IMMUNOLOGY AND MICROBIOLOGY

Changed Homing of Hemopoietic Precursor Cells after Long-Term Treatment with Parathyroid Hormone

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 142, No. 7, pp. 97-100, July, 2006
Original article submitted January 31, 2006

Changes in the capacity of hemopoietic stromal microenvironment to promote homing of hemopoietic stem cells from different hierarchical compartments were evaluated in mice treated with parathyroid hormone by determining their 24-h precipitation factor. This parameter did not change for splenic short-living hemopoietic stem cells (splenic CFU) and considerably decreased for the bone marrow of mice treated with parathyroid hormone. For earlier long-living hemopoietic stem cells (cells forming the cobblestone area on day 28) the precipitation factor after injections of parathyroid hormone did not change in the bone marrow and decreased in the spleen. These data suggest that parathyroid hormone decreases the efficiency of homing of short-living hemopoietic stem cells in the bone marrow.

Key Words: *parathyroid hormone; hemopoietic stem cells; precipitation factor; stromal microenvironment*

Parathyroid hormone (PTH) regulating calcium metabolism and osteogenesis is the only known for today systemic regulator of stromal hemopoietic microenvironment. In stromal cultures PTH causes activation and stimulates proliferation of osteoblasts [1], an important cell component of the hemopoietic "niche" (the site of hemopoietic microenvironment maintaining the existence of hemopoietic stem cells, HSC) [7]. Reciprocal interactions between HSC and osteoblasts and other hemopoietic microenvironment cells play the key role in initiation and maintenance of the hemopoietic "niche" in the bone marrow [6]. Osteoblasts can produce secondary signals for bone resorption. They express factors attracting osteoclasts to sites of mineralization and modulate the expression of adhesion molecules. The count of HSC carrying Sca1 and c-kit markers increases in the bone marrow of mice with elevated expression of type I PTH receptor [1]. Long-term PTH treatment leads to a 2-fold increase in $Sca1^{+}c\text{-}kit^{+}$ HSC population in mice.

Recently, PTH has been actively used in clinical practice for bone growth stimulation in patients with osteoporosis [3]. In pharmacological concentrations PTH activates osteoclasts and, consequently, bone resorption, while in low doses it stimulates the formation of cortical and trabecular bone characterized by high mechanical strength. The effects of this hormone on hemopoietic precursor cells of different degree of maturity also deserve special investigation. PTH effect on hemopoiesis can be directly evaluated by analyzing the adhesion capacity of hemopoietic precursors in the bone marrow of animals injected with PTH. Here we studied changes in the precipitation factor for HSC derived from different hierarchical compartments of hemopoiesis 24 h after injection of bone marrow cells to lethally irradiated mice pretreated with PTH. Precipitation

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factor (f-24) for short-living polypotent stem cells forming colonies in the spleen of lethally irradiated mice (CFUs) and for long-living polypotent stem cells, whose incidence reflects that of stem cells capable of restoring hemopoiesis in lethally irradiated mice was also studied. These precursor cells form the cobblestone areas 28-35 days after seeding on the stromal feeder (CFCA-28) [5].

MATERIALS AND METHODS

The study was carried out on 9-26-week-old female $(CBA\times C57Bl/6)F_1$ mice. The experimental animals were injected with synthetic rat PTH (1-34) (Bachem) in a dose of 10 or 80 μg/kg intraperitoneally 5 times a week for 4 weeks. Evaluation of f-24 for CFUs was performed routinely. Bone marrow cell pool from 8 donors was used after 2-h adhesion to plastic (for maximum elimination of stromal cells and macrophages). In order to evaluate the initial concentration of CFUs, the recipients were irradiated (total dose 10 Gy) on a ^{137}Cs IPK device (Hematology Research Center) by two equal doses at a 3-h interval; after 1-2 h bone marrow cells (2×10^4) were injected intravenously (control group). After irradiation in a lethal dose a mixture of bone marrow cells (group 1 recipients) was intravenously injected (16×10^6) to animals treated and not treated with PTH and after 24 h the spleen and bone marrow were collected. The spleen was crushed in a homogenizer and a single-cell suspension was prepared from the bone marrow from the femoral and crural bones. The resultant cells in volumes equivalent to $^1/_{30}$ spleen or $^1/_5$ one femoral+crural bone marrow were injected to lethally irradiated recipients (group 2 recipients). On day 8 colonies were counted under a binocular magnifying glass in spleens fixed in Bouin's fluid in recipients of the control group and group 2. The factor was estimated by the formula: f-24=(a/N) ×100, where a is the mean number of colonies in the spleens of group 2 recipients and N is the total number of CFUs injected to group 1 recipient.

CFCA-28 precipitation factor was evaluated similarly. CFCA-28 were counted in the initial bone marrow by the Ploemacher's method [5]. To this end, MS5 cells [4] were inoculated (3000 cells per well) in αMEM (ICN) with 10% FCS (HyClone), 2 mM L-glutamine (ICN), 100 U/ml penicillin (Ferane), and 50 μg/ml streptomycin (Ferane) in 60 wells of a 96-well flat-bottom plate for culturing 24 h before the experiment. In order to prevent drying and contamination, 200 μl 0.1 M NaCl (ICN) was pipetted into all marginal wells. The plates were cultured at 33°C and 5% CO_2 in a humid atmosphere. In order to evaluate CFCA-28 incidence, $^1/_{24}$ of the spleen or $^1/_4$ crural+femoral bone marrow cells from group 1 recipients were inoculated per well in the first dilution, after which 3 more 2-fold dilutions were made and culturing was carried out in a nutrient medium consisting of 80% Fisher's medium (ICN), 2 mM glutamine (ICN), 10^{-6} M hydrocortisone (Sigma), 20% serum ($^1/_3$ FCS (HyClone) and $^2/_3$ equine serum (GibcoBRL)), 100 U/ml penicillin (Ferane), and 50 μg/ml streptomycin (Ferane). Half of the medium was replaced once a week. The content of the wells was analyzed under an inverted microscope 4 weeks after cell inoculation. Negative (containing no CFCA) wells were counted. The probability of the well containing no CFCA is described by Poisson's distribution: $P_0=e^{-m}$, where m is the mean number of CFCA in a sample. This probability is evaluated as P=r/n, where n is the number of wells with specified dilution and r the number of negative wells in this dilution, and hence m=-ln(r/n). The mean number (m) of CFCA in a studied sample can be thus estimated. Inoculation of many dying cells from irradiated group 1 recipients and subsequent analysis of CFCA gives distorted results, and therefore measurement errors in these experiments are high.

RESULTS

The part of hemopoietic precursor population reaching the spleen after intravenous injection is determined by the method of exogenous splenic colonies. It varies depending on the quality of injected donor cells and status of stromal microenvironment in this organ. PTH does not modify splenic stroma, because the percentage of precipitating CFUs did not change after treatment with the hormone in the studied concentrations (Fig. 1, *a*). This result could be expected, because there are no cells carrying PTH receptors in the spleen. Receptors of two types are known. Type 1 are receptors on osteoblasts (but not osteoclasts), type 2 receptors were detected in the brain, pancreas, kidneys, and testicles. The CFUs precipitation factor decreased more than 3-fold in the bone marrow of mice treated with PTH in both concentrations (Fig. 1, *b*). This unexpected result indicates that characteristics of the bone marrow stroma changed significantly. Short-living polypotent hemopoietic precursors, to which CFUs belong, are worse retained in the bone marrow of PTH-treated animals compared to the bone marrow of control animals.

No changes in CFCA-28 precipitation factor were detected in the bone marrow of PTH-treated animals (Fig. 2, *b*). Despite great errors in these experiments, we assert that PTH exhibits no nega-

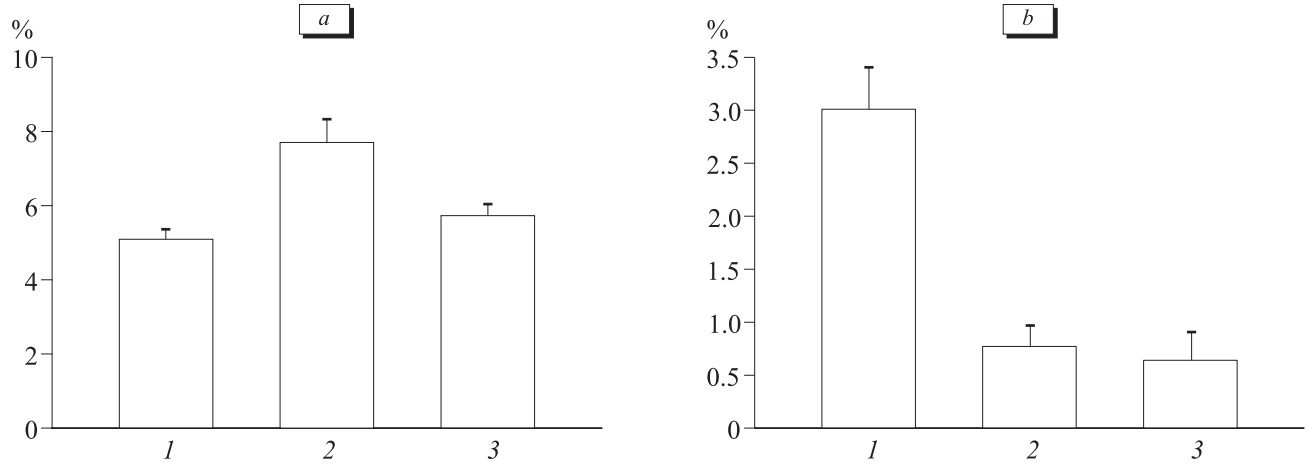

Fig. 1. CFUs precipitation factor in the spleen (*a*) and bone marrow (*b*) of intact and PTH-treated mice. Here and in Fig, 2: *1*) control; *2*) PTH, 10 μg; *3*) PTH, 80 μg.

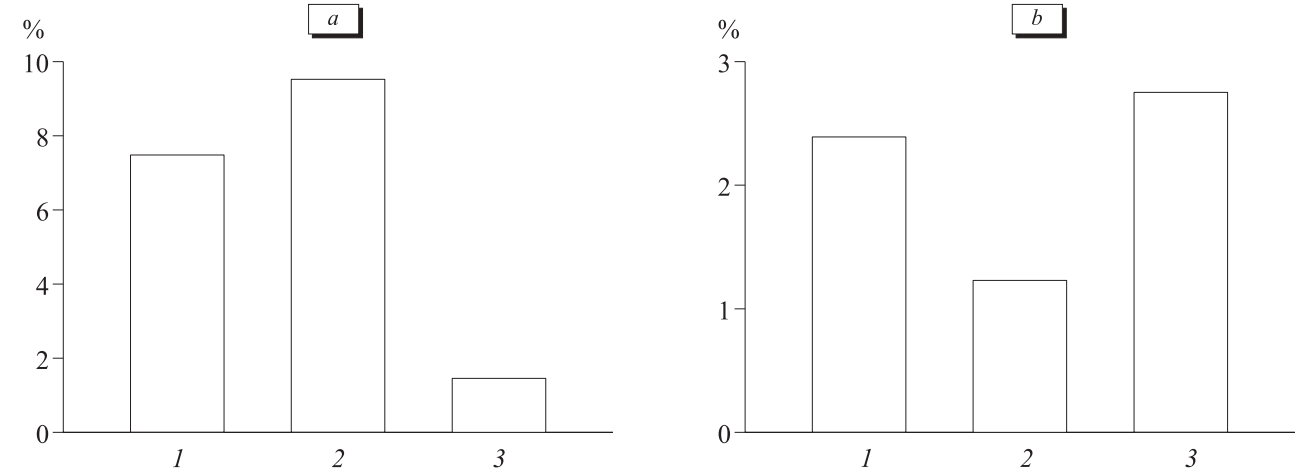

Fig. 2. CFCA-28 precipitation factor in the spleen (*a*) and bone marrow (*b*) of intact and PTH-treated mice.

tive impact on HSC capacity to adsorb on the bone marrow stroma. However, with consideration for previous findings [1], we expected enhanced precipitation of early hemopoietic precursors in the bone marrow of mice treated with PTH. Hence, increased count of osteoblasts in the "niches" for

TABLE 1. Distribution of CFUs and CFCA-28 in Mouse Bone Marrow and Spleen

PTH; organ		Precipitation, %	
		CFUs	CFCA-28
Control	bone marrow	43	34
	spleen	5	7
	not reached hemopoietic organs	52	59
PTH 10 μg	bone marrow	11	18
	spleen	8	10
	not reached hemopoietic organs	81	72
PTH 80 μg	bone marrow	9	39
	spleen	6	1
	not reached hemopoietic organs	85	60

HSC does not directly depend on the capacity of hemopoietic precursor cells to precipitate in the bone marrow. Presumably, homing molecules, chemoattractants, and adhesion molecules for HSC are located on different cells. The f-24 parameter for CFCA-28 in the spleen of mice receiving a low dose of PTH does not differ from that in the control group (Fig. 2, *a*). A drop of f-24 for CFCA-28 in the spleen of mice treated with PTH in a dose of 80 μg/kg can hardly be caused by changes in the stromal microenvironment. The distribution of mouse hemopoietic cells in the entire bone marrow was determined [2]. According to previous estimation [2], 14% of all bone marrow cells are located in the femoral and crural bones. Hence, we can estimate how many HSC reached the hemopoietic areas over 24 h after irradiation (Table 1). Under conditions of our experiments about 40% injected CFCA-28 reached the hemopoietic areas in control and PTH-treated mice. Control and experimental groups differed by distribution in the studied territories, the differences being not reliable.

PTH treatment of mice led to an almost 30% decrease in f-24 for later precursors (CFUs). This treatment can lead to imbalance in the hemopoietic system and have a negative impact on short-living stem cells.

These results necessitate further studies of PTH effect on hemopoiesis. The aftereffects of PTH in the studied concentrations should be thoroughly studied in patients with osteoporosis, treated with this hormone for a long time.

The study was partially supported by the Russian Foundation for Basic Research (grant No. 05-04-48242-a).

REFERENCES

1. L. M. Calvi, G. B. Adams, K. W. Weibrecht, *et al.*, *Nature*, **425**, 841-846 (2003).
2. G. A. Colvin, J. F. Lambert, M. Abedi, *et al. Leukemia*, **18**, 575-583 (2004).
3. J. S. Finkelstein, A. Hayes, J. L. Hunzelman, *et al.*, *N. Engl. J. Med.*, **349**, 1216-1226 (2003).
4. L. Kobari, A. Dubart, F. Le Pesteur, *et al.*, *J. Cell. Physiol.*, **163**, 295-304 (1995).
5. R. E. Ploemacher, J. P. van der Sluijs, J. S. Voerman, *et al.*, *Blood*, **74**, 2755-2763 (1989).
6. R. S. Taichman, *Blood*, **105**, 2631-2639 (2005).
7. J. Zhang, C. Niu, L. Ye, *et al. Nature*, **425**, 836-841 (2003).