Plasma Hydroxyproline in Renal Osteodystrophy

Z VARGHESE, J F MOORHEAD, G L V TATLER, R A BAILLOD, M R WILLS
Royal Free Hospital, London, UK

Hydroxyproline is a non-essential amino acid which is found almost exclusively in collagen where it constitutes approximately 14 per cent of the amino acid residues. Collagen is probably the most abundant species of protein in the body and approximately 50 per cent of the total body collagen is present in bone: more than 90 per cent of the organic matrix of bone is composed of collagen (Hahn & Avioli, 1970). In view of the findings that bone collagen is metabolically more active than skin collagen (Smith, 1970), and that parathyroid hormone (PTH) induces bone collagen breakdown without altering skin collagen metabolism (Laitinen, 1967), urine hydroxyproline excretion has been used as a biochemical marker in a number of studies relating to bone collagen turnover (Prockop & Kivirikko, 1967; Smith, 1969). There are, however, only limited studies relating to the diagnostic value of plasma hydroxyproline measurement (Bishop & Smith, 1971). In uraemic osteodystrophy, where bone destruction with excessive osteoclastic activity is a dominant feature, changes in plasma hydroxyproline concentration would be expected to provide a useful index of the degree and extent of bone involvement. The present study reports an evaluation of the usefulness of plasma hydroxyproline measurement in patients with renal osteodystrophy.

MATERIALS AND METHODS

Patients: Biochemical measurements were carried out on two groups of patients.

Group 1: 88 patients who had been receiving maintenance haemodialysis treatment (MHT) for up to 9 years. The patients were dialysed three times a week for a total of 30 hours per week: the dialysis fluid contained 3.75 mEq/l of calcium. In this group of patients radiological assessment had been performed at six-monthly intervals while on MHT. On the basis
of radiological criteria (Tatler et al, 1973) for renal osteo-
dystrophy this group of patients was further divided into
three sub-groups:

Group 1A: 50 patients on MHT who had no consistent radiological evi-
dence of bone disease.

Group 1B: 16 patients on MHT who had varying degrees of skeletal
involvement, and which included fractures and soft tissue,
vascular and periarticular calcification.

Group 1C: 22 patients who had osteitis fibrosa.

Group 2: Consisted of 28 non-dialysed patients with varying degrees
of renal impairment on the basis of their radiographs.
These patients were further divided into two sub-groups:

Group 2A: 16 patients with no radiological evidence of bone disease.

Group 2B: 12 patients with osteitis fibrosa.

Control Group: Comprised of 39 normal subjects ranging in age from 19 to 60
years with no history of metabolic bone disease or renal
disease. The group comprised 17 men and 22 women.

METHODS

In all the patient and control subjects studied, venous blood specimens were
collected without stasis after fasting from the previous evening. Before
blood collection the patients were rested in the supine position for one hour.
In the patients on MHT the specimens were collected between 36 and 48
hours after the last dialysis. All foods containing gelatin were excluded
from the diet for the 5 days before collection of blood.

Plasma hydroxyproline was measured as the 'ethanol extractable' fraction
based on the modification of Bishop (1972, personal communication) of the
method of Prockop and Udenfriend (1960). Optimum conditions were estab-
lished to increase the speed, accuracy and precision of the method.

Total plasma calcium and inorganic phosphate concentrations were measured
by autoanalyser method N-26.

Plasma alkaline phosphatase activity was measured by autoanalyser method
N-28.

Total plasma protein was measured by autoanalyser method N-27.

Plasma albumin was measured by autoanalyser using Bromocresol green
(Northam & Widowson, 1967).

RESULTS AND DISCUSSION

Group 1: Patients on MHT:
The values obtained for biochemical variables measured in this study are
shown in Table I.
<table>
<thead>
<tr>
<th>Plasma concentrations</th>
<th>Control Group (n = 38)</th>
<th>Group 1 - Patients on MHT</th>
<th>Group 2 - Non-dialysed patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1A (n = 50) 1B (n = 16) 1C (n = 22)</td>
<td>2A (n = 16) 2B (n = 12)</td>
</tr>
<tr>
<td>Calcium (mg/100 ml)</td>
<td>Mean 9.66 S.E.M. 0.06</td>
<td>10.48*** 10.49*** 9.96*</td>
<td>8.86*** 9.90‡</td>
</tr>
<tr>
<td>Phosphate (mg/100 ml)</td>
<td>Mean 3.59 S.E.M. 0.07 4.70** 3.95‡ 4.83***</td>
<td>5.48*** 7.15***</td>
<td></td>
</tr>
<tr>
<td>Magnesium (mg/100 ml)</td>
<td>Mean 2.15 S.E.M. 0.02 2.88*** 2.97*** 2.90***</td>
<td>- -</td>
<td></td>
</tr>
<tr>
<td>Total proteins (g/100 ml)</td>
<td>Mean 7.58 S.E.M. 0.10 7.22* 7.16* 7.04**</td>
<td>6.61*** 6.83**</td>
<td></td>
</tr>
<tr>
<td>Albumin (g/100 ml)</td>
<td>Mean 4.37 S.E.M. 0.06 4.02*** 3.89*** 4.03**</td>
<td>3.55*** 3.69***</td>
<td></td>
</tr>
<tr>
<td>Alkaline Phosphatase (KA.u/100 ml)</td>
<td>Mean 6.00 S.E.M. 0.30 7.70** 12.30*** 31.90***</td>
<td>11.20*** 23.70***</td>
<td></td>
</tr>
<tr>
<td>Hydroxyproline (mg/l)</td>
<td>Mean 1.60 S.E.M. 0.10 4.09*** 5.76*** 10.50***</td>
<td>2.96*** 18.24**</td>
<td></td>
</tr>
</tbody>
</table>

Significance value of 't' for the groups of patients on comparison with the control subjects by students 't' index:

*** P < 0.001  ** P < 0.005  * P < 0.05  ‡ not significant
When compared with the control subjects, the mean plasma calcium concentration was significantly raised in the three groups of patients on MHT (Table I: Groups 1A, 1B, 1C). There was no significant difference between the mean plasma calcium concentration of the patients in groups 1A and 1B. In both of these groups, the calcium concentrations were significantly higher than in patients with osteitis fibrosa (Group 1C). \( P = < 0.05 \). The mean plasma phosphate concentrations were significantly higher than in the control subjects in two of the groups of patients (1A, 1C), but did not differ significantly in the other group (1B). In all three groups of patients on MHT, the mean plasma magnesium concentrations were significantly higher than in the control subjects: there was no significant difference between the three groups. The mean total plasma protein concentration was significantly lower than that of the control subjects in all three groups of patients, and this was associated with a significant decrease in albumin concentration in the three groups. There was no significant difference in either total protein or albumin concentrations between the three groups.

The mean plasma alkaline phosphatase activity in the control group of subjects was 6.0 KA units per 100 ml \((SD = \pm 1.6)\) with a range \((Mean \pm 2 SD)\) of 2.8 to 9.2 KA units per 100 ml. This range is somewhat lower than the generally accepted one of 3 to 13 KA units. Alkaline phosphatase in all three patient groups was significantly higher than in the control subjects. In the patients with some degree of skeletal involvement (Group 1B) alkaline phosphatase was higher than in those without radiological bone disease. However, in this group (1B) the value for the enzyme was significantly lower than in those with osteitis fibrosa (Group 1C). A plot of the individual results (Figure 1) shows that of the 50 patients without radiological bone disease (Group 1A), there were 4 with values above the accepted normal range and 10 with values above the range for the control subjects studied here. Of the 16 patients in Group 1B and the 22 in Group 1C with radiological skeletal changes, there were 7 and 16 patients respectively with values above the accepted normal range, and 8 and 21 patients respectively with values above the range found in the control subjects. The findings suggest that the plasma alkaline phosphatase activity is of limited value in the diagnostic assessment of skeletal involvement in patients with chronic renal failure on MHT.

The mean \((\pm 2 SD)\) plasma hydroxyproline concentration in the control subjects was 1.60 \(\pm\) 1.2 mg per litre (range 0.4 to 2.8 mg per litre). In all three groups of patients the mean values were significantly increased when compared with the control subjects. The mean value in the patients with osteitis fibrosa (Group 1C) was significantly higher \((P = < 0.001)\) than in those with some skeletal involvement (Group 1B), and the mean value in the
latter group was significantly higher ($P < 0.05$), than in those without radiological bone disease (Group 1A). The individual results for the patients on MHT (Figure 1) show that in 30 of the 50 patients in Group 1A, in 13 of the 16 in Group 1B, and in all of the 22 patients in Group 1C, the values were above the range found in the control subjects. The findings suggest that the plasma hydroxyproline concentration in patients with chronic renal failure on MHT is of considerable value as an index of skeletal involvement. The increased values in the patients without radiological bone disease (Group 1A) may be attributed either to an impairment of hydroxyproline catabolism, a manifestation of uraemia (Avioli et al, 1969), or to the presence of bone disease which is not detectable by radiological techniques.

Group 2: Non-dialysed patients:

The mean plasma calcium concentration in the patients without radiological bone disease (Group 2A) was significantly lower than in the control subjects, while in the patients with osteitis fibrosa (Group 2B), the difference was not significant (Table 1). In both of the groups of non-dialysed patients the mean plasma phosphate concentration was increased: the differences were significant when compared with the control subjects, although there was no significant difference between the two groups. The total protein and albumin
The plasma hydroxyproline concentration was significantly increased in both of the groups of undialysed patients when compared with the control subjects. Of the 16 patients without radiological evidence of bone disease (Figure 2) (Group 2A), there were 8 with values above the range found in the control subjects, and of these there were 4 with a concentration of 4 mg per litre or more (4.0, 4.1, 4.4 and 4.5 mg/l). All of the 12 patients with osteitis fibrosa (Group 2B) had plasma hydroxyproline concentrations of 4 mg per litre or above (Figure 2). In the two groups of undialysed patients there was evidence of a relationship between the increase in plasma hydroxyproline concentration and endogenous creatinine clearance (Figure 3). In the one patient with a markedly increased plasma hydroxyproline concentration (11.7 mg/l) and a creatinine clearance of 28.0 ml per minute, the diagnosis was primary hyperparathyroidism with secondary renal failure. As in the group of patients on MHT, plasma hydroxyproline concentration appears to be a valuable index of skeletal involvement. In the patients without radiological evidence of bone disease, the abnormal values for plasma hydroxyproline were found in those patients with an endogenous creatinine clearance of 16.0 ml per minute or less.

EFFECT OF PARATHYROIDECTOMY

Two patients with primary hyperparathyroidism and secondary renal impairment, and three patients on MHT with severe renal osteodystrophy underwent parathyroidectomy. The two patients with primary hyperparathyroidism and one of the patients on MHT (Table II, case no. 1, 2 and 3) postoperatively required treatment with calcium supplements and vitamin D to restore the
Table II. Changes in plasma calcium, alkaline phosphatase activity and hydroxyproline concentrations following parathyroidectomy

<table>
<thead>
<tr>
<th>Case Number</th>
<th>Diagnosis</th>
<th>Plasma Concentrations</th>
<th>Before Parathyroidectomy</th>
<th>Days after parathyroidectomy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Calcium (mg/100 ml)</td>
<td>15.0</td>
<td>11.7 9.9 9.0 8.1 - 7.0 6.9 8.0 8.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Alk. phosphatase (KA.u/100 ml)</td>
<td>49.0</td>
<td>74.0 75.0 87.0 92.0 - 121.0 105.0 106.0 80.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hydroxyproline (mg/l)</td>
<td>11.5</td>
<td>4.9 3.9 3.0 4.2 - 4.2 2.5 4.2 4.9</td>
</tr>
<tr>
<td>1</td>
<td>Primary hyperparathyroidism</td>
<td>Calcium (mg/100 ml)</td>
<td>12.0</td>
<td>9.4 8.0 7.2 7.2 6.8 - 6.8 6.8 - 7.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Alk. phosphatase (KA.u/100 ml)</td>
<td>50.0</td>
<td>39.0 53.0 44.0 44.0 52.0 - 56.0 44.0 - 29.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hydroxyproline (mg/l)</td>
<td>14.6</td>
<td>6.7 3.1 4.6 4.0 2.7 - 1.1 7.7 - 8.7</td>
</tr>
<tr>
<td>2</td>
<td>Primary hyperparathyroidism</td>
<td>Calcium (mg/100 ml)</td>
<td>10.2</td>
<td>6.4 5.9 5.9 6.3 6.5 6.8 7.5 8.8 - 10.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Alk. phosphatase (KA.u/100 ml)</td>
<td>70.0</td>
<td>69.0 69.0 67.0 64.0 68.0 75.0 67.0 75.0 - 41.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hydroxyproline (mg/l)</td>
<td>36.5</td>
<td>7.5 6.0 3.6 3.1 5.2 5.0 4.5 7.3 - 6.9</td>
</tr>
<tr>
<td>3</td>
<td>Tertiary hyperparathyroidism</td>
<td>Calcium (mg/100 ml)</td>
<td>12.3</td>
<td>10.7 11.6 - 12.0 - 10.6 10.8 10.1 9.8 9.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Alk. phosphatase (KA.u/100 ml)</td>
<td>72.0</td>
<td>76.0 78.0 - 82.0 - 79.0 77.0 93.0 82.0 80.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hydroxyproline (mg/l)</td>
<td>10.8</td>
<td>9.6 11.4 - - 8.7 7.7 10.1 10.8 10.3</td>
</tr>
<tr>
<td>4</td>
<td>Tertiary hyperparathyroidism</td>
<td>Calcium (mg/100 ml)</td>
<td>10.6</td>
<td>10.4 10.5 10.8 - 10.9 - 10.1 11.5 11.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Alk. phosphatase (KA.u/100 ml)</td>
<td>50.0</td>
<td>49.0 49.0 50.0 - 48.0 - 45.0 81.0 89.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hydroxyproline (mg/l)</td>
<td>82.0</td>
<td>82.5 93.5 88.0 - 77.0 - 100.0 78.9 93.5</td>
</tr>
</tbody>
</table>
plasma calcium concentration. In these three patients there was a dramatic fall in plasma hydroxyproline concentration within the first 48 hours after parathyroidectomy. Plasma hydroxyproline levels were either in or just above the normal range within three to five days. The plasma alkaline phosphatase activity, showed an increase from pre-operative levels (Table II). On treatment with vitamin D and presumably with increased collagen synthesis and new bone formation, the plasma hydroxyproline concentration showed a subsequent increase and remained elevated for a few weeks and then once again fell back into normal range. These three patients showed radiological improvement during this period.

In 2 other cases (Table II, case no 4 and 5) where the complete surgical removal of the parathyroid glands was unsuccessful, there were no significant changes in plasma calcium, alkaline phosphatase and hydroxyproline. This dissociation between the plasma alkaline phosphatase activity and hydroxyproline concentration in the immediate post-operative period suggest that, in this situation, these two variables reflect alterations in osteoblastic and osteoclastic activity respectively. The secondary increase in plasma hydroxyproline concentration with vitamin D treatment and radiological healing can be attributed to an increase in collagen synthesis during new bone formation.

CONCLUSIONS

Although plasma calcium, phosphate, magnesium, total protein, and albumin concentrations of MHT patients and non-dialysed patients were significantly different from the control group, they were insufficiently sensitive in detecting differences between the various patient groups. Alkaline phosphatase activity in all 5 patient groups was higher than in the control group. The activity of this enzyme showed a progressive rise with increase in severity of bone lesion in both MHT and undialysed patients. These differences in the enzyme activity between these various patient groups were statistically significant. Nevertheless, alkaline phosphatase activity in general is difficult to interpret because of wide variations in its isoenzyme distributions.

Plasma hydroxyproline in the present study correlated well with the radiological findings. Our results in non-dialysed patients, and in patients who have undergone parathyroidectomy indicate the specificity of plasma hydroxyproline as a biochemical marker in renal osteodystrophy. The finding that 30 of the 50 patients with no apparent radiological lesion had plasma hydroxyproline values above the control value, probably indicates the sensitivity of this parameter in indicating early changes in skeletal metabolism. On the other hand, this elevation could be attributed to a reduction in the catabolism of plasma hydroxyproline because of inhibition of plasma hydroxyproline oxidase activity. However, a significant proportion (40 per cent) of patients in Group 1A had normal values for plasma
hydroxyproline. Giordano (1972) reported that there was a gradual rise in plasma hydroxyproline values with time on MHT. The concentration of plasma hydroxyproline was not affected during the first year of treatment: this suggests an association between the rise in plasma hydroxyproline values and the development of renal osteodystrophy.

REFERENCES

Bishop, M. C. and Smith, R. (1971) Clinica chimica acta, 33, 403
Hahn, T. J. and Avioli, L. V. (1970) Archives of Internal Medicine, 126, 382
Laitinen, O. (1967) Endocrinology, 80, 815
Procop, D. J. and Udenfriend, S. A. (1960) Analytical Biochemistry, 1, 228
Smith, R. (1970) Proceedings of the Royal Society of Medicine, 63, 331

OPEN DISCUSSION

M KAYE (Montreal): Could I confirm first that you are measuring non-protein bound hydroxyproline?

VARGHESE: Yes, free plus peptide bound.

KAYE: We looked at plasma hydroxyproline and compared it with the bone fraction of alkaline phosphatase and found a very good correlation between the two. It is a lot easier to do than measuring hydroxyproline. Do you have any comments on that?

VARGHESE: We looked at the isoenzyme pattern in some of these patients, and to our surprise found most of these alkaline phosphatases to be of intestinal origin. We don't know the significance of this.

R. SMITH (Oxford): I wonder if you would comment if you have any bone histology. Our group produced similar data 2 years ago with fewer numbers, but exactly the same conclusion.

VARGHESE: We are looking at histology and will have the data soon.