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# Effect of Growth Hormone and Pamidronate on Bone Blood Flow, Bone Mineral and IGF-I Levels in the Rat

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## Summary

So far it is not known whether the growth hormone (GH) has an effect on the local blood circulation in bones. Using male rats we studied the local blood circulation in the tibia and the distal end of the femur (by means of the uptake of $^{85}$Sr-microspheres), the density and ash weight of the tibia, the urinary excretion of pyridinoline (PD) and deoxypyridinoline (DPD) as an indicator of bone resorption and the blood levels of IGF-I after the administration of human GH (4 mg/kg s.c. daily for 4 weeks) and/or bisphosphonate pamidronate (Aredia, CIBA-Geigy, administered in the dose of 3 mg/kg i.p. on day 1, 2, 9 and 10). The rats were divided into four groups: 1. controls, 2. GH, 3. pamidronate, 4. GH plus pamidronate. After the administration of GH, we observed a significant increase in bone blood flow (and in the uptake of $^{85}$Sr-microspheres), a decrease in the density and ash weight of the tibia and increased urinary excretion of PD and DPD; IGF-I levels in the blood were non-significantly elevated. Simultaneously administered pamidronate inhibited all significant effects of GH and it also decreased the IGF-I levels in rats treated with GH. After the administration of pamidronate itself the bone density and ash weight of the tibia were increased and urinary DPD excretion was decreased. In view of the known vascular effects of IGF-I, we assume that the increase in bone blood flow after the administration of GH and its reduction after simultaneous administration of pamidronate could be mediated by the changes of IGF-I blood levels, although the effect of pamidronate on IGF-I is still not clear. Regarding the role of blood circulation in rat bones, we consider that our present results are further evidence for the relationship between the blood circulation in bones and bone resorption, although these results do not show how active is bone blood circulation in the regulation of bone tissue metabolism.

**Key words**
Bone blood flow • Bone mineral • IGF-1 • Growth hormone • Pamidronate • Rat

## Introduction

We have previously been studying the local blood flow and mineral content in rat bones in various model situations usually induced by hormonal influences, e.g. the deficiency of sex hormones and their administration (Kapitola *et al.* 1995). We try to explain the mutual relationship between bone blood flow and bone mineralization state especially with regard to the possible role of local circulation in bone tissue metabolism.



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Recently, we directed our attention to the growth hormone (GH). However, the situation here is rather complicated. The effects of GH on bones are complex and have not yet been fully clarified. GH can stimulate osteoblasts and osteoclasts, it can enhance bone formation and also bone resorption and thereby influence the bone tissue by various mechanisms (Spencer *et al.* 1991, Wüster 1995, Nishiyama *et al.* 1996, Holmes and Shalet 1996).

Almost nothing is known concerning the bone blood flow. We therefore present the results of experiment in male rats which had been administered the growth hormone and/or bisphosphonate pamidronate in order to ascertain the possible interaction of bisphosphonate with growth hormone. In addition to the usual observation of the blood circulation and mineral content in bones, we also determined bone resorption indicators, i.e. urinary excretion of pyridinoline (PD) and deoxypyridinoline (DPD), and blood levels of insulin-like growth factor I (IGF-I).

## Material and Methods

Forty-three male rats aged 70 days (Research Institute of Pharmacy and Biochemistry, Konárovice, Czech Republic) were used in the experiment. The animals were kept in a room with regulated temperature (23 °C) and were fed a standard pelleted diet (Bergman, Jesenice, Czech Republic) with water *ad libitum*.

The rats were divided into four groups: group 1 – controls, group 2 – growth hormone (GH), group 3 – pamidronate, group 4 – GH plus pamidronate.

The growth hormone was extracted from human pituitaries isolated according to the method described by Lumley-Jones *et al.* (1979), with hormonal activity of 3 IU/mg. It was purchased from the Center for Hormonal Proteins and Peptides, Medical Faculty, Charles University, Hradec Králové, Czech Republic, (Head Dr. J. Lomský). The GH dissolved in PBS was injected to appropriate groups of animals s.c. daily for 28 days before the experiment in a dose of 4 mg/kg body weight (the other groups were given PBS in the same manner). The bisphosphonate pamidronate (Aredia, CIBA-Geigy, Switzerland) was injected i.p. on day 1, 2, 9 and 10 in the dose of 3 mg/kg body weight (the other groups were given physiological saline in the same manner).

Circulatory values were determined using microspheres labeled with radioactive strontium $^{85}Sr$ (Rudolph and Heymann 1967, Kapitola *et al.* 1987). The rats, anesthetized with thiopental, were given an i.v. injection of heparin. A catheter connected to a Type 304 peristaltic pump (Zalimp, Warszawa, Poland), fulfilling the function of an artificial organ for determining cardiac output, was introduced into the right femoral artery. Another catheter, connected to an LMP 160 pressure transducer, an LDP 186 pressure recorder and an LKM 210 cardiomonitor (Tesla, Valašské Meziříčí, Czech Republic) was introduced into the right carotid artery and from there, controlled by the pressure curve, into the left heart ventricle. Through this catheter we injected a dose of about 18.5 kBq, i. e. 0.5 μCi $^{85}Sr$-microspheres (diameter 15 μm, NEN, Boston, MA, USA) over 10 s and immediately washed through with saline. One minute later, the rat was sacrificed by decapitation, the left tibia and femur were dissected out (from the femur an approximately 7 mm long segment of the distal end was separated), cleaned and weighed. Together with blood samples from the femoral artery and with $^{85}Sr$-microsphere standards, the bone samples were measured in an NA 3601 automatic gamma-counter (Tesla, Vrchlabí, Czech Republic). The $^{85}Sr$-microsphere content in the bone samples was expressed as the percentage of the dose per gram of tissue (which is actually an indicator of specific local circulatory changes, excluding the influence of simultaneous changes in cardiac output).

Cardiac output and local blood flow were computed by the following formulas:

$$\text{CO (ml/min)} = \frac{\text{withdrawal of blood (ml/min)} \cdot 100}{^{85}\text{Sr in blood sample (\% of dose)}}$$

$$\text{BF (ml/min/g)} = \frac{\text{CO (ml/min)} \cdot {}^{85}\text{Sr in bone (\% dose/g)}}{100}$$

Bone density was computed on the basis of Archimedes' principle, after weighing the tibia on a PRLT TW2 torsion balance (Tecniprot, Pruszkow, Poland) under water and in the air. Ash weight was determined after burning the tibia for about 18 h at 800 °C in a muffle furnace, by weighing it on a TS120 balance (OHAUS Corp., Florham Park, NJ, USA), and was expressed in grams of ash per ml of bone volume.

For determination of IGF-I, we used DSL-2900 Rat IGF-I RIA kits (Diagnostic Systems Laboratories Inc., Webster, Texas, USA). Pyridinoline and

deoxypyridinoline in the urine of rats was detected by high-performance liquid chromatography (Eyre *et al.* 1984, Ezzat *et al.* 1993). We are grateful to Ing. Špaček and Ing. Hulejová from the Rheumatological Institute in Prague (Head Assoc. Prof. Dr. K. Pavelka) for performing the measurements.

Statistical significance of the results was evaluated using one-way analysis of variance with the LSD test.

## Results

After the administration of growth hormone the blood flow and the uptake of $^{85}$Sr-microspheres were significantly increased in the tibia and in the distal end of the femur (Fig. 1). Pamidronate itself did not have any significant influence on the local circulation in bones. However, the effect of GH was fully suppressed by the simultaneous administration of pamidronate.

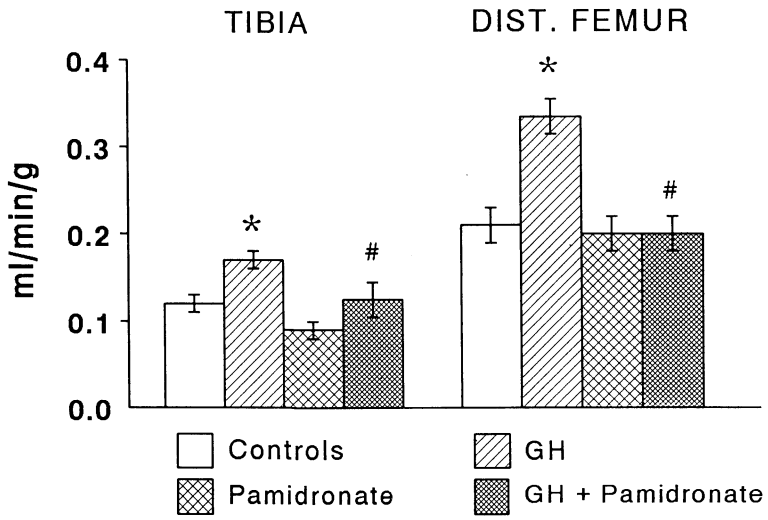


**Fig. 1.** *Effects of the administration of growth hormone (GH, 4 mg/kg s.c. daily for 4 weeks before the experiment) and/or pamidronate (Aredia, 3 mg/kg i.p. on day 1, 2, 9 and 10) on the bone blood flow in the tibia and distal femur of male rats. Significantly different ($p<0.05$): * from controls, # from GH-treated group.*

Both the density and ash weight of the tibia (expressed per ml of bone volume) were decreased after GH treatment and increased after pamidronate treatment (Fig. 2). In rats treated with GH, the simultaneous administration of pamidronate significantly increased both the density and ash weight when compared with the group on GH only and with the controls.

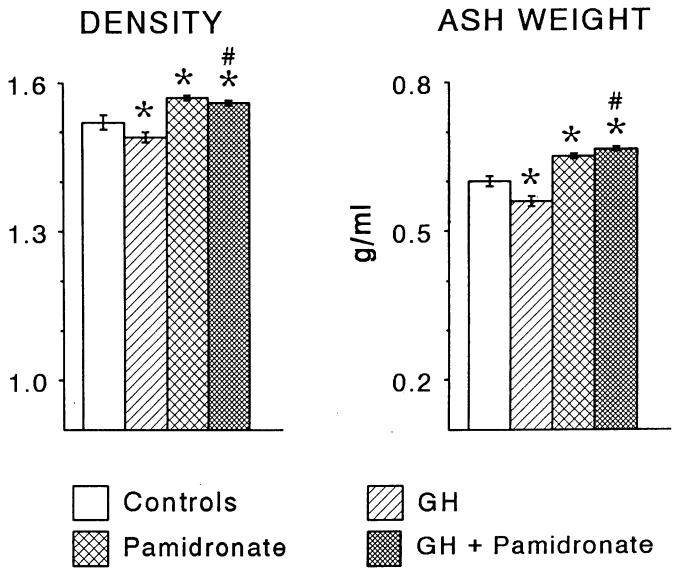


**Fig. 2.** *Effects of the administration of growth hormone (GH, 4 mg/kg s.c. daily for 4 weeks before the experiment) and/or pamidronate on the density and ash weight of the tibia in male rats. Significantly different ($p<0.05$): * from controls, # from GH-treated group.*

Table 1 shows that the urinary excretion of PD and DPD was significantly increased after GH, both increases being inhibited by the simultaneous administration of pamidronate (in the case of DPD below values of the controls). The DPD excretion was also significantly decreased after the administration of pamidronate alone. The level of IGF-I in the rats, that were given GH, was only non-significantly higher. However, the simultaneous administration of pamidronate with GH led to a significant decrease of IGF-I in comparison with the group that had been administered GH only. After pamidronate itself the level of IGF-I was not significantly changed (Table 1).

The body weight of rats was slightly but significantly increased after the administration of GH. The values of cardiac output were not significantly changed in any of the group investigated (Table 1).

**Table 1.** Effects of growth hormone (GH) and/or pamidronate in male rats.

| Groups | Controls | GH | Pamidronate | GH + pamidronate |
|---|---|---|---|---|
| *Number of rats* | 11 | 11 | 11 | 10 |
| *Body weight (g)* | 339±4 | 367±4* | 334±4 | 352±7 |
| *Cardiac output* | | | | |
| *(ml/min)* | 57.0±2.3 | 63.8±2.6 | 63.3±4.9 | 67.2±3.0 |
| *(ml/min per 100 g)* | 16.9±0.8 | 17.3±0.7 | 18.7±1.4 | 19.1±0.9 |
| *$^{85}$Sr-microsphere uptake (% dose/g)* | | | | |
| *tibia* | 0.35±0.02 | 0.51±0.04* | 0.25±0.02 | 0.30±0.04$^{\#}$ |
| *distal femur* | 0.66±0.05 | 0.96±0.06* | 0.54±0.05 | 0.47±0.06$^{\#}$ |
| *Urinary pyridinoline* | | | | |
| *(nmol/mmol creat.)* | 56.0±2.6 | 71.6±3.5* | 51.6±1.9 | 57.4±2.1$^{\#}$ |
| *Urinary deoxypyridinoline* | | | | |
| *(nmol/mmol creat.)* | 29.5±1.6 | 39.0±2.4* | 15.1±1.1* | 18.6±1.1*$^{\#}$ |
| *IGF-I (ng/ml)* | 1425±140 | 1748±224 | 951±126 | 783±137$^{\#}$ |

*Data are means ± S.E.M. Significant differences ($p<0.05$): * from controls, $^{\#}$ from GH-treated rats.*

## Discussion

As was briefly mentioned in the Introduction, the effects of growth hormone on bone metabolism can vary quite considerably. Various effects of GH on local blood circulation in bones could therefore be expected. Available reports are rather sparse. Using the $^{18}$F-method, normal blood supply of the skeleton was ascertained in patients with acromegaly (VanDyke *et al.* 1971). We disclosed a significant decrease in tissue clearance of intravenously injected $^{133}$Xe in the greater trochanter of the femur in 30 hormonally active acromegalics (Kapitola *et al.* 1986).

In the present experiment, the basic effect of GH on rat bone metabolism was to increase bone resorption. We cannot explain the reason for this finding. Methodological factors (animals used, mode of GH administration) might play an important role, but to eliminate the different experimental conditions possibly involved is beyond our possibilities.

The most important result of our experiment, i.e. the increase in bone blood flow after the administration of GH, is in agreement with the metabolic effect of GH, i.e. increased bone resorption. The increase in blood flow through rat bones observed in the present experiment does not correspond with our results in acromegalics

cited above, however, these two situations and the methods employed are very different.

The suppressive effect of bisphosphonates on bone resorption is known and is therapeutically used. However, it turns out that bisphophonate can also influence blood circulation in bones. In a recent experiment, pamidronate suppressed the uptake of $^{85}$Sr-microspheres in the bones of female rats, which was increased after oophorectomy (Kapitola and Žák 1998).

In the present experiment, we also measured IGF-I levels. Besides the known role of IGF-I in bone physiology, we also anticipated its possible relationship to the problem studied. It has been found that circulating IGF-I levels are increased after ovariectomy and decreased after estradiol, indicating a possible IGF-I participation in the regulation of bone turnover (Sato *et al.* 1993, Kalu *et al.* 1994). Vasodilatory effects of IGF-I have been demonstrated in various tissues (but not in bones) (Haylor *et al.* 1991, Copeland and Sreekuran 1994, Walsh *et al.* 1996) with participation of NO as a mediator (Haylor *et al.* 1991). Consequently, we have considered that IGF-I might take part in the regulation of blood circulation in bones.

The main results of the present experiment can be briefly summarized as follows. In rats, growth hormone enhances bone blood flow and bone resorption but the increase of IGF-I is only non-significant. On the other hand, pamidronate decreases bone blood flow, bone resorption and IGF-I levels in rats treated with GH.

There are two important questions concerning the observed local changes in bone blood circulation as the main focus of our interest: a) by what mechanism do these changes occur, and b) what is the relationship of local circulation in bones to the other observed changes.

We think that the administration of GH might increase the blood flow in bones through IGF-I action. The cited vascular effects of IGF-I, including NO as a mediator, support this idea. Similarly, the decrease of blood flow in bones after the simultaneous administration of GH and pamidronate can be associated with the decrease of IGF-I levels. However, the pamidronate effect on IGF-I is surprising, because it is not clear by which mechanism the decrease of IGF-I occurs after the administration of pamidronate. It is not likely to be due to its direct influence, but it could be a part of complex mechanisms regulating bone metabolism.

The relationships between the changes in bone blood flow and changes in other parameters are very important from the point of view of the specific role of local blood flow in the bone tissue. In our experiment on rats, bone blood flow and bone resorption indicators are increased after growth hormone administration, whereas the increase of bone flow and resorption are suppressed when pamidronate is administered at the same time. These results convincingly support the earlier assumption about the relationship between bone blood circulation and bone resorption (Schoutens *et al.* 1984, Kapitola *et al.* 1995). Unfortunately, even these results still do not show whether the local circulation changes are an active regulatory factor or only a part of the metabolic changes in bone tissue.

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