ORIGINAL ARTICLE

Bone and cartilage condition in experimental osteoarthritis and hypothyroidism

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ABSTRACT

Aim To investigate changes in bone and cartilage tissue during the use of non-steroidal anti-inflammatory drugs and paracetamol in experimental osteoarthritis and hypothyroidism by the markers CTX-I and CTX-II.

Methods The experiments were performed on 75 white nonlinear rats of both sexes, which recreated osteoarthritis and hypothyroidism. Experimental osteoarthritis was performed by single intra-articular administration of monoiodoacetic acid solution in the knee joint and experimental hypothyroidism was reconstructed by enteral administration of a solution of carbimazole. After the formation of the experimental models on the 42nd day of the experiment, the animals were divided into 14 groups and drug administration began daily for 5 days. The quantitative level of markers of blood serum was performed by specific kits, which are based on ELISA on 42 and 47 days of the experiment.

Results The degree of influence on degenerative-dystrophic processes in bone tissue, which was assessed by the level of the marker CTX-I in the serum of rats, the studied drugs were as follows: diclofenac sodium > ibuprofen > nimesulide = meloxicam > celecoxib > paracetamol. According to the degree of influence on degenerative-dystrophic processes in cartilage tissue, which were assessed by the level of marker CTX-II in the serum of rats, the studied drugs were as follows: nimesulide > celecoxib > meloxicam > ibuprofen > diclofenac sodium > paracetamol.

Conclusion Determination of the levels of CTX I and CTX II allows the evaluation of the bone and cartilage condition in experimental osteoarthritis and hypothyroidism.

Key words: anti-inflammatory agents, cartilage, enzyme-linked immunosorbent assay, hypothyroidism, knee joint, osteoarthritis

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INTRODUCTION

To date there has been a clear need to identify biomarkers that could predict the patient's response to treatment for osteoarthritis (OA), especially in comorbid conditions. It is known that hypofunction of the thyroid gland leads to metabolic disorders that adversely affect the condition of bone and cartilage tissue, causing the development of OA (1,2).

One of the manifestations of OA is a pathological change of the subchondral bone, which responds to the disease by the formation of sclerosis, marginal bone growths and the formation of deformation of the articular surfaces due to the destruction of bone and cartilage (1,2). Although non-steroidal anti-inflammatory drugs (NSAIDs) are effective in reducing pain and disability in patients with OA, it is still unclear to what extent these drugs can affect joint metabolism and therefore joint structure, especially in the setting of thyroid dysfunction (3,4).

Peculiarities of OA treatment with concomitant hypothyroidism is in the appointment of basic hormone replacement therapy and NSAIDs, but the effects of NSAIDs on bone and cartilage and the interaction of these drugs in comorbid pathology in our opinion are insufficiently studied (3-5).

CTX-I (C-telopeptide of collagen type I) and CTX-II (C-telopeptide of collagen type II) are markers of the destruction of bone and cartilage tissues, respectively, the level of which can investigate the state of anabolic and catabolic processes in the joint. According to the literature, the markers CTX-I and CTX-II are widely used in the experimental study of joint pathology, but the effect of NSAIDs and paracetamol has not been evaluated (5-7).

The aim of this work was to investigate changes in bone and cartilage tissue during the use of NSAIDs and paracetamol in experimental osteoarthritis and hypothyroidism by the markers CTX-I and CTX-II in rats.

MATERIALS AND METHODS

Materials and study design

The study was performed on 75 white nonlinear rats weighing 200-250 g, which were kept under standard conditions of the vivarium of the Dnipro State Medical University, Ukraine, in the period from January 2020 to August 2020. Experimental studies were carried out in accordance with the "General Ethical Principles of Animal Experimentation" (8) "Bioethical Examination of Preclinical and other Animal Research" (9) and the provisions of the "European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes" (10). When conducting the research, the regulation was approved by the Ethics Committee of the Dnipro State Medical University (protocol number 5 - 02.09.2020).

Methods

Experimental osteoarthritis (EOA) was reproduced at the beginning of the study by administering 0.1 mL of monoiodoacetic acid solution (Iodoacetic acid reagent ≥98.0%; No. I4386 (Sigma-Aldrich Chemie GmbH, Germany) to the knee joint. The solution of monoiodoacetic acid was prepared at the rate of 3 mg per 50 **µ**L of sterile saline (11, 12) and from the same day began the formation of experimental hypothyroidism (EH) by enteral administration of 0.02% solution of carbimazole (Esparma GmbH, Germany) in tablets 5 mg or 10 mg, which was prepared at the rate of 5 mg per 250 mL of saline solution and given with the animals' diet for 6 weeks (13).

On the 42nd day of the experiment animals were divided into 14 research groups of 5 rats in each group (n=5): group I - rats with EOA+ EH "without treatment"; group II – EOA + EH + L-thyroxine (T) in a dose of 1.5 mcg/kg (intragastric); group III – EOA + EH + diclofenac sodium (D) (PJSC Plant "Red star, Ukraine) at a dose of 10 mg/kg (intragastric); group IV – EOA + EH + D+T in the appropriate dosages and routes of administration; group V – EOA + EH + ibuprofen (I) (PJSC Borshagovskiy chemical pharmaceutical plant, Ukraine) at a dose of 5 mg/kg (intragastric); group VI – EOA + EH + I+T in the appropriate dosages and routes of administration; VII group – EOA + EH + meloxicam (Mel) (PJSC Kiev Vitamin Plant, Ukraine) at a dose of 10 mg/kg (intragastric); group VIII – EOA + EH + Mel+T in the appropriate dosages and routes of administration; group IX - EOA + EH + paracetamol (P) (PJSC Pharmaceutical Company "Darnitsa", Ukraine) at a dose of 150 mg/kg (intragastric); group X – EOA + EH + P+T in the

appropriate dosages and routes of administration; group XI - EOA + EH + nimesulide (N) (PJSC Lekhim-Kharkov, Ukraine) at a dose of 80 mg/kg (intragastric); group XII – EOA + EH + N+T in the appropriate dosages and routes of administration; group XIII - EOA + EH + celecoxib (C) (Health, Ukraine) at a dose of 50 mg/kg (intragastric); group XIV – EOA + EH + C+T in the appropriate dosages and routes of administration. The choice of drugs was based on the requirements of plants for primary health care for the treatment of OA and hypothyroidism (14).

The drugs were administered daily from day 42 of the experiment for 5 days in doses and modes listed above. To obtain a homogeneous suspension for intragastric administration tablets used solution of Tween-80 (Polysorbate 80, Ukraine) (15).

The quantitative level of CTX-I and CTX-II in serum was performed by specific kits (Elabscience, Inc., Houston, Texas, USA), which are based on ELISA (enzyme-linked immunosorbent assay) for cross linked C-telopeptide of type I and II collagen *in vitro* twice (on 42 and 47 days of the experiment) using the enzyme immunoassay system (Elabscience, Inc., USA) according to the manufacturer. Standard curves for CTX-I and CTX-II were generated by using reference cytokine concentrations supplied by the manufacturer. The sensitivity of kit for cross linked C-telopeptide of type I collagen is 18.75 pg/mL, detection range is 123.5-10000 pg/mL. The sensitivity of kit for cross linked C-telopeptide of type II collagen is 0.10 pg/mL, and the detection range is 35.2-10000 pg/mL. For the reference level of CTX-I and CTX-II adopted the values obtained in intact rats (n=5) (Table 1). Blood samples were obtained from the tail vein of the rats by means of puncture using a vacuum system on the 42nd and 47th day of the experiment (15).

The duration of administration of the drugs was 5 days and by the 47th day of the experiment. After the collection of biological material all animals were removed by decapitation under general anaesthesia (15,16).

Statistical analysis

Statistical differences included calculations of arithmetic mean values (M) and their errors (±m). The probability of difference of arithmetic mean (p) values was determined using nonparametric Mann-Whitney U-test. Probabilities of intragroup and between group differences were determined using Student's parametric t-test and ANOVA. The differences were considered statistically significant at $p \leq 0.05$. Before applying the parametric criteria, the hypothesis of a normal law of distribution of random variables was tested (17).

RESULTS

Changes in the level of CTX-I and CTX-II in the serum of rats under the influence of NSAIDs and paracetamol occurred unequally (Table 1).

Thus, in group III EOA + EH + diclofenac sodium a significant decrease in the level of CTX-I by 1.32 times ($p<0.05$) was determined on the 47th day of the experiment. When co-administered with diclofenac sodium with L-thyroxine (group IV) for 47 days on the background of experimental pathologies, there was a significant decrease in the level of CTX-I in 1.42 times ($p<0.05$).

When prescribed ibuprofen (V group EOA + EH + ibuprofen) for 47 days a significant decrease in the level of CTX I 1.24 times ($p<0.05$) was found. Co-administration of ibuprofen and L-thyroxine (group VI) on day 47 revealed a significant reduction in the level of CTX I by 1.28 times ($p<0.05$).

In group VII EOA + EH + meloxicam for 47 days a significant decrease in the level of CTX I of 1.1 times ($p<0.05$) was found. In group VIII EOA + EH + meloxicam + L-thyroxine for 47 days a significant decrease in the level of CTX I of 1.17 times was found ($p<0.05$).

Appointment of paracetamol (IX group EOA + EH + paracetamol) on 47 days led to a significant decrease in the level of CTX I by 1.01 times ($p<0.05$). When co-administered with L-thyroxine (X group EOA + EH + paracetamol + L-thyroxine) a significant decrease in the level of CTX I of 1.04 times was found ($p<0.05$).

In the XI group EOA + EH + nimesulide on 47 days of the experiment, a significant decrease in the level of CTX I of 1.1 times was found ($p<0.05$). When prescribed nimesulide and L-thyroxine (XII group EOA + EH + nimesulide + L-thyroxine) a significant reduction in the level of CTX I of 1.17 times ($p<0.05$) was found.

Administration of celecoxib (group XIII EOA + EH + celecoxib) determined a significant reducti-

Table 1. Indicators of serum CTX-I and CTX-II in rats against the background of non-steroidal anti-inflammatory drugs (NSAIDs) and paracetamol under conditions of osteoarthritis and hypothyroidism

Group, drug and dose	Arithmetic mean (±SD)			
	The level of CTX-I at 42 days (pg/mL)	The level of CTX-I at 47 days (pg/mL)	The level of CTX-II at 42 days (pg/mL)	The level of CTX-II at 47 days (pg/mL)
Intact rats (IR) (n=5)	-	-	-	-
I group EOA+EH "without treatment" (n=5)	122.4 (±0.72)	122.76* (±0.56)	294.9 (±1.27)	295.5* (±0.88)
II group EOA+EH + L-thyroxine (T) 1.5 mcg/kg (n=5)	122.08 (±0.99)	117.96† (±1.22)	294.8 (±0.95)	282.8† (±2.63)
III group EOA+EH + diclofenac sodium (D) 10 mg/kg (n=5)	122.04 (±1.11)	92.74*† (±2.44)	295.0*† (±1.0)	232.2*† (±1.38)
IV group EOA+EH + diclofenac sodium (D) + L-thyroxine (T) (n=5)	121.78 (±1.28)	85.76*† (±1.10)	294.9 (±0.91)	227.0*† (±1.08)
V group EOA+EH + ibuprofen (I) 5 mg/kg (n=5)	121.7 (±1.18)	97.84*† (±2,34)	295.3*† (±0.91)	220.4*† (±1.55)
VI group EOA+EH + ibuprofen (I) + L-thyroxine (T) (n=5)	122.1 (±1.10)	95.04*† (±1.06)	295.2*† (±1.23)	216.0*† (±1.05)
VII group EOA+EH + meloxicam (Mel) 10 mg/kg (n=5)	121.64 (±1.45)	110.8*† (±1.40)	295.3*† (±1.09)	213.4*† (±1.96)
VIII group EOA+EH + meloxicam (Mel) + L-thyroxine (T) (n=5)	122.38 (±1.05)	104.42*† (±1.65)	295.1*† (±1.24)	207.6*† (±1.18)
IX group EOA+EH + paracetamol (P) 150 mg/kg (n=5)	121.86 (±0.89)	120.74† (±1.21)	295.0* (±1.18)	256.8*† (±1.93)
X group EOA+EH + paracetamol (P) + L-thyroxine (T) (n=5)	121.58 (±1.20)	117.1† (±1.24)	294.6*† (±1.17)	246.9*† (±0.99)
XI group EOA+EH + nimesulide (N) 80 mg/kg (n=5)	122.12 (±0.93)	110.98*† (±1.07)	295.1*† (±0.8)	197.4*† (±1.0)
XII group EOA+EH + nimesulide (N) + L-thyroxine (T) (n=5)	121.84 (±1.17)	104.48*† (±1.47)	294.5*† (±0.7)	193.6*† (±1.19)
XIII group EOA+EH + celecoxib (C) 50 mg/kg (n=5)	122.02 (±1.12)	114.42† (±1.51)	295.3*† (±0.8)	204.0*† (±2.02)
XIV group EOA+EH + celecoxib (C) + L-thyroxine (T) (n=5)	122.54 (±0.73)	112.82*† (±1.22)	294.8 (±1.08)	201.3*† (±0.54)

*values are significant ($p<0.05$) relative to the corresponding group I indicator; †values are significant ($p<0.05$) with respect to the corresponding intact rats (IR) indicator;
EOA, experimental osteoarthritis; EH, experimental hypothyroidism

on in the level of CTX I by 1.07 times ($p<0.05$). When prescribed celecoxib and L-thyroxine (XIV group EOA + EH + celecoxib + L-thyroxine) a significant reduction in the level of CTX I of 1.09 times was found ($p<0.05$) (Table 1).

The changes in the level of the final C-telopeptide of collagen type II (CTX II) in the serum of rats under the influence of NSAIDs, paracetamol, and L-thyroxine occurring differently were noticed. Thus, for 42 days in all experimental groups, there was a high level of the marker CTX II, which was not determined in the group of intact rats, which reflects the development of pathological changes under the influence of experimental models and the ability of experimental models to affect the level of CTX II serum in rats ($p<0.05$) (Table 1).

On the 47 days of the experiment in group I EOA + EH "without treatment" compared with 42 days, the level of CTX II increased 1.002 times ($p<0.05$).

In group II EOA + EH + L-thyroxine for 47 days, at the end of 5-day administration of the studied drugs, against the background of the appointment of L-thyroxine, there was a significant decrease in the levels of CTX II 1.04 times ($p<0.05$) compared with 42 days. The obtained data reflect the effect of L-thyroxine on the experimental pathology - EOA + EH (Table 1).

Thus, in group III EOA + EH + diclofenac sodium on 47 days of the experiment a significant decrease in the level of CTX II 1.27 times ($p<0.05$) was determined. When co-administered diclofenac sodium with L-thyroxine (group IV) for 47 days on the background of experimental pathologies, a significant decrease in the level of CTX II in 1.3 times was found ($p<0.05$) (Table 1).

When prescribed ibuprofen (V group EOA + EH + ibuprofen) for 47 days, a significant decrease in the level of CTX II 1.34 times was found ($p<0.05$). Co-administration of ibuprofen and L-thyroxine (group VI) for 47 days revealed a significant decrease in the level of CTX II by 1.37 times ($p<0.05$).

In group VII EOA + EH + meloxicam for 47 days a significant decrease in the level of CTX II of 1.38 times ($p<0.05$) was found. In group VIII EOA + EH + meloxicam + L-thyroxine for 47 days a significant decrease in the level of CTX II of 1.42 times was found ($p<0.05$).

Administration of paracetamol (group IX EOA + EH + paracetamol) for 47 days led to a significant reduction in the level of CTX II by 1.15 times ($p<0.05$). When co-administered with L-thyroxine (X group EOA + EH + paracetamol + L-thyroxine) a significant decrease in the level of CTX I of 1.19 times was found ($p<0.05$).

In group XI EOA + EH + nimesulide on 47 days of the experiment, a significant reduction in the level of CTX II by 1.49 times was found ($p<0.05$). When prescribed nimesulide and L-thyroxine (XII group EOA + EH + nimesulide + L-thyroxine) a significant reduction in the level of CTX II of 1.52 times was found ($p<0.05$).

Administration of celecoxib (group XIII EOA + EH + celecoxib) determined a significant reduction in the level of CTX II by 1.45 times ($p<0.05$). When prescribed celecoxib and L-thyroxine (XIV group EOA + EH + celecoxib + L-thyroxine) a significant reduction in the level of CTX II of 1.46 times was found ($p<0.05$) (Table 1).

The degree of influence on degenerative-dystrophic processes in bone tissue, which was assessed by the level of the marker CTX-I in the serum of rats, the studied drugs were as follows: diclofenac sodium > ibuprofen > nimesulide = meloxicam > celecoxib > paracetamol ($p<0.05$). According to the degree of influence on degenerative-dystrophic processes in cartilage tissue, which were assessed by the level of marker CTX-II in the serum of rats, the studied drugs were as follows: nimesulide > celecoxib > meloxicam > ibuprofen > diclofenac sodium > paracetamol ($p<0.05$).

DISCUSSION

To date, the diagnosis of OA is based on data from clinical and additional research methods, among which X-ray research methods play a leading role. However, laboratory markers of cartilage metabolism are known, which are able to reflect the degree of its destruction, and due to the cost of their determination, this method of diagnosing OA is used more often in experimental works (5-7).

One such marker is CTX-II - is a C-terminal telopeptide of type II collagen, which is an important biomarker of type II collagen degradation in various diseases of the musculoskeletal system and indicates degradation of cartilage tissue and can be detected in synovial fluid, plasma and serum, urine, and other tissues (18-22). The biochemical marker of CTX-II is a reflection of the intensity of cartilage tissue breakdown by the level of type II collagen breakdown products that enter the blood serum and other environments of the body and tissues (23,24). According to many authors, the level of CTX-II is sensitive to changes in the early stages of OA (25,26), correlates with other biochemical markers of inflammation and decay of cartilage and bone tissue (27-31), bone mineral density (18,26,32), progression of OA (33), reflects the degree of cartilage destruction (25,31,33,34), correlates with changes in oxidative stress (35) and changes in MRI (33), sensitive to changes during pharmacotherapy of OA (36,37).

Biochemical markers formed by the destruction of type I collagen have not been widely used as selective indicators of OA due to the specificity of the pathological process, which is more relevant to tissues formed by type II collagen (5). They are more widely used in pathologies of pure bone tissue, such as osteoporosis, osteonecrosis, and heterotopic ossification (5, 6). However, diclofenac sodium is known to affect the number of subchondral bone osteophytes in OA (38, 39) and has the ability to inhibit bone resorption by anti-inflammatory action (40).

Unfortunately, no other data on the effect of NSAIDs on the level of the CTX-I marker in OA and concomitant hypothyroidism have been found in the available literature.

In conclusion, determination of the level of markers CTX I and CTX II makes it possible to assess the severity of degenerative-dystrophic changes in bone and cartilage tissue, respectively, against the background of experimental osteoarthritis and hypothyroidism when prescribing NSAIDs and paracetamol, based on the level of breakdown products of type I and II collagen. In terms of the degree of influence on the degenerative-dystrophic process in bone tissue according to the level of CTX I marker, the most active were non-selective inhibitors of cyclooxygenase of types 1 and 2 - diclofenac sodium and ibupro-

fen. By the degree of influence on the degenerative-dystrophic process in the cartilage tissue, according to the CTX II marker, the most active were predominantly selective type 2 cyclooxygenase inhibitors - nimesulide and celecoxib. Paracetamol had the least activity in terms of the degree of influence on the degenerative-dystrophic process in bone and cartilaginous tissue in terms of the level of CTX I and CTX II markers.

The revealed different effect of non-selective type 1 and type 2 cyclooxygenase inhibitors and predominant type 2 cyclooxygenase inhibitors on bone and cartilage tissue in experimental osteoarthritis and hypothyroidism indicates the presence of a specific effect of NSAIDs on various joint structures.

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