

Impact of Antihypertensive Therapy on the Skeleton: Effects of Enalapril and AT₁ Receptor Antagonist Losartan in Female Rats

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Summary

No data are available about the effects of AT₁ receptor antagonist losartan on the skeleton and there is also little information on the activity of an ACE inhibitor enalapril on bone metabolism. It is widely believed that the vasculature plays an important role in bone remodeling under normal and pathological conditions. We treated 14-week-old female Wistar rats with losartan, enalapril or saline. Administration of the ACE inhibitor enalapril and angiotensin II antagonist losartan had no effect on total malondialdehyde (MDA) in the blood and on urinary excretion of some eicosanoids and their metabolites. The administration of enalapril and losartan in a dose recommended for the treatment of hypertension did not cause significant changes in bone density, the ash and mineral content or morphometric parameters of the femur compared to the values found in control female rats.

Key words

Enalapril • Losartan • Female rats • Bone metabolism

Introduction

Angiotensin converting enzyme inhibitors or AT₁ receptor antagonists are widely used for treating hypertension in hypertensive women after menopause. The ACE inhibitor enalapril has been widely studied in laboratory animals for hemodynamic and hypertensive activity, but there is little information on the activity of this agent on bone metabolism. No data are available whether AT₁ receptor antagonist losartan affects the skeleton. Enalapril lowers the vascular resistance and blood pressure by relaxing peripheral arterioles (Fouad-Tarazi 1994). It is widely believed that the vasculature

plays an important role in bone remodeling under normal and pathological conditions.

The reason for investigating the effect of ACE inhibitor and AT₁ receptor antagonist on bone turnover is based on findings that osteoclasts, the primary inducers of postmenopausal bone loss, are in close proximity to the blood capillaries of the metaphyseal bone marrow. They are target cells for substances released by the endothelium of the microvasculature such as endothelin (Sasaki and Hong 1993), nitric oxide (MacIntyre *et al.* 1991) and prostaglandins (Jee *et al.* 1990). This suggests that, in addition to systemic hormonal regulation, osteoclasts might also be locally regulated by the endothelial products. *In vitro* experiments and animal

experiments have suggested that prostaglandins are important mediators of bone metabolism (Zusman 1981, Jee *et al.* 1990).

In the present study, we investigated the effects of the ACE inhibitor enalapril and AT₁ receptor antagonist losartan on the bone density and bone morphology in female Wistar rats.

Material and Methods

The experiment was carried out on 14-week-old female Wistar rats. Twenty-four animals weighing about 200 g were subdivided into three groups: A: control animals (8 animals treated daily with intraperitoneally administered saline), B: 8 animals treated daily with losartan (2 mg/kg b.w. i.p., MSD, USA), and C: 8 animals treated daily with enalapril (0.4 mg/kg b.w. i.p., Lachema, Czech Republic).

We used a dose for enalapril and losartan relevant to those used for the treatment of hypertension. The dose used in our experiment for enalapril (0.4 mg/kg/day i.p.) was similar to the dose used by Ma *et al.* (1997) for moexipril (10 mg/kg/day orally). Moexipril lowered the blood pressure by 12 %. In the case of losartan (2 mg/kg/day i.p.) we used a dose which produced a reduction in blood pressure by 27 % (Inada *et al.* 1999).

Blood samples were withdrawn before the beginning of the experiment and 30 days after drug administration. The biochemical parameters were measured by standard assays on a biochemical analyzer Cobas Mira plus (Roche Switzerland) at the beginning and 30 days after the beginning of the experiment.

For more precise detection of total malondialdehyde (MDA), HPLC with a spectrophotometric detector was used (Carbonneau *et al.* 1991).

For the determination of urinary eicosanoids, 0.1 ml of normal saline containing 50 mmol/l Na₂EDTA.2H₂O and 2 mmol/l indomethacin were added to each 1 ml of collected urine. Samples were pipetted into aliquots of 0.5 ml for each method and then frozen and stored at -20 °C. The solution containing 5.8 g/l NaCl, 2.2 g/l KCl and 15.0 g/l urea was used for dilution of samples immediately before the measurement. 6-keto-prostaglandin F_{1α} (a stable metabolite of prostacycline), thromboxane B₂ (a stable metabolite of TxA₂), bicyclo-PGE₂ (a stable metabolite of PGE₂ – using this assay PGE₂ and all its metabolites were converted to bicyclo-PGE₂ thus representing total

production of PGE₂ in the kidney) and 8-isoprostane were determined by the ELISA method in competitive arrangement on kits from Cayman Chemical (Ann Arbor, USA). In one step, complexes of labeled and unlabeled analyte with primary polyclonal antibody are fixed to the surface of pits with the help of secondary monoclonal antibody. Labeling is carried out with the link of analyte on acetylcholinesterase from the electrical organ of the ray. Ellman reagents (5,5'-dithiobis- (2-nitrobenzoate) serves as chromogen, detection is by spectrophotometric assay (412 nm). Before the determination of 8-isoprostane, prepurification of samples with chromatographic extraction on 400 mg columns of silicagel SGX C₁₈ 60 T (Tessek Czech Republic) followed by chromatography on thin layer of silicagel (plates Whatman 4865-821) after addition of (³H)-PGF_{2α} as internal standard was carried out. Because of the lack of serum we did not measure parameters of bone metabolism.

All rats were sacrificed and their femurs were removed, cleaned and weighed on a torsion balance. The femurs were stripped of soft tissue, and placed in an unstoppered glass vial filled with deionized water, and the vial was put in a exsiccator. The bones were suspended on a fine wire mesh and weighed in air and water to an accuracy of 0.1 mg. The volume and density of the femurs were calculated from the weight in air and water by the Archimedes principle (Kalu *et al.* 1994). The bones were then dried.

Standardized roentgenographs were made using Philips Mamo Diagnost 3000 X-ray machine at controlled exposures of 26 kV at 5.5 mAs. Morphometric measurements were performed directly on the X-rays after magnification with a fine caliper. On the roentgenographs at 40 % of the total length, starting from the distal end the external bone diameter, inner bone diameter and cortical width were measured after magnification with a fine caliper, by the method of Beall *et al.* (1984) and Vanderschueren *et al.* (1992).

The dry femur was incinerated for 24 h at 600 °C to white ash, which was weighed. The ash weight was expressed per unashed dry femurs. Bone ashes were then dissolved in 3 mol/l hydrochloric acid before the determination of calcium and phosphorus content. Calcium was determined by the method of Gitelman (1967) and bone phosphorus according to Kraml (1966).

The differences between groups were compared statistically by analysis of variance followed by Duncan's multiple-range test (Duncan 1955).

Results

Enalapril or losartan treatments exerted no significant effect on the body weight. Animals treated either with enalapril or losartan exhibited no increase in urinary excretion of 6-keto PGF_{1α} and thromboxane B₂. Animals treated with enalapril had increased urinary excretion of bicyclo-PGE₂ but no such effect was observed in animals treated with losartan (Table 1). This

enalapril-induced increase of urinary excretion of bicyclo-PGE₂ was possibly mediated by kinins. Urinary excretion of 8-isoprostane was not elevated in animals treated with losartan and enalapril. In animals treated with enalapril or losartan there was no increase of blood MDA as the best available measure of global peroxidation in comparison with control animals. There was also no difference between the groups concerning proteinuria and blood creatinine (Table 1).

Table 1. Total malondialdehyde in blood and urinary excretion of some eicosanoids and their metabolites in control and enalapril- and losartan-treated animals.

	Controls N=8	Losartan n=8	Enalapril n=8
Urinary 6-keto-PGF _{1α} (ng/mmol creat)	18.8±4.2	20.1±4.0	19.0±2.6
Urinary thromboxane (ng/mmol creat)	28.7±5.6	27.4 ±13.1	36.1±9.1
Urinary bicyclo PGE ₂ (ng/mmol creat)	1.66±0.81	2.45±0.90	4.32±0.62*
Urinary 8-isoprostane (ng/mmol creat)	23.7±7.6	30.6±7.5	36.0±8.2
Proteinuria (g/mmol creat)	0.15±0.02	0.16±0.03	0.16±0.02
Serum total MDA (μmol/l)	1.12±0.28	0.84±0.0.8	1.27±0.09
Urinary creatinine (μmol/l)	47.1 ±7.5	34.1±7.0	42.9±10.0

Data are means ± S.D. **p*<0.01 vs control animals

The administration of losartan and enalapril to intact female rats did not cause any significant changes in bone density and the ash and mineral content of the femur compared to the control animals (Table 2).

No significant differences between the controls and animals treated with losartan or enalapril were found in the midshaft outer diameter, inner diameter or cortical width (Table 3).

Table 2. Body weight, bone density and bone mineral content in control and enalapril- and losartan-treated animals.

	Controls n=8	Losartan n=8	Enalapril n=8
Initial body weight (g)	203±4	201±5	206±6
Final body weight (g)	218±12	255±15	217±12
Femur dry weight (mg)	370±36	345±14	329±15
Femur volume (μl)	402±30	404±20	395±23
Femur density (g/ml)	1.44±0.03	1.45±0.02	1.44±0.05
Femur ash content (g/ml)	0.55±0.07	0.51±0.05	0.50±0.08
Femur calcium (mg/ml)	208±11	196±8	190±12
Femur phosphorus (mg/ml)	94±5	87±5	84±6

Data are means ± S.D.

Table 3. Morphometric parameters of the femur midshaft in control and enalapril- and losartan-treated animals.

	Controls n=8	Losartan n=8	Enalapril n=8
<i>Length (mm)</i>	37.6±0.84	37.0±1.12	36.8±1.12
<i>Outer diameter (mm)</i>	4.20±0.22	4.15±0.36	4.10±0.39
<i>Inner diameter (mm)</i>	2.84±0.31	2.85±0.28	2.79±0.33
<i>Cortical thickness (mm)</i>	1.36±0.11	1.30±0.08	1.31±0.56

Data are means ± S.D.

Discussion

Bone metabolism is closely regulated by hormones and cytokines which have effects on both bone resorption and deposition. Under physiological conditions, these processes are carefully coordinated so that deposition is coupled to resorption.

Enalapril administration *in vitro* was shown to stimulate the release of prostaglandin PGE₂ from cultured rabbit renomedullary interstitial cells (Zusman 1981). Oral administration of enalapril up to 40 mg/day in hypertensive patients had no effect on urinary concentration of 6-keto-PGF₁ or thromboxane (Vlasses *et al.* 1983). On the contrary, Mittman *et al.* (1985) reported significant increases in the urinary excretion of PGE₂, and 6-keto-PGF₁ during enalapril therapy.

The available evidence in experimental animals has indicated that prostaglandins are produced by skeletal tissue and that prostaglandins (particularly of the E series) cause resorption of cultured fetal rat long bones (Dietrich *et al.* 1975). These findings provided evidence that the predominant effect of prostaglandins derived from skeletal tissue in rats is to produce bone resorption and that endogenously produced prostaglandins modulate ongoing bone resorption.

However, our results do not support the idea that enalapril or losartan in pharmacological doses would be able to stimulate the production of prostaglandins. We also did not observe increased oxidative stress as a result of the administration of enalapril and losartan. Plasma MDA used as a marker of lipid peroxidation and oxidative stress did not differ in these experimental groups.

The local circulation in the bone is considered to be important for supplying minerals and other substances (including humoral regulatory factors) for production of the osseous tissue. Blood flow plays an important role in the metabolism of bone. Bone is a living tissue relatively

well-supplied with blood vessels which, in long bones, consist of nutrient, metaphyseal and epiphyseal vessels (Ma *et al.* 1997). Mineral exchange depends on the blood flow in the bone. Bones receive about 10 % of the cardiac output. The size and shape of the mature Haversian system depends to a large extent on the size and shape of the resorbed cavity. However, the limiting factors for the maximum distance traveled by the resorption front in the centrifugal direction most likely depend on the demand of the most remote cells for sufficient supply of oxygen and nutrients and for the efficient removal of waste products (Broulik *et al.* 1982). From these aspects, the bone cells depend on the metabolic activity of the cells and on diffusion processes between the cells and the capillary loops which follow the advancing cutting cone and the variations in regional blood flow. Enalapril is known to lower vascular resistance and blood pressure by relaxing peripheral arterioles. Kapitola *et al.* (1994) considered a possible role of prostaglandins in the regulation of bone blood flow. PGE₂ production was raised after oophorectomy in rats indicating a striking parallel to changes in local blood flow in bones which rises after oophorectomy.

The results of Hatton *et al.* (1992) identify angiotensin I and II as potent stimulators of osteoclastic bone resorption and speak in favor of the possibility that bones might contain a tissue renin-angiotensin system that might play a role in the regulation of bone resorption. On the contrary, angiotensin II stimulates the proliferation of osteoblast-rich populations of cells obtained from calvariae of newborn rats (Hiruma *et al.* 1997). This effect was completely inhibited by an antagonist of the AT₁ receptor. Angiotensin II might play an important role in coordinating capillary cell growth and osteoblastic bone formation during bone remodeling (Lamparter *et al.* 1998). Endothelium has been identified as both a target for and a source of renin-angiotensin activity. Osteoclasts are target cells for substances

released by the endothelium of the bone microvasculature such as prostaglandins (Jee *et al.* 1990).

On the basis of the reports mentioned above, it seems possible that inhibition of the production of angiotensin II by enalapril or blocking angiotensin action on AT₁ receptors by losartan may be important in bone metabolism and may play a role in the development of postmenopausal osteoporosis. In animal studies, losartan blocked virtually all of the known effects of angiotensin II. Captopril did not produce any significant changes in the blood levels of ionized calcium or phosphorus and did not alter the serum levels of PTH and metabolites of vitamin D (Townsend *et al.* 1991).

In our study we were not able to demonstrate any significant influence of either losartan or enalapril on the urinary excretion of eicosanoids. Administration of losartan or enalapril in a dose recommended for the

treatment of hypertension did not affect the skeleton in intact female rats. Our study on enalapril and losartan use in female rats suggests that regular use of these two drugs is not associated with changes in bone density. Our results are in good agreement with the work of Stimpel *et al.* (1995) on the angiotensin converting enzyme inhibitor moexipril on the ovariectomy-induced cancellous bone loss in young rats.

However, the clinical relevance of these findings has yet to be confirmed. Further verification of our results and completion of the experiments with regards to the problems mentioned is desirable.

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