The cardioprotective effects of parathyroid hormone are independent of endogenous granulocyte-colony stimulating factor release

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Aims
Parathyroid hormone (PTH) administration after myocardial infarction (MI) is known to attenuate ischaemic cardiomyopathy. This effect mainly resulted from an increase in mobilization and homing of CD34+/CD45+ cells into the ischaemic myocardium. PTH-related stem cell mobilization was shown to be related to endogenous granulocyte-colony stimulating factor (G-CSF) release. The aim of our study is to determine the role of G-CSF on the cardioprotective effects of PTH.

Methods and results
G-CSF+/+ (C57BL/6) and G-CSF−/− mice were treated with PTH for 6 days after inducing a MI. The myocardial homing factor stromal cell-derived factor-1 (SDF-1) was analysed on day 2 with enzyme-linked immunosorbent assay. Stem cell populations in peripheral blood and hearts were examined by FACS on days 6 and 2, respectively. Cardiac function and immunohistochemistry were investigated on day 6 and day 30. PTH treatment resulted in a significant increase in CD45+/CD34+ cells in peripheral blood in G-CSF+/+ but not in G-CSF−/− mice. However, a significant increase in SDF-1 and enhanced migration of CD45+/CD34+ cells into the ischaemic myocardium was revealed after PTH administration in both G-CSF+/+ and G-CSF−/− mice. Enhanced stem cell homing was associated with improved cardiac function and post-MI survival after PTH treatment. Furthermore, infarct size, wall thickness, and neovascularization showed a significant improvement in both groups 30 days after MI.

Conclusion
The cardioprotective effects of PTH were shown to be independent of endogenous G-CSF release and therefore from stem cell mobilization. This puts more emphasis on the role of stem cell homing into ischaemic myocardium.

Keywords
PTH • G-CSF • Homing • Stem cells • SDF-1 • Regenerative medicine

1. Introduction
In recent years, using bone marrow-derived cells (BMCs) has become a novel approach for the treatment of ischaemic disorders such as myocardial infarction (MI).1,2 As the number of circulating BMCs was shown to independently predict the outcome in patients with cardiovascular disease, strategies to increase circulating BMCs after MI are warranted.3 Cytokines, notably Granulocyte-Colony Stimulating Factor (G-CSF), are potent stimuli for mobilization of BMCs into peripheral blood.4 Preclinical studies using G-CSF after MI could demonstrate beneficial effects on cardiac function, remodelling, and survival.5–7 However, randomized clinical trials failed to demonstrate efficacy of G-CSF treatment after MI.8,9 One explanation for the disappointing results in clinical trials was the impaired homing behaviour of circulating BMCs after G-CSF administration.10 This is caused by the G--CSF-dependent cleavage of chemokine receptor type 4 (CXCR4) on mobilized BMCs resulting in loss of chemotaxis in response to the myocardial homing factor Stromal Cell-Derived Factor-1 (SDF-1).11

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An alternative substance inducing stem cell mobilization is parathyroid hormone (PTH), a hormone which is mainly known to act on the homeostasis of calcium and phosphate. In recent years, it was demonstrated that PTH also affects the bone marrow (BM) stem cell niche. In patients with primary hyperparathyroidism, we could show increased numbers of circulating BMCs in the peripheral blood. Furthermore, PTH administration in healthy mice effectively induced BMC mobilization, suggesting to be a promising substance in the therapy of ischaemic diseases. Administration of PTH after MI resulted in beneficial effects attenuating ischaemic cardiomyopathy. These effects were associated with increased numbers of circulating BMCs and improved migration of these cells into the ischaemic myocardium.

The mechanism of PTH-induced mobilization is based on the activation of osteoblasts within the BM. This activation results in a strengthening of survival and self-renewal of haematopoietic stem cells and in a secretion of cytokines, including G-CSF. We could demonstrate that PTH treatment in healthy mice is associated with increased G-CSF serum levels. Moreover, blocking G-CSF after PTH administration results in a reduction of circulating BMCs.

It is currently unknown whether this G-CSF-dependent mobilization behaviour of PTH is essential for the beneficial effects after MI. Therefore, we investigated the cardioprotective effects of PTH in a transgenic mouse model of G-CSF deficiency.

2. Methods

2.1 Animal model

MI was induced in 8–10-week-old male G-CSF knockout (KO) or C57BL/6 wildtype (WT) mice (Charles River Laboratories, Sulzbach, Germany) by surgical occlusion of the left anterior descending (LAD) artery through a left anterolateral approach. Mice were anaesthetized by an intraperitoneal (i.p.) injection of a mixture of medetomidine (0.714 mg/kg, Domitor, Pfizer, Berlin, Germany), midazolam (7.14 mg/kg, Midazolam ratsipharma, Ratiopharm, Ulm, Germany), and fentanyl (0.07 mg/kg, Fentanyl-Janssen, Janssen-Cilag, Neuss, Germany). For the surgical procedure, mice were intubated, and artificially ventilated by a mouse ventilator (MiniVent, HUGO SACHS, March, Germany) with 200 strokes/min and 200 μl/stroke. Anaesthetic monitoring included responsiveness to painful stimuli, character and rate of respiration, heart rate, body temperature, and mucus membrane colour. We received G-CSF-heterozygote breeding pairs from the laboratory of Prof. Erwin Wagner (Centro Nacional de Investigaciones Oncológicas (CNIO) in Madrid, Spain). Animal care and all experimental procedures were performed in strict accordance to the German and National Institutes of Health animal legislation guidelines and were approved by the local animal care committees (Regierung von Oberbayern). The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

2.2 Administration of PTH

Mice were randomly divided into the following groups: infarcted WT mice receiving either saline (0.9% NaCl) or PTH daily for six consecutive days (80 μg/kg/day i.p. Amgen Biologicals); and G-CSF KO mice receiving either saline (0.9% NaCl) or PTH daily for six consecutive days. Treatment was initiated immediately after the surgical procedure. This PTH treatment regime was chosen, because we could previously show that a longer administration of PTH has no additional effect on stem cell mobilization.

2.3 Functional parameters

For evaluation of pressure–volume relationships in vivo, mice were anaesthetised with midazolam, medetomidine, and fentanyl (see above), fixated on a temperature controlled plate, intubated, and ventilated (MiniVent, HUGO SACHS, March, Germany). We introduced an impedance micro-manometer catheter (Millar Instruments, Houston, TX, USA) into the left ventricle via the right carotid artery and recorded pressure volume loops. Raw conductance volumes were corrected for parallel conductance by the hypertonic saline dilution method as described previously. Haemodynamic measurements as well as data analyses were performed using PVAN analysis software (HUGO SACHS, March, Germany).

2.4 Histology and immunohistochemistry

We performed histological studies on day 6 and day 30. After anaesthesia with midazolam, medetomidine, and fentanyl (see above), hearts were excised and flushed with formaline. After fixation in 4% phosphate-buffered formalin, the hearts were cut transversally into four 2 mm thick slices, processed, and embedded in paraffin by standard methods; 4 μm thick sections were cut and mounted on positively charged glass slides. Standard histological procedures (haematoxylin/eosin and Masson trichrome) and immunostaining were performed. Infant size and wall thickness were determined according to the previous description. For immunostaining, we used a CD 31 antibody (goat anti-mouse, Santa Cruz) to determine the extent of neovascularization. Apoptotic cells were detected using the TUNEL assay (ApopTag, MP Biomedicals). Digital photographs were taken at a magnification of ×400, and 10 random high-power fields (HPFs) from the border zone of each heart sample (n = 3) were analysed. Quantification of blood vessels was assessed by CD31+ immunohistochemistry in the border zone. The border zone was defined as a 0.5 mm wide area around the site of infarction. The numbers of CD31+ capillaries were quantified from 10 random HPFs with ×400 magnification. To quantify the granulation tissue, the fraction of the granulation tissue was related to the total area of infarction.

2.5 Flow cytometry of peripheral blood and non-myocyte cardiac cells

At day 6 after MI, 1 mL of peripheral blood was harvested from each mouse by aspirating the carotid artery. Mononuclear cells were separated by density-gradient centrifugation using 1.077 g/mL Histopaque solution (Sigma Chemicals), purified, and resuspended in phosphate-buffered saline (PBS) containing 1% bovine serum albumin (BSA). Cells were incubated for 40 min in the dark at 4°C with the following fluoresceinisothiocyanate (FITC)-, phycoerythrin (PE), and peridininchlorophyll-protein (PerCP) conjugated monoclonal antibodies: CD45-PerCP, CD34-FITC, CD31-PE, and CXCR-4 (all from BD Pharmingen). Matching isotype antibodies (BD Pharmingen) served as controls. Cells were analysed by three-colour flow cytometry using a Coulter Epics XL-MCLTM flow cytometer (Beckman Coulter). Each analysis included 50 000 events.

Cardiac cells were analysed 48 h after MI. Therefore, a ‘myocyte-depleted’ cardiac cell population was prepared, incubating minced myocardium in 0.1% collagenase IV (GIBCO BRL) 30 min at 37°C, lethal to most adult mouse cardiomyocytes. Cells were then filtered through a 70 μm mesh. To exclude spurious effects of enzymatic digestion, BM cells with or without collagenase treatment were stained, revealing no significantly changed staining of labelled cell antigens (data not shown). Cells were stained and analysed as described above.

2.6 Enzyme-linked immunosorbent assay of hearts

Hearts were excised 48 h after MI. After digestion in 0.1% collagenase for 45 min, cells were lysed by ultrasonic pulse echo instrument. The SDF-1
protein was determined using a commercially available Quantikine kit (R&D systems, MCX 120) according to the manufacturer’s instructions.

2.7 Enzyme-linked immunosorbent assay of bone marrow
After extracting and cleaning the scapulae, the extremity and the pelvic bones 48 h after MI, we crashed them in a mortar with PBS containing 2% BSA. In the next steps, molecular cells were separated by density-gradient centrifugation using Histopaque solution (1.077 g/mL, Sigma Chemicals), purified, and resuspended in BSA (1%). Cells were lysed by an ultrasonic pulse echo instrument afterwards. The SDF-1 protein was determined using a commercially available Quantikine kit (R&D systems, MCX 120) according to the manufacturer’s instructions.

2.8 Statistical analyses
Results were expressed as mean ± SEM. Multiple group comparisons were performed by one-way analyses of variance followed by the Bonferroni procedure for comparison of means. Comparisons between two groups were performed using the unpaired Student’s t-test. Data were considered statistically significant at a value of $P \leq 0.05$. Mortality was analysed by the Kaplan–Meier method.

3. Results

3.1 Enhanced mobilization of BMCs from bone marrow into peripheral blood after PTH treatment in WT but not in G-CSF KO mice
To show the extent of BMC mobilization, FACS analyses from peripheral blood samples of WT and G-CSF KO mice were performed. PTH treatment after MI in WT mice revealed a significantly increased mobilization of CD45+/CD34+ BMCs compared with saline-treated WT controls. Subpopulations of these BMCs expressing CD31 (an endothelial marker) or CXCR-4 (the ligand of the homing factor SDF-1) were also significantly increased. However, PTH administration in G-CSF KO mice showed no effect on mobilization. Comparing WT and G-CSF KO control mice, G-CSF deficiency showed lower numbers (not significant) of circulating BMCs (Figure 1A).

3.2 Enhanced recruitment of CD45+/CD34+ BMCs to the ischaemic myocardium after PTH treatment in WT and in G-CSF KO mice
To analyse the extent of BMC migration into ischaemic myocardium, we isolated a myocyte-depleted fraction of cardiac cells and performed flow cytometry. The number of CD45+/CD34+ cells in heart tissue was significantly increased after PTH treatment in both WT and G-CSF KO mice. There was no significant difference between WT and G-CSF KO mice, neither in control nor in PTH-treated mice. However, PTH-treated G-CSF KO mice revealed slightly (not relevant) reduced numbers of migrated BMCs compared with WT mice. These effects were shown in all analysed BMC subpopulations (Figure 1B).

Before induction of ischaemia, the investigated cells in the hearts are significantly lower than in infected hearts. There was no significant difference between WT and G-CSF KO mice before ischaemia (CD45+/CD34+ cells: $0.74 \pm 0.15$ vs. $0.84 \pm 0.11\%$, $P = 0.60$; CD45+CD34+CD31+ cells: $0.58 \pm 0.19$ vs. $0.59 \pm 0.15\%$, $P = 0.96$; CD45+CD34+ cells: $0.42 \pm 0.11$ vs. $0.50 \pm 0.07\%$, $P = 0.64$; each group $n = 8$).

3.3 No increased inflammatory cell infiltration after PTH treatment in WT and in G-CSF KO mice
We analysed whether the enhanced homing behaviour after PTH treatment also affects inflammatory cells. Therefore, we analysed the granulation tissue related to the total area of infarction. The results showed no difference between all groups, suggesting that there is no increased inflammatory cell infiltration after PTH treatment (WT+MI+ saline vs. WT+MI+PTH: $85.6 \pm 0.5$ vs. $87.2 \pm 1.2\%$, $P = 0.29$; KO+MI+ saline vs. KO+MI+PTH: $85.2 \pm 3.4$ vs. $86.6 \pm 3.2\%$, $P = 0.84$; each group $n = 8$).

3.4 Increased myocardial levels of the SDF-1 protein after PTH treatment is preserved in G-CSF KO mice
Since improved homing of circulating BMCs into ischaemic myocardium after PTH treatment is mediated by the SDF-1/CXCR-4 axis,17 we analysed the protein levels of SDF-1 in ischaemic myocardium. Therefore, we performed enzyme-linked immunosorbent assay (ELISA) measurements of lysates from the hearts. SDF-1 protein levels of heart lysates from infected WT mice showed augmented SDF-1 levels after PTH treatment compared with saline-treated controls. This increased SDF-1 expression is preserved in G-CSF KO mice. There was no difference in SDF-1 levels between WT and G-CSF KO mice, suggesting no effect of endogenous G-CSF on SDF-1 expression (Figure 2).

To analyse whether PTH-induced stem cell mobilization is driven by modified SDF-1 expression in the bone marrow, we performed ELISA measurements of bone marrow cell lysates. There was no significant difference between saline and PTH treatment ($0.18 \pm 0.02$ vs. $0.22 \pm 0.04$ pg/mg, $P = 0.38$; each group $n = 8$).

3.5 Endogenous G-CSF has no impact on infarct remodelling after MI
To analyse the changes in infarct remodelling after induced MI, we performed histology. At day 6 after LAD occlusion, LV-infarct sizes were comparable among the groups, which altered at day 30, when the sizes of LV infarction (scar tissue) were smaller in the PTH-treated groups. This effect was revealed in both WT and G-CSF KO mice. LV-infarct sizes were comparable between WT and G-CSF KO mice (Figure 3A and B). A similar effect could be revealed regarding the thickness of the left ventricular wall. PTH treatment significantly ameliorated the thickness of the left ventricular wall in WT and in G-CSF KO mice (Figure 3D and E).

3.6 Increased neovascularization and reduced apoptosis after PTH treatment in infarcted WT and G-CSF KO mice
To analyse the effect on neovascularization, immunohistochemistry was performed. Consistent with the attenuated infarct remodelling, heart sections of PTH-treated groups (WT and G-CSF KO mice) revealed a significantly increased number of CD31+ capillaries at the infarct border zone at day 6 (Figure 4). G-CSF deficiency had no relevant effect on the number of CD31+ endothelial cells.
Cardioprotective parathyroid hormone effect is independent of granulocyte-colony stimulating factor

Figure 1  Mobilization and homing of CD45+/CD34+ BMCs. Bar graphs representing the percentage of CD45+/CD34+ cells, CD45+/CD34+/CD31+ cells, and CD45+/CD34+/CXCR-4+ cells in (A) peripheral blood or (B) hearts of infarcted WT and G-CSF KO mice treated either with saline or with PTH (each group \( n = 8 \)); n.s., not significant.
To analyse apoptosis of cardiomyocytes as a major target for prevention of ischaemic cardiomyopathy, TUNEL staining in the infarct border zone was performed. The number of apoptotic cardiomyocytes was significantly reduced in PTH-treated groups. Comparing WT and G-CSF KO mice, we found a significant higher number of apoptotic myocytes in G-CSF KO mice (Figure 5).

### 3.7 Improved survival and cardiac function after PTH treatment in WT and G-CSF KO mice

At day 6 and day 30 after MI, pressure volume relations were measured in vivo. PTH treatment of WT or G-CSF KO mice revealed a significantly improved systolic function, reflected by an increased ejection fraction (Figure 6A and B) and cardiac output (Figure 6C and D). Interestingly, at day 6, G-CSF KO mice showed a slightly (not significant) reduced cardiac output compared with WT mice in both saline- and PTH-treated mice, but there was no difference between WT and G-CSF KO mice at day 30 (Figure 6C and D).

Finally, PTH treatment showed a significantly increased survival rate compared with saline-treated controls in WT mice (80 vs. 65%) as well as G-CSF KO mice (75 vs. 55%). The survival rate of G-CSF KO mice was reduced (not significant) compared with WT mice. Mor-

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**Figure 2** PTH treatment after MI increased protein levels of the homing factor SDF-1 in ischaemic hearts. Histograms representing SDF-1 protein levels of saline or PTH-treated WT and G-CSF KO mice (each group n = 8); n.s., not significant.

**Figure 3** Attenuated infarct remodelling after PTH treatment in WT and G-CSF KO mice. (A) Bar graphs representing the size of infarction at day 6 and (B) at day 30 after induced MI. (C) Bar graphs representing the thickness of the left ventricular wall at day 6 and (D) at day 30 after MI (each group n = 8); n.s., not significant. (E) Representative Masson Trichrome stainings of controls and PTH-treated WT and G-CSF KO mice 30 days after MI.
Figure 4 Increased neovascularization after PTH treatment in WT and G-CSF KO mice. (A) Bar graphs representing the number of CD31+ cells at the infarct border zone after saline or PTH treatment in WT and G-CSF KO mice at day 6. Data represent mean ± SEM (each group n = 8); HPF, high power field; n.s., not significant. (B) Representative immunohistochemical staining of CD31 (brown) in infarcted hearts 6 days after MI. Arrows show endothelial lining of small vessels with CD31+ cells.
Figure 5  PTH treatment decreased apoptotic cell death in WT and G-CSF KO mice. (A) Bar graph representing the number of TUNEL-positive CMs in the border zone 2 days after MI. Data represent mean ± SEM (each group n = 8); HPF, high power field; n.s., not significant. (B) Representative TUNEL staining (brown nuclei) in infarcted hearts 2 days after MI.
tality was high within the first 6 days, but declined thereafter in all groups (Figure 6E).

4. Discussion

In the present study, we focused on the impact of endogenous G-CSF in PTH-treated infarcted mice. Our main findings were the following: (i) PTH-induced mobilization of BMCs is inhibited in G-CSF KO mice, (ii) PTH-induced migration of BMCs into ischaemic myocardium is preserved in G-CSF KO mice due to increased SDF-1 levels, (iii) attenuated myocardial remodelling as well as improved neovascularization after PTH treatment is not affected by endogenous G-CSF, (iv) G-CSF KO mice revealed an increased number of apoptotic cardiomyocytes; (v) slightly impaired cardiac function and survival in G-CSF KO mice is equalized in the long term.

Besides its function in the regulation of calcium and phosphate homeostasis, PTH was shown to have cardioprotective properties. In a mouse model of MI, PTH treatment resulted in improved cardiac function.16 This effect could be explained by enhanced BMC mobilization and by improved migration of BMCs into the ischaemic myocardium.15,17 Since PTH-induced mobilization was shown to be mediated by endogenous G-CSF release,15 it remained to be elucidated if the cardioprotective effects of PTH are completely dependent from endogenous G-CSF. To address this question, we compared the effect of PTH after induced MI in WT and in G-CSF KO mice.

As previously demonstrated by our group, our current data also showed increased numbers of circulating BMC population as a sign of mobilization from bone marrow into peripheral blood.15–17 This mobilization property is completely inhibited in G-CSF KO mice, suggesting that PTH-induced mobilization to be dependent from endogenous G-CSF. Beside our findings in PTH-treated animals, it was demonstrated that the physiological increase in circulating CD34+ cells in patients with acute MI is strongly correlated to endogenous G-CSF serum levels.21

Concerning the impact on stem cell homing, our data demonstrate an enhanced migration of circulating BMCs in both WT and G-CSF KO mice. This suggests that homing of BMCs to ischaemic myocardium is independent of endogenous G-CSF. Exogenous G-CSF administration was shown to result in an impaired homing capacity, which is explained by a cleavage of CXCR-4 on mobilized BMCs resulting in loss of chemotaxis in response to the myocardial homing factor SDF-1.10,11 Different from G-CSF, PTH treatment results in a strengthening of the SDF-1/CXCR-4 axis by inhibition of dipeptidyl-peptidase IV (DPP IV/CD26), which is one of the major enzymes to be involved in the process of SDF-1 degradation.17,22 In vitro experiments could demonstrate a direct PTH-mediated effect of DPP IV inhibition.17 Furthermore, PTH administration results in increased protein levels of SDF-1, which is not abolished by G-CSF deficiency. Compared with WT mice, in PTH treated G-CSF KO mice, the number of migrated BMCs was not significantly different. A slight decrease may be explained by a loss of G-CSF-mediated supply of mobilized BMCs.
Since there was no increased inflammatory cell infiltration after PTH treatment, our data suggest that enhanced recruitment of BMC majorly affects protective progenitor cells.

In our previous study, we could demonstrate that PTH treatment after MI results in an ameliorated remodelling, which may be explained by increased neovascularization and reduced apoptosis. Our present data confirm these findings showing a reduced size of infarction, ameliorated left ventricular wall thickness as well as increased numbers of CD31+ endothelial cells and reduced apoptotic cells. The number of CD31+ cells was slightly, but not significantly decreased in G-CSF KO mice compared with WT mice, which is in accordance with our finding on migrated BMCs. This suggests that migration of BMCs plays a major role for ameliorating myocardial remodelling. Our data are supported by other reports showing that the number of CD31+ cells contribute to enhanced neovascularization and reduced apoptosis after MI. In addition, improvement of BMC homing mechanisms like DPP IV inhibition could reveal higher mortality within the first 6 days. These differences are not explained by transdifferentiation of recruited BMCs but through paracrine mechanisms. To investigate the contribution of direct receptor-dependent effects on neovascularization and reduced apoptosis, further studies are necessary.

The beneficial G-CSF-independent effects were reflected by the cardiac function. PTH treatment resulted in an improved cardiac function in WT and in G-CSF KO mice. Furthermore, there was no significant difference between WT and G-CSF KO mice. However, at day 6 after MI, cardiac function was slightly impaired in G-CSF KO mice, which is also reflected by a slightly reduced survival caused by a higher mortality within the first 6 days. These differences are not significant but remarkable. This may be explained by a slightly reduced number of migrated BMCs. Furthermore, it may result from direct receptor-mediated effects of endogenous G-CSF, which reduces apoptosis by activating the Jak-Stat pathway in cardiomyocytes. This explains that the number of apoptotic cardiomyocytes was significantly increased in G-CSF KO mice compared with WT mice. However, 30 days after MI, differences concerning cardiac function and survival between WT and G-CSF KO mice completely disappeared suggesting a predominant role of PTH-induced migration of BMCs for the beneficial effects. In conclusion, we could demonstrate that the cardioprotective effects of PTH are independent of endogenous G-CSF release and therefore from stem cell mobilization. This puts more emphasis on the role of stem cell homing into the ischemic myocardium.

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Conflict of interest: The Ludwig Maximilians University is the holder of a pending patent (‘Remedies for ischemia’ EP 2007/003272 and US 60/792 943) claiming a second medical use of PTH to treat ischemic organ failure.

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References


