THE INTERRELATIONSHIPS BETWEEN SERUM 25-HYDROXYCHOLECALCIFEROL, SERUM PARATHYROID HORMONE AND BONE CHANGES IN ANTICONVULSANT OSTEOMALACIA

By
Leif Mosekilde, Merete Sanvig Christensen, Birger Lund, Ole Helmer Sørensen and Flemming Melsen

ABSTRACT

Serum 25-hydroxycholecalciferol (25-HCC) and serum parathyroid hormone (iPTH) were measured in 59 randomly selected adult epileptic outpatients receiving chronic anticonvulsant therapy. Quantitative morphometric analysis of iliac crest biopsies was performed. A mild degree of osteomalacia was found which was inversely correlated to dietary vitamin D intake. Serum 25-HCC was reduced in the epileptic patients compared to a control group, although dietary intake of vitamin D was higher than the mean daily intake in the Danish population. Serum 25-HCC was positively correlated to dietary vitamin D intake, but not correlated to the severity of bone changes, indicating that other factors than circulating 25-HCC are responsible for the development of anticonvulsant osteomalacia. Serum 25-HCG was inversely correlated to serum iPTH in patients with a low dietary calcium intake. The mean value of serum iPTH was not increased, and there was no correlation between serum iPTH and bone morphometry.
Several investigators have reported low serum levels of 25-hydroxycholecalciferol (25-HCC) in patients receiving anticonvulsant treatment. In the present study we have investigated the relationships between serum 25-HCC, serum parathyroid hormone (iPTH) and bone morphometry in a group of epileptic patients receiving chronic anticonvulsant therapy, in order to evaluate the importance of low serum 25-HCC concentration for the development of anticonvulsant osteomalacia and secondary hyperparathyroidism.

**MATERIAL AND METHODS**

The investigation comprised 59 randomly selected adult epileptic outpatients: 30 men aged 18–50 years (mean 27 years) and 29 women aged 18–47 years (mean 26 years). All patients received diphenylhydantoin, some of them in combination with other anticonvulsant drugs, and all had been treated for more than 10 years. None of the patients had symptoms of bone disease.

Serum 25-HCC was measured by a competitive protein-binding assay described by Haddad & Chyu (1971) with a modification in the extraction procedure and the chromatographic step. The coefficient of variation of repeated measurements at the level of 15 ng was 13.5%. The sensitivity in the routine assay was 1.5 ng/ml. Serum 25-HCC was measured in the epileptics and in 52 healthy controls in January and February.

Serum iPTH was measured by a radioimmunoassay after extraction of the hormone from serum by absorption to and elution from a microfine precipitated silica (Quso G 32), providing a hormone concentration 3.2 times greater in extract than in serum (Christensen 1976). The antibody used was antiovine PTH (AS 211/32) and bovine PTH was used for labelling. The coefficient of variation for measurements within normal range (30–105 pg/ml bovine equivalents, MRC bPTH standard 71/324) was 16%, and the sensitivity 10 pg bPTH present in the incubation mixture.

Bone biopsies were performed by transfixing the right iliac crest after double-labelling with tetracycline. The following parameters were measured on decalcified and undecalcified bone sections: absolute volume of trabecular bone (AVTB) in per cent of total bone area; ostecoid surfaces (OS) in per cent of total trabecular bone surfaces; relative ostecoid volume (OV) in per cent of trabecular bone volume; trabecular osteoclastic resorption surfaces (RS) in per cent of total trabecular bone surfaces; mean volume of peri-osteocytic lacunae (POL) in \( \mu m^3 \), and calcification rate (CR) in \( \mu m \) per day, as the mean distance between the fluorescent tetracycline lines in all double-labelled zones.

A detailed history of dietary calcium and vitamin D intake throughout the year was obtained from each patient using a standard questionnaire. Fasting serum values of magnesium, phosphorus and alkaline phosphatase were determined by standard laboratory methods. Serum calcium was corrected for individual variation in serum protein concentration.

Statistical significance of differences in group means was determined by Wilcoxon test for two samples and correlation coefficients by Spearman's rank correlation. Linear regression analysis was made after testing for normal distribution.
RESULTS

The morphometric bone parameters in the epileptic patients were compared to normal controls (Table 1). In the epileptics the percentage of osteoid surfaces, the relative osteoid volume, the trabecular osteoclastic resorption surfaces and the mean of volume of peri-osteocytic lacunae were significantly increased \((P < 0.01)\), whereas the calcification rate was decreased \((P < 0.05)\).

The serum levels of 25-HCC are given in Fig. 1. The mean value in the epileptic patients was significantly \((P < 0.001)\) lower than the mean value of the control group, but there was a considerable overlap. The mean daily intake of vitamin D in the epileptics was 287 ± 39 IU. Serum 25-HCC was positively correlated to the daily vitamin D intake \((R = 0.44, P < 0.001)\). Table 2 correlates dietary vitamin D, serum 25-HCC and bone morphometry in the epileptic patients. Dietary vitamin D was positively correlated to the calcification rate \((P < 0.02)\) and inversely correlated to the percentage of osteoid surfaces \((P < 0.05)\) and to the relative osteoid volume \((P < 0.01)\). No significant correlations were found between serum 25-HCC and bone morphometry.

In the epileptic patients the mean value of serum iPTH was not significantly different from that of normal controls. However, 7 patients had slightly elevated levels. A significant inverse correlation \((R = -0.38, P < 0.01)\) was found between serum 25-HCC and serum iPTH (Fig. 2). No correlation was found between dietary vitamin D and serum iPTH \((R = -0.21, P > 0.05)\). Fig. 3 shows the correlations between serum 25-HCC and serum iPTH in two groups of epileptics with a dietary calcium intake of above and below 900 mg per day respectively. The inverse correlation between serum 25-HCC and serum iPTH was significant only in the group with low calcium intake. There was no correlation between serum iPTH and bone morphometry or between dietary calcium intake and bone morphometry.

Table 1.
Bone morphometry in epileptic patients and normal controls.

<table>
<thead>
<tr>
<th></th>
<th>AVTB</th>
<th>OS</th>
<th>OV</th>
<th>CR</th>
<th>RS</th>
<th>POL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>(\bar{x}) 22.9</td>
<td>9.2</td>
<td>1.5</td>
<td>0.88</td>
<td>3.3</td>
<td>52.0</td>
</tr>
<tr>
<td></td>
<td>se (n) 1.0 (16)</td>
<td>1.4 (16)</td>
<td>0.3 (16)</td>
<td>0.07 (12)</td>
<td>0.1 (16)</td>
<td>1.0 (16)</td>
</tr>
<tr>
<td>Epileptics</td>
<td>(\bar{x}) 23.4</td>
<td>14.9</td>
<td>4.1</td>
<td>0.73</td>
<td>5.0</td>
<td>71.6</td>
</tr>
<tr>
<td></td>
<td>se (n) 0.6 (59)</td>
<td>1.0 (58)</td>
<td>0.3 (57)</td>
<td>0.02 (39)</td>
<td>0.2 (59)</td>
<td>0.9 (59)</td>
</tr>
<tr>
<td>(P)</td>
<td>ns</td>
<td>(&lt; 0.01)</td>
<td>(&lt; 0.01)</td>
<td>(&lt; 0.05)</td>
<td>(&lt; 0.01)</td>
<td>(&lt; 0.01)</td>
</tr>
</tbody>
</table>

For explanation of signatures of bone morphometry, see text.

561
Serum levels of 25-HCC in normal subjects and in epileptic patients. Mean ± sd indicated by solid and interrupted lines.

Table 2.
Correlation between dietary vitamin D, serum 25-HCG and bone morphometry in epileptic patients. (Spearman's rank correlation).

<table>
<thead>
<tr>
<th></th>
<th>AVTB</th>
<th>OS</th>
<th>OV</th>
<th>CR</th>
<th>RS</th>
<th>POL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary vitamin D</td>
<td>R = 0.02</td>
<td>R = −0.30</td>
<td>R = −0.38</td>
<td>R = 0.40</td>
<td>R = 0.05</td>
<td>R = 0.01</td>
</tr>
<tr>
<td></td>
<td>ns</td>
<td>P &lt; 0.05</td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.02</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Serum 25-HCC</td>
<td>R = −0.03</td>
<td>R = 0.04</td>
<td>R = 0.06</td>
<td>R = 0.18</td>
<td>R = 0.04</td>
<td>R = −0.17</td>
</tr>
<tr>
<td></td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

For explanation of signatures of bone morphometry, see text.

562
Fig. 2.
Correlation between serum levels of 25-HCC and iPTH. \( R = -0.38, \ P < 0.01 \).

Fig. 3.
Correlation between logarithmic values of serum 25-HCC and of serum iPTH in epileptic patients with a dietary calcium intake above and below 900 mg per day.
The serum level of calcium, magnesium, phosphorus, alkaline phosphatase and the urinary phosphorus excretion was not correlated to bone morphometry, dietary vitamin D, serum 25-HCC or serum iPTH. The urinary calcium excretion (non restricted diet) was positively correlated to the vitamin D intake ($R = 0.33, P < 0.02$), not correlated to serum 25-HCC or bone morphometry and inversely correlated to serum iPTH ($R = -0.38, P < 0.01$).

DISCUSSION

The bone morphometry showed a mild degree of osteomalacia in the epileptic patients as demonstrated by an increased amount of osteoid and a decreased calcification rate, reported in detail elsewhere (Melsen & Mosekilde 1976). In accordance with previous reports (Hahn et al. 1972b, 1975; Stamp et al. 1972; Belsey et al. 1974) the mean value of serum 25-HCC was lower in the epileptics than in normal controls. However, the levels of serum 25-HCC were higher than those reported elsewhere both in epileptics and controls. This is undoubtedly due to a high vitamin D intake in the Danish population, about 200 IU per day (Hansen 1973), and an even higher average intake in the present epileptic group. In accordance with other studies we found a positive correlation between serum 25-HCC and the vitamin D intake (Hahn et al. 1972b, 1975).

Anticonvulsant drug-induced osteomalacia is considered to stem from induction of hepatic P-450 microsomal enzyme activity, resulting in decreased plasma $[^3]$Hcholecalciferol half life, increased formation rate of 25(OH) cholecalciferol and more polar metabolites, increased biliary excretion of these metabolites and reduced tissue levels of biologically active vitamin D metabolites (Hahn et al. 1972a; Silver et al. 1974). The present study shows that the degree of osteomalacia is related to the mean yearly dietary vitamin D intake, but not to the actual serum level of 25-HCC. This inconsistency might be explained in several ways. Firstly, the serum level of 25-HCC will mainly reflect the vitamin D intake of the last few days or weeks, whereas the dietary vitamin D intake in this study was calculated as the mean daily intake throughout the year. It is comprehensible that the bone morphology rather reflects the longterm vitamin D effect than the short-term effect. Secondly, disturbed metabolism of 1,25(OH)$_2$cholecalciferol may well contribute to the anticonvulsant osteomalacia, since it has been shown that liver microsomes from animals treated with phenobarbitone convert 1,25 (OH)$_2$ cholecalciferol to more polar metabolites (Hahn et al. 1973). Furthermore phenobarbitone treatment results in markedly lower quantities of 1,25 (OH)$_2$ cholecalciferol in chick bone (Norman et al. 1976).
Serum 25-HCC was inversely correlated to iPTH. A similar relation has been reported in patients with advanced renal failure (Offermann & Dittmar 1975) and in primary hyperparathyroidism (Arnaud et al. 1975). In the present study the correlation was significant only in patients on low dietary calcium intake, indicating than an adequate supply of vitamin D is essential for the adaptation to a low calcium intake. Serum iPTH was slightly elevated in 12% of the patients, but the mean value was not significantly increased compared to that of normal controls. The normal range of iPTH here reported is lower than generally presumed, but is in agreement with the normal range recently reported by Hannsjoerg et al. (1975). There was no correlation between serum iPTH and morphological bone changes, and the increase in trabecular osteoclastic resorption surfaces and mean volume of periosteocytic lacunae could not be explained by increased serum levels of iPTH.

ACKNOWLEDGMENTS

This investigation was supported by grants from the Danish Medical Research Council and from the Medical Research Council for Copenhagen, Faroe Islands and Greenland.

REFERENCES


Received on April 26th, 1976.