Pharmacological Reports 66 (2014) 93–99

Contents lists available at ScienceDirect

Pharmacological Reports

journal homepage: www.elsevier.com/locate/pharep

Original research article

Modifications of histamine receptor signaling affect bone mechanical properties in rats

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ARTICLE INFO

Article history:
Received 31 July 2012
Received in revised form 1 August 2013
Accepted 13 August 2013
Available online 1 February 2014

Keywords:
Histamine receptor antagonists
Loratadine
Ranitidine
Betahistine
Bone mechanical properties

ABSTRACT

Histamine receptors are expressed on bone cells and histamine may be involved in regulation of bone metabolism. The aim of the present study was to investigate the effects of loratadine (an H_1 receptor antagonist), ranitidine (an H_2 receptor antagonist) and betahistine (an H_3 receptor antagonist and H_1 receptor agonist) on bone mechanical properties in rats.

Loratadine (5 mg/kg/day, *po*), ranitidine (50 mg/kg/day, *po*), or betahistine dihydrochloride (5 mg/kg/day, *po*), were administered for 4 weeks to non-ovariectomized and bilaterally ovariectomized (estrogen-deficient) 3-month-old rats, and their effects were compared with appropriate controls. Serum levels of bone turnover markers, bone mineralization and mechanical properties of the proximal tibial metaphysis, femoral diaphysis and femoral neck were studied.

In rats with normal estrogen level, administration of loratadine slightly favorably affected mechanical properties of compact bone, significantly increasing the strength of the femoral neck ($p < 0.05$), and tending to increase the strength of the femoral diaphysis. Ranitidine did not significantly affect the investigated parameters, and betahistine decreased the strength of the tibial metaphysis (cancellous bone, $p < 0.01$). There were no significant effects of the drugs on serum bone turnover markers. In estrogen-deficient rats, the drugs did not significantly affect the investigated skeletal parameters.

In conclusion, the effects of histamine H_1, H_2 and H_3 receptor antagonists on the skeletal system in rats were differential and dependent on estrogen status.

Introduction

There is growing evidence that histamine may be involved in the regulation of bone metabolism. Histamine receptors are expressed on osteoblastic and osteoclastic cells [2,6,14].

Histamine is a biogenic amine playing a fundamental role as a mediator of allergic reactions (mainly through H_1 receptors) and in regulation of gastric acid secretion (through H_2 receptors) [27]. It is also a central nervous system neurotransmitter, where it acts through H_1, H_2 and H_3 receptors [26]. H_3 receptors are presynaptic autoreceptors located on axon terminals of histaminergic neurons, which stimulation decreases the synthesis and release of histamine, or heteroreceptors located on other neurons, modulating the release of their neurotransmitters [31,32]. There are also H_4 receptors located, among others, in bone marrow and on eosinophils and mast cells, affecting their chemotaxis [26,27]. All four known histamine receptors are G protein-coupled receptors. Since the histamine receptors exhibit constitutive activity, their antagonists may be reclassified as inverse agonists [13,31]. However, to simplify the nomenclature, in the present study we use the term "antagonist" for the investigated drugs.

Drugs counteracting peripheral effects of histamine are widely used in the treatment of allergic (H_1 receptor antagonists) and upper gastrointestinal (H_2 receptor antagonists) diseases [31]. Moreover, betahistine, an H_3 receptor antagonist and weak H_1 receptor agonist, is used in the treatment of Ménière's disease [21].

Little is known on the effects of drugs affecting histamine signaling on the skeletal system in humans. The data on the effects of H_2 antagonists are conflicting [4,5,8,18,20,43], and, to our knowledge, only one population-based study and one clinical study addressed the effect of H_1 antagonists on bone mineral density or content [18,41].

However, the increased bone fracture prevalence has been observed in postmenopausal women suffering from pollen-allergy [9]. Mastocytosis may lead to osteoporosis, and histamine is the most abundant mediator released by mast cells [1,3,16,28,36]. In experimental conditions, *in vitro*, histamine has been shown to promote osteoclastogenesis; all known histamine receptors were reported to be expressed on bone cells [2,6,14], and histamine to be synthesized in osteoclast precursors [2]. *In vivo*, it was demonstrated that both H_1 and H_2 receptors modulate osteoclastic bone resorption [2,7] and that mice lacking histamine due to histidine

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http://dx.doi.org/10.1016/j.pharep.2013.08.010

decarboxylase gene deletion had increased bone formation and were protected against ovariectomy-induced bone loss [10]. The effects of H_2 antagonists (cimetidine and famotidine) on the rat skeletal system have already been studied; the authors reported the antiresorptive effect in estrogen-deficient rats [22–24]. Also favorable effects of promethazine, an H_1 antagonist, on the skeletal system of old mice [42] and ovariectomized rats [34] have been reported. However, promethazine is a non-selective, sedating antihistamine. There are no data on effects of currently used, selective and non-sedating (second generation) H_1 receptor antagonists on development of osteoporosis. The effects of betahistine, a drug increasing histamine signaling, on the skeletal system have also not been investigated. Moreover, to our knowledge, no data on the effects of histamine receptor antagonists on bone mechanical properties have been reported so far.

The aim of the present study was to investigate the effects of loratadine (a second generation H_1 receptor antagonist), ranitidine (an H_2 receptor antagonist) and betahistine (an H_3 receptor antagonist and H_1 receptor agonist) on bone mechanical properties in female rats, both with normal estrogen levels, and with estrogen deficiency induced by bilateral ovariectomy (developing osteoporosis).

Materials and methods

The experiments were performed on mature 3-month-old female Wistar rats obtained from the Center of Experimental Medicine, Medical University of Silesia. The rats were fed a standard laboratory diet Labofeed B (Wytwórnia Pasz "Morawski", Poland) *ad libitum*. The diet contained 0.9% Ca and 0.7% P. The protocol for the experiments on animals was approved by Local Ethics Commission, Katowice, Poland.

Drugs used: loratadine (Loratadyna Galena, tablets 10 mg, Galena, Poland), ranitidine hydrochloride (Ranitydyna Sanofi, tablets containing 150 mg of ranitidine, Sanofi-Aventis, Poland), betahistine dihydrochloride (Betahistine Pliva, tablets 16 mg, Pliva Kraków, Poland). The drugs were prepared for administration by triturating and suspending tablets in tap water. Loratadine was administered to the rats at a dose of 5 mg/kg, ranitidine at a dose of 50 mg/kg, and betahistine dihydrochloride at a dose of 5 mg/kg, by a stomach tube (*po*), once daily for 4 weeks, at a volume of 2 ml/kg. The doses were chosen from the range previously used in rat experimental studies [25,35,38].

The experiments were carried out in two experimental models: non-ovariectomized (NOVX) and ovariectomized (OVX) rats. The NOVX rats were divided into following groups: Ia, NOVX control rats ($n = 18$); IIa, NOVX rats receiving loratadine ($n = 9$); IIIa, NOVX rats receiving ranitidine ($n = 9$); IVa, NOVX rats receiving betahistine ($n = 9$), and the OVX rats were divided into: Ib, OVX control rats ($n = 18$); IIb, OVX rats receiving loratadine ($n = 9$); IIIb, OVX rats receiving ranitidine ($n = 9$); IVb, OVX rats receiving betahistine ($n = 9$). The mean initial body mass of rats in those groups was in the range: 233 ± 5 to 240 ± 4 g.

Bilateral ovariectomy was performed seven days before the administration of the drugs started, under ketamine–xylazine anesthesia. All of the animals were weighed three times a week to adjust the drug dosing.

The rats were fasted overnight after the last drug administration. The next day, the animals were anesthetized with ketamine and xylazine, blood samples were collected by heart puncture, and the animals were sacrificed. The serum was stored at −80 °C until analyzed. The tibias, femurs and L-4 vertebra, as well as the uterus and thymus were excised. In the left tibias and femurs, the mass and macrometric parameters were determined (length and diameter of the diaphysis at mid-length). The vertebrae were weighed. The left femur, tibia, and proximal part of the right femur from each rat were wrapped in gauze soaked in 0.9% NaCl solution and stored below −20 °C until the mechanical tests were performed on thawed bones [40]. The mass of the uterus and thymus was also determined.

Bone mechanical properties studies

Mechanical properties of the femoral diaphysis and tibial metaphysis in bending tests, and the femoral neck in a compression test, were studied using an Instron 3342 500N apparatus. The data were analyzed by Bluehill 2 version 2.14 software (Instron).

Mechanical properties of the diaphysis of the left femurs were studied using a bending test with three-point loading as previously described [11,12]. The distance between the supporting points was 20 mm. The load was applied perpendicularly to the long axis of the femur at the bone mid-length. After pre-conditioning to obtain steady positioning, the mechanical test was started (displacement rate of 0.01 mm/s, sampling rate of 100 Hz). The load–displacement curves obtained for each bone were analyzed. Maximum load, displacement for the maximum load and energy for the maximum load were assessed. The same parameters were determined for the fracture point. The intrinsic parameters: stress and Young's modulus were also determined. To determine moment of inertia in the break-section, necessary for the calculations, the transverse cross-sections of the right femoral diaphysis were made in the bone mid-length, and the inside and outside diameters were measured, according to [17], using Osteomeasure software for histomorphometric measurements.

The mechanical strength of the femoral neck was determined using a compression test. The bone was prepared for measurement by fixing the diaphysis, which was cut at the femur mid-length, in a methacrylate plate [33]. The load was applied to the head of the femur along the long axis of the femur (preload of 1 N, displacement rate of 0.01 mm/s). The load causing the fracture of the femoral neck (maximum load) was measured.

Mechanical properties of the proximal metaphysis of the left tibia were studied using a bending test with three-point loading according to [37], as previously described [11], after a preload of 1 N, with a displacement rate of 0.01 mm/s. The same parameters as for the femoral diaphysis were determined. To estimate moment of inertia in the break-section, the mean diameter of the metaphysis was measured (assuming that the tibial metaphysis was a circular beam).

Bone mineralization studies

The L-4 vertebra, left tibia and femur were lyophilized for 7 days to determine the dehydrated bone mass, and then mineralized at 640 °C for 48 h in a muffle furnace and weighed to determine the mass of bone mineral. To specify whether the changes in bone mass parameters were related to the changes in the body mass caused by the treatments, results concerning bone mass, lyophilized bone mass and mass of bone mineral were also determined as ratios to body mass. The ratios of bone mineral mass to bone mass and to lyophilized bone mass were treated as substitutes for bone mineral density measurements.

Calcium and phosphorus content in the mineralized bones dissolved in 6 M HCl and then diluted in distilled water was determined colorimetrically, using kits produced by Pointe Scientific, Inc.

Biochemical studies

Serum osteocalcin levels were determined using an enzyme immunoassay (Rat-MID Osteocalcin EIA). Serum osteoclast-derived tartrate-resistant acid phosphatase form 5b (TRACP 5b)