of six months and the variation has not been at all as great as one sees here, so I think this is a response attributable to 1 alpha rather than to the natural history or the natural change in calcitonin.

BONE (Liverpool) I would like to ask Dr Kanis how reliable he thinks it is to draw conclusions from the administration of calcitonin in pharmacological quantities, to the pathophysiology of endogenous calcitonin. In a much smaller series of patients, a group with high bone turnover and erosive bone disease, and a second group with no erosions and low normal levels of PTH, we found preliminary results were rather the converse; that the patients with erosive bone disease and high PTHs had the higher levels rather than as your thesis would suggest.

KANIS I would be very interested to see that data. Certainly there are differ-
ences between various calcitonin assays - of that there is no doubt - not only in absolute levels but in some important qualitative differences such as the detection of high calcitonin levels in patients with a variety of carcinomas by some workers, whereas others don't. All I can say is that the assay that is used here does relate at least to histological and indirect indices of bone cell turnover, suggesting that even if we are not measuring something that is biologically active it does at least have biological relevance. Now to return to your first question, I was very careful not to invoke pathological importance in the pathophysiology of renal bone disease to the argument about the exogenous use of calcitonin.