ALTERAÇÕES NO METABOLISMO DE FOSFORO-CÁLCIO NO HIPOTIROIDISMO EXPERIMENTAL

CHANGES IN PHOSPHORUS-CALCIUM METABOLISM IN EXPERIMENTAL HYPOTHYROIDISM

ИЗМЕНЕНИЕ СОСТОЯНИЯ ФОСФОРНО-КАЛЬЦИЕВОГО ОБМЕНА В КОСТНОЙ ТКАНИ ПРИ ЭКСПЕРИМЕНТАЛЬНОМ ГИПОТИРЕОЗЕ

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Received 09 December 2019; received in revised form 30 January 2020; accepted 11 February 2020

RESUMO

O objetivo deste trabalho foi estudar mudanças no estado do metabolismo fósforo-cálcio, indicadores de certos hormônios, perfil de citocinas e estrutura histológica do tecido ósseo no hipotireoidismo experimental em ratos. O hipotireoidismo experimental foi causado pela administração intragástrica diária de tiamazol na dose de 2,5 mg / 100 g de peso corporal por 21 dias. O método colorimétrico foi utilizado para determinar o teor sérico de cálcio, fósforo e magnésio no sangue. Marcadores do metabolismo ósseo, nível de tireotropina, T3 e T4 total, testosterona, hormônios folículo-estimulantes (FSH) e luteinizante (LH), hormônio paratireóide (PTH), interleucina-1 β (IL-1 β) e -6 (IL-6), os tumores alfa de fator de necrose (TNF- α) foram estudados usando um imunoensaio enzimático. Para determinar a estrutura histológica do tecido ósseo, seções preparadas da diáfise femoral foram estudadas sob um microscópio da série MZ-300 (Áustria). Verificou-se que o desenvolvimento do hipotireoidismo é confirmado pela diminuição do teor de tiroxina e triiodotironina no contexto do aumento da secreção do hormônio estimulador da tireóide. O hipotireoidismo experimental é acompanhado por uma diminuição na concentração de fosfatase alcalina óssea no soro sanguíneo, mantendo os telopeptídeos C-terminais tipo 1 de colágeno, refletindo processos de remodelação prejudicados, há também uma diminuição na secreção de testosterona, um aumento nas gonadotrofinas, hormônio da paratireóide, citocinas pró-inflamatórias (IL-1 β , IL-6 e TNF- α). Sinais característicos do desenvolvimento da osteoporose displásica são revelados histologicamente na diáfise dos ossos tubulares.

Palavras-chave: *hipotireoidismo experimental, fosfatase alcalina óssea, telopeptídeos C-terminais tipo 1 de colágeno, interleucinas, ossos tubulares.*

ABSTRACT

The aim of this work was to study changes in the state of phosphorus-calcium metabolism, indicators of certain hormones, cytokine profile, and histological structure of bone tissue in experimental hypothyroidism in rats. Experimental hypothyroidism was caused by daily intragastric administration of Thiamazole in a dose of 2.5 mg / 100 g body weight for 21 days. The colorimetric method was used to determine blood serum calcium, phosphorus, and magnesium content. Bone metabolism markers, thyrotropin level, total T3 and T4, testosterone, follicle-stimulating (FSH) and luteinizing (LH) hormones, parathyroid hormone (PTH), interleukin-1 β (IL-1 β) and -6 (IL-6), necrosis factor tumors-alpha (TNF- α) were studied using an enzyme immunoassay. To determine the histological structure of bone tissue, prepared sections of the femoral diaphysis were studied under a microscope of the MZ-300 series (Austria). It was found that hypothyroidism development is confirmed by decreased content of thyroxine and triiodothyronine on the background of the increased secretion of thyroid-stimulating hormone. Experimental hypothyroidism is accompanied by a decrease in the concentration of bone alkaline phosphatase in the blood serum while maintaining the collagen C-terminal telopeptides type 1, reflecting impaired remodeling processes, there is also a decrease in testosterone secretion, an increase in gonadotropins, parathyroid hormone, pro-inflammatory cytokines (IL-1 β , IL-6, and TNF- α). Signs characteristic for the developing dysplastic osteoporosis are histologically revealed in the diaphysis of the tubular bones.

Keywords: *experimental hypothyroidism, bone alkaline phosphatase, collagen C-terminal telopeptides type 1, interleukins, tubular bones.*

АННОТАЦИЯ

Целью исследования явилось изучение изменений состояния фосфорно-кальциевого обмена, показателей некоторых гормонов, цитокиновый профиль и гистоструктуры костной ткани при экспериментальном гипотиреозе у крыс. Экспериментальный гипотиреоз, вызвали путём ежедневного внутривидового введения мерказолила в дозе 2,5 мг/100 г массы тела в течение 21 дня. В сыворотке крови крыс в сыворотке крови колориметрическим методом исследовали содержание общего кальция, фосфора, магния. Содержание маркеров костного обмена, уровень тиреотропина, общий Т3 и Т4, тестостерона, фолликулостимулирующего (ФСГ) и лютеинизирующего (ЛГ) гормонов, паратгормона (ПТГ), интерлейкинов-1 β (IL-1 β) и - 6 (IL-6), фактора некроза опухолей- альфа (TNF- α) изучали методом иммуноферментного анализа. Для определения гистологической структуры костной ткани изучали подготовленные срезы диафиза бедренной кости под микроскопом серии МЗ-300 (Австрия). Было выявлено, что развитие гипотиреоза подтверждается снижением содержания тироксина и трийодтиронина на фоне увеличения секреции тиреотропного гормона. Экспериментальный гипотиреоз сопровождается уменьшением концентрации в сыворотке крови костной щелочной фосфатазы при сохранении С-концевых телопептидов коллагена типа 1, отражая нарушение процессов ремоделирования, также происходит снижение секреции тестостерона, увеличение - гонадотропинов, паратиреоидного гормона, провоспалительных цитокинов (IL-1 β , IL-6 и TNF- α). В диафизах трубчатых кости гистологически выявляются признаки, характерные для развивающегося диспластического остеопороза.

Ключевые слова: *экспериментальный гипотиреоз, костная щелочная фосфатаза, С-концевые телопептиды коллагена типа 1, интерлейкины, трубчатые кости.*

1. INTRODUCTION

Iodine deficiency diseases are among the most common noncommunicable diseases and is a serious problem in protecting public health. In more than 50% of the Russian Federation territory, there is an iodine deficit, and about 100 million people live in these regions in a state of chronic insufficiency. The territory of the Bashkortostan Republic is a region with iodine deficiency and associated goiter endemic. Iodine deficiency endemic conditions include a wide range of pathologies characterized by a decrease in the functional activity of the thyroid gland. Iodine deficiency manifests in a variety of pathologies from defects in physical and intellectual development, reproductive disorders to specific thyroid diseases (Kazimirko *et al.*, 2006; Farkhutdinova, 2005; Eriksen, 1986).

Hypothyroidism and hyperthyroidism are associated with a risk of osteoporosis (Marova, 2003; Nochevnaya *et al.*, 2011; Cardoso *et al.*, 2014; Wong and Steffes, 1984). Expression of the receptors of iodinated thyroid hormones was found both in osteoblasts and osteoclasts (Platonova and Troshina, 2015; Meunier *et al.*, 2005). Hypothyroidism is accompanied by a decrease in the number of basic multicellular

units in the bones in which local resorption and bone formation are associated with time. The duration of the phases of the remodeling cycle is increased, and the duration of the mineralization time of the newly formed osteons is significantly prolonged (Eriksen, 1986; Belyakov, 2014). The process of bone remodeling is a wonderful mechanism that simultaneously contributes to maintaining calcium homeostasis and bone integrity, and it's adaptive restructuring according to mechanical stimuli, and the needs of the body. Imbalance of remodeling cycles in hypothyroidism ultimately leads to a decreased bone mass, microarchitecture violation with an increase in bone tissue fragility and risk of fractures (Pavlova *et al.*, 2012; Bassett *et al.*, 2010; Nicholls *et al.*, 2012). There are literary data that in hypothyroidism, the spongy bone mainly affected, and excessive mineralization occurs in the cortical bone (Gouveia *et al.*, 1997; Schwarz *et al.*, 2006; Zimmermann and Anderson, 2011), which, however, does not reduce the risk of fractures. Mechanisms of these phenomena are not fully understood (Cardoso *et al.*, 2014; Wong and Steffes, 1984). Probably thyroid hormones affect the bioavailability and action of estrogens and / or androgens (Freitas *et al.*, 2003; Krassas *et al.*, 2010; Zhang and Lazar, 2000). Other authors (Nicholls *et al.*, 2012; Zhdo

et al., 2012) associated disturbances in the course of remodeling under the action of thyroid hormones with a change of D2 deiodinases (activator of T3 formation) in bone tissue activity and D3 (T3 inactivator) in osteoblasts.

Aim of the study: to characterize changes in phosphorus-calcium metabolism and bone tissue, its structure in experimental hypothyroidism in rats.

2. MATERIALS AND METHODS

The 26 white mongrel sexually mature male rats weighing 200-210 g were involved in experiments. Requirements and ethical standards on humane attitude to laboratory animals (order of the Ministry of Health of the Russian Federation dated 06/19/2003 No. 267 "On approval of laboratory practice." The animals were kept in standard vivarium conditions with a natural light mode, on a standard laboratory animal diet (GOST 50258-92). In the experimental group of animals, hypothyroidism was modeled by daily intragastric administration of Thiamazole at the rate of 2.5 mg / 100 g of body weight for three weeks by a metallic probe (Kozlov, 2006). On day 22, animals were decapitated under ether anesthesia. The content of total calcium, phosphorus, magnesium was studied in blood serum, using colorimetric method HUMAN company (determination of Ca - using D-cresolphatein-complexone, ammonium P-molybdenum acid, Mg - 1- (2-hydroxyazo) -2-naphthol-3- (2,4-dimethyl) -carboxiamide). Enzyme multiplied immunoassay was used to analyze the content of bone metabolism markers - bone alkaline phosphatase (reagents "Metra BAFKit Quidel Corporation), C - terminal collagen telopeptides type 1 (Serum Cross Laps Elisa reagents, Nordie Biosince Diagnostic A / S), and levels of thyrotropin (TSH), total T3 and T4, testosterone, follicle-stimulating (FSH) and luteinizing (LH) hormones, parathyroid hormone (PTH, reagents of Vector-Best CJSC, Russia), interleukins-1 β (IL-1 β) and - 6 (IL-6), tumor necrosis factor-alpha (TNF- α , reagents LLP "Cytokine circuit ", Russia) using Stat Fox 2100 analyzer (USA) and the Uniplan semi-automatic analyzer (Russia) according the producer's protocols. To characterize the histological structure of bone tissue, the femoral pieces were fixed in 10% neutral formalin, decalcified for 3 weeks in a 7% solution of nitric acid (with a change of solution every week), washed, poured paraffin into blocks, made slices 7-8 microns

thick. Sections were stained with hematoxylin and eosin. Microscopic sections were studied under a microscope of the MZ-300 series (Austria), micrographs were made using a Nikon Coolpix 4500 camera.

Statistical processing was carried out using the Statistica 6.0 software package (Stat Soft), with the calculation of arithmetic mean (M), standard error of arithmetic mean (m) with the estimation of the significance of intergroup differences by Student t-criterion.

3. RESULTS AND DISCUSSION

Table 1 shows the content of thyrotropin and thyroid hormones in the blood serum of white male rats in intoxication with Thiamazole. The results were obtained by enzyme multiplied assay.

Table 2 presents the indicators of mineral metabolism and markers of bone metabolism in experimental Thiamazole hypothyroidism in male rats, determined by the colorimetric method.

PTH, testosterone, FSH, LH, and cytokines blood serum content in male rats is shown in Table 3.

Figures 1 - 4 show microphoto-histostructures of femoral bones diaphysis in control and experimental groups of animals

Determination of thyroid-stimulating hormone (TSH), total thyroxine (tT4) and total triiodothyronine (tT3) in the blood serum of the experimental group of animals confirmed the development of thyroid hypofunction with a decrease in hormone secretion on the background of increased TSH at the end of 3-week Thiamazole administration (Table 1)

The results of determination of mineral metabolism indicators in blood serum and bone remodeling (Table 2) showed that with the development of Thiamazole hypothyroidism, statistically significant changes in serum calcium, phosphorus and magnesium are not observed, but there is a tendency to a decrease in the level of Ca and P (Marova, 2003; Bassett and Williams, 2009; Pantazi and Papapetrou, 2000).

In hypothyroidism, an increase in the secretion of parathyroid hormone has been established as an adaptive reaction to a slowdown in bone remodeling with the development of hypocalcemia, a decrease in calcitonin levels and an increase in calcitriol

(Kazimirko *et al.*, 2006; Vanderpump *et al.*, 2011).

The content of bone resorption marker - C - terminal collagen telopeptides type 1 (β -Cross Laps) in rats of the experimental group decreases slightly, and the bone formation marker - bone alkaline phosphatase decreases statistically significantly. The obtained results show the disturbance of bone remodeling with significant inhibition of bone tissue formation phase. This results in the disturbance of bone tissue structure, which was found in its histological study. Figures 1-4 show the microphoto-histostructure of the femoral bone diaphysis of animals in the control and experimental groups.

Thyroid hormones are likely to influence the metabolism of bone tissue and mineral metabolism indirectly, affecting other systemic hormones and tissue regulatory factors. In addition to the established counter-regulatory phenomenon between thyroid hormones and parathyroid hormone, it was shown that T_3 and calcitriol synergistically affects osteoclastogenesis (Samsonova, 2007; Gruber *et al.*, 1999; Wang and Sum, 1980). There are indications of changes in the levels of growth hormone, insulin-like growth factor, interleukin-6, prostaglandin- E_2 , sex hormones under the influence of T_3 (Bassett and Williams, 2009; Gruber *et al.*, 1999). Analysis of the level of certain hormones and cytokines in the serum of rats with Thiamazole hypothyroidism also indicates the effect of thyroid hormones on the hormonal status of the body (Table 3). In the experimental group, all the studied parameters (PTH, FSH, LH, IL-1 β , IL-6, and TNF- α) were significantly increased except for the main sex hormone in the male body, testosterone. An increased level of gonadotropic hormones of the pituitary gland, on the background of a slight decrease in testosterone, indicates that in rats of the experimental group with hypothyroidism, the central regulation of the synthesis and secretion of androgens and reciprocal physiological relationships between the components of the hormonal axis hypothalamus-pituitary gonad is preserved.

It should be stressed that in the case of Thiamazole intoxication, the content of pro-inflammatory cytokines in the blood serum increases in animals with hypothyroidism: IL-1 β , IL-6, and TNF- α . Pro-inflammatory cytokines in bone tissue induce the production of matrix metalloproteinases, inhibit the synthesis of their inhibitors, increase the production of RANKL, inhibit the migration of osteoblasts, induce

apoptosis of osteocytes and activate osteoclastogenesis and osteoclast functions (Cardoso *et al.*, 2014; Wei *et al.*, 2005). Whether the increased secretion of pro-inflammatory cytokines is associated with the development of hypothyroidism under the action of Thiamazole or the direct effect of the toxicant on the cells – producers of the studied cytokines requires further research. Nevertheless, the results of the studies show that with the development of hypothyroidism, disturbance of bone remodeling and mineral metabolism is a consequence of not only the direct influence of thyroid hormones on osteoblasts and osteoclasts, but they can also be associated with other mechanisms of their action.

In rats of the control group (Figure 1), the cortical bone is represented by a developed network of multidirectional havers channels with different calibers. Under the periosteum and endostomy, the outer and inner layers of the compact substance formed by the system of bone plates are located parallel to the bone surface. Osteocytes located between the outer and inner common plates have a process shape. Osteons are interconnected by insertion plates. Blood vessels are located in the center of the osteon, perforating canals are found.

In experimental hypothyroidism, a significant change in the histostructure of the diaphysis of the tubular bones is determined. The thickness of the wall of the femur is uneven. There is an alternation of a fairly thick section with a section of bone thinning. The cementing (joint) lines located between common plates and osteons and between packages of bone plates undergo a particularly significant change (Figure 2). Loss of bone mass is seen. Bone plates, combined in bone packs, have, in some places, a bright zone, sometimes exfoliate from each other, indicating the presence of dystrophic changes with demineralization of bone tissue. There are deformed sections of common (general) plates (Figure 3.). The disorder of bone tissue formation (bone formation) is revealed, which is manifested by focal changes in the mass of bone structures. At the same time, the narrowing of osteons gaps is observed (Figure 4).

Thus, in the group of animals with experimental Thiamazole hypothyroidism, changes characteristic of dysplastic osteopenia were observed in the bone tissue.

4. CONCLUSIONS

1. Three-weekly daily intragastric administration of Thiamazole in a dose of 2.5 mg / 100 g of the animal mass causes the development of changes characteristic to hypothyroidism in white mongrel rats: decreased blood serum level of thyroid hormones and increase in the level of thyrotropin.

2. In animals, experimental hypothyroidism is accompanied by insignificant changes (decrease) in blood serum levels of phosphorus and total calcium, a significant reduction in bone alkaline phosphatase levels while maintaining C-terminal telopeptides of collagen type 1 concentration, reflecting disturbances in remodeling processes.

3. Experimental Thiamazole hypothyroidism in male rats is accompanied by a decrease in testosterone secretion, an increase of gonadotropins, parathyroid hormone, pro-inflammatory cytokines (: IL-1 β , IL-6, and TNF- α), thus showing a complex mechanism for the development of bone and mineral metabolism disorders.

4. The study of the histostructure of the tubular bone diaphysis in experimental hypothyroidism, reveals signs characteristic for the developing dysplastic osteoporosis.

The work has been approved by the Local Ethics Committee of the Bashkir State Medical University Ministry of Health of the Russian Federation

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Table 1. Blood serum thyrotropin and thyroid hormones content in white male rats in Thiamazole intoxication

Hormones	Control group n=10	Experimental group n=8	P
TTH, ml ME/l	1.11 ± 0.105	1.98 ± 0.166	<0.01
tT4, nmol/l	76.6 ± 3.33	61.9 ± 3.30	< 0.05
tT3, nmol/l	3.11 ± 0.19	1.8 ± 0.156	< 0.01

Table 2. The level of indicators of mineral metabolism and markers of bone metabolism in experimental Thiamazole hypothyroidism in male rats

Indicators	Control group	Experimental group n=8	P
Total Ca, mmol/l	2.24 ± 0.087	2.01 ± 0.166	> 0.2
P, mmol/l	1.81 ± 0.054	1.74 ± 0.065	> 0.5
Mg, mmol/l	0.86 ± 0.063	0.88 ± 0.053	> 0.5
ABP, E/l	6.1 ± 0.46	4.8 ± 0.36	< 0.05
β- Cross Laps, mgr/ml	0.87 ± 0.06	0.78 ± 0.04	> 0.1

Table 3. Content of some hormones and cytokines in rat blood serum in experimental hypothyroidism

Indicators	Control group, n=10	Experimental group, n=16	P
PTH	16.4 ± 0.22	22.1 ± 0.19	0.003
Testosterone nmol/l	23.6 ± 0.88	20.5 ± 0.72	0.048
FSH, ME/l	2.86 ± 0.116	3.70 ± 0.245	0.024
LH, ME/l	2.26 ± 0.195	2.98 ± 0.161	0.051
IL-1β, pg/ml	12.1 ± 1.07	18.7 ± 0.81	0.036
IL-6, pg/ml	15.2 ± 0.48	18.2 ± 0.39	0.050
TNF-α, pg/ml	15.9 ± 0.79	23.1 ± 0.91	0.026

Figure 1. *Periosteum, compact bone, and endostasis of tubular bone diaphysis in the control group of animals. Hematoxylin and eosin stain. Microphoto, OK10, 40x*

Figure 2. *Dilatation of tubular bones cementing lines of during hypothyroidism in experimental animals. Hematoxylin and eosin stain. Microphoto, OK10, 40x*

Figure3. Deformation zone of tubular bones structures in hypothyroidism in experimental animals. Hematoxylin and eosin stain. Microphoto, OK10, 40x

Figure 4. Narrowing of osteons gaps of with disturbance of the soldering line of the tubular bone in hypothyroidism in experimental animals. Hematoxylin and eosin stain. Microphoto, OK10, 40x