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Prooxidant - Antioxidant Status and PTGS2, NOS2 Genes Expression In Rat Cartilage With Osteoarthritis And After The Treatment Of Chondroitin Sulfate.

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ABSTRACT

In the experiment on rats with osteoarthritis induced by sodium monoiodoacetate in knee cartilage tissue we found an increase of gene expression of inflammatory mediators - cyclooxygenase-2 (Ptgs2 gene) and inducible nitric oxide synthase (Nos2 gene), an activation of reactive oxygen species (ROS) (O_2^-, H_2O_2) synthesis and high xanthine oxydase activity, an increased content of TBA-active products of lipid peroxidation and activation of antioxidant enzymes (superoxide dismutase and catalase). After the therapy with the drug "Drastop" based on chondroitin sulfate these biochemical parameters were brought close to norm: the gene expression of pro-inflammatory enzymes was reduced, the synthesis of ROS and lipid peroxidation products was inhibited and the activity of antioxidant enzymes lowered. The results indicate that chondroprotector "Drastop" possess anti-inflammatory and antioxidant properties that make it effective in the treatment of osteoarthritis.

Keywords: cartilage, osteoarthritis, rats, chondroitin sulfate, oxidation

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INTRODUCTION

Osteoarthritis (OA) is a degenerative joint disease characterized by inflammation of the synovial membrane, erosion of cartilage, and resorption of subchondral bone [1]. The degeneration of cartilage starts with biomechanical and biochemistry pathological changes: injury, muscle weakness or obesity, genetics, dietary intake, estrogen use and bone density [2]. These factors can cause the excess synthesis of proinflammatory cytokines interleukin-1-beta and tumor necrosis factor-alpha that stimulate the expression of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS), two potent proinflammatory enzymes that lead to increased synthesis of prostaglandin E2 (PGE2) and nitric oxide (NO) [3]. Enzymes and metabolites of arachidonic acid cascade, including enzyme COX-2 (encoded by the gene - *Ptgs2*) and its product - PGE2, are known to be important mediators of inflammatory response. A rapid change in expression level *Ptgs2* and the content of proinflammatory cytokines is specifically shown human synovial tissue samples in early OA [4]. It was detected that iNOS ex vivo in cartilage samples of patients with OA is active even without additional stimulation by interleukin-1 or lipopolysaccharide, NO inhibits collagen and proteoglycan synthesis, enhances apoptosis, and inhibits B1 integrin-dependent adhesion to the extracellular matrix [5]. Activated articular chondrocytes produce as much, if not more, NO than any other cell in the body [6]. Pharmacological therapy, including nonsteroidal anti-inflammatory drugs, immunosuppressive agents, and tetracyclines, attenuate the activity of NO and PGE2. These pleiotropic mediators and enzymes that produce them are targets for pharmacologic intervention [7]. Enzymatic peroxidation is carried out by heme-containing cycloxygenase and non-heme iron lipoxygenase, which substrate is arachidonic acid. The heme-containing cyclooxygenase forms endopereoxides of arachidonate in the biosynthesis of prostaglandins, prostacyclins and thromboxanes. The reactivity of the reactive oxygen species *(*ROS) can vary depending on the place of their generation. Thus, the activity of the superoxide anion radical increases in the hydrophobic environment. Biological activity of reactive forms of oxygen is associated with the synthesis of prostaglandins, leukotrienes, thromboxanes, as well as with oxidative modification of proteins, nucleic acids, lipids and articular chondrocytes are likely to be the major intraarticular source of ROS synthesis. Peroxides and nitric oxide cause peroxide oxidation of membrane phospholipids, which causes pronounced disturbances of membrane transport and leads to oxidative stress. The degree of expression of the oxidative modification of macromolecules under the influence of ROS depends on the ratio of the capacity of the antioxidant system and the intensity of pro-oxidant formation [8, 9].

Modern treatment with non-steroidal anti-inflammatory drugs has pronounced side effects on the digestive and cardiovascular system [10]. That's why today therapists and OA patients try to consider complementary approaches to control pain and to improve quality of life. Drugs based on chondroitin can suppress inflammation, lipid peroxidation and restore antioxidant system in the cell. Chondroprotectors are used in medicine for a long time, but the raw material for them present a mixture of molecules of chondroitin sulfate with different lengths and variations in the position of sulfated groups and have different levels of purification, which causes different effects that you need to keep in mind when assigning original products and generics [11, 12]. That's why the purpose of the study was to determine changes in the expression level of mediators of the inflammatory response and the state of oxidative-antioxidant system in the cartilage tissue of rats with sodium monoiodoacetate induced OA and after using chondroitin sulfate as a therapy.

RESEARCH METHODS

White nonlinear rats of both sexes weighing 180-240 g were used in the experiment, animals were kept on a standard diet. While working with animals we complied with the general ethical principles of animal experiments approved by the First National Congress of Ukraine on Bioethics (September 2001) and international agreements in this field. Each group consisted of 7 animals of each sex. The experiment was put under the scheme: the group "Control" - control rats that on the first day were injected with stabbed 50 ml 0.9 % solution of NaCl in both knees through knee ligaments; the group "MIA" (sodium monoiodoacetate) - rats, that on the first day were injected into the right knee with 3 mg of sodium monoiodoacetate dissolved in 50 ml 0.9% NaCl solution, in the left knee - 50 ml 0.9% NaCl solution through the knee ligament; the group "Drastop" served as the negative control where animals on the first day were injected with stabbed 50 ml 0.9% NaCl solution in both knees through knee ligaments and further got intramuscularly the drug "Drastop" 1time per day during 25 days; animal group "MIA + Drastop" on the first day was injected into the right knee with 3 mg sodium monoiodoacetate dissolved in 50 ml 0.9 % NaCl solution, in the left knee - 50 ml 0.9 % NaCl solution through knee ligaments and got the therapy with "Drastop" 1 time per day during 25 days. Conversion factors

dose (mg/kg) to the animals from humans were used to calculate the dosage for animals [7]. The slaughter of rats was performed 30 days after the experiment began. The hyaline cartilage of the knee were removed and stored at -20 ° C prior to the study.

In cartilage homogenate the following parameters were determined. The protein content was measured by Lowry [13]. The content of superoxide anion radical was determined using spectrophotometrical method with HTT (2,3-bis (2-methoxy-4-nitro-5-sulfofenil) -2H-tetrazol-5-karboksanilid) as the electron acceptor [14, 15], the activity of xanthine oxidase was determined by the formation of uric acid with xanthine [16], the content of hydrogen peroxide was measured spectrophotmetrically using ksylenol orange [17]. The content of diene conjugates was determined in heptane-izopropanol extract using spectrophotometric method, schiff bases – using fluorescence method [18, 19]. The content of TBA-active products was determined by reaction with thiobarbituric acid [20].

Real-time reverse-transcription polymerase chain reaction (RT-PCR). RNA was obtained by the method of Chomczynski [21]; The synthesis of cDNA and quantitative polymerase chain reaction in real time (real-time qPCR) with the help of commercial set «Thermo Scientific Verso SYBR Green 1-Step QRT-PCR ROX Mix» («Thermo Scientific», Lithuania), using Mmol / L of each primer, was carried out under the temperature witch was recommended by the manufacturer: synthesis of cDNA 50 ° C - 30 min; initiating denaturation 95 ° C - 15 minutes; then 40 cycles: denaturation of DNA 95 ° C - 15 c; hybridization of primers 50º C - 35 sec; completion of the chain 72 ° C - 30 sec ; final elongation 72 ° C - 5 min.

In the reactions the following sequences of primers were used: for *Ptgs2* - forvard - TGCTGTTCCAACCCATGTCA and reverse - TGTCAGAAACTCAGGCGTAGT; for *Nos2* - forvard - GTGTTCCACCAGGAGATGTTG and reverse - CTCCTGCCCACTGACTTCGTC; for *Actb* (β-actin gene was used as an internal control of the reaction due to its constitutive expression) - forvard - TGGGACGATATGGAGAAGAT and reverse - ATTGCCGATAGTGATGACCT. The results of amplification were tested in parallel repeating experiments of qPCR on samples of the RNA from all animals using each primer at least three times. After each amplification cycle fluorescence dye SYBR Green I was detected, and after reaction melting curve was built to control the formation of dimers and primer specificity of the reaction. The initial amount of mRNA was calculated using comparative C_T method «$\Delta\Delta C_T$ Method», the effectiveness of PCR reactions was similar Ex = ($10^{-1 / slope}$) -1, slope <0,1. The relative amount of mRNA of these genes was normalized to mRNA *Actb*.

Statistical analysis of research results. Mathematical and statistical analysis of research results was carried out using a computer software package «GraphPad Prism 5.04» («GraphPad Software Inc.», USA). They were tested for normal distribution using the Shapiro-Wilkie test. Further calculation was performed using unidirectional analysis of variance (one-way ANOVA) with Tukey post-test. The results are shown as the arithmetic mean ± standard deviation (variance) - SD. The results considered significant when $p \leq 0,05$.

RESULTS

We found that in the group of rats with MIA - induced OA in homogenates of rat cartilage the content of superoxide anion radicals increased in 3.1 times, the activity of xanthine oxidase - in 3.2 times, and the level of hydrogen peroxide - 1.7 times respectively, compared with controls (Table. 1). After the therapy the content of superoxide anion radical decreased 1.6 times, the activity of xanthine oxidase decreased by 2 times and the amount of hydrogen peroxide decreased 1.4 times relative to the respective parameters group "MIA". The negative control confirmed that the drug based on chondroitin sulfate didn't affect the shift of prooxidant-antioxidant balance in animals, the study showed no changes in the contents of superoxide anion radical, activity of xanthine oxidase and quantity of hydrogen peroxide in the cartilage of animals from the group "Drastop" regarding the relevant indicators of the control group.

We found the growing content of lipid peroxidation products in rat cartilages after administration of sodium monoiodoacetate: the content of TBA - active products increased 2.3 times relative to control (Table. 2). Negative control on the drug didn't show an increase in the content of TBA - active products relative to the control. The drug "Drastop" in cartilage homogenates reduced the content of secondary lipid peroxidation products 1.5 times relative to the group "MIA".

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Table 1. The content of ROS and xanthine oxydase activity in the cartilage of rat knee joints with OA (M ± m, n = 7)

Indicator Groups of animals	O^{2-} content, μmol × mg protein^{-1}	Xanthine oxydase activity nmol × min^{-1} · mg protein-1	Content of H_2O_2, μmol × mg protein^{-1}
Control	8,03±0,76	2,02±0,18	12,83±1,15
MIA	24,95±2,17*	6,39±0,54*	22,39±2,08*
Drastop	9,21±0,85*	2,51±0,23*	11,96±1,07*
MIA + Drastop	15,32±1,38#	3,18±0,29#	15,71±1,53#

* - p < 0,05, relative control
# - p < 0,05, relative to group "MIA"

Table 2. The content of TBA - active products in the cartilage of rat knee joints with OA (M ± m, n = 7)

Indicator Groups of animals	TBA-active products, nmol × mg protein^{-1}
Control	65,21±6,18
MIA	149,97±12,36*
Drastop	72,83±6,95*
MIA + Drastop	98,35±9,51#

* - p < 0,05 relative to control
# - p < 0,05 relative to animals injected only with MIA

We established that in rat cartilages affected with chronic joint inflammation the activities of antioxidant enzymes - superoxide dismutase and catalase significantly increased: superoxide dismutase activity showed 4 times increase and catalase activity - 4.7 times increase compared to control. Negative control showed a weak growth of superoxide dismutase activity in rat cartilage - 1.2 times relative to control. After the therapy with chondroitin sulfate enzyme activities decreased, superoxide dismutase activity - 1.9 times, catalase activity - 3.1 times compared to MIA group (Table. 3).

Table 3. The activities of antioxidant enzymes in the cartilage of rat knee joints with OA (M ± m, n = 7)

Indicator Groups of animals	Superoxide dismutase activity units × min-1 × mg protein-1	Catalase activity, nmol × min^{-1} × mg protein^{-1}
Control	0,321 ± 0,029	14,21 ± 1,25
MIA	1,281 ± 0,105 *	67,49 ± 6,38 *
Drastop	0,383 ± 0,035*	12,92 ± 1,23*
MIA + Drastop	0,672 ± 0,064#	21,87 ± 2,09#

* - p < 0,05, relative to control
# - p < 0,05, to animals injected only with MIA

The experimental studies have shown that the level of *Ptgs2* gene expression in the group of animals with disorders was higher by 8 times (p ≤ 0,001) compared to intact animals (Fig. 1). The levels of expression of this gene in the first and second (negative control: the introduction of saline intramuscularly and orally with distilled water) group of rats were not significantly different.

At the same time, after the introduction of both MIA and Drastop the corresponding mRNA level was 3,9 (p ≤ 0,001) times lower than in the third group of animals.

Further experimental studies have shown that the level of *Nos2* gene expression in the group of animals with disorders was 5.8 times (p ≤ 0,001) higher compared to intact animals (Fig. 2). The levels of expression of this gene in the first and second (negative control: the introduction of saline intramuscularly and orally with distilled water) group of rats were not significantly different.

At the same time, after the introduction of both MIA and Drastop corresponding mRNA level was 2,8 (p ≤ 0,001) times lower than in the third group of animals.

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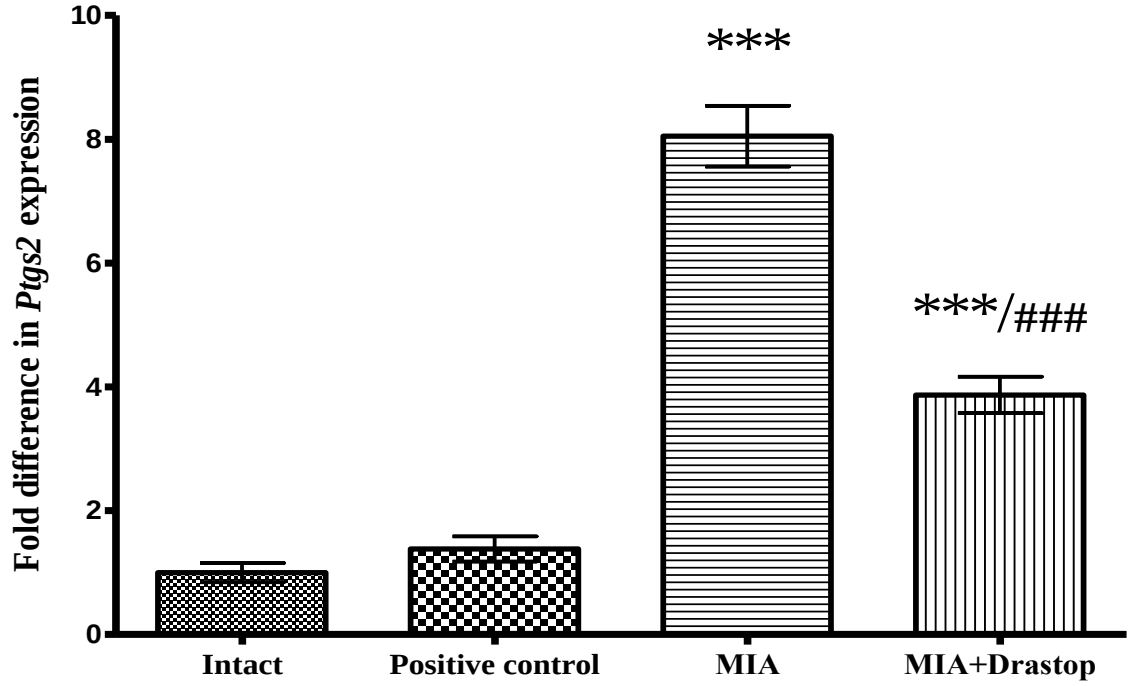

Fig.1. level of *Ptgs2* gene expression in the cartilage of rat knee joints with OA and with the treatment of Drastop. 1 - intact; 2 - positive control; 3 - MIA; 4 - MIA + Drastop; * - p ≤ 0,001 relative to intact animals (control); ### - p ≤ 0,001 relative to animals injected only with MIA.**

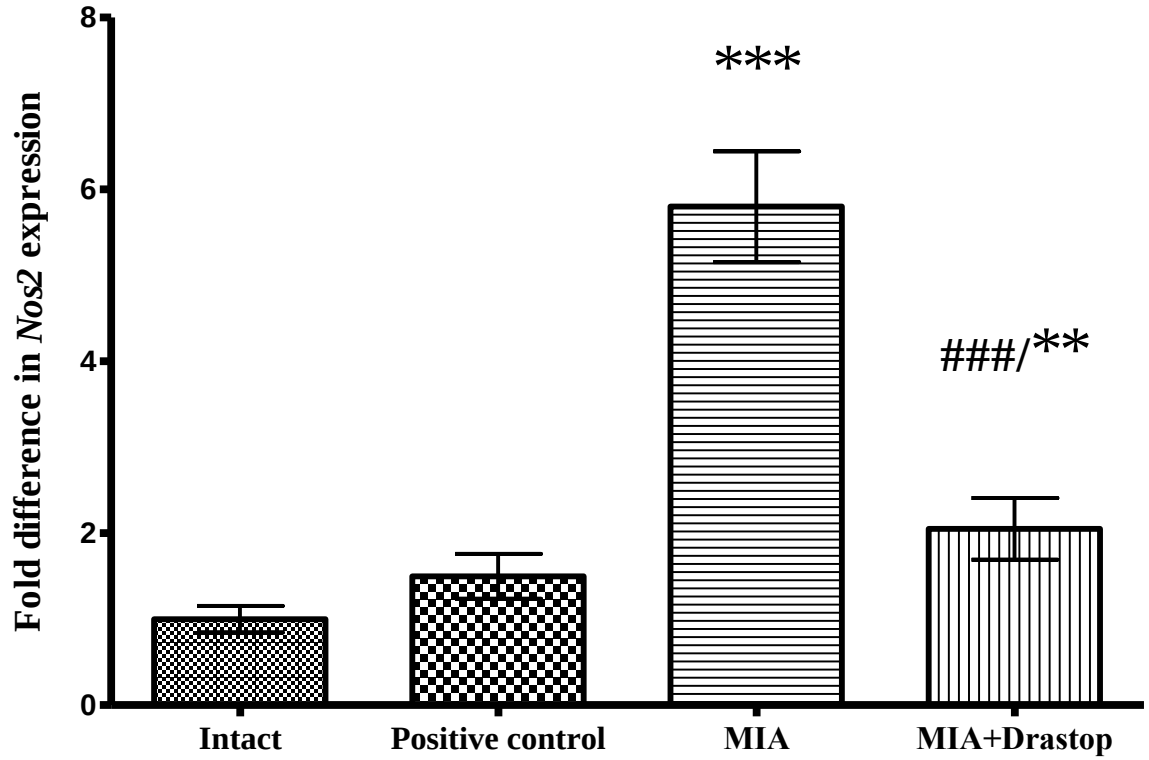

Fig. 2. Level of *Nos2* gene expression in the cartilage of rat knee joints with OA and with the treatment of Drastop. 1 - intact; 2 - positive control; 3 - MIA; 4 - MIA + Drastop; * - p ≤ 0,001, ** - p ≤ 0,01 relative to intact animals (control); ### - p ≤ 0,001 relative to animals injected only with MIA.**

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DISCUSSION

It has been reported that ROS (O^{2-} and peroxynitrite) directly injure the guanine repeats in the telomere DNA, indicating that oxidative stress directly leads to telomere erosion, regardless of cell active division [22]. These findings suggest that oxidative stress could induce chondrocyte telomere instability, leading to chondrocyte senescence.

In the articular cavity damaged and necrotic cartilage and bone fragments are subjected to phagocytosis by leukocytes with the release of inflammatory mediators, ROS (superoxide anion (O2−•), hydroxyl radical (•OH), hydrogen peroxide (H_2O_2), and hypochlorous acid (HOCl)) and lysosomal enzymes, which is clinically manifested by synovitis of the joint and the development of immunological reactions on decay products [23]. In chondrocytes ROS regulate intracellular signaling molecules such as receptor tyrosine kinases, the MAP kinases (ERK1/2, JNK, p38), lipid pathways (phospholipases, PKC, and the PI3-kinase/Akt pathway), phosphatases, and transcription factors (NFIB, p53, and AP-1) that are involved in signaling networks that regulate cartilage matrix synthesis and degradation, ROS can promote the extended activity of certain signaling through reversible inactivation of specific phosphatases and increased ROS level activate catabolic signaling, cause oxidative stress and inhibit anabolic signaling through inhibition of IGF-1 depended proteoglycan synthesis [24, 25]. Xanthine oxidase plays a crucial role in ischemia-reperfusion injury. During ischemia, ATP is degraded to hypoxanthine and xanthine dehydrogenase is converted to xanthine oxidase. During reperfusion, xanthine oxidase catalyses the reaction of hypoxanthine or xanthine and molecular oxygen to superoxide radicals. These radicals rapidly react with nitric oxide, peroxynitrite, and other reactive species [26].

ROS although can cause peroxidation of polyunsaturated fatty acids in the membrane and produce lipid peroxidation products: malonaldehyde as well as 4-hydroxy-2(E)-nonenal are common markers of oxidation in cells [27].

The production of endogenous ROS (generated by NADPH oxidase, xanthine oxidase (XO), or cytochrome P450, etc) are balanced by the actions of cellular antioxidant defense systems, including enzymes (superoxide dismutase (SOD) and catalase (CAT) [28].

The study showed an increase in the activity of xanthine oxidase, an increase in the content of free radicals and TBA-active products in OA. Also, a high activity of antioxidant enzymes was observed, which indicates the development of oxidative stress in cartilage tissues. The use of the drug based on chondroitin sulfate reduced biochemical manifestations of oxidation and reduced the burden on the enzymes of the antioxidant system.

The results indicate significant activation of *Ptgs2* and *Nos2* expression which is typical for the development of inflammation in cartilage tissues. Normally iNOS is active in immune cells and provides antimicrobial and tumoricidal activity but when overexpressed, iNOS-generated NO can cause tissue injury by its cytotoxic effects on resident cells [29]. It is known, that human OA-affected cartilage release substantial amounts of nitrite for up to 72 h in ex vivo organ cultures but authors predict that OA-NOS may be a unique isoform expressed by the chondrocyte under pathological conditions [30]. NO was shown to suppress proteoglycan synthesis, by decreasing metabolic incorporation of sulfate ($^{35}SO_4^{2-}$) into glycosaminoglycans[31], to activate matrix metalloproteinases in cartilage and chondrocytes [32] and to inhibit collagen II synthesis [33] . iNOS is upregulated by IL-1 and TNF-a. Without cytokine stimulation, basal OA cartilage controls did not express messenger RNA for *Nos2* when analyzed by real-time reverse-transcription polymerase chain reaction (RT-PCR) [34]. COX or prostaglandin H (PGH) synthase, like NO synthase, is although regulated by IL-1. It was shown, that COX in bovine chondrocytes can be activated by NO through interaction with the iron catalytic site but after specific inhibition of iNOS COX still remained active and PGE2 levels were further elevated [35, 36]. As the administration of the drug based on chondroitin sulfate reduced the expression of *Nos2* and *Ptgs2* genes, it may be considered as a suppressor of inflammation and the development of OA.

Chondroitin sulfate is the main component of proteoglycans, a component of the cartilage matrix. It is a sulfated glycosaminoglycan, which consists of long unbranched chains of repeating residues of N-acetylgalactosamine and glucuronic acid. Most acetylgalactosamine have N-sulfated residues in the 4th and 6th positions. Chondroitin sulfate molecules are involved in the processes of water, amino acids and lipid

transport in the areas of avascular cartilage [37]. On animal models of chronic OA it was observed that the therapy with chondroitin sulfate reduced gene expression and synthesis of cyclooxygenase-2 and chemokine ligand 2, decreased infiltration of the synovial membrane by immune cells and lowered the degree of proliferation in the synovial membrane [38]. It is known that inflammatory cytokines IL-1 β and TNF - α stimulate the synthesis of IL-8 and -6, activate matrix metalloproteinases 1, 8 and 13 that destroy cartilage collagen type 2, cytokines activate cyclooxygenase 2 in fibroblast-like synoviocytes and chondrocytes, which generates inflammatory mediators prostaglandins, that increase inflammation. Some studies in vivo showed that chondroitin sulfate may reduce concentrations of IL-1 β in joint tissues [39] and other inflammatory mediators (IL-6, nitric oxide synthase, prostaglandin E2) and inhibit induced expression of matrix metalloproteinase 3 9 and 13 in chondrocytes [40, 41]. Chondroprotectors protect cells by inhibiting oxidation of proteins, lipids and synthesis of free radicals. Some studies suggest the inhibition of apoptosis of chondrocytes [42, 43]. Chondroitin sulfate inhibits the activation and nuclear translocation of factor NFκβ in chondrocytes and synoviotcytes. NFκβ, binding to the promoter of corresponding gene activates the transcription of proinflammatory cytokines, nitric oxide synthase, cyclooxygenase 2, phospholipase A2, matrix metalloproteinases, which deepens the destructive processes in deformed osteoarthritic joints. Chondroitin sulfate inhibits the expression of RANKL and activates the synthesis of osteoprotegerin regulating the subchondral bone remodeling [44].

CONCLUSIONS

The biochemical analysis of rat cartilage tissue with monoyodatsetat-induced OA showed an increase in the expression levels of *Ptgs2* and *Nos2*, an increase of ROS production and activation of xanthine oxidase, high levels of TBA-active products and increased activities of antioxidant enzymes - superoxide dismutase and catalase compared with the corresponding control groups of animals. This indicates the activation of inflammatory processes, abuse redox balance and the development of oxidative stress and lipid peroxidation in the tissues. The usage of the drug based on chondroitin sulfate brought the specified biochemical parameters closer to normal compared with experimental OA, indicating the anti-inflammatory and antioxidant properties of the drug "Drastop" that make it effective in the treatment of OA.

REFERENCES

[1] Au RY, Al-Talib TK, Au AY, Phan PV, Frondoza CG. Osteoarthritis and Cartilage 2007; 15(11): 1249-1255.
[2] Felson DT, Lawrence RC, Dieppe PA, Hirsch R, Helmick CG, Jordan JM. Annals of Internal Medicine 2000; 133 (8): 635–646.
[3] Goldring SR, Goldring MB. Clinical Orthopaedics and Related Research 2004; 427 (Suppl): S27–S36.
[4] Benito MJ, Veale DJ, FitzGerald O, van den Berg WB, Bresnihan B. Annals of the rheumatic diseases 2005; 64 (9): 1263-1267.
[5] Amin AR, Abramson SB. Current opinion in rheumatology 1998; 10(3): 263-268.
[6] Stadler J1, Stefanovic-Racic M, Billiar TR, Curran RD, McIntyre LA, Georgescu HI, Simmons RL, Evans CH. The Journal of Immunology 1991;147: 3915–20.
[7] Amin AR, Attur M, Abramson SB. Current opinion in rheumatology 1999; 11(3): 202-209.
[8] Madrigal JL, Moro MA, Lizasoain I, Lorenzo P, Fernández AP, Rodrigo J, Boscá L, Leza JC. Neuropsychopharmacology 2003; 28 (9): 1579 - 88.
[9] Jang D, Murrell GAC. Free Radical Biology and Medicine 1998; 24(9): 1511-1519.
[10] Barnes EV, Edwards NL. The Southern Medical Journal 2005; 98 (2): 205–209.
[11] Volpi N. Journal of Pharmacy and Pharmacology 2009; 61(10): 1271–80.
[12] Lauder RM. Complementary Therapies in Medicine 2009; 17(1), 56–62.
[13] Hartree EF. Analytical biochemistry 1972; 48(2): 422-427.
[14] Sutherland MW, Learmonth BA. Free radical research 1997; 27(3): 283-289.
[15] Able AJ, Guest DI, Sutherland MW. Plant Physiology 1998;117(2): 491-499.
[16] Hashimoto S. Analytical biochemistry 1974; 62(2): 426-435.
[17] Gay CA, Gebicki JM. Analytical biochemistry 2003; 315(1): 29-35.
[18] Gavrilov VB, Gavrilova AR, Hmara NF. Laboratornoe delo 1988; 2: 540-546.
[19] Kolesova OE, Markin AA, Fedorova TN. Laboratornoe delo 1984; 9: 60-63.
[20] Orekhovich V.N. Sovremennye metody v biohimii, Medicina, M, 1977, pp. 62-68.
[21] Chomczynski P, Sacchi N. Analytical Biochemistry 1987; 162(1): 156-159.

[22] Yermilov V, Rubio J, Ohshima H. FEBS Letters 1995; 376: 207-210.
[23] Karrej HLF. Osteoartroz. Klinicheskaya revmatologiya, Medicina, M, 1990, pp. 121-128.
[24] Kamata H, Hirata H. Cell Signal 1999; 11: 1-14.
[25] Starkman BG, Cravero JD, Delcarlo M, Loeser RF. Biochemical Journal 2005;389: 723-9.
[26] Akyol O, Herken H, Uz E, Fadillioğlu E, Unal S, Söğüt S, Ozyurt H, Savaş HA. Progress in Neuro-Psychopharmacology and Biological Psychiatry 2002; 26(5): 995-1005.
[27] Grigolo B, Roseti L, Fiorini M, Facchini A. The Journal of rheumatology 2003; 30(2): 345-347.
[28] Ozgocmen S, Ardicoglu1 O, Erdogan H, Fadillioglu E, Gudul H. Annals of Clinical & Laboratory Science 2005; 35 (2):137-143.
[29] Nathan CF, Hibbs JB. Current Opinion in Immunology 1991; 3: 65–70.
[30] Amin AR, Di Cesare PE, Vyas P, Attur M, Tzeng E, Billiar TR, Stuchin SA, Abramson SB. The Journal of Experimental Medicine 1995; 182:2097–2102.
[31] Taskiran D, Stefanovic-Racic M, Georgescu H, Evans C. Biochemical and Biophysical Research Communications 1994; 200:142–148.
[32] Murrell GAC, Jang D, William RJ. Biochemical and Biophysical Research Communications 1995; 206:15–21.
[33] Cao M, Westerhausenlarsen A, Niyibizi C, Kavalkovich K, Georgescu HI, Rizzo CF, Hebda PA, Stefanovic-Racic M, Evans CH. Biochemical Journal 1997; 324: 305–310.
[34] Rediske JJ, Hoehne CF, Zhang B, Lotz M. Osteoarthritis Cartilage 1994; 2: 199–206.
[35] Manfield L, Jang D, Murrell GAC. Inflammation Research 1996; 45: 254–258.
[36] Tsai AL, Wei C, Kulmacz RJ. Archives of Biochemistry and Biophysics 1994; 313: 367–372.
[37] Conte A, Volpi N, Palmieri L, Bahous I, Ronca G. Arzneimittel Forschung - Drug Research 1995; 45: 918–925.
[38] Largo R, Roman–Blas JA, Moreno–Rubio J. Osteoarthritis Cartilage 2010; 18 (Suppl. 1): 17–23.
[39] Pelletier JP, Martel–Pelletier J, Abramson SB. Arthritis & Rheumatology 2001; 44(6): 1237–1247.
[40] Chan PS, Caron JP, Rosa GJ, Orth MW. Osteoarthritis Cartilage 2005; 13(5): 387–394.
[41] Cho SY, Sim JS, Jeong CS, Chang SY, Choi DW, Toida T, Kim YS. Biological and Pharmaceutical Bulletin 2004; 27(1): 47–51.
[42] Caraglia M, Beninati S, Giuberti G, D'Alessandro AM, Lentini A, Abbruzzese A. Experimental & Molecular Medicine 2005; 37: 476–481.
[43] Jomphe C, Gabriac M, Hale TM, Héroux L, Trudeau LE, Deblois D, Montell E, Vergés J, du Souich P. Basic & Clinical Pharmacology & Toxicology 2008; 102(1): 59-65.
[44] Tat Kwan S, Pelletier JP, Lajeunesse D, Fahmi H, Lavigne M, Martel-Pelletier J. Clinical and experimental rheumatology 2008; 26(2): 295.