Parathyroid hormone is a DPP-IV inhibitor and increases SDF-1-driven homing of CXCR4⁺ stem cells into the ischaemic heart

Bruno C. Huber†, Stefan Brunner†, Alexander Segeth, Petra Nathan, Rebekka Fischer, Marc M. Zaruba, Marcus Vallaster, Hans D. Theiss, Robert David, Armin Gerbitz, and Wolfgang-Michael Franz*

1Department of Internal Medicine I, Ludwig-Maximilians-University, Campus Grosshadern, Marchioninistr. 15, D-81377 Munich, Germany; and
2Department of Internal Medicine V, Friedrich-Alexander-University, Erlangen, Germany

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Aims
Parathyroid hormone (PTH) has been shown to promote stem cell mobilization into peripheral blood. Moreover, PTH treatment after myocardial infarction (MI) improved survival and myocardial function associated with enhanced homing of bone marrow-derived stem cells (BMCs). To unravel the molecular mechanisms of PTH-mediated stem cell trafficking, we analysed wild-type (wt) and green fluorescent protein (GFP)-transgenic mice after MI with respect to the pivotal stromal cell-derived factor-1 (SDF-1)/chemokine receptor type 4 (CXCR4) axis.

Methods and results
WT and GFP-transgenic mice (C57BL/6J) were infarcted by coronary artery ligation and PTH (80 μg/kg/day) was injected for 6 days afterwards. Number of BMCs was analysed by flow cytometry. SDF-1 protein levels and activity of dipeptidyl peptidase-IV (DPP-IV) were investigated by ELISA and activity assay. Functional analyses were performed at day 30 after MI. PTH-treated animals revealed an enhanced homing of CXCR4⁺ BMCs associated with an increased protein level of the corresponding homing factor SDF-1 in the ischaemic heart. In vitro and in vivo, PTH inhibited the activity of DPP-IV, which cleaves and inactivates SDF-1. Functionally, PTH significantly improved myocardial function after MI. Both stem cell homing as well as functional recovery were reversed by the CXCR4 antagonist AMD3100.

Conclusion
In summary, PTH is a DPP-IV inhibitor leading to an increased cardiac SDF-1 level, which enhances recruitment of CXCR4⁺ BMCs into the ischaemic heart associated with attenuated ischaemic cardiomyopathy. Since PTH is already clinically used our findings may have direct impact on the initiation of studies in patients with ischaemic disorders.

Keywords
Parathyroid hormone • Stem cells • SDF-1/CXCR4 • Regenerative medicine

1. Introduction
Recently, it was demonstrated that the bone marrow (BM) stem cell niche can be activated by parathyroid hormone (PTH), a peptide hormone secreted from the parathyroid glands, which mainly acts on bone and kidney cells and is involved in systemic calcium homeostasis. PTH promoted the proliferation of lin⁻/c-kit⁺/Sca-1⁺ stem cells by activation of PTH receptor positive osteoblasts and increased homing of haematopoietic stem cells to the BM after lethal irradiation. In patients with primary hyperparathyroidism, we were able to show an increased number of circulating bone marrow-derived stem cells (BMCs) in the peripheral blood. In addition, intermittent PTH administration effectively induced progenitor cell mobilization in mice. The increase of circulating stem cells was associated with an augmented granulocyte-colony stimulating factor (G-CSF) serum level, which may also cause mobilization. These data suggested that PTH is an attractive substance to mobilize BMCs into peripheral blood.

In the past few years, regeneration of ischaemic myocardial tissue has been attempted using BMCs. Mobilization of BMCs using
growth factors such as G-CSF offers an alternative to the direct injection of stem cells.\textsuperscript{7} Circulating mobilized stem cells can be recruited from the blood pool into the damaged myocardial tissue and this homing behaviour is suggested to play a key role for myocardial regeneration.\textsuperscript{9} In contrast to preclinical studies demonstrating beneficial effects of G-CSF administration after myocardial infarction (MI),\textsuperscript{10–12} larger randomized and double-blinded clinical trials failed to demonstrate efficacy of G-CSF treatment after MI.\textsuperscript{13,14} One explanation for the poor clinical outcome after G-CSF administration was the diminished stem cell homing caused by the N-terminal cleavage of chemokine receptor type 4 (CXCR4) on mobilized haematopoietic progenitor cells resulting in loss of chemotaxis in response to stromal cell-derived factor-1 (SDF-1/CXCL12).\textsuperscript{15} Thus, new drug therapies not only have to concentrate on stem cell mobilization, but rather have to focus on the process of cardiac stem cell homing as a pivotal element to advance cell therapies in the future. SDF-1 is a 7.977 kDa chemokine, which is secreted from endothelial cells in ischaemic tissue\textsuperscript{16} and binds to the corresponding homing-receptor CXCR4 in its active form (1–68).\textsuperscript{17} The interaction between SDF-1 and CXCR4, which is expressed on many circulating progenitor cells, has been shown to play an essential role in stem cell migration to ischaemic organs.\textsuperscript{18–20} SDF-1 is N-terminally cleaved at position-2 proline by the cell surface protein dipeptidyl peptidase-IV (DPP-IV/CD26).\textsuperscript{21} The truncated form of SDF-1 not only looses its chemotactic properties, but also blocks chemotaxis of full length SDF-1.\textsuperscript{22} DPP-IV is expressed on many haematopoietic cell populations and is present in a catalytically active soluble form in the plasma.\textsuperscript{23}

On the basis of its anabolic effects on the BM stem cell niche\textsuperscript{1} and based on the fact that PTH has already been clinically approved in patients with osteoporosis,\textsuperscript{24} PTH has been suggested a promising agent for tissue repair and protection. In this context, PTH treatment after MI in mice was demonstrated to improve cardiac function associated with increased neovascularization and cell survival.\textsuperscript{25} Using the innovative SPECT technique, we were recently able to show increased cardiac perfusion in ischaemic hearts associated with increased numbers of BMCs in the myocardium after PTH administration.\textsuperscript{26} Although it has been shown in several studies that PTH affects the stem cell niche and promotes cardioprotection after MI, it is currently unknown how these effects on stem cell mobilization and homing after MI are orchestrated. Therefore, the present study focused on molecular mechanisms of PTH-mediated stem cell trafficking especially with respect to the pivotal SDF-1/CXCR4 axis in a preclinical mouse model.

2. Methods

2.1 Animal model

For wild-type (wt) experiments MI was induced in 8–12 weeks old male C57BL/6 mice (Charles River Laboratories, Sulzbach, Germany) by surgical occlusion of the left anterior descending (LAD) artery through a left anterolateral approach as described previously.\textsuperscript{25} For the generation of BM-chimeric animals also 8–12-week-old C57BL/6 mice (Charles River Laboratories, Sulzbach, Germany) were used. Briefly, mice were irradiated and underwent BM-transplantation thereafter. Donor mice (C57BL/6) expressing a transgene encoding for green fluorescent protein (GFP) under control of the human ubiquitin C promoter (UBI-GFP/BL6)\textsuperscript{27} were bred in our facility. BM was harvested from the femurs and tibias of donor mice. Cell mixtures of erythrocyte-lysed 5 × 10\textsuperscript{6} BM cells were supplemented with 1 × 10\textsuperscript{6} splenic cells from donors, resuspended in Leibovitz L-15 medium and transplanted into recipients via tail vein infusion (0.25 mL total volume). Before transplantation, host mice received 12 Gy of total body irradiation. MI was induced 10 weeks after BM-transplantation (18–22 weeks, see Supplementary material online, Figure S1). 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, No. 55.2-1-54-2531-73-09). 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 and AMD3100

Mice were randomly divided into the following groups: (i) intraperitoneal (ip.) administration of saline for daily 6 days (n = 6); (ii) administration of PTH daily for six consecutive days (80 μg/kg/day ip., Amgen Biologicals, n = 6); (iii) PTH + AMD3100 (1.25 mg/kg/day ip.) for 6 days (n = 6). PTH and/or AMD3100 treatment was started 30 min after ligation of the LAD. Because stimulation with PTH for 6 days showed no differences regarding stem cell mobilization compared with a 14 day administration,\textsuperscript{25} we used the short PTH treatment protocol.

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

Peripheral blood and hearts of WT and GFP-transgenic mice (n = 6 per group) were investigated by flow cytometry (FACS). At day 6, 1 mL of peripheral blood was harvested from each mouse by aspirating the carotid artery. Mononuclear cells were separated by density-gradient centrifugation using Histopaque solution (1.077 g/mL, Sigma Chemicals), purified and resuspended in phosphate-buffered saline containing 1% bovine serum albumin (BSA). For cardiac FACS analyses infarcted hearts of the mice were explanted at day 6 and retrogradely perfused with saline (0.9% NaCl) to wash out circulating blood cells. Thereafter, 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 from peripheral blood and hearts were incubated for 40 min in the dark at 4°C with the following fluorescence isothiocyanate (FITC), phycoerythrin (PE), and peridininchlorophyll-protein (PerCP) conjugated monoclonal antibodies: CD45-PerCP, CD34-FITC, and CXCR4-PE (all from BD Pharmingen). A matching isotype antibody served as control. Cells were analysed by three-colour flow cytometry using a Coulter\textsuperscript{48} Epics\textsuperscript{49} XL-MCLTM flow cytometer (Beckman Coulter). Each analysis included 50 000 events.

2.4 SDF-1 protein levels by ELISA

It has been shown previously that the time course of SDF-1 up-regulation is depicted by a peak of SDF-1 expression at 24–48 h after acute MI followed by a subsequent decline.\textsuperscript{18,28} Therefore, we decided to investigate SDF-1 expression at day 2 after MI. After extracting the hearts (n = 6 per group) were digested in 0.1% collagenase solution for 45 min and cells were lysed by ultrasonic pulse echo instrument afterwards. SDF-1 protein was determined using a commercially available Quantikine kit (R&D systems, MCX 120) according to the manufacturer’s instructions. Serum levels of SDF-1 protein were also determined by ELISA using a commercially available kit (R&D systems, MCX 120).

2.5 DPP-IV activity assays

Enzyme activity of DPP-IV in vivo was measured according to Scharpe et al.\textsuperscript{29} with minor modifications: its activity was defined as substrate turnover over time (H-Gly-Pro-AMC; 353 nmem, 442 nmex) and one reaction was performed with 30 μM of substrate and 1.25 mg/kg/day i.p., Amgen Biologicals, n = 6). PTH and/or AMD3100 treatment was started 30 min after ligation of the LAD. Because stimulation with PTH for 6 days showed no differences regarding stem cell mobilization compared with a 14 day administration,\textsuperscript{25} we used the short PTH treatment protocol.

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2.5 DPP-IV activity assays

Enzyme activity of DPP-IV in vivo was measured according to Scharpe et al.\textsuperscript{29} with minor modifications: its activity was defined as substrate turnover over time (H-Gly-Pro-AMC; 353 nmem, 442 nmex) and one reaction well contained 0.5 mM H-Gly-Pro-AMC with 10 μl sample (BM, heart) respectively 60 μL sample (blood) in 100 mM Tris–HCl; pH 8. The
surge of this kinetic curve was defined as the activity. The fluorescence signal was converted into amount of product via conversion of the maximal fluorescence signal after complete substrate turnover. The activity was referred to the protein amount of the samples (BSA protein standard curve, n = 6 per group).

For the in vitro assays the activity of DPP-IV was likewise defined as substrate rate—time curve (H-Gly-Pro-AMC, 353 nmem, 442 nmex). Yet, we modified the assay as follows.

As seminal control the turnover from 0.5 mM H-Gly-Pro-AMC by 0.12 U DPP-IV (Calbiochem, recombinant) in 100 mM Tris—HCl; pH 8 was determined. For measurement of the effect of PTH on the enzyme activity 12.5 and 25fmol PTH were added and the slope was calculated (n = 6). Additional controls contained BSA instead of PTH at the same concentrations to rule out any unspecific or PTH-independent activity of DPP-IV.

2.6 Cardiac function

For evaluation of myocardial function, mice of the previously described groups (each n = 6) were investigated using an impedance-micromanometre catheter. The method, as well as data analyses, were performed as previously described in the literature. Briefly, after catheterization via the right carotid artery, a 1.4 French impedance-micromanometre catheter (Millar Instruments, Houston, TX, USA) was introduced into the left ventricle and pressure—volume loops were recorded. The method was based on measuring the time-varying electrical conductance signal of two segments of blood in the left ventricle from which total volume is calculated. Raw conductance volumes were corrected for parallel conductance by the hypertonic saline dilution method.

2.7 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 ≤ 0.05.

3. Results

3.1 Enhanced recruitment of BMCs into the ischaemic heart after treatment with PTH in wt mice

To show the effect of PTH treatment on mobilization and migration of BMCs, FACS analyses of cell populations from peripheral blood and extracted heart tissue were performed. PTH treatment after MI revealed a significantly increased mobilization of CD45+/CD34+ BMCs to the blood pool compared with saline-treated control animals (Figure 1A). To analyse the extent of BMC migration into the ischaemic heart, we isolated a myocyte-depleted fraction of cardiac cells was performed (in the ischaemic heart after PTH administration).

3.2 Enhanced mobilization and homing of CXCR4+ cells after PTH treatment in GFP-transgenic mice

To prove the hypothesis that CXCR4+ BMCs may be the predominant fraction migrating from the BM via the blood pool into the ischaemic heart after PTH stimulation, we used a chimeric mouse model to track BMCs by ubiquitously expression of EGFP under control of the ubiquitin C promoter. First, we confirmed the efficiency of BM transplantation by performing flow cytometry of BM cells from transplanted chimeric mice. More than 99% of BM cells were positive for EGFP. FACS analysis of EGFP+/CXCR4+ BMCs in peripheral blood showed a 2.9-fold increase of these cells after PTH treatment (Figure 2A). To analyse the impact of PTH on cell migration of EGFP+/CXCR4+ cells into the ischaemic heart, flow cytometry of a myocyte-depleted fraction of cardiac cells was performed (in the same manner as in WT mice). In PTH-treated mice the number of migrated EGFP+/CXCR4+ cells was significantly increased in comparison to the control group (Figure 2B).

3.3 Elevation of SDF-1 protein in the ischaemic heart after PTH administration

After showing increased expression of CXCR4 on mobilized BMCs after PTH stimulation, we addressed the question whether SDF-1/CXCL12, the corresponding ligand of CXCR4 is also regulated by PTH. Therefore, we performed ELISA measurements of blood serum and lysates from the heart of PTH-treated and control mice. SDF-1α protein levels in the peripheral blood showed no significant differences between sham-operated mice, infarcted control mice, and PTH-treated mice (Figure 3A). In contrast, analysis of heart lysates from infarcted control mice showed augmented SDF-1α levels compared with sham-operated mice. Moreover, treatment with PTH significantly increased SDF-1α protein levels in lysates derived from infarcted hearts when compared with untreated controls (Figure 3B) suggesting an increased SDF-1α gradient towards the ischaemic heart.

3.4 DPP-IV inhibition after PTH treatment in vitro

It has been clearly shown that SDF-1 is N-terminally cleaved by the cell surface protein DPP-IV/CD26, which is expressed on many hematopoietic cell populations. Thus, we established an enzymatic activity assay in vitro to investigate whether the elevated levels of SDF-1 protein in the ischaemic heart after PTH stimulation may be due to changes of DPP-IV activity in vitro. We used a recombinant version of DPP-IV expressed in insect cells, bearing a His-Tag at the N-terminus activity of DPP-IV. Its activity was defined as a substrate rate—time curve. To rule out unspecific activity of DPP-IV in the in vitro samples, we included controls adding BSA in the increasing concentrations as PTH. Thereby, addition of 12.5 fmol PTH led to a significant inhibition of DPP-IV enzyme activity. The effect was even higher, when 25 fmol PTH was used, the amount that corresponds to the total PTH administration in mice. In contrast, substrate turnover remained unaltered after BSA administration at the same concentrations (Figure 4).
3.5 DPP-IV inhibition in the ischaemic heart after PTH treatment

After demonstrating DPP-IV inhibition mediated by PTH in vitro, we wondered whether these effects are also relevant in vivo. PTH-treated mice revealed significant decreased proteolytic DPP-IV activity in the blood serum 2 days after MI compared with infarcted control animals (Figure 5A). In the ischaemic heart PTH stimulation resulted in an even more pronounced DPP-IV inhibition reflected by very low levels of DPP-IV activity compared with controls (Figure 5B).

3.6 Neutralization of enhanced cardiac stem cell homing and improved cardiac function after PTH treatment by CXCR4 antagonist AMD3100

In order to investigate whether the observed enhanced stem cell homing into the ischaemic heart and functional improvement after PTH treatment is dependent on an intact SDF-1/CXCR4 axis, the CXCR4 antagonist AMD3100 was administered along with PTH after MI. In fact, the number of CD34+/CD45+ cells was significantly decreased in mice treated with PTH and AMD3100 compared with animals treated solely with PTH (1.43 ± 0.15 vs. 2.56 ± 0.33%, P < 0.05; Figure 6A). Furthermore, 4 weeks after LAD occlusion, pressure–volume relations were measured in vivo from surviving sham-operated, control, or PTH-treated (+AMD3100) mice. Compared with infarcted control mice, PTH-treated animals revealed a significantly improved cardiac function, reflected by an increased dP/dt max and cardiac output (Figure 6B and C). Interestingly, administration of AMD3100 completely neutralized the improvement of cardiac function in the PTH group (dP/dt max: 3599.1 ± 173.7 vs. 4740.4 ± 394.0 mmHg/s, P < 0.05; cardiac output 3392.2 ± 541.1 vs. 4980.3 ± 650.0 μL/min, P < 0.05).

4. Discussion

In the present study, we focused on the molecular mechanisms of PTH facilitating migration of BMCs into the ischaemic heart. Our main findings were the following: (i) PTH administration significantly increased homing of CXCR4+BMCs to the ischaemic heart. (ii) Protein levels of SDF-1, the ligand of CXCR4, were increased after PTH stimulation in the ischaemic heart. (iii) PTH significantly inhibited DPP-IV activity in vitro and in vivo. (iv) The CXCR4 antagonist
AMD3100 diminished the number of CXCR4\(^+\) BMCs and neutralized beneficial effects on cardiac function after PTH treatment.

### 4.1 PTH and the SDF-1/CXCR4 axis

Besides its function in regulating calcium and phosphorus concentration in extracellular fluid, PTH was shown to exert cardioprotective effects. After induced MI in a mouse model, PTH administration resulted in improved cardiac function.\(^25\) On the one hand, this effect could be explained by enhanced stem cell mobilization, which is mediated by endogenous G-CSF release.\(^5\) On the other hand, PTH was shown to improve stem cell migration into the ischaemic heart.\(^25\) However, PTH-mediated homing mechanisms are not well understood. Since therapeutic regimes utilizing cytokines solely capable of stimulating mobilization of stem cells have failed in the clinical setting, the understanding of complex extra- and intracellular signalling pathways that govern cell homing is considered critically important for myocardial repair.\(^9,31\) Concerning the latter, the SDF-1/CXCR4-axis represents the major interacting partners for the recruitment of circulating BMCs to the site of ischaemia.\(^18,32\) In several studies, SDF-1 was targeted to improve cardiac function after acute ischaemia. Transplantation of SDF-1-expressing fibroblasts or adenoviral SDF-1 gene delivery revealed cardioprotective effects.\(^18,28\) However, a potential limitation for the therapeutic use of SDF-1 derives from its sensitivity to cleavage by proteases, predominantly by DPP-IV/CD26. Consequently, intramyocardial delivery of a protease-resistant form of SDF-1 after MI enhanced the recruitment of CXCR4\(^+\) cells and improved cardiac function.\(^33\) Likewise, stabilization of the active form of SDF-1 by genetic or pharmacologic inhibition of DPP-IV resulted in increased migration of mobilized BMCs.\(^34\) The specific receptor of the chemokine SDF-1 is the membrane receptor CXCR4, which is expressed on several populations of BMCs. Several studies demonstrated its crucial role for cardiac development and repair. A knockout model of CXCR4, which is lethal, revealed a significant impairment of haematopoietic stem cells to populate the embryonic BM niche, which was associated with essential defects in cardiogenesis and vasculogenesis. A recent study could show that the injection of CXCR4\(^+\) but not CXCR4\(^-\) BMCs resulted in blood flow recovery after acute ischaemia.\(^35\)

A first hint that PTH improves the homing capacity via the SDF-1/CXCR4-axis was provided by analyses of osteoblasts in BM. The administration of PTH resulted in an up-regulation of SDF-1.\(^36\) Our FACS analyses investigating the numbers of circulating CD45\(^+\)/CD34\(^+\) cells in peripheral blood and heart were consistent with our previous findings showing that circulating CD45\(^+\)/CD34\(^+\) BMCs were increased after PTH treatment.\(^5,25\) CD45\(^+\)/CD34\(^+\) cells were further characterized by analysing the CXCR4\(^+\) fraction. CD45\(^+\)/CD34\(^+\)/CXCR4\(^+\) cells were significantly increased after PTH treatment in peripheral blood and in infarcted hearts. The fraction of CD45\(^+\)/CD34\(^+\) cells expressing CXCR4 significantly increased from 51% in control mice to 82% in PTH-treated animals. In addition, CD45\(^+\)/CD34\(^+\)/CXCR4\(^+\) cells obtained from peripheral blood revealed a much lower expression of CXCR4. These findings suggest that mainly the CXCR4\(^+\) fraction of BMCs migrated from peripheral blood to the ischaemic heart and this fraction was further increased.
after PTH treatment. Since the analyses regarding the homing of BMCs relied on the expression of the pan-haematopoietic marker CD45 in non-transgenic animals, we created a chimeric mouse model expressing constitutive GFP in all BM cells to prove the origin of migrated cells in the heart. In this model, EGFP+/CXCR4+ cells were significantly increased in peripheral blood and hearts after PTH treatment, confirming our previous findings.

Analyses of SDF-1 concentrations in PTH-treated animals revealed an increase of SDF-1 levels in ischaemic hearts, which is in accordance with the results by Jung et al. showing increased expression of SDF-1 in PTH-treated osteoblasts. SDF-1 levels in serum remained unchanged resulting in a chemotactic gradient towards the site of ischaemia. However, measured SDF-1 levels contain active and inactive forms of the chemokine. As described previously, the recruitment can be limited by proteases cleaving the active form of SDF-1.

4.2 Effects of PTH on activity of DPP-IV in vitro and in vivo
As a major enzyme being involved in this process DPP-IV/CD26 has been identified. As a new discovery with respect to the SDF-1/CXCR4 axis, we could show in vitro that PTH significantly reduced the enzymatic activity of DPP-IV in a dose-dependent manner. The most distinctive inhibition could be achieved with the administration of 25 fmol PTH, a dose corresponding to our in vivo experiments and in previous preclinical studies. Investigating the enzyme activity of DPP-IV in vivo, PTH treatment resulted in a significant inhibition of the activity in different organs. The extent of inhibition was most prominent in infarcted hearts after PTH treatment.

The lower grade of inhibition in the in vitro when compared with the in vivo situation in the heart may be explained by the fact that we had to use a recombinant version of DPP-IV expressed in Spodoptera frugiperda insect cells, which bears a His-Tag at the N-terminus. In addition, the in vitro setting does not provide potential co-factors, which may be required for optimal DPP-IV activity. The in vitro effect is, however, specific and clearly PTH dependent as control samples with equal amounts of BSA instead of PTH showed unaltered substrate turnover (Figure 4).

These in vitro and in vivo findings suggest that ischaemia may trigger the PTH-mediated effects on DPP-IV/CD26 inhibition, resulting in facilitated recruitment of CXCR4+ cells to the infarcted heart. The effect of enhanced mobilization of BMCs and improved homing via inhibition of DPP-IV/CD26 was achieved by a ‘dual stem cell...
therapy’ in a previous study by our group. In this study G-CSF was used to mobilize stem cells and Diprotin A to inhibit the activity of DPP-IV/CD26. In the present study, we could show for the first time that the administration of PTH combines the properties of both substances. Similar to our previous study, the enhanced migration of CXCR4+ cells after PTH treatment was associated with an improved cardiac function after MI.

4.3 Influence of the SDF-1/CXCR4 axis on PTH-mediated cardioprotection

In a final step, we intended to adduce evidence that the demonstrated effects on the SDF-1/CXCR4-axis are crucial for enhanced homing of BMCs and improved cardiac function. Therefore, we injected the CXCR4 antagonist AMD3100 along with PTH in infarcted animals. Co-administration of AMD3100 with PTH significantly reduced the number of CD45+/CD34+ cells in the infarcted heart. Moreover, the beneficial effects of PTH on cardiac function were inverted by blocking CXCR4 as well. These results strongly suggest that SDF-1/CXCR4 interactions regulate PTH-mediated migration of BMCs and (consequently) account for the cardioprotective effects. The latter may occur through BMCs-mediated paracrine repair mechanisms leading to enhanced neovascularization and prevention of apoptosis. Our findings demonstrate a crucial participation of the SDF-1/CXCR4-axis in these effects after PTH treatment, which is consistent with previous data.

It was the goal of our study to investigate the influence of PTH on stem cell migration and homing and to analyse the molecular mechanisms of these effects. In order to match our previous protocols, we decided to use total hearts to quantify the extent of migration. With this method the exact region of the invaded cells remains unexplored and it will be the goal of further studies to further unravel this question.
4.4 Clinical perspective

In summary, our data suggest a cardioprotective mechanism of PTH treatment after MI. We showed for the first time that PTH is a DPP-IV inhibitor leading to an increased cardiac SDF-1 protein level, which enhances recruitment of regenerative CXCR4+ BMCs into the ischaemic heart associated with improved cardiac function (Figure 7). On the basis of the fact that PTH has already been clinically approved in patients with osteoporosis, our data suggest PTH as a promising agent for tissue repair and protection in ischaemic disorders. In this regard, the present study may pave the way for the initiation of clinical studies in patients with MI.

Supplementary material

Supplementary material is available at Cardiovascular Research online.

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References


