Primary hyperparathyroidism is associated with increased circulating bone marrow-derived progenitor cells

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Submitted 9 May 2007; accepted in final form 27 September 2007

BONE MARROW-DERIVED PROGENITOR CELLS (BMC) are the source of all mature blood cells. They reside in niches of bone marrow and can be released into the circulation (1, 38). Mobilization of BMC into peripheral blood occurs in response to different stimuli, including chemokines, hormones, and growth factors (2, 6, 11, 12, 14, 16, 19, 24, 32, 33). Endothelial progenitor cells (EPC) are a subpopulation of BMC known to contribute to vessel formation and restoration (8, 13, 34, 36). Mobilization of EPC can also be induced by traumatic vascular injury or other forms of physiological stress like tissue ischemia or hypoxia (17, 23, 24, 27, 39). Besides their vasculoprotective function, EPC can also play a maladaptive role in tumor progression (17, 25, 26). It has been demonstrated (25, 26) that EPC may infiltrate tumors and give rise to up to 16% of the tumor neovascularization.

Starting in the late 1950s, an effect of parathyroid hormone (PTH) on the hematopoietic system was shown. Administration of PTH stimulated proliferation of murine colony-forming unit spleen and bone marrow cells via cAMP, leading to an increased survival of X-irradiated mice (37). Recently, Calvi et al. (4) were able to demonstrate that PTH strengthens survival and self-renewal of hematopoietic BMC by activating osteoblasts. In addition, activated osteoblasts were shown (18, 22, 29–31) to increase the production of growth factors known to enhance mobilization of BMC into the peripheral blood. In the present study we wanted to investigate the effect of primary hyperparathyroidism (PHPT), a condition of sustained PTH stimulation, on mobilization of BMC.

PATIENTS AND METHODS

Patients. Twenty-four patients with untreated PHPT and 10 controls were enrolled into the study prospectively. The patients with PHPT included in the study (15 women and 9 men) were recruited from the Department of Surgery at the Hospital Martha-Maria in Munich, Germany. The inclusion criteria for the PHPT group comprised an increased total serum concentration of calcium (>2.65 mmol/l), an inappropriately high serum level of PTH (>67 pg/ml), serum creatinine <1.3 mg/dl, postoperative normalization of serum calcium, and sonographic and histological confirmation of parathyroid adenoma (3, 10). The mean age of the patients was 58.7 ± 17.3 yr (range 26–80 yr), and their mean total serum calcium concentration was 2.88 ± 0.04 mmol/l before parathyroid surgery. Laboratory findings are summarized in Table 1.

Eight patients were on antihypertensive or antianginal treatment in various combinations before surgery. Three had ß-blockers, one had a calcium channel blocker, five had angiotensin-converting enzyme inhibitors, and three had diuretics. Besides typical concomitant PHPT-dependent disorders, patients suffered from the following disorders: one had non-insulin-dependent diabetes mellitus, two suffered from coronary artery disease without signs or symptoms of congestive heart failure, 13 patients had a multinodular goiter with normal levels of TSH, free triiodothyronine (FT3), and free thyroxine (FT4), and two suffered from papillary thyroid carcinoma. Clinical characteristics are presented in Table 2.

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The control group was age matched and unpaired. Control subjects had no severe disorders. However, seven of the controls receiving β-blockers, angiotensin-converting enzyme inhibitors, and/or diuretics suffered from arterial hypertension.

Initially, 24 patients with PHPT were assessed for the study. However, two did not fulfill inclusion criteria. Blood samples were taken 12–24 h before surgery. Fourteen of 22 samples were utilisable for cytometric analyses. Eight patients agreed to followup examinations, which were performed 16.7 ± 2.3 mo after surgery.

The purpose, nature, and potential risks of the study were explained to the patients before their written, informed consent was obtained. The protocol was approved by the Ethics Committee on Human Research of the Ludwig-Maximilians University of Munich, Germany.

**Quantification of BMC.** Cytometric analyses were performed using a flow cytometer (FACScan; Becton Dickinson, Heidelberg, Germany) according to International Society of Hematotherapy and Graft Engineering guidelines and to a standard protocol (21). Each analysis included 100,000 events. For immunophenotyping, the following monoclonal antibodies were used: CD45 (conjugated with peridinin-chlorophyll-protein, clone 2D1, no. 345809; BD Biosciences, Heidelberg, Germany), CD34 (conjugated with FITC, clone 581, no. IM 1870; Beckman Coulter Immunotech, Marseille, France), CD31 (conjugated with phycoerythrin (PE), clone WM-59, no. 555446; BD Pharnmcien, Heidelberg, Germany), CXCR4 (conjugated with PE, clone 12G5, no. 555974; BD Pharnmcien, Heidelberg, Germany), and c-kit (conjugated with PE, clone 9S3, no. PN IM 1360 U; Beckman Coulter, Krefeld, Germany). The following subpopulations of BMC were analyzed: CD45+/CD34+/CD31+ cells representing endothelial progenitor cells, CD45+/CD34+/c-kit+ cells representing hematopoietic stem cells, and CD45+/CD34+/CXCR4+ cells representing progenitor cells with the homing receptor CXCR4. Samples were excluded from evaluation in case of high amounts of cell detritus or smudge cells (>20%), no exact delimitable population in any dot plot, or high numbers of positive cells in the isotype control (>2%). Exact numbers of evaluated subjects are included in the figures and tables.

**Biochemical measurements.** Prior to the surgery the following routine laboratory parameters were assessed: serum concentration of intact PTH, total calcium, inorganic phosphate, sodium, potassium, creatinine, urea nitrogen, aminotransferases, gammaglutamyl transferase, alkaline phosphate, glucose, complete blood count (including Hb), Hct, leukocytes, and platelets. In addition, thyroid parameters TSH, FT₃, and FT₄ were measured. Serum levels of stem cell factor (SCF), stromal cell-derived factor 1 (SDF-1), VEGF, erythropoietin (EPO), and granulocyte colony-stimulating factor (G-CSF) were analyzed using ELISA (R & D Systems, Wiesbaden, Germany).

**Statistical analysis.** Results are expressed as means ± SE as indicated. Comparisons between two groups were performed using the unpaired t-test. Values of P < 0.05 were considered statistically significant. Comparison of categorical variables was generated by the χ² Pearson test. Statistics were calculated using SPSS for Windows (release 14.0; SPSS, Chicago, IL).

**RESULTS**

**Clinical characteristics.** Patients with PHPT showed characteristic laboratory findings with increased concentrations of PTH (164.8 ± 14.4 pg/ml), increased total calcium levels (2.88 ± 0.04 mmol/l), and decreased levels of inorganic phosphate (2.42 ± 0.11 mg/dl). All other measured parameters were within normal limits (see Table 1).

The patients suffered from the following typical concomitant PHPT-dependent disorders: eight from nephrolithiasis (36%), eight from osteopenia (36%), one from gastritis (4.5%), and one from gallstones (4.5%), and one from acral paresthesia (4.5%). In addition, 13 patients had multinodular goiter (59%), two had papillary thyroid carcinoma (9%), eight had arterial hypertension (36%), two had coronary artery disease (9%), and one had diabetes mellitus (4.5%) (see Table 2).

**BMC in peripheral blood.** In patients suffering from PHPT, CD45+/CD34+/c-kit+ (1.78 ± 0.26 vs. 0.62 ± 0.06 cells/µl, P < 0.05) and CD45+/CD34+/CXCR4+ cells (0.89 ± 0.10 vs. 0.46 ± 0.14 cells/µl, P < 0.05) were significantly increased in peripheral blood compared with the control levels. Levels of CD45+/CD34+/CD31+ cells were increased in trend (2.26 ± 0.54 vs. 1.60 ± 0.21 cells/µl, P = 0.18) but did not reach statistical significance (Fig. 1). In eight patients who agreed to followup examinations (16.7 ± 2.3 mo after surgery), the numbers of all characterized subpopulations were reduced significantly compared with the preoperative BMC levels (Fig. 2). The menopausal status did not affect the number of circulating BMC. Seven postmenopausal patients showed comparable numbers of BMC with the other patients (data not shown).

**Cytokine serum levels.** Serum levels of SDF-1 were significantly elevated in patients with PHPT compared with controls (1.781.5 ± 105.9 vs. 1.431.3 ± 40.8 pg/ml, P < 0.05). In addition, VEGF serum concentrations were significantly elevated (80.2 ± 9.5 vs. 46.5 ± 8.0 pg/ml, P < 0.05). In contrast, serum levels of G-CSF were significantly decreased (16.8 ± 1.5 vs. 30.9 ± 5.0 pg/ml, P < 0.05). Serum levels of SCF and EPO remained unchanged [SCF: 746.0 ± 62.8 vs. 741.8 ± 63.9 pg/ml; EPO: 170.8 ± 40.5 vs. 170.5 ± 31.9 pg/ml, P > 0.05].

**Table 1. Laboratory parameters**

<table>
<thead>
<tr>
<th></th>
<th>PHPT Patients (n = 22)</th>
<th>Controls (n = 10)</th>
<th>Normal Limits</th>
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<tbody>
<tr>
<td>PTH, pg/ml</td>
<td>164.9 ± 14.4</td>
<td>27.7 ± 2.9</td>
<td>11.0–67.0</td>
</tr>
<tr>
<td>Ca, mmol/l</td>
<td>2.88 ± 0.04</td>
<td>2.39 ± 0.03</td>
<td>2.05–2.65</td>
</tr>
<tr>
<td>PO₄, mg/dl</td>
<td>2.42 ± 0.11</td>
<td>3.98 ± 0.12</td>
<td>2.5–4.5</td>
</tr>
<tr>
<td>Cr, mg/dl</td>
<td>0.64 ± 0.05</td>
<td>0.88 ± 0.03</td>
<td>0.5–1.0</td>
</tr>
<tr>
<td>BUN, mg/dl</td>
<td>27.2 ± 3.9</td>
<td>35.8 ± 1.9</td>
<td>9–50</td>
</tr>
<tr>
<td>TSH, µIU/l</td>
<td>0.92 ± 0.15</td>
<td>1.29 ± 0.17</td>
<td>0.40–4.00</td>
</tr>
<tr>
<td>FT₃, pg/ml</td>
<td>3.31 ± 0.17</td>
<td>2.97 ± 0.16</td>
<td>1.80–4.20</td>
</tr>
<tr>
<td>FT₄, ng/dl</td>
<td>1.41 ± 1.10</td>
<td>1.31 ± 0.06</td>
<td>0.80–1.90</td>
</tr>
</tbody>
</table>

Values are means ± SE for n = 22 primary hyperparathyroidism (PHPT) patients and 10 controls. Relevant laboratory parameters in our study population. PTH, parathyroid hormone; Ca, calcium; PO₄, phosphate; Cr, creatinine; BUN, blood urea nitrogen; FT₃, free triiodothyronine; FT₄, free thyroxine.

**Table 2. Clinical characteristics of the study population showing PHPT-related disorders and additional diseases**

<table>
<thead>
<tr>
<th>PHPT (n = 22)</th>
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<tbody>
<tr>
<td>Nephrolithiasis</td>
</tr>
<tr>
<td>Osteopenia</td>
</tr>
<tr>
<td>Gallstones</td>
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<tr>
<td>Gastritis</td>
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<tr>
<td>Paresthesia</td>
</tr>
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</table>

**Additional diseases**

| Cardiovascular diseases | 8 (36%) |
| Arterial hypertension  | 8 (36%) |
| Coronary artery disease| 2 (9%)  |
| Thyroid diseases       | 15 (68%) |
| Multinodular goiter    | 13 (59%) |
| Papillary thyroid carcinoma | 2 (9%) |
| Diabetes mellitus      | 1 (4.5%) |
Fig. 1. Bone marrow-derived progenitor cell (BMC) populations in peripheral blood. All investigated subpopulations of BMC show elevated levels in patients with primary hyperparathyroidism (PHPT; black bars). A: histogram shows that circulating CD45^+CD34^+CXCR4^+, CD45^+CD34^+c-kit^+ and CD45^+CD34^+CD31^+ are increased in patients with PHPT compared with controls; *P < 0.05. B: representative FACScan data demonstrate an increased level of CD34^+ cell populations in peripheral blood of PHPT patients. Error bars designated as SE. n, Number of patients; NS, not significant.

Fig. 2. BMC populations before and after surgery; 8 patients agreed to followup examinations, which were performed 16.7 ± 2.3 mo after surgery. A–C: mononuclear cell populations of circulating BMC in each enrolled individual before (pre-OP) and after (post-OP) surgery. D: bar graphs representing mean values of BMC subpopulations before and after surgery; *P < 0.05. Error bars designated as SE.
Correlation of PTH serum levels with circulating BMC. Serum levels of PTH in PHPT patients were positively correlated with the number of BMCs. In addition, PTH serum concentrations correlated with the number of CD45<sup>+</sup>/CD34<sup>+</sup>/c-kit<sup>+</sup> (r = 0.73, P < 0.05), CD45<sup>+</sup>/CD34<sup>+</sup>/CXCR4<sup>+</sup> (r = 0.85, P < 0.05), and CD45<sup>+</sup>/CD34<sup>+</sup>/CD31<sup>+</sup> cells (r = 0.71, P < 0.05; see Fig. 4). No correlation was detected between cytokine serum levels and circulating BMC or PTH (Table 3).

DISCUSSION

In our prospective study we showed a significant effect on mobilization of BMC in patients suffering from parathyroid adenoma. Serum levels of PTH were positively correlated with the number of circulating progenitor cells, which decreased to normal levels after removal of the adenoma. In addition, we demonstrated increased serum levels of SDF-1 and VEGF in PHPT patients. However, there was no correlation between cytokine serum levels (SDF-1, VEGF) and circulating BMCs. Serum levels of G-CSF, EPO, and SCF known to mobilize BMC were even decreased or remained unchanged, suggesting a direct effect of PTH on stem cell mobilization.

PTH is a key hormone regulating calcium and phosphate homeostasis. Recently, Calvi et al. (4) demonstrated direct effects of PTH on the hematopoietic system in a mouse model. Overexpression of the human PTH/PTH-related peptide receptor under control of the osteoblast-specific α1(I) collagen promoter resulted in an increase of Sca-1 and c-kit-positive stem cells in the bone marrow. PTH treatment of lethally irradiated wild-type mice resulted in an improved survival after bone marrow transplantation. The impact of PTH on mobilization of BMC into peripheral blood was not investigated. Here, we used a natural model of permanent PTH stimulation in humans suffering from parathyroid adenomas. Our prospective trial showed an increase of circulating BMC in these patients for the first time.

To investigate the mechanism for mobilization we analyzed several cytokines, such as G-CSF, VEGF, SDF-1, SCF, and EPO, that are known to stimulate bone marrow for release of stem cells into the circulation (2, 11, 24). Activated osteoblasts, which are among other structural cells in the hematopoietic stem cell niche, are able to produce growth factors such as G-CSF and SDF-1 (18, 22, 29–31). In our study, G-CSF was even downregulated, and serum levels of SCF and EPO remained unchanged. Interestingly, our data showed a significant increase of SDF-1 and VEGF in peripheral blood. This is consistent with a study from Lazaris et al. (20) showing an upregulation of VEGF and VEGF receptor in immunostaining of parathyroid adenomas. However, SDF-1 and VEGF did not correlate with the number of circulating BMC. In contrast, PTH showed a significant positive correlation with mobilized BMC. This strongly indicates that PTH is the effector for BMC mobilization.

BMC are known to contribute to neovascularization and vessel repair, as revealed in studies showing increased mobilization of EPC in conditions of ischemia and vessel injury (8, 9, 13, 19, 28, 32, 34, 36). Our results showed that numbers of circulating BMC significantly decreased to normal levels after removal of the adenoma, suggesting that mobilization of BMC occurs as a consequence of high PTH concentrations and thus may play a role in the pathophysiology of the formation of parathyroid adenomas. It has been demonstrated (25, 26) that EPC may infiltrate tumors and give rise to up to 16% of the tumor neovascularization. The mobilization of EPC in PHPT may also contribute to angiogenesis in parathyroid adenomas, and thus PTH-dependent mobilization of BMC may promote adenoma growth.

Our study showed for the first time that elevated PTH serum levels result in increased mobilization of BMCs into peripheral
blood. This may enhance angiogenesis in parathyroid adenoma and support tumor growth. However, further studies are necessary to evaluate the exact role of mobilized BMC in PHPT.

In summary, our results offer new insights into the function of PTH and pathophysiology of PHPT and thus may provide new options for diagnosis and treatment of the disease. Furthermore, our data may pave the way for PTH as a novel substance for mobilization of BMCs.

ACKNOWLEDGMENTS

We thank J. Arcifa and K. Angermüller (financed by the Fritz-Bender-Stiftung) for excellent technical assistance. Furthermore, we thank Drs. H. Diem and M. Adam, Institute of Clinical Chemistry, Ludwig-Maximilians University, for great support with flow cytometry analyses.

GRANTS

This work was supported by grants from the Fritz-Bender-Stiftung, Munich, Germany. Additional financial support for flow cytometry was provided by Dr. Helmut Legerlotz-Stiftung, Munich, Germany.

REFERENCES


Table 3. Correlations of cytokines with levels of circulating BMC

<table>
<thead>
<tr>
<th>Cells</th>
<th>CD45+/CD34+/c-kit+</th>
<th>CD45+/CD34+/CXCR4+</th>
<th>CD45+/CD34+/CD31+</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTH</td>
<td>0.73*</td>
<td>0.85*</td>
<td>0.71*</td>
</tr>
<tr>
<td>SDF-1</td>
<td>0.18</td>
<td>−0.23</td>
<td>−0.19</td>
</tr>
<tr>
<td>VEGF</td>
<td>0.25</td>
<td>−0.19</td>
<td>−0.02</td>
</tr>
<tr>
<td>SCF</td>
<td>0.02</td>
<td>−0.03</td>
<td>0.07</td>
</tr>
<tr>
<td>EPO</td>
<td>0.29</td>
<td>−0.16</td>
<td>−0.35</td>
</tr>
<tr>
<td>G-CSF</td>
<td>0.24</td>
<td>0.38</td>
<td>0.49</td>
</tr>
</tbody>
</table>

BMC, bone marrow-derived progenitor cells; SDF-1, stromal cell-derived factor 1; SCF, stem cell factor; EPO, erythropoietin; G-CSF, granulocyte colony-stimulating factor. Correlations of PTH and cytokine serum levels with circulating BMC. Nos. indicate correlation coefficient. *P < 0.05.

Fig. 4. Parathyroid hormone (PTH) serum levels related to the levels of BMC populations in the study group. PTH serum levels significantly correlated with the no. of all investigated mobilized subpopulations of BMC. A: correlation of PTH serum levels with no. of CD45+/CD34+/CD31+ cells. B: correlation of PTH serum levels with no. of CD45+/CD34+/c-kit+ cells. C: correlation of PTH serum levels with no. of CD45+/CD34+/CXCR4+ cells. r, Correlation coefficient.