Parathyroid stimulation after bleeding in man

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A role for the PTH-calcium axis in the normal bone-marrow response to bleeding or erythropoietin administration has been demonstrated in rats. We studied 20 autologous blood donors, each donating two units of blood, who served as a human bleeding model. Fifteen patients completed the study. Blood donations were followed by a significant increase in serum intact PTH (2.15 ± 0.67 to 2.81 ± 0.84 pmol/l; p = 0.0003) and protein-corrected total calcium (2.43 ± 0.09 to 2.49 ± 0.08 mmol/l; p = 0.02). All the individual values remained within the normal range. PTH weakly correlated with the reticulocyte count, but not with the corrected serum calcium. We conclude that moderate bleeding in humans is followed by a physiological increase in serum PTH and calcium.

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Studies in rats demonstrated that the increased mitotic activity in the bone marrow in response to bleeding or exogenous erythropoietin was associated with mild elevation of serum calcium levels, and was dependent on the presence of intact parathyroid glands (1, 2). Following bleeding, a parathyroid-dependent increase in the number of osteoclasts in rat bones was observed (3). A 30% increase in serum PTH levels was found in patients undergoing skeletal surgery accompanied by a moderate blood loss (4); however, the relative contribution of bleeding and skeletal trauma could not be separated.

The purpose of the present study was to examine in man whether stimulation of marrow activity, without bone injury, is associated with changes in PTH and related biochemical indices. To this end, autologous blood donors, each giving two units of blood, were studied before and following blood donations.

Patients and methods

We studied twenty consecutive autologous blood donors (age 15–79 years, 16 females, 18 caucasian) without an overt bone or mineral metabolic disorder, each having an initial hematocrit level of > 35%. All patients were scheduled for elective surgical procedures: hip arthroplasty (N = 5), knee arthroplasty (N = 5), spinal surgery (N = 5), hysterectomy (N = 2), mammoplasty, aortic bypass and prostatectomy (one each). One patient was later found to have mild hyperparathyroidism, probably secondary to long-term treatment with hydantoin, and was excluded from further analysis. Two patients had increased serum alkaline phosphatase which could be attributed to known biliary disease. Serum calcium and inorganic phosphate levels were normal in all patients.

Three patients were on thyroid replacement therapy; they were all euthyroid. One of them also received low dose glucocorticoid (7.5 mg/day prednisone) for rheumatoid arthritis, but had no evidence of secondary hyperparathyroidism. All patients provided informed consent to participate in the study.

The patients donated one unit of blood (approximately 450 ml) on two separate occasions, within four weeks prior to surgery. At each blood donation, additional blood was drawn for study purposes (t0 and t1); a final specimen was obtained on the day of admission, prior to the surgery (t2). All study samples were obtained between 09.00 and 15.00. The third specimen was not available in four patients, in whom the surgery was postponed for various reasons. Of the remaining 15 patients, the time interval between blood sample acquisition was one week in six (group a) and two weeks in nine (group b) (Table 1).

Hematocrit (Hct) and reticulocyte count (RTC) were determined by standard laboratory methods. Serum total calcium, inorganic phosphate, alkaline phosphatase, creatinine, total protein, albumin and globulin (by difference) were measured by SMAC (sequential multiple analyzer-computer). Total calcium was corrected for serum proteins using the formula (5):

$$\text{Corrected calcium} = \text{measured calcium} + [0.029 \times (45.2 - \text{albumin})] + [0.07 \times (25.9 - \text{globulin})]$$

(albumin and globulin values in g/l). Ionized calcium was measured by ICA–Ionized Calcium Analyzer (Radiometer, Copenhagen). Intact PTH was determined by immunoradiometric assay using an Allegro Intact PTH kit (Nichols Institute Diagnostics, San Juan Capistrano, CA) and calcitriol by a radioreceptor assay kit (INCSTAR, Stillwater, MN). Serum samples for the determination of PTH and calcitriol were stored frozen (−20°C) and
Table 1. Time sequence of sample acquisition.

<table>
<thead>
<tr>
<th>Time interval (weeks)</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>x</td>
</tr>
<tr>
<td>1</td>
<td>x</td>
</tr>
<tr>
<td>2</td>
<td>x</td>
</tr>
<tr>
<td>3</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

- Three samples measured (15 patients): 6 (Group a)
- Two samples measured (4 patients): 1

\(^{a}\) Another patient from this group was excluded from analysis because hyperparathyroidism was subsequently diagnosed.

analyzed in two batches for each; we have previously demonstrated that such storage has no significant effect on the results.

Repeated measures analysis of variance (ANOVA) was used to test the effect of bleeding and whether the difference in time-interval between measurements (i.e. group a vs. group b) affected the change in each variable over time (interaction effect) (6). When an interaction effect was observed, two sample \( t \)-tests were performed between groups (a and b) at each time and a separate repeated measures ANOVA was performed for each time-interval group. If a bleeding effect was found with ANOVA, paired \( t \)-tests, with a Bonferroni adjusted \( p \) value of 0.017 (=0.05/3), were done to determine when those effects occurred. Correlations were computed for each primary variable, as well as each time change, using Pearson’s correlation coefficient with a Bonferroni adjusted \( p \)-value of 0.017.

Results

At baseline, PTH weakly correlated with RtC \((r=0.41; p=0.08)\), but not with Hct \((r=0.15)\) or calcium \((r=-0.05)\). The mean \((\pm SD)\) time intervals from baseline to second measurement were 11.8±3.7 days and from baseline to third measurement 22.1±5.6 days. Further statistical analysis was confined to the 15 patients who completed the study.

The mean \((\pm SD)\) values of the biochemical and hematological variables at the three time points are given in Table 2. Except for serum creatinine, there was no interaction between bleeding effect and group effect, thus both groups (a and b) were combined. The repeated measures ANOVAs revealed that bleeding was followed by a highly significant decrease in Hct \((p=0.0001)\), but the changes in RtC were only suggestive of a bleeding effect \((p=0.10)\), presumably due to the large variance of the measured values. Of the biochemical variables, there

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Table 2. The effect of autologous blood donations on blood measurements (mean±SD).

<table>
<thead>
<tr>
<th>Variable</th>
<th>(N)</th>
<th>(t_0)</th>
<th>(t_1)</th>
<th>(t_2)</th>
<th>ANOVA</th>
<th>(t_1) vs (t_0)</th>
<th>(t_2) vs (t_1)</th>
<th>(t_2) vs (t_0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematocrit (%)</td>
<td>15</td>
<td>39.2±3.9</td>
<td>36.3±3.8</td>
<td>35.6±4.0</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.027</td>
<td>0.0002</td>
</tr>
<tr>
<td>Reticulocytes (%)</td>
<td>10</td>
<td>1.47±0.94</td>
<td>1.85±0.84</td>
<td>2.24±1.69</td>
<td>0.10</td>
<td>0.023</td>
<td>0.05</td>
<td>0.017</td>
</tr>
<tr>
<td>PTH (pmol/l)</td>
<td>15</td>
<td>2.15±0.67</td>
<td>2.31±0.75</td>
<td>2.81±0.84</td>
<td>0.0017</td>
<td>0.197</td>
<td>0.013</td>
<td>0.0003</td>
</tr>
<tr>
<td>Total calcium (mmol/l)</td>
<td>13</td>
<td>2.43±0.09</td>
<td>2.43±0.08</td>
<td>2.49±0.08</td>
<td>0.02</td>
<td>1.003</td>
<td>0.02</td>
<td>0.027</td>
</tr>
<tr>
<td>Ionized calcium (mmol/l)</td>
<td>13</td>
<td>1.27±0.05</td>
<td>1.29±0.05</td>
<td>1.30±0.04</td>
<td>0.31</td>
<td>0.654</td>
<td>0.019</td>
<td>0.06</td>
</tr>
<tr>
<td>Phosphate (mmol/l)</td>
<td>13</td>
<td>1.15±0.25</td>
<td>1.12±0.21</td>
<td>1.26±0.22</td>
<td>0.05</td>
<td>0.194</td>
<td>0.013</td>
<td>0.0003</td>
</tr>
<tr>
<td>Alkaline phosphatase (U/l)</td>
<td>13</td>
<td>115±50</td>
<td>105±38</td>
<td>109±34</td>
<td>0.85</td>
<td>0.654</td>
<td>0.019</td>
<td>0.06</td>
</tr>
<tr>
<td>Calcitriol (pmol/l)</td>
<td>9</td>
<td>59.8±9.1</td>
<td>65.5±15.1</td>
<td>61.9±14.2</td>
<td>0.46</td>
<td>0.194</td>
<td>0.013</td>
<td>0.0003</td>
</tr>
<tr>
<td>Creatinine (pmol/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Weekly (Group a)</td>
<td>6</td>
<td>99.0±23.0</td>
<td>85.7±18.6</td>
<td>101.7±27.4</td>
<td>0.25</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Biweekly (Group b)</td>
<td>7</td>
<td>78.7±16.8</td>
<td>94.6±13.3</td>
<td>93.7±19.4</td>
<td>0.10</td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^{a}\) \(t_0\), \(t_1\), \(t_2\) = see explanations in the text. \(^{b}\) PTH = intact serum parathyroid hormone. \(^{c}\) Total calcium = corrected for protein binding.
was a highly significant increase in serum PTH (p = 0.017) and corrected total serum calcium (p = 0.02) but not serum ionized calcium (p = 0.31; Table 2). There was a mild increase in serum phosphate (p = 0.05).

Paired t-test revealed that the decrease in Hct and the increase in RtC took place mainly after the first blood donation, while the increases in serum PTH, corrected total calcium and phosphate occurred mainly after the second donation (Table 2). The individual PTH values in all the 19 patients, including the four in whom only the first two measurements were available, are plotted in Fig. 1: the values at last measurement were higher than at baseline in all but two subjects.

In addition to the correlation observed at baseline, PTH correlated significantly with RtC also at t1 (N = 10; r = 0.67; p = 0.02), but not t2 (N = 10; r = 0.10); no significant correlation was found between the changes over time in PTH vs RtC. Neither the absolute values nor the changes between measurements of PTH correlated with the corresponding values of Hct or corrected total serum calcium.

Discussion

Our study did not include a control group, so we cannot exclude the possibility that some non-specific factor such as apprehension of major surgery could have influenced the results. With that proviso, we demonstrated that moderate bleeding in humans was followed by a statistically significant physiological increase in serum PTH and calcium levels of similar magnitude to that observed following orthopedic surgeries accompanied by a similar decrease in blood loss (4). Our results indicate that bone trauma probably did not contribute to the increase in PTH levels in the earlier study.

Blood loss may be followed by acute depression of renal function, but our sampling was delayed for at least one week, by which time no change in serum creatinine was observed. Furthermore, the intact PTH assay is unaffected by reduced renal clearance of inactive C-terminal fragments of PTH (7). Of the major physiological stimuli to increased PTH secretion (8), our results exclude both reduced serum calcitriol and hypocalcaemia; indeed, the rise in corrected serum calcium was likely the result of the increase in PTH. Serum magnesium was not measured, but no fall in serum magnesium following blood loss has been reported, or would be expected.

It is unlikely that volume depletion, or physiological consequences such as adrenergic stimulation (9), influenced the PTH levels, since our patients were studied at least one week after the last blood donation and none of them had hypotension or tachycardia. Furthermore, rats in which blood was removed and immediately replaced with an equal volume of homologous plasma still became hypercalcemic, while those in which replacement was by blood had no change in serum calcium, suggesting that the mild hypercalcemia was the consequence of loss of blood cells rather than hypovolemia (1).

The relationships between the stimulation of marrow activity, increase in serum erythropoietin and increases in serum PTH and calcium are complex and not yet fully understood. An early fall in serum ionized calcium was observed in rats after blood loss (1), probably the result of tissue anoxia, which could stimulate the parathyroids; however, when parathyroid-dependent marrow stimulation was elicited by exogenous erythropoietin, no decrease in serum calcium preceded the hypercalcemic response (2). In both experiments, the concomitant increase in bone marrow activity and in serum calcium was abolished by previous parathyroidectomy and, in the former, restored by injecting PTH (1).

Based on these results, it has been proposed that erythropoietin, or some erythropoietic agent, stimulates PTH secretion, leading to a rise in serum calcium which in turn promotes cell division in the bone marrow (10), thus allowing the erythrocyte deficit to be repaired more rapidly than would be possible in the absence of parathyroid gland (1). Our results are consistent with this theory. The increase in serum PTH was not due to any of the well established causes, and was accompanied by a significant increase in corrected total calcium. As expected from previous studies of blood donors (11), there was an increase in RtC of similar magnitude to the increase in serum PTH; although the increase was not quite statistically significant, there was a modest correlation between PTH and RtC at t0 and t1, but not t2. Measurement of serum erythropoietin, which was not done in this study, could probably shed some light on the assumption that the increase in serum PTH following blood donation was indeed caused by increased erythropoietin release.

In summary, we have demonstrated that moderate bleeding was followed by an increase, within the physiological range, in serum intact PTH, and (presumably as a result) an increase in serum calcium. The relationship between marrow regeneration and the parathyroid-calcium axis in man merits further investigation.

References


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