CLINICAL STUDY

Immediate changes in biochemical markers of bone turnover and circulating interleukin-6 after parathyroidectomy for primary hyperparathyroidism

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Abstract

Objective: The time course of the immediate change in bone turnover after parathyroidectomy (PTX) for primary hyperparathyroidism (PHPT) is not clear. It is uncertain whether circulating interleukin-6 (IL-6) plays a role in mediating the acute withdrawal of the effects of parathyroid hormone (PTH) on bone turnover after PTX. The aims of this study were to determine the time course of immediate changes in biochemical markers of bone turnover after PTX and whether circulating IL-6 is involved in the immediate changes of bone turnover after PTX.

Design and Methods: IL-6 and bone turnover markers were measured in eight women (aged 55 ± 11 years, mean $\pm$ S.D.) with PHPT at baseline and at 1–2 h, and 1, 2, 5, 7 and 12 days after PTX. We compared the results with those from eight individually matched women (healthy controls) and five subjects undergoing major surgery (surgical controls).

Results: At baseline, serum levels of IL-6 and bone turnover markers were higher in PHPT than those in healthy controls ($P < 0.05$). Serum levels of procollagen propeptides increased by 22 and 27% at days 2 and 5, respectively, compared with baseline ($P < 0.05$). Serum tartrate-resistant acid phosphatase decreased by 2 days after PTX, and urinary collagen crosslinks decreased significantly by 21–41% within 24 h ($P < 0.05$). Serum IL-6 levels increased immediately in both PHPT and surgical controls at postoperative follow-up (repeated measures ANOVA).

Conclusions: (1) PTX decreases bone resorption immediately and (2) circulating IL-6 is not involved in the changes in bone turnover immediately after PTX.

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Introduction

The immediate effects of parathyroid hormone (PTH) on bone could be examined by injecting PTH or by studying changes immediately after parathyroidectomy (PTX). It has been reported that PTH infusion increases urinary pyridinoline (Pyd), deoxypyridinoline (Dpd) (1) and hydroxyproline (OHP) (1–4), and decreases serum procollagen type I C-terminal propeptide (PICP) (5, 6) and osteocalcin (bone Gla-protein, BGP) (4). Bone turnover is increased in patients with primary hyperparathyroidism (PHPT). The increased bone turnover may lead to osteopenia which is the most common skeletal abnormality in PHPT even though it is debated whether mild to moderate PHPT causes osteopenia (7–9). PTX may restore bone turnover to normal. However, the time course of the restoration in bone turnover immediately after PTX is unclear.

It is generally agreed that PTH does not act directly on osteoclasts to stimulate bone resorption. PTH increases

the number and activity of osteoclasts by acting on osteoblasts which release cytokines such as interleukin-6 (IL-6) (10). Recently, a study reported that serum circulating levels of IL-6 and IL-6 soluble receptors were increased significantly in untreated patients with PHPT and fell into the normal range after surgery (11). It is unknown whether the changes in biochemical markers of bone resorption are explained by the changes in circulating IL-6 immediately after surgery. The aims of this study were to determine the time course of immediate changes in biochemical markers of bone turnover after PTX and whether circulating IL-6 is involved in the immediate changes in bone turnover after PTX.

Subjects

Eight women (aged 55 ± 11 years, mean $\pm$ S.D.) with PHPT were recruited from the Department of Surgery

at the Northern General Hospital, Sheffield. Five of the eight patients were postmenopausal. These women were 2–21 years postmenopause (8 ± 5 years). Patients had no other diseases known to affect bone metabolism or history of fracture in the preceding 2 years. Patients were not taking any medication known to affect bone metabolism, such as oestrogen, glucocorticoids, anti-convulsants, vitamin D or calcium supplements. The diagnosis of PHPT was based on preoperative clinical and laboratory criteria and postoperative pathology. Serum Ca levels ranged from 2.70 to 2.95 mmol/l (2.80 ± 0.12 mmol/l) and PTH levels ranged from 34 to 134 pg/ml (110 ± 55 pg/ml). Serum PTH levels were above the upper limit of reference interval (63 pg/ml) in seven of the eight patients. Eight healthy controls individually matched for age and sex were used for comparison to baseline values. Five patients (four women and one man, aged 33–76 years) who underwent cholecystectomy, sigmoid colectomy or hemicolectomy were recruited as surgical controls. All control subjects were free from diseases and were not taking any medication known to affect bone metabolism. All participants gave written informed consent, and the study was approved by the North Sheffield Local Research Ethics Committee.

Methods

Serum levels of IL-6, BGP, bone alkaline phosphatase (BAP), PICP, N-terminal of type I procollagen (PINP) and tartrate-resistant acid phosphatase (TRAP) were measured in patients with PHPT at baseline and at 1–2 h, and 1, 2, 5, 7 and 12 days after PTX. Serum levels of biochemical markers of bone turnover and IL-6 were also measured in the five surgical controls at baseline and 2, 7 and 14 days after operation. Biochemical markers of bone turnover and IL-6 were measured only at baseline in the eight age-matched women as healthy controls. Baseline samples (preoperative) were collected twice from five of the eight patients with PHPT. In these five patients, baseline values were taken as the mean of the two baseline collections. All blood samples, except those taken 1–2 h after PTX, were taken in the fasting state. All blood samples were centrifuged for 30 min at 2000 *g* after they were taken and the serum was separated and stored at -70°C . Serum samples were measured at the end of the study in duplicate in a single analytical batch.

Two hour urine samples (second morning void) were collected in PHPT group and healthy control group in this study. Urinary levels of total Pyd, total Dpd and cross-linked N-telopeptide of type I collagen (NTx) were measured at the same times as measured for blood samples except at 1–2 h after PTX.

Serum IL-6 was measured by ELISA (Quantikine, R&D Systems, Inc., Minneapolis, USA). Serum BGP was measured by ELISA (NovoCalcin, Metra Biosystems, Inc., Mountain View, USA). Serum BAP was measured

by the wheat germ lectin precipitation method (12). Serum PICP and PINP were measured by RIA (Orion Diagnostica, Espoo, Finland). Urinary levels of total Pyd and Dpd were measured by HPLC after acid hydrolysis (13). Urinary NTx was measured by ELISA (Osteomark, Ostex International, Inc., Seattle, WA). Urinary creatinine (Cr) was measured in the Department of Clinical Chemistry at Northern General Hospital, Sheffield, using a dry slide chemistry autoanalyser (Johnson and Johnson, Ektachem 950). Urinary levels of Pyd, Dpd and NTx were expressed as a ratio to urinary level of Cr. The overall intra- and inter-assay coefficients of variation were 4 and 6% for IL-6, 7 and 10% for BGP, 3 and 5% for BAP, 6 and 8% for PICP, 6 and 8% for PINP, 2 and 4% for TRAP, 5 and 7% for NTx, 7 and 8% for Pyd and 9 and 10% for Dpd, respectively. The minimal detectable concentration of IL-6 was determined to be less than 0.70 pg/ml by adding two standard deviations to the mean optical density value of 20 zero standard replicates and calculating the corresponding concentration. The assay recognises both natural and recombinant human IL-6 and no significant cross-reactivity with any factors related to or associated with IL-6 has been observed.

Non-parametric statistical analysis was used in this study due to the small sample size. The Wilcoxon signed rank test was used to compare the difference between patients with PHPT and age- and sex-matched healthy controls at baseline. The repeated measures Friedman test with multiple comparison (Dunn's test, $P < 0.05$) was used to compare the changes between different time points for patients with PHPT and surgical controls. Area under the curve (AUC) and Kruskal–Wallis tests were used to compare the percentage change between different biochemical markers of bone resorption after PTX. Spearman's rank correlation was used to examine the relationship between biochemical markers of bone turnover and serum IL-6 at baseline.

Results

Baseline

Serum levels of IL-6, BGP, BAP, PINP, and urinary ratios of Pyd/Cr, Dpd/Cr and NTx/Cr were significantly higher PHPT than in healthy controls (Wilcoxon signed rank test $P = 0.001, 0.02, 0.06, 0.04, 0.01, 0.005$ and 0.007 , respectively, Figs 1 and 2). The serum level of IL-6 did not correlate significantly with any biochemical marker of bone turnover.

Follow-up

Serum IL-6 levels increased immediately (by 1–2 h) with a maximum increase 1 day after PTX in PHPT, and had increased by 2 days after operation in surgical controls (repeated measures Friedman's test with Dunn's test at $P < 0.05$, Fig. 3).

Figure 1 Serum IL-6 concentration at baseline in patients with PHPT and in age- and sex-matched controls (upper panel) and percentage changes after operation in patients with PHPT (middle panel) and surgical controls (lower panel). Asterisks indicate $P < 0.05$. Solid lines represent mean change $\pm$ S.E.M. Fine lines represent change for individual patients.

Serum levels of BGP and BAP decreased transiently from day 1 after PTX and then began to increase on days 5 and 12, respectively. Compared with baseline, serum levels of BGP and BAP did not change significantly even though there was a tendency to decrease within 5 days of PTX. Serum levels of PICP and PINP began to increase on day 2 and day 5, respectively (repeated measures Friedman's test, Fig. 3). The maximum increase was found to occur on day 5 for PINP (22%) and day 12 for PICP (27%). Biochemical markers of bone formation did not change significantly in the five surgical controls during follow-up (repeated measures Friedman's test $P > 0.05$, Fig. 4). However, serum levels of BGP and BAP showed a tendency to decrease, and PICP and PINP showed a tendency to increase.

There was no significant difference between biochemical markers of bone resorption measured in healthy controls and these markers measured at any time in patients with PHPT after PTX. Compared with baseline, serum TRAP decreased significantly from day 2 after PTX whereas urinary Pyd/Cr, Dpd/Cr and NTx/Cr decreased significantly from day 1 after PTX (repeated measures Friedman's test, Fig. 5). The maximum decrease was found to occur on day 7 (21%) for serum TRAP and on day 1 for urinary Pyd/Cr (21%), Dpd/Cr (33%) and NTx/Cr (41%) after PTX. Serum TRAP did not change significantly in the surgical controls (not shown). We did not find significant differences in postoperative change (AUC) between serum TRAP, urinary Pyr/Cr, Dpyr/Cr and NTx/Cr after PTX in patients with PHPT (one-way ANOVA $P > 0.05$).

Discussion

PTH infusion studies have shown that PTH increases bone resorption (1–4) and decreases bone formation (4–6). Therefore, a decreased bone resorption and an increased bone formation would be expected shortly after PTX. The interesting finding in the present study was that all collagen-derived markers of bone resorption decreased and normalised immediately within 24 h whereas serum levels of BAP and BGP did not change significantly after PTX, which contrasts with PTH infusion studies and some previous PTX studies (14,15) but not all (16–20).

Indeed, serum levels of BGP and BAP decreased slightly shortly after PTX in the present study but similar changes were found in the surgical controls. This could be explained by the stress of surgery. Elevated levels of serum cortisol have been reported for up to 3 days after major abdominal surgery (21, 22). Serum levels of PICP and PINP increased significantly in PHPT after PTX in the present study. However, serum levels of PICP and PINP also showed a tendency to increase in surgical controls. Following major abdominal surgery, increased procollagen synthesis has been found within 2–4 days at the site of the wound

Figure 2 Biochemical markers of bone turnover at baseline in patients with PHPT and healthy controls. Asterisks indicate $P < 0.05$.

(23–25), and serum PICP started to increase from day 4 (25) with a maximum increase occurring between days 10 and 21 (26, 27). Serum PICP has been reported to be increased 3–5 days after PTX (18, 19) which is consistent with the present study. In the present study, both PICP and PINP increased about 20% from 5 to 7 days after surgery in surgical controls. This result suggests that in PHPT after PTX, the increased procollagen synthesis in the early phase represents increased bone matrix synthesis whereas in the late phase represents wound healing.

Urinary levels of Pyd/Cr, Dpd/Cr and NTx/Cr decreased significantly within 24 h after PTX. The immediate decrease in bone resorption cannot be explained by a slowing down of osteoclast recruitment

which would last days or weeks. The rapid decrease in bone resorption could be explained by the immediate release of existing osteoclasts from their stimulation by PTH. It has been suggested that PTH acts later in the osteoclast lineage rather than in the formation of new osteoclasts (10). This finding also supports the hypothesis that PTH acts on the osteoclast itself, although this is controversial (10).

Garton and colleagues (15) showed that urinary Dpd decreased by 48 h after PTX in ten patients (no samples were taken within 24 h after PTX in that study) whereas urinary Pyd did not change significantly during 6 months follow-up after PTX. Urinary Pyd has been reported to be less specific to bone than urinary Dpd since urinary Pyd may originate from

Figure 3 Changes in biochemical markers of bone formation in patients with PHPT before and after PTX. Asterisks indicate $P < 0.05$ compared with baseline.

Figure 4 Changes in biochemical markers of bone formation in surgical controls before and after surgery. Asterisks indicate $P < 0.05$ compared with baseline.

Figure 5 Changes in biochemical markers of bone resorption in patients with PHPT before and after PTX. Asterisks indicate $P < 0.05$ compared with baseline.

tissues containing type II collagen such as cartilage. Previous studies reported that the maximum decrease in serum TRAP level occurred between 5 and 10 days after PTX in patients with PHPT (16, 19). This is consistent with the present study. Serum TRAP as an enzyme released from osteoclasts represents osteoclast numbers whereas pyridinium crosslinks of collagen represent osteoclast function. Osteoclast apoptosis may take a few days. Another possibility is that the half-life for TRAP in serum may be longer than that for crosslinks.

It is likely that most of the decrease in collagen degradation occurs in the first postoperative day. We found a decrease of 41% in NTx/Cr on day 1, similar to the 52% decrease we reported previously for up to 20 months follow-up after PTX (7). In a study with up to 24 months follow-up after PTX, Seibel and colleagues (28) reported that although urinary Dpd decreased by 37% in 0.5–6 months after PTX, a further decrease (up to 53%) was found in 7–12 months after PTX compared with baseline. Nevertheless, our results showed that significant decrease of bone resorption occurred within 24 h even though a further small decrease in long-term follow-up may occur.

PTH receptors in bone cells are found only in osteoblasts or osteoblast precursors and the mechanisms of the effect of PTH on osteoclasts are generally considered to be mediated by cytokines such as IL-6 (10). A recent study has reported that serum levels of IL-6 and IL-6 soluble receptors were increased significantly in untreated patients with PHPT and fell into the normal range after surgery (11). We confirmed high baseline IL-6 levels in PHPT. If IL-6 were the main determinant of bone resorption, we would have expected a immediate decrease of serum IL-6 after PTX. However, the effects of PTH on bone resorption are unlikely to be mediated by IL-6 since serum levels of IL-6 increased in both patients with PHPT and surgical controls after surgery. This result indicated that the increased IL-6 concentration was not related to bone turnover directly. The concentration of IL-6 in serum and plasma has been shown to increase in response to surgery and has been proposed as a sensitive marker of tissue damage (29–31). Since local IL-6 levels in bone may not parallel circulating IL-6 levels, we cannot entirely rule out the possibility that the increased circulating IL-6 level induced by surgical trauma masks a decrease in bone IL-6 levels induced by a decrease in PTH level.

We conclude that (1) PTX results in an immediate decrease in bone resorption and (2) circulating IL-6 is not involved in the changes of bone turnover immediately after PTX.

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