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ORIGINAL ARTICLE

Effect of Hypothyroidism on Bone Repair in Mature Female Rats

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Thyroid hormones are major regulators of bone metabolism and development. In hyperthyroidism, bone resorption is increased, however, the mechanism by which thyroid hormones increase osteoclasts activity and its growth remains unknown. This research was designed to study the effect of hypothyroidism on the bone repair.

Materials and Methods: Sixty mature female rats were randomly divided into two groups: control and methimalole treated groups. In the methimazole treated group, hypothyroidism was induced. Medial surfaces of right tibia of control and methimazole treated groups were drilled; all the rats were killed after three weeks by choloroform inhalation. Bone samples were obtained from defected regions and were subjected to histomorphometric study.

Results: The weight, length and periosteum thickness of tibia of the rats were significantly decreased in methimazole treated group as compared to the controls. There was significant decrease in osteoblast numbers (19.2±3.6 vs 34.1±3.1, p<0.001) and increase in the numbers of osteoclasts (5.9 ± 1.6 vs 2.3±1.1, p<0.001) of the methimazole treated group compared to the control group.

Conclusion: This study demonstrates that hypothyroidism delays bone remodeling and repair in rat.

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Introduction

Thyroid hormones are major regulators of bone metabolism and development.[1,2] In hyperthyroidism, bone resorption is increased, however, the mechanism by which thyroid hormones increase osteoclasts activity and its growth remains unknown.[3] It has been reported that the effect of thyroid homones on bone resorption is maintained through osteoblast mediatory functions, achieved in the presence of osteoblasts.[3] Some studies on hypothyroid female rats have revealed that the growth plates of tibia to be grossly disorganized and contained an abnormal matrix.[4,5]

Bone remodeling occurs at different rates in the trabecular and cortical bones and in differing anatomical locations. The rate at which given sites undergo remodeling is known as the bone formation rate, or activation frequency, and the major factor that determines total bone turnover. In hypothyroidism, the time taken for bone resorption, matrix deposition and mineralization are prolonged, the activation frequency is reduced and the phases of the remodeling cycle are markedly prolonged. The final resorption depth is reduced.[6,7] The mineralization lag time is prolonged because of a more pro-

nounced reduction in the osteoid appositional rate.[6] Therefore we conducted the present study to investigate the effect of hypothyroidism on bone repair in mature female rats.

Materials and Methods

Sixty female adult albino rats weighing 150 – 200 g, kept in separate cages and fed ad libitum, were used in the experiment. The rats were randomly divided into two equal groups, one the methimazole treated group and the seconed the control group. Rats were weighed, and blood samples were obtained from their canthus to determine thyroid hormone levels. Serum T_4 and TSH were measured using a commercial radioimmunoassay kit (Diagnostic products Ltd, Abingdon, UK). In rats of the experimental group, hypothyroidism was induced by ingestion of 4mg powdered methimazole (Taheran Iran Hormone) dissolved in 100 cc of distilled water; duration of ingestion was two weeks. Rats were anesthetized by intramuscular injection of 50 mg/kg ketamine hydrocholoride with 5 mg/kg diazepam under general anesthesia and sterile conditions. In all the rats a 2 mm hole with a 3 mm depth was made in the medial surface of tibia of right hind limb.

Three weeks after the operation, all rats were killed by means of choloroform inhalation. The right tibia was extracted and soft tissue was removed from it. The weight of all rats were measured before and after induction of hypothyroidism by a sensitive scale in a blind fashion. The whole length of tibia from proximal end (superior surface of its condyles) to distal end (level of medial malleolus), and maximum breadth of the mid shaft of the bone were measured by a colis in a blind fashion. Osteoblasts were identified according to the following features: their location on the surface of bone, some cuboidal and others pyramidal and were frequently in a continous layer such as a epithelial layer. The nucleus was large and the cytoplasm exhibited marked basophilia. Osteoclasts were diagnosed according to the following features: they were multinucleated giant cells, varying greatly in size and number of nuclei they posses, they were found in close association with surface of bone. Their cytoplasms appeared faintly basophill and granular.[8]

After macroscopic studies, specimens were taken from the defected bone regions and were fixed in formaline saline, decalcified in EDTA, processed, sectioned and stained with H and E and Massons trichorme methods for microscopic analysis. Ten zones from each sample were examined morphometerically using a calibrated ocular eye piece on a Nikon light microscope at a magnification of 400×for the counting of osteoblasts and osteoclasts.

Data were analyzed using student t test and a p value below than 0.05 was considered significant.

Results

The mean body weights of the methimazole treated and control groups were 134±9.2 g and 181±11.9 g respectively ($p<0.001$).

We observed decreased serum T4 and increased TSH levels after ingestion of methimazole which confirmed induction of hypothyroidism Plasma T_4 and TSH concentrations were 61.50±0.80 nmol/lit and 0.56±0.06 μg/ml in control group and 3.20±0.05 nmol/lit and 5.20±0.80 μg/mL in hypothyroid group. The weights of the tibias were 274±17.5 and 334±14.1 mg in the experimental and control groups, respectively ($p<0.001$).

The number of osteoblasts was significantly increased in the reparative tissue of the rats in the control rats as compared to methimazole treated rats: 34.1±3.11 vs 19.2±3.62 respectively ($p<0.001$).

The number of osteoclasts were significantly increased in the reparative tissue of the rats in the methimazole treated group as compared to the controls: 5.9±1.6 vs 2.3±1.1 respectively ($p<0.001$).

Table. 1 Mean tibial length and diameter and thickness (mean±SD) in experimental and control groups

Group	Tibial length (mm)	Tibial diameter (mm)	Periosteum thickness (mm)
Experimental	25.7±2.7*	2.9± 0.09	27.3±17
Control	30.5±1.8	2.9±0.07	38.2±3.2

* P<0.001 compared to control group.

The tibial length and diameter and periosteum thickness of study the groups are shown in table 1. Tibial length and thickness of the periosteum of the control group was significantly higher in comparison with those seen in the methimazole treated group (p<0.001). However no significant difference in the tibial diameter of the two groups was seen (Table 1).

Discussion

The rat is a suitable model for studying endocrine disorders, especially those dealing with thyroid gland.[9-15] In the present research we found that experimental hypothyroidism is associated with body weight loss and decreased weight and length of tibia. Mudde et al have evaluated the effects of methimazole in hyperthyroid women and concluded that excessive bone loss and osteoporosis is mainly prevented by methimazole treatment in hyperthyroid women.[16] Loftus and Peterson reported a patient with a lack of adequate amounts of the thyoid hormone.[17] He had a fracture of the mandible which failed to heal in the 2 years following surgical treatment. When thyroid hormone supplementation was begun, the fracture progressed to union. They concluded that lack of adequate thyroid hormone may interfere with fracture healing. Recent studies in mature male rats have indicated that increased thyroid hormone concentration is accompanied by increased bone turnover, weight loss and decreased length of tibia.[4,14] Urabe et al, using a rat fracture model, investigated the effects of decreased serum level of thyroid hormone on the fracture - repair process by ghene expression and mechanical evaluation methods.[18] Rats were divided into the following groups: (a) controls, (b) those treated with methimazole for the duration of study, and (c) those treated with methimazole and thyroxine, receiving both for the same duration. Three weeks after the initiation of pharmacologic treatment, closed femoral fracture was produced. The formation of cartilage tissue in the fracture callus in all rats was not obviously different on day 7 after fracture. In rats treated with methimazole, differentiation from proliferating to hypertrophic chondrocyted in the fracture callus was less advanced and vascular invasion was clearly inhibited on day 12. Ghene expression of alkaline phosphatase and osteocalcin in the callus was significantly lower, in these rats than in the controls on days 10, 12 and 14. The mechanical properties of the fracture callus were also significantly weaker in these animals than in the controls on day 21, resulting in impaired fracture repair. Urabe, et al demonstrated that hypothyroidism inhibits enchondral ossification, resulting in an impaired fracture - repair process. L-thyroxine replacement in rats treated with methimazole caused the impaired repair process to revert to normal concluded The authors that thyroid hormone is one of the critical systemic factors for fracture repair.[18] Our results have revealed that in hypothyroid rats, osteogenesis activity is decreased in defected bone regions. This could result in less periosteum thickness, decreased bone weight and lack of bone development and growth.

We conclude that hypothyroidism is associated with delayed bone remodeling, and delayed bone growth and development.

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