PART X

NEPHROLOGY—BONE DISEASE

Chairmen: U Binswanger
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Plasma Immunoreactive Calcitonin and Bone Disease in Patients on Haemodialysis

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Summary

Among patients with chronic renal failure on haemodialysis, those with elevated plasma alkaline phosphatase (AP) or histological evidence for increased bone turnover had significantly lower plasma levels of immunoreactive calcitonin (iCT) than patients without bone disease. Plasma levels of iCT and parathyroid hormone (iPTH) were each correlated with other indices of skeletal metabolism (plasma AP, and osteoblastic activity, calcification front and marrow fibrosis in bone biopsies). The use of the ratio iPTH/iCT allowed a better discrimination between patients with increased and normal bone turnover than the use of either assay alone. These studies suggest an important role for calcitonin in renal bone disease.

Introduction

In experimental animals the major effect of calcitonin (CT) is to inhibit bone resorption and it may also serve to minimise the potentially hypercalcaemic effects of dietary calcium (Munson, 1967). There have been considerable advances in our knowledge of the chemistry and physiology of this hormone in various animal species, and the effect of exogenous CT has been extensively studied in man (for review see Queener & Bell, 1975), so that CT is now widely used in the treatment of Paget’s disease (Shai et al, 1971; Kanis et al, 1974). However the physiological role of CT in man has remained uncertain. The present study provides evidence suggesting that CT may have important effects on skeletal turnover in patients with chronic renal disease.
METHODS

Blood samples were taken for plasma measurements from 28 normal subjects and 52 patients with chronic renal failure treated with thrice weekly intermittent haemodialysis (Kii multipot 15–24h/wk, dialysate calcium = 1.53mmol/l). A transiliac bone biopsy was taken at the same time for quantitative histology of cancellous bone in 26 of the patients. No patient had received barbiturates, anticonvulsants, vitamin D, its analogues or metabolites for at least 1 year.

With the exception of the hormone assays, biochemical measurements were done on the Vickers autoanalyser. The assay for immunoreactive CT (iCT — Heynen & Franchimont, 1974) measures both small CT (corresponding to the elution profile of synthetic human CT on Sephadex G-50 column chromatography) and big CT (Heynen et al, 1975). Plasma immunoreactive parathyroid hormone (iPTH) was measured using an assay based on that of Berson et al (1963). The antibody (Wellcome Laboratories, London, AS 211/32) is thought to have predominantly N-terminal specificity (Woo & Singer, 1974; Franchimont & Heynen, 1976) though Barling et al (1975) showed it to be equally reactive to the N- and C-terminal portions of the molecule.

RESULTS

Spontaneous Variations of Plasma iPTH and iCT

iPTH and iCT were measured in plasma taken just before a dialysis treatment from 10 of the patients on several occasions at intervals of 4 to 11 months. There was a high degree of consistency in the plasma concentrations (log transformed) of both iPTH (mean difference between samples from the same patient, +0.6, range, −3.7 to +4.3% of initial value) and iCT (mean difference, −1.4, range −16.6 to +11.5%) over the time intervals studied.

Relationship between Plasma iPTH and iCT

There was a positive correlation between plasma iPTH and iCT in the 28 normal subjects (r = +0.72; P < 0.001, Figure 1). In contrast in the 52 dialysed patients the relationship was inverse (r = −0.41; P < 0.005).

Inter-relationships of Plasma iPTH, iCT and Alkaline Phosphatase (AP)

In the dialysed patients plasma AP correlated positively with levels of iPTH (r = +0.72; P < 0.001) and negatively with plasma iCT (r = −0.40; P < 0.005). On the basis of a bimodal distribution of plasma AP patients were divided into two groups according to their activity of AP ('high AP group'; 'low AP group').

There was no difference in plasma calcium or phosphate between the two groups though the relationships of iPTH with iCT differed markedly (Figure 1).
Figure 1. The relationships between immunoreactive parathyroid hormone (iPTH) and calcitonin (iCT) in normal subjects (+) and in dialysed patients with normal (○) and increased (●) activities of plasma alkaline phosphatase. Hormone concentrations are plotted on logarithmic scales. The lines show the common regression (dashed) with 95% confidence limits (continuous) of values from normal subjects and those renal patients with normal levels of plasma alkaline phosphatase.

In the ‘low AP group’ the correlation of iPTH with iCT was positive (r = +0.40; P < 0.05) and the regression lines seen in this group and normal subjects did not differ. A common regression with 95% confidence limits was calculated (Figure 1).

In the ‘high AP group’ the relationship of iPTH with iCT differed from the ‘low AP group’ and normal subjects in two important respects. This (‘high AP’) group had significantly lower plasma levels of iCT (2.49 ± SEM 0.10 and 3.11 ± 0.08 log_{10} ng/l respectively; t = 4.93, P < 0.001) and higher levels of iPTH (3.62 ± 0.10 and 2.99 ± 0.07 log_{10} ng/l respectively; t = 5.17, P < 0.001), than the ‘low AP group’. Secondly, there was a negative correlation between plasma iPTH and iCT (r = 0.46; P < 0.02, Figure 1) in contrast with the direct relationship seen in the other groups. The use of a ratio of iPTH over iCT gave a better discrimination of patients with normal and increased AP than the use of iPTH or iCT alone (t = 5.58; P < 0.001).

Relationship of Plasma iCT and iPTH with Bone Histology

The intercorrelations of plasma AP, iCT and iPTH with several histological indices of bone turnover are summarised in Table I. As was seen in the case of plasma
<table>
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<tr>
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<th>Osteoblastic activity (% osteoid surface)</th>
<th>Osteoblastic activity (% trabecular surface)</th>
<th>Resorbing surface (% trabecular surface)</th>
<th>Calcification front (% osteoid surface)</th>
<th>Index of fibrosis</th>
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<tbody>
<tr>
<td>Plasma AP</td>
<td>+ 0.55</td>
<td>+ 0.51</td>
<td>+ 0.57</td>
<td>+ 0.60</td>
<td>+ 0.59</td>
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<tr>
<td>Log\textsubscript{10} plasma ICT</td>
<td>- 0.52</td>
<td>- 0.59</td>
<td>- 0.33</td>
<td>- 0.53</td>
<td>- 0.46</td>
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<td>(&lt;0.01)</td>
<td>(&lt;0.005)</td>
<td>(NS)</td>
<td>(&lt;0.01)</td>
<td>(&lt;0.02)</td>
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<tr>
<td>Log\textsubscript{10} plasma iPTH</td>
<td>+ 0.73</td>
<td>+ 0.70</td>
<td>+ 0.62</td>
<td>+ 0.79</td>
<td>+ 0.81</td>
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Footnote

Histological measurements (10 μm sections) were made using a squared eyepiece graticule (Chalkley point array) and point counting technique (400 points of intersection in each biopsy). The proportion of the trabecular surface occupied by active-looking (plump) osteoblasts was expressed both as a percentage of the osteoid and total trabecular surface. The resorbing surface was the creased surface not occupied by osteoid. The calcification front was defined on the basis of staining with Toluidine Blue in the osteoid lamella closest to the mineralised surface. Bone fibrosis was graded in 4 ways as follows: No fibrosis; Fibrosis confined to resorbing surfaces; Fibrosis present on resorption and some of the osteoid surfaces; and Fibrosis over the resorption and all of the osteoid surface.

AP, patients with increased osteoblastic activity and fibrosis of bone had higher iPTH and lower ICT levels than more normal patients. The presence or absence of osteomalacia was not associated with any difference in the levels of these hormones.

The distribution of measurements of osteoblastic activity, and log\textsubscript{10} iPTH and ICT did not differ significantly from normal, and partial correlations showed that the correlations of osteoblastic activity with plasma iPTH and ICT were independent of each other, but that the apparent correlation of iPTH with ICT (\(r = -0.2\), NS) was dependent upon osteoblastic activity (Figure 2).

**DISCUSSION**

**Biological Relevance of Hormone Assays**

Interpretation of the results of radioimmunoassay may be difficult due to the uncertain nature of the immunoreactive fragments. The assay for iPTH used in this study, which is probably N-terminal selective, can distinguish normal from hyperparathyroid serum (Woo & Singer, 1974) and plasma levels are in direct proportion to weight of parathyroid tissue in patients with primary hyperparathyroidism (Franchimont & Heynen, 1976). Similarly plasma levels of ICT, using the present assay, are related to other biological measurements such as plasma.
Figure 2. The correlations between osteoblastic activity (osteoblastic surface X100/total trabecular surface) with (a) plasma immunoreactive parathyroid hormone (iPTH), (b) plasma immunoreactive calcitonin (iCT). Hormone concentrations are plotted on a logarithmic scale and the correlation coefficients and significance levels are based on log transformed values. The partial correlation coefficients were: iPTH vs osteoblastic activity, $r = +0.56$ (P < 0.01); iCT vs osteoblastic activity, $r = -0.40$ (P < 0.05); iPTH vs iCT, $r = -0.2$ (NS)

phosphate, and change appropriately with calcium or EDTA infusion and pentagastrin injection (Franchimont & Heynen, 1976). In this study striking correlations of both hormones were found with histological and indirect estimates of bone turnover and the small spontaneous variation in pre-dialysis plasma levels over many months suggest the suitability of using single samples taken immediately before a dialysis session.

These data suggest that both radioimmunoassays are measuring fragments of biological relevance, even though the fragments may not themselves possess biological activity.

**General Conclusions**

In patients with chronic renal failure, high plasma levels of iPTH and iCT may both be found. In normal subjects and in dialysed patients without renal bone disease plasma levels of iPTH and iCT were directly proportional to each other. The increased plasma iCT associated with high levels of iPTH might serve to protect skeletal tissue from excessive resorption. There are several observations which support this idea.

Thus, patients with renal bone disease, as judged by histological methods or
by plasma AP, characteristically had higher plasma levels of iPTH and lower levels of iCT than patients or normal subjects with low bone turnover. Secondly, the negative correlation between plasma iCT and osteoblastic activity (Figure 2) was not spurious in the sense that partial correlation analysis confirmed that the correlation was independent of iPTH and indeed that plasma iCT contributed to the significance of the relationship of iPTH with osteoblastic activity. Finally, a 70-fold range of plasma iPTH and a 40-fold range of iCT were found in patients with normal bone turnover. Thus observations that plasma concentrations of iPTH do not accurately reflect histological findings (Ritz et al, 1975) may be due in part to a failure to account for the secretion of CT. In support of this, the use of an iPTH/iCT ratio allowed a finer discrimination of patients with and without bone disease than did the measurement of iPTH or iCT alone.

These observations suggest the possible importance of endogenous calcitonin in regulating osteoblastic function and hence bone turnover. Renal bone disease is probably the result of several adverse factors which may include deficiency of CT relative to the prevailing rate of secretion of PTH.

Acknowledgments

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