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# Effect of Pamidronate on Bone Blood Flow in Oophorectomized Rats

J. KAPITOLA, J. ŽÁK[1]

*Laboratory for Endocrinology and Metabolism and [1]Third Medical Department, First Faculty of Medicine, Charles University, Prague, Czech Republic*

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## Summary

In connection with the known inhibitive action of bisphosphonates on bone resorption we were interested in their possible influence on bone blood flow (BBF). We determined BBF ($^{85}$Sr-microsphere uptake in the tibia, distal femur and diaphysis of femur), cardiac output, density and ash weight of the tibia, as well as 24-h incorporation of $^{45}$Ca and $^{3}$H-proline into the tibia. Pamidronate (Aredia, CIBA-Geigy, Switzerland) was administered to sham-operated or oophorectomized (OOX) female rats in doses of 0.6 mg i.p. 2 days a week for 4 weeks. $^{85}$Sr-microsphere uptake was increased after OOX in the tibia and distal femur, simultaneous pamidronate administration significantly suppressed this increase below the control level. In addition, pamidronate inhibited the 24-h incorporation of $^{45}$Ca and $^{3}$H-proline in sham-operated females and suppressed the incorporation of $^{3}$H-proline that was increased after OOX. Bone density and ash weight were significantly increased after pamidronate administration in both sham-operated and OOX rats. The results of both experiments showing a significant effect of pamidronate on BBF and incorporation of $^{45}$Ca and $^{3}$H-proline require further verification and elucidation.

**Key words**
Bone blood flow – Bisphosphonates – Pamidronate – Oophorectomy – Rat

## Introduction

Bone blood flow (BBF) is usually influenced in experimental model situations with altered bone turnover (mainly bone resorption), e.g. BBF rises after castration and declines after oestradiol or testosterone administration (Schoutens *et al.* 1984, Kapitola *et al.* 1995, Kapitola 1996). Bisphosphonates, when used therapeutically, inhibit bone turnover and especially bone resorption. It is not clear, whether BBF is influenced or not. Schoutens *et al.* (1988) found that diphosphonate APD administration to paraplegic rats prevented the decrease of bone mass in the tibia and femur but did not influence increased BBF (provoked by paraplegia). Similarly, Laroche *et al.* (1996) observed that tiludronate administration prevented loss of bone mass in female OOX rats but did not influence the increased area of sinusoidal capillaries in the trabecular tissue of lumbar vertebra. We studied the possible influence of a bisphosphonate on BBF and on 24-h incorporation of $^{45}$Ca and $^{3}$H-proline in OOX female rats. Positive results of two experiments are presented in this paper.



Fax +420 2 293 894
E-mail physres@sun1.biomed.cas.cz
http://www.lf2.cuni.cz/physiolres/physiolres.htm

## Material and Methods

The experiments were performed on 84 Wistar female rats (Research Institute of Pharmacy and Biochemistry, Konárovice, Czech Republic) fed pelleted food (Bergman, Jesenice, Czech Republic) and drinking water *ad libitum*. In both experiments, the rats were divided into four groups: group 1: controls, group 2: oophorectomy (OOX), group 3: pamidronate, group 4: OOX + pamidronate. Oophorectomy was performed by the dorsal approach 4 weeks before the actual experiment (group 1 and 3 were sham-operated). Pamidronate (Aredia, CIBA-Geigy, Switzerland) was injected i.p. to the rats twice a week for 4 weeks before the experiment in a dose of 0.6 mg in 0.2 ml solution *per* rat (the medium effective dose according to Reitsma *et al.* 1980). Groups 1 and 2 received an injection of physiological saline in the same way.

The local blood flow and cardiac output were determined by means of microspheres labelled with radioactive strontium $^{85}Sr$ (Rudolph and Heymann 1967, Kapitola *et al.* 1987). Under thiopental anaesthesia (Research Institute of Antibiotics and Biotransformations, Roztoky, Czech Republic) the rats were given an i.v. injection of 200 IU heparin in 0.2 ml. A catheter was introduced into the right femoral artery and connected to a Type 304 peristaltic pump (Zalimp, Poland), which acted as an artificial organ for the determination of cardiac output. Another catheter, which was connected to LMP 160 pressure transducer with LDP 186 blood pressure recorder and LKM 210 cardiomonitor, was introduced *via* the right carotid artery into the left heart ventricle under control of the pressure curve. Through this tubing, we injected a dose of approximately 37 kBq, i.e. 1 $\mu$Ci $^{85}Sr$-microspheres (diameter 15 microns, NEN, USA) and immediately rinsed the catheter with saline. The rat was decapitated after about one minute and the following samples were removed: left tibia, an approximately 7 mm segment of the distal end of the left femur and diaphysis of the left femur. The samples were weighed on a TS 120 balance (OHAUS, USA) and their radioactivity was measured together with blood samples and $^{85}Sr$-microsphere standards using Gamaautomat Na 3601 (Tesla, Czech Republic). The uptake of $^{85}Sr$-microspheres was expressed as the percentage of the dose per 1 g of tissue and the bone blood flow and cardiac output were computed according to generally employed formulas (Kapitola *et al.* 1987).

The 24-h incorporation of $^{45}Ca$ and $^{3}H$-proline, as an indicator of mineralization and of the formation of the organic matrix, was determined after Globus *et al.* (1986). 7.4 kBq, i.e. 0.2 $\mu$Ci $^{45}Ca$, in the form of $CaCl_2$ (Polatom, Poland) and 185 kBq, i.e. 5 microCi $^{3}H$-proline (Amersham, England) per 100 g body weight were injected i.p. in a single dose. The rats were sacrificed on the next day, their left tibia was dissected out, cleaned and dissolved in concentrated HCl. Some of the diluted material was measured in a liquid scintillator (Insta-Gel Packard, USA) on a 1219 Rackbeta Liquid Scintillation Counter (LKB, Finland). The results are given as the number of dpm per mg osseous tissue.

**Table 1.** Effect of pamidronate administration on the cardiac output and bone blood flow in female sham-operated or oophorectomized (OOX) rats.

Experiment A

| Group | 1 Controls (sham-op.) | 2 OOX | 3 Pamidronate (+sham-op.) | 4 OOX +pamidronate |
|---|---|---|---|---|
| Number of rats | 11 | 11 | 11 | 11 |
| Body weight (g) | 218±3 | 256±5[a] | 217±2[b] | 267±4[a,c] |
| Cardiac output (ml/min) | 47.2±3.5 | 45.7±2.5 | 46.2±3.1 | 57.5±2.3 |
| (ml/min per 100 g) | 21.5±1.5 | 17.8±1.0 | 21.4±1.5 | 22.0±1.0 |
| Blood flow (ml/min per g) | | | | |
| Tibia | 0.13±0.02 | 0.15±0.01 | 0.09±0.01[b] | 0.11±0.01 |
| Distal femur | 0.27±0.03 | 0.31±0.02 | 0.18±0.03[b] | 0.25±0.02 |
| Diaphysis (femur) | 0.11±0.01 | 0.09±0.01 | 0.08±0.01 | 0.10±0.01 |

*Means ± S.E.M., significantly different ($p<0.05$), a – from group 1, b – from group 2, c – from group 3.*

Bone density was computed on the basis of the principle of Archimedes, after weighing the tibia on a PRLT TW2 torsion balance (Tecniprot, Poland) under water and in the air. The ash weight was determined after incinerating the bone for about 18 h in a muffle furnace at 800 °C by weighing the ash on a TS 120 balance (OHAUS, USA). The results are given in mg of ash per ml bone volume.

*Statistics*

The significance of group differences was tested using one-way analysis of variance after checking the normality of distribution of the data and equality of variances in the groups.

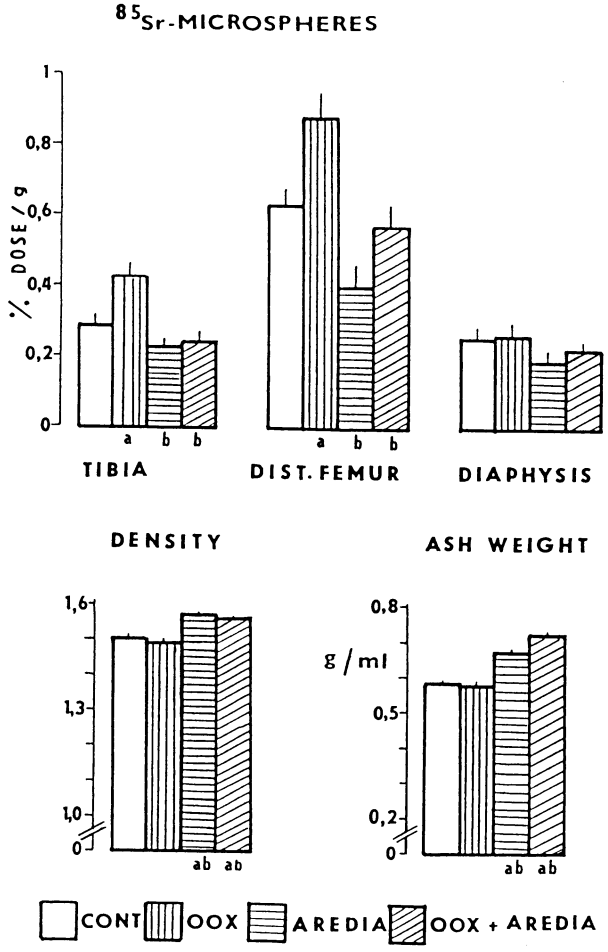


**Fig. 1.** *Effect of pamidronate administration on $^{85}$Sr-microsphere uptake in the tibia, distal femur and diaphysis of the femur and on the density and ash weight of the tibia of female sham-operated or oophorectomized (OOX) rats. Experiment A. Means ± S.E.M, statistically different ($p<0.05$): a – from controls, b – from OOX group, c – from Aredia group.*

## Results

*Experiment A* – BBF in the tibia, distal femur and diaphysis of the femur (Figure 1, Table 1). Uptake of $^{85}$Sr-microspheres was significantly increased after OOX in the tibia and distal femur. Pamidronate administration inhibited this increase below the level of control values. No significant changes of the uptake of $^{85}$Sr-microspheres were found in the femur diaphysis. Blood flow in ml/min per g was not significantly changed in the examined bone samples. Density and ash weight of the tibia were significantly increased after pamidronate in the sham-operated control group and in the group after OOX. The weight of rats was increased in both OOX groups. No significant differences were observed in their cardiac output.

*Experiment B* – incorporation of $^{45}$Ca and $^{3}$H-proline in the tibia (Table 2). After OOX, the incorporation of $^{3}$H-proline was significantly increased. Pamidronate administration decreased the incorporation of both substances in sham-operated rats and suppressed the increase in incorporation of $^{3}$H-proline after OOX.

## Discussion

Interpretation of the local blood flow results presented in this paper is based, in particular, on the values of $^{85}$Sr-microsphere uptake in the tissue, normalized for the body weight ($^{85}$Sr-microsphere uptake in % dose/g multiplied by the cardiac output which gives the blood flow value in ml/min per g). Therefore, the $^{85}$Sr-microsphere uptake value corresponds to local blood flow in ml/min per g divided by cardiac output. It is a similar expression as, for example, the "blood flow index", which was used by MacPherson and Tothill (1978) to assess blood flow through bones in rats of different ages. Although $^{85}$Sr-microsphere uptake in percentage of the dose in grammes is not a physiological value, it is advantageous because it discloses local changes, does not include cardiac output and is not therefore influenced by changes in cardiac output.

The results of the blood flow experiment demonstrates convincing effects of pamidronate: the increased uptake of $^{85}$Sr-microspheres in the tibia and distal femur after oophorectomy was completely suppressed by pamidronate administration. In the same situation, the 24-h incorporation of labelled proline in the females, either sham-operated or oophorectomized was suppressed by pamidronate ($^{45}$Ca incorporation only in the sham-operated animals). The density and ash weight of the tibia was, however, higher after pamidronate.

The discrepancy with the results of Laroche *et al.* (1986), mentioned in the Introduction, can be explained by the different methodology and also by the

examined object. The results of Schoutens *et al.* (1984) were obtained by the same method as ours but only blood flow values were presented.

Therefore, according to our results, pamidronate is another substance which concomitantly inhibits bone resorption, bone loss, as well as elevated bone blood flow in OOX rats. At present, we do not know why and how pamidronate acts on bone blood flow. Verification of the results and possibly the explanation of the significance and mechanism of bone blood flow changes in this experimental situation will be the subject of our future work.

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**Table 2.** Effect of pamidronate administration on 24-h incorporation of $^{45}Ca$ and $^{3}H$-proline into the tibia of female sham-operated or oophorectomized (OOX) rats.

Experiment B

| Group | 1 Controls (sham-op.) | 2 OOX | 3 Pamidronate (+sham-op.) | 4 OOX +pamidronate |
|---|---|---|---|---|
| Number of rats | 12 | 10 | 9 | 11 |
| Body weight (g) | 226±2 | 268±4[a] | 213±2[a,b] | 261±3[a,c] |
| 24-h incorporation (dpm/mg) | | | | |
| $^{45}Ca$ | 2.88±0.06 | 3.08±0.08 | 2.46±0.03[a,b] | 2.85±0.04c |
| $^{3}H$-proline | 2.07±0.02 | 2.54±0.09[a] | 1.56±0.03[a,b] | 1.81±0.05[a,b,c] |

*Means ± S.E.M., significantly different ($p<0.05$), a – from group 1, b – from group 2, c – from group 3.*

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**Reprint requests**
Dr. J. Kapitola, Laboratory for Endocrinology and Metabolismm, Third Medical Department, First Faculty of Medicine, Charles University, U nemocnice 1, 128 21 Prague 2, Czech Republic.