Biomedicine & Pharmacotherapy 94 (2017) 124–128

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Original article

Effects of long-term oral administration of methimazole on femur and tibia properties in male Wistar rats

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ARTICLE INFO

Article history:
Received 31 March 2017
Received in revised form 27 June 2017
Accepted 21 July 2017

Keywords:
Rat
Femur
Tibia
Methimazole
Densitometry
Quantitative computed tomography

ABSTRACT

Physiological concentrations of thyroid hormones are crucial for skeletal growth and development, physiological bone turnover and bone homeostasis maintenance. Methimazole (1-methyl-2-mercaptoimidazole) is an antithyroid drug used for the treatment of the hyperthyroidism in humans and animals. The aim of the study was to determine effects of long-term oral methimazole treatment in male Wistar rats on biochemical bone metabolism markers, as well as morphological, geometric, densitometric and mechanical properties of femur and tibia. Experimental rats were subjected to 90-day-long oral treatment with 0.05% water solution of methimazole and were kept under identical environmental conditions and received the same diet *ad libitum* as the control group. Serum concentration of osteocalcin (OC) and C-terminal telopeptides of type I collagen (CTX-I) was determined. Femur and tibia were evaluated using quantitative computed tomography (QCT), peripheral QCT (pQCT) and three-point bending test. Final body weight of the experimental group was significantly decreased by 30% ($P = 0.01$). Methimazole treatment significantly decreased serum OC concentration by 21% ($P = 0.02$) and increased CTX-I concentration by 17% ($P = 0.06$). Methimazole decreased morphological, geometric and densitometric parameters of femur and tibia in rats. Mechanical evaluation of bones has shown significantly decreased maximum elastic strength and ultimate strength of femur in rats treated with methimazole by 36% and 40% when compared to the control group ($P < 0.05$). In conclusion, this study has shown that long-term treatment with methimazole inhibits bone formation and accelerates bone resorption processes. The observed negative effects of methimazole treatment on body weight gain and skeletal properties may be considered as additional possible side effects in living organisms to those reported in the previous studies. It may be suggested that long-term antithyroid treatment should be combined with prevention of the negative effects of methimazole on bone tissue and whole body metabolism.

Abbreviations: Methimazole, 1-methyl-2-mercaptoimidazole; IX_CRT_A, axial moment of inertia of the cortical bone; BMC, bone mineral content; CTR_CNT, bone mineral content in the cortical bone; TRAB_CNT, bone mineral content in the trabecular bone of femur; TOT_CNT, bone mineral content of proximal metaphysis of tibia; BMD, bone mineral density; CRT_A, cortical bone area; CI, cortical index; A, cross-sectional area; CTX-I, C-terminal telopeptides of type I collagen; DEXA, dual-energy X-ray absorptiometry; ENDO_C, endosteal circumference at the midshaft; GH, growth hormone; IGF-I, insulin-like growth factor I; IL-1, interleukin 1; IL-6, interleukin 6; Wy, maximum elastic strength; MRWT, mean relative wall thickness; MvBMD, mean volumetric bone mineral density; OC, osteocalcin; PERI_C, periosteal circumference at the midshaft; pQCT, peripheral quantitative computed tomography; IP_CRT_A, polar moment of inertia of the cortical bone; RP_CRT_A, polar moment of resistance of the cortical bone; QCT, quantitative computed tomography; Ix, second moment of inertia; SSI or RP_CM_W, strength-strain index; TSH, thyroid-stimulating hormone; TRH, thyrotropin releasing hormone; T4 or 3,5,3′,5′-Tetraiodo-L-thyronine, tthyroxine; Bvol, total bone volume; TRAB_A, trabecular bone area; T_3 or 3,5,3′-Triiodo-L-thyronine, triiodothyronine; TNF-α, tumor necrosis factor alpha; Wf, ultimate strength; VOI, volume-of-interest; CRT_DEN, volumetric bone mineral density of the cortical bone; TOT_DEN, volumetric bone mineral density of the proximal metaphysis of tibia; TRAB_DEN, volumetric bone mineral density of the trabecular bone of femur.

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http://dx.doi.org/10.1016/j.biopha.2017.07.107
0753-3322/

1. Introduction

Thyroid gland releases thyroid hormones under control of the hypothalamic paraventricular nucleus through thyrotropin releasing hormone (TRH) and anterior lobe of pituitary through thyroid-stimulating hormone (TSH) secretion. Thyroid follicular cells are stimulated via TSH receptors for cell proliferation as well as synthesis and secretion of thyroxine (T_4 or 3,5,3′,5′-Tetraiodo-L-thyronine) and triiodothyronine (T_3 or 3,5,3′-Triiodo-L-thyronine). Physiological euthyroid status is maintained by a negative feedback loop establishing inverse relationship between TSH and circulating T_3 and T_4 levels [1]. Physiological concentrations of thyroid hormones are crucial for skeletal growth and development, physiological bone turnover and to sustain bone homeostasis. Thyrotoxicosis is the causative factor for secondary osteoporosis development and disturbed thyroid hormone signaling was reported as a risk factor for osteoarthritis development [2,3]. T_3 exerts an anabolic action during growth and catabolic effects in adult skeleton. Most of T_3 is generated by deiodination of T_4 in peripheral tissues. Circulating free T_4 concentration is 4-fold greater than free T_3; however, thyroid hormone-binding affinity for T_3 is 15-fold higher than affinity for T_4 that determines its higher biological activity [4]. Juvenile hypothyroidism causes growth inhibition, delayed bone formation and mineralization, while T_4 replacement induces catch-up growth before the closure of the growth plates and skeletal growth termination [5,6]. Thyrotoxicosis in children accelerates bone formation with premature closure of the growth plates and skull sutures resulting in short stature and craniosynostosis [7]. Thyroid hormones, especially T_3, are considered as modulators of normal bone metabolism including balanced skeletal growth and development, repair and bone remodelling [8]. Histomorphometric studies in rats with hypothyroidism have shown also that thyroid hormones are required for normal bone turnover [9].

Methimazole (1-methyl-2-mercaptoimidazole) is an antithyroid drug used for the treatment of the hyperthyroidism in humans [10–12] and animals [13–15]. Its mechanism of action is similar to that of the other thioamides blocking the activity of thyroperoxidase and leading to reduction of the biosynthesis of thyroid hormones. Methimazole overdosing results in hypothyreosis [16]. Experimental treatment of young rats with methimazole was shown to increase the trabecular bone volume of the subchondral spongiosa [17] and decrease calcium and phosphorus content in bone [18]. Thus, the aim of this study was to determine effects of long-term oral methimazole treatment in male Wistar rats on morphological, geometric, densitometric and mechanical properties of femur and tibia. Furthermore, biochemical bone formation and resorption markers in serum were evaluated in this study.

2. Materials and methods

The experimental procedures used in this study were approved by The II Local Ethics Committee on Animal Experimentation of the University of Life Sciences in Lublin, Poland - permission number 44/2011.

2.1. Experimental design

The study was performed on Wistar male rats (initial body weight 220–260 g) divided into the weight-matched control (N = 6) and experimental groups (N = 6) at the beginning of the experiment. During the experiment, all animals were kept in an identical environmental conditions with free access to fresh water and a standard diet fed *ad libitum* (commercial diet for laboratory animals, Agropol, Motycz, Poland). The vivarium room with animals were air-conditioned (temperature 22–23 °C) with an average humidity between 45% and 47%, and 12/12 h light/dark cycle. Prior to the beginning of the experiment, the rats were acclimatized to new environmental conditions for 14 days. After the acclimatization period, the experimental rats (9 weeks of age) had free access to tap water containing 0.05% methimazole (Sigma-Aldrich, St. Louis, USA) solution. Water solution containing methimazole was prepared each day for the experimental group of rats. After 90 days of the experiment, all animals were anaesthetised using ketamine (80 mg/kg of body weight *i.m.*) and their blood was collected by cardiac puncture into sterile tubes for a clot. Collected blood samples were centrifuged at 3000 rpm for 30 min to obtain serum that were kept at −70 °C until biochemical analysis. Moreover, left and right femur and tibia were isolated postmortally from the control and experimental animals. The isolated bones were cleaned from soft tissues, their length and weight were determined and were kept frozen until further analysis.

2.2. Biochemical evaluation of serum

Serum osteocalcin (OC) concentration was measured using Micro-Vue Osteocalcin EIA Kit (Quidel Corp, San Diego, CA, USA). The determination of the bone collagen degradation products – C-terminal telopeptides of type I collagen (CTX-I) in serum was performed using Serum CrossLaps ELISA (Immunodiagnostic Systems Ltd, Boldon, Tyne & Wear, UK). Results of biochemical evaluation of bone tissue metabolism markers were obtained using Benchmark Plus microplate spectrophotometer supplied with MicroplateManager Software Version 5.2.1 (Bio-Rad Laboratories Inc, Hercules, CA, USA).

2.3. Morphological and densitometric evaluation of bones using quantitative computed tomography

Quantitative computed tomography (QCT) method and Somatom Emotion Siemens apparatus (Siemens, Erlangen, Germany), equipped with Somaris/5 VB10B software (version B10/2004A) were used to determine total bone volume (Bvol) and mean volumetric bone mineral density (MvBMD) of femur and tibia. The values of MvBMD and Bvol of femur and tibia were determined using volume-of-interest (VOI) limited by minimum and maximum density of the investigated samples at 0 and 3071 Hounsfield units, respectively. The measurements of MvBMD and Bvol were performed for the whole bones and the results obtained reflect the parameters for all the anatomical structures of femur and tibia, including trabecular and cortical bone compartments. Using cross-sectional computed tomography scans positioned at 50% of femur length and at 60% of tibia length (measuring from the proximal bone extremity), the geometrical parameters such as cross-sectional area (A), second moment of inertia (Ix), mean relative wall thickness (MRWT) and cortical index (CI) were determined. The values of these parameters were derived on the basis of the measurements of horizontal and vertical diameters (both external and internal) of the bone shafts.

2.4. Densitometric and geometrical evaluation of bones using peripheral quantitative computed tomography

Peripheral quantitative computed tomography (pQCT) and XCT Research SA Plus apparatus (Stratec Medizintechnik GmbH, Pforzheim, Germany) were used to determine densitometric and geometrical properties of femur and tibia in terms of volumetric bone mineral density of the trabecular bone of femur (TRAB_DEN), bone mineral content in the trabecular bone of femur (TRAB_CNT), trabecular bone area (TRAB_A), volumetric bone mineral density of proximal metaphysis of tibia (TOT_DEN), bone mineral content of