ORIGINAL ARTICLE

EXPERIMENTAL RESEARCH OF THE EFFECT OF COXIBS ON THE CERULOPLASMIN LEVEL IN RAT SERUM ON THE FORMALIN-INDUCED EDEMA MODEL

DOI: 10.36740/WLek202209103

Ganna O. Syrova, Olena V. Savelieva, Tetyana S. Tishakova, Larysa V. Lukianova
KHARKIV NATIONAL MEDICAL UNIVERSITY, KHARKIV, UKRAINE

ABSTRACT

The aim: To estimate anti-inflammatory action of coxibs (3-(4-methylsulfonylphenyl)-4-phenyl-2H-furan-5-one, 2,3,5,6-tetradeuterio-4-[5-(4-methylphenyl)-3-(trifluoromethyl) pyrazol-1-yl]benzenesulfonamide) compared to reference drug – 2-[(2,6-Dichlorophenyl)amino]benzeneacetic acid sodium salt.

Materials and methods: The anti-inflammatory effect of studied substances was investigated using the ceruloplasmin test as serum ceruloplasmin is a routinely investigated biochemical index. Formalin-induced hind paw edema was used as the most commonly used animal model to simulate acute inflammation. 3-(4-methylsulfonylphenyl)-4-phenyl-2H-furan-5-one (1.5 mg/kg) and celecoxib (5 mg/kg) were administrated intragastrically in 4 hours after induction of inflammation with formalin. The ceruloplasmin level in the serum was investigated using the Ravin's method.

Results: The levels of serum ceruloplasmin under conditions of formalin edema was 3.11 ± 0.02 μmol/L, that is 2.5 times greater than in intact animals. It was shown that at the injection of 3-(4-methylsulfonylphenyl)-4-phenyl-2H-furan-5-one serum ceruloplasmin level demonstrated a statistically significant reduction in comparison with formalin edema. There is no statistically significant difference between groups. 3-(4-methylsulfonylphenyl)-4-phenyl-2H-furan-5-one affected the serum ceruloplasmin levels in rats under the conditions of formalin edema effectively. 2,3,5,6-tetradeuterio-4-[5-(4-methylphenyl)-3-(trifluoromethyl)pyrazol-1-yl]benzenesulfonamide had only tendency to decrease the inflammatory marker ceruloplasmin in serum of rats in reference to formalin edema.

Conclusions: Results of biochemical research of the effect of coxibs on the serum ceruloplasmin level in rats show that 3-(4-methylsulfonylphenyl)-4-phenyl-2H-furan-5-one has marked anti-inflammatory activity while 2,3,5,6-tetradeuterio-4-[5-(4-methylphenyl)-3-(trifluoromethyl)pyrazol-1-yl]benzenesulfonamide has only tendency to decrease the inflammatory marker ceruloplasmin in serum of rats.

KEY WORDS: 3-(4-methylsulfonylphenyl)-4-phenyl-2*H*-furan-5-one, 4-[5-(4-methylphenyl)-3-(trifluoromethyl)pyrazol-1-yl]benzenesulfonamide, 2-[(2,6-Dichlorophenyl) amino]benzeneacetic acid sodium salt, ceruloplasmin, acute inflammation

Wiad Lek. 2022;75(9 p1):2065-2069

INTRODUCTION

Inflammation is a process how your body fights infection, the damage of cells or presence of irritants. This process seeks to repair injured tissues and eliminate unwanted elements. Inflammation is a key element of most pathological processes and is accompanied by certain reactions: redness, pain, swelling, fever and dysfunction.

The first inflammatory response is characterized by the presence of large numbers of neutrophils at the site of injury, which serve to handle foreign materials and pathogens, utilizing their phagocytic, enzymatic, and respiratory functions [1, 2]. The research of molecular mechanism of interaction between the participants of inflammatory reactions could be useful for the development of techniques for diagnosing the severity of inflammation and for the assess the adequacy of anti-inflammatory treatment as well as for the design and development of new anti-inflammatory drugs.

It is known that positive acute-phase proteins increase in plasma concentration in response to inflammation. Concentration of some proteins, such as C-reactive protein and serum amyloid in the blood plasma increase over 300-fold during first days of inflammation reaction, reaching 0.03μM. One of the prevailing acute-phase proteins is a ceruloplasmin (CP) [3, 4].

It was found that the CP level in the blood serum changes significantly in various conditions including acute and chronic inflammatory processes accompanied by destructive and necrotic changes in tissues, malignancy, hereditary aceruloplasminemia, uremia, and other inflammatory and infectious diseases [4, 5]. Acute-phase protein CP has ability to normalize coagulation hemostasis and functional activity of platelets by improving secretory processes in platelets and increasing aggregation rate. This can be regarded as response on inflammation [6]. CP possesses significant oxidase activity towards Fe (II) and numerous aromatic amines and phenols. Its ferroxidase activity has led to the discovery that it is a molecular link between copper and iron metabolism. It activates oxidation of ascorbic acid, noradrenaline, serotonin and sulfhydryl

compounds. CP was shown to be a much more effective scavenger of peroxide radicals than superoxide dismutase, deferoxamine, and albumin, it prevents lipid peroxidation. Lack of ceruloplasmin causes of yield of copper ions into the extravascular space (serum copper level also decreases). Copper deficiency in the blood (due to the CP deficiency) results in the raise of resorption of copper ions in the intestine. Low CP might also mean you have: nephrotic syndrome, or kidney problems [7].

Coxibs (also known as COX-2 inhibitors) are a type of non-steroidal anti-inflammatory drug (NSAID). Coxibs, like other NSAIDs, relieve pain and reduce inflammation. The selective COX–2 inhibitors (coxibs) were originally developed to minimize the adverse effects of conventional NSAIDs while maintaining the same analgesic and anti–inflammatory properties. According the chemical structure these compounds are derivatives of 4-aminobenzenesulfonamide. This group of medications includes 2,3,5,6-tetradeuterio-4-[5-(4-methylphenyl)-3-(trifluoromethyl)pyrazol-1-yl]benzenesulfonamide, 3-(4-methylsulfonylphenyl)-4-phenyl-2H-furan-5-one, 4-(5-methyl-3-phenyl-1,2-oxazol-4-yl)benzene-1-sulfonamide, sodium;[4-(5-methyl-3-phenyl-1,2-oxazol-4-yl)phenyl] sulfonyl-propanoylazanide, 5-chloro-2-(6-methylpyridin-3-yl)-3-[4-(trideuteriomethylsulfonyl)phenyl]pyridine, 2-[2-(2-chloro-6-fluoroanilino)-5-methylphenyl] acetic acid [8, 9] and 4-[2-(4-ethoxyphenyl)-4-methylpyrrol-1-yl]benzenesulfonamide [8].

4-[4-(methylsulfonyl)phenyl]-3-phenyl-2(5H)-furanone) and 2,3,5,6-tetradeuterio-4-[5-(4-methylphenyl)-3-(trifluoromethyl)pyrazol-1-yl]benzenesulfonamide (4-[5-(4-methylphenyl)-3-(trifluoromethyl)-pyrazole-1-yl]benzenesulfonamide work by inhibiting the enzyme cyclo-oxygenase (COX), responsible for prostaglandin synthesis. 3-(4-methylsulfonylphenyl)-4-phenyl-2H-furan-5-one and 2,3,5,6-tetradeuterio-4-[5-(4-methylphenyl)-3-(trifluoromethyl) pyrazol-1-yl]benzenesulfonamide are selective COX-2 inhibitors that have originally been approved for the treatment of acute pain [9]. The two selective COX-2 inhibitors, 2,3,5,6-tetradeuterio-4-[5-(4-methylphenyl)-3-(trifluoromethyl)pyrazol-1-yl]benzenesulfonamide and 3-(4-methylsulfonylphenyl)-4-phenyl-2H-furan-5-one, are about 100–1000 times more selective on the COX-2 than on the COX-1 isoform. Today 3-(4-methylsulfonylphenyl)-4-phenyl-2H-furan-5-one is indicated for the symptoms and signs of osteoarthritis, whereas 2,3,5,6-tetradeuterio-4-[5-(4-methylphenyl)-3-(trifluoromethyl) pyrazol-1-yl]benzenesulfonamide is indicated for both osteoarthritis and rheumatoid arthritis. The major clinical interest of these drugs has been related to the lower incidence of gastrointestinal bleeding which, with the conventional COX-1/COX-2 agents has been a source of hospitalization, disablement and death. Compared to COX-1 active center of COX-2 is created by a long hydrophobic channel that is the site of non-steroidal anti-inflammatory drug binding. The isoleucine at position 523 (Iso 523) in COX-1 is replaced by a valine in COX-2. This substitution creates a side pocket of the active binding site of COX-2, not present in COX-1, and a somewhat wider binding site in COX-2. These differences between the COX active sites have major implications for the selectivity profile of inhibitors. All the coxibs have a rigid sidechain, such as a sulfone group or a sulfonamide group. Coxibs are limited in their binding capacity to COX-1 since the larger isoleucine (compared to valine in COX-2) at position 523 blocks access of the rigid sidechain of coxibs to the allosteric site [9].

THE AIM

The aim of this work was to estimate anti-inflammatory action of coxibs (3-(4-methylsulfonylphenyl)-4-phenyl-2H-furan-5-one, 2,3,5,6-tetradeuterio-4-[5-(4-methylphenyl)-3-(trifluoromethyl)pyrazol-1-yl]benzenesulfonamide) compared to reference drug – 2-[(2,6-Dichlorophenyl)amino]benzeneacetic acid sodium salt.

MATERIALS AND METHODS

Experimental biochemical studies were performed on male albino WAG rats weighing 280-300 g to compare anti-inflammatory activity of coxibs (3-(4-methylsulfonylphenyl)-4-phenyl-2H-furan-5-one, 2,3,5,6-tetradeuterio-4-[5-(4-methylphenyl)-3-(trifluoromethyl) pyrazol-1-yl]benzenesulfonamide) in contrast to reference drug – 2-[(2,6-Dichlorophenyl)amino]benzeneacetic acid sodium salt [10, 11].

Anti-inflammatory activity of investigated drugs was studied using the formalin-induced edema model. Animals were divided into 5 groups (6 animals per group). The inflammation was induced by injecting formalin in their paws. Group 1 animals (control subjects) were injected intragastrically with 3% starch mucilage (2 ml/200 g body weight); group 2 animals were injected subplantarly with 2% formalin solution and were injected intragastrically with 3% starch mucilage. Groups 3-5 animals were injected with investigated drugs subcutaneously: group 3 animals received 4-[4-methylsulfonyl)phenyl]-3-phenyl-2(5H)-furanone at a dose of 1.5 mg/kg (body weight), group 4 animals received 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-pyrazole-1-yl] benzenesulfonamide at a dose of 5 mg/kg (body weight), group 5 animals received reference drug – 2-[(2,6-Dichlorophenyl)amino]benzeneacetic acid sodium salt 8 mg/kg (body weight). Maximal inflammation was measured in 4 hours after formalin injection. The investigated medicinal products were given 1 h prior to formalin injection. The animals of all groups were decapitated under ether anesthesia [12, 13]. Rats were kept in the vivarium and treated according to high ethical standards and regulations of humane treatment of experimental animals. This study was carried out in accordance with European Convention for the protection of Vertebrate Animals used for Experimental and Scientific purposes [14] and in accordance with resolution of First National Congress on Bioethics [15].

Anti-inflammatory action of abovementioned medications was studied using the biochemical index of CP

Table I. The level of serum ceruloplasmin after injection of coxibs under the conditions of formalin edema in rats

S. №	Groups of animals (n = 6)	CP, μmol/L
1.	Control	1.13±0.04
2.	Formalin edema	3.11±0.02*
3.	3-(4-methylsulfonylphenyl)-4-phenyl-2H-furan-5-one + formalin edema	1.39±0.02**/****
4.	2,3,5,6-tetradeuterio-4-[5-(4-methylphenyl)-3-(trifluoromethyl)pyrazol-1-yl] benzenesulfonamide + formalin edema	2.51±0.01*/***/*****
5.	2-[(2,6-Dichlorophenyl)amino]benzeneacetic acid sodium salt + formalin edema	1.57±0.03**/****

Note 1. (mean ± error of mean) * - difference is relevant compared to control group, $p < 0.05$;
Note 2. (mean ± error of mean) ** - difference is relevant compared to formalin edema, $p < 0.05$;
Note 3. (mean ± error of mean) *** - difference is relevant compared to 3-(4-methylsulfonylphenyl)-4-phenyl-2H-furan-5-one $p < 0.05$;
Note 4. (mean ± error of mean) **** - difference is relevant compared to 2,3,5,6-tetradeuterio-4-[5-(4-methylphenyl)-3-(trifluoromethyl)pyrazol-1-yl] benzenesulfonamide, $p < 0.05$;
Note 5. (mean ± error of mean) ***** - difference is relevant compared to 2-[(2,6-Dichlorophenyl)amino]benzeneacetic acid sodium salt, $p < 0.05$.

because ceruloplasmin is an acute phase reactant. Ceruloplasmin is an α-globulin that means it is an acute-phase plasma protein produced by hepatocytes and activated macrophages, and has ferroxidase with bactericidal activities. It is known that serum ceruloplasmin concentrations are elevated by acute inflammation, stress, contagious and autoimmune diseases.

The level of CP was determined as per the Ravin's method using the test-kit "Determination of ceruloplasmin in the blood serum" (Private Joint Stock Company "Reagent") [16, 17].

At pH 5.5 ceruloplasmin catalyzes the oxidation of paraphenylenediamine (PPD) to yield a colored product [18]. Reaction mixture includes 1.58 mL of 0.5M sodium-acetate buffer (pH = 5.5), 0.2 mL of freshly prepared solution of dihydrochloride PPD (0.5%) and 0.02 mL of investigated sample, containing CP. The reaction mixture was then placed into water bath at 37°C for an hour. The reaction was stopped by the addition of 0.2 mL of 0.5% sodium aside solution. Reaction mixture with 0.2 mL of 0.5% sodium aside solution added before incubation was taken as blank sample. This method is used both for determination of PPD oxidase activity and for determination of CP concentration. The concentration of serum ceruloplasmin was determined spectrophotometrically by using spectrophotometer SF 46 with absorbance filter for 530 nm. The concentration of ceruloplasmin was calculated according to the following formula: $C_{CP} = A \times 5.83$, where 5.83 is a density factor (according to Ravin›s method) in μmol of active protein per 1 liter (μmol/L) [19, 20].

The obtained results were statistically processed using the standard software package of «*STATISTICA 6.0*». The validity of the test data was determined using Student›s t-test [21].

RESULTS

Experimental research was performed on laboratory animals to study anti-inflammatory properties of coxibs (4-[4-(methylsulfonyl)phenyl]-3-phenyl-2(5H)-furanone and 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-pyrazole-1-yl]benzene sulphonamide). Famous nonsteroidal anti-inflammatory drug – 2-[(2,6-Dichlorophenyl)amino] benzeneacetic acid sodium salt was chosen as reference drug. Investigation of coxibs' anti-inflammatory activity was carried out in laboratory rats under the conditions of formalin edema.

Results of biochemical research of the anti-inflammatory effect of coxibs on the level of ceruloplasmin in the rats' blood serum are given in the table I.

The levels of serum ceruloplasmin under conditions of formalin edema was 3.11 ± 0.02 μmol/L (group 2), that is 2.5 times greater than in intact animals (group 1). Biochemical research showed that investigated coxibs (group 3, 4) affected the level of serum ceruloplasmin and decreased it in reference to formalin edema (group 2).

As Table I shows that at the injection of 3-(4-methylsulfonylphenyl)-4-phenyl-2H-furan-5-one serum CP level demonstrated a statistically significant reduction in comparison with formalin edema. There is no statistically significant difference between groups. 3-(4-methylsulfonylphenyl)-4-phenyl-2H-furan-5-one affected the serum ceruloplasmin levels in rats under the conditions of formalin edema. When 2,3,5,6-tetradeuterio-4-[5-(4-methylphenyl)-3-(trifluoromethyl)pyrazol-1-yl]benzenesulfonamide was given under the conditions of formalin edema, decrease in serum CP level was insignificant. That's why 3-(4-methylsulfonylphenyl)-4-phenyl-2H-furan-5-one is considered to be a leader of our research.

After injection of 2,3,5,6-tetradeuterio-4-[5-(4-methylphenyl)-3-(trifluoromethyl)pyrazol-1-yl]benzenesulfonamide (group 4) serum ceruloplasmin level decreased by 1.2 times compared to formalin edema that is significantly inferior to the reference drug – 2-[(2,6-Dichlorophenyl) amino]benzeneacetic acid sodium salt.

DISCUSSION

Inflammation is one of the main body reactions, ensuring the vital activity of the organism. It is a body response

to injury and infection, and also body's way of signaling the immune system to heal and repair damaged tissue, as well as defend itself against foreign invaders, such as viruses and bacteria [22, 23]. The liver is a main organ which responds to tissue damage and inflammation. Hepatocytes synthetize proteins and supply them to the blood influencing the inflammation process. Tissue damage and inflammatory reaction is accompanied by quite dramatic changes in the plasma level of several proteins participating in the body response on damage. Appearance of these proteins in the blood and its increased level is an indicator of inflammation. That's why such proteins are called acute phase reactants [24-26].

One of the acute-phase reactants is a ceruloplasmin, and its level increases in cases of inflammation, infection, and trauma. Ceruloplasmin has both prooxidant and antioxidant properties and has been described as a "moonlighting protein" due to its many and varied activities. Acute phase reactant ceruloplasmin is able to decrease proaggregatory properties of thrombocytes that can be understood as feedback in the implementation of the inflammation process. Ceruloplasmin antioxidant function is mainly related to its ferroxidase activity; it activates oxidation of ascorbic acid, noradrenaline, serotonin and sulfhydryl compounds as well as inactivates reactive oxygen intermediates, preventing lipid peroxidation. Therefore, determination of ceruloplasmin level in blood plasma at the acute phase of inflammation is very relevant today.

Every physician face with problem of inflammation in his practice. It is necessary to understand development mechanism of inflammation and know properties of non-steroidal anti-inflammatory drugs (NSAIDs) in order to help patient to beat pain appearing due to inflammation. As usual, NSAIDs are used to stop inflammatory process. Inhibition of cyclooxygenase (COX) enzyme, which takes part in the biosynthesis of prostaglandins (PGs) and thromboxane (TX), is the mechanism of action of NSAIDs. The more is selectivity of NSAID for COX-2, the more is efficient its anti-inflammatory activity. [4, 26].

NSAIDs are typically divided into three groups based on their chemical structure and selectivity. First group includes non-selective NSAIDs (ibuprofen, diclofenac, indomethacin etc.), that inhibit COX-1 more than COX-2 and prevent the development of not only pathological but also physiological prostaglandins in tissues. This explains their side effects, such as, ulcerative lesion of GI tract, kidney failure. A relatively selective COX-2 inhibitors, such as meloxicam, belong to second group of NSAIDs. Meloxicam has been found to be at least as effective as other NSAIDs, but with a greatly reduced incidence of gastrointestinal side-effects. [27]. Third group is the highly selective cox-2 inhibitors (coxibs). The use of these drugs, relative to conventional NSAIDs, is associated to a significantly lesser gastroduodenal ulcer rate and to fewer clinically relevant complications, as well as to a smaller rate of treatment discontinuation due to gastrointestinal (GI) symptoms.

CONCLUSIONS

1. Results of biochemical research of the effect of coxibs on the serum CP level in rats show that 2,3,5,6-tetradeuterio-4-[5-(4-methylphenyl)-3-(trifluoromethyl) pyrazol-1-yl]benzenesulfonamide and 3-(4-methylsulfonylphenyl)-4-phenyl-2H-furan-5-one affect the level of ceruloplasmin but with varying degrees of activity.
2. 2,3,5,6-tetradeuterio-4-[5-(4-methylphenyl)-3-(trifluoromethyl)pyrazol-1-yl]benzenesulfonamide 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-pyrazole-1-yl]benzene sulphonamide) under the conditions of formalin edema has tendency to decrease the inflammatory marker CP in serum of rats in reference to formalin edema.
3. The leader of research is 3-(4-methylsulfonylphenyl)-4-phenyl-2H-furan-5-one (4-[4-(methylsulfonyl)phenyl]-3-phenyl-2(5H)-furanone, statistically significantly reduced the level of CP and was more effective than reference drug.

ABBREVIATIONS

CP – ceruloplasmin,
COX - cyclo-oxygenase,
NSAID – non-steroidal anti-inflammatory drug,
PPD – p-phenylenediamine

REFERENCES

1. Cassatella Marco A. Social networking of human neutrophils within the immune system. Blood. 2014;124(5):710-9. doi: 10.1182/blood-2014-03-453217.
2. Hazeldine J., Harris P., Chapple Iain L. et al. Impaired neutrophil extracellular trap formation: a novel defect in the innate immune system of aged individuals: Aging Cell. 2014;13:690-698. doi: 10.1111/acel.12222.
3. Aleshkin V.A., Novikova L.I., Motov A.G. et al. Belki ostroy fazyi i ih klinicheskoe znachenie [Acute phase proteins and their clinical significance]. Clinical medicine. 2001; 8(66):39-48 (in Russian).
4. Panasenko O. M., Chekanov A. V., Vlasova I. I. et al. Vliyaniye tseruloplazmina i laktoferrina na khloriruyushchuyu aktivnost' leykotsitarnoy miyeloperoksidazy. Izucheniye metodom khemilyuminestsentsii [A study of the effect of ceruloplasmin and lactoferrin on the chlorination activity of leukocytic myeloperoxidase using the chemiluminescence method]. Biophysics. 2018;53:573-581. (In Russian).
5. Vavilova T.P., Gusarova Yu.N., Koroleva O.V. et al. Rol tseruloplazmina pri razvitii neoplasticheskih protsessov [The role of ceruloplasmin in the development of neoplastic processes]. Biomed. chemistry. 2005; 51(3): 263-275 (in Russian).
6. Ermolaeva E. N., Krivokhizhina L.V., Kantyukov S.A. et al. Tseruloplazmin – endogennyy regulyator funktsional'nogo sostoyaniya trombotsitov [Ceruloplasmin – an endogenous regulator of the functional state of platelets]. Chelyabinsk: 1GBOU VPO "South Ural State Medical University" of the Ministry of Health of the Russian Federation, 2014; 7: 492-495 (in Russian).
7. Ang M.T.C., Gumbau-Brisa R., Allan D.S. et al. A 3-hydroxypyridin-4-one chelator iron-binding polymer with enhanced antimicrobial activity. Medchemcomm. 2018; 9(7):1206-1212. doi: 10.1039/c8md00192h.

8. Kirane A., Toombs E.J., Ostapoff K. et al. Apricoxib, a novel inhibitor of COX-2, markedly improves standard therapy response in molecularly defined models of pancreatic cancer. Clinical Cancer Research. 2012;18(18):5031-42. doi: 10.1158/1078-0432.
9. Ratsionalnost primeneniya rofekoksiba (denebol) pri travmah oporno-dvigatelnogo apparata [The rationality of the use of rofecoxib (denebol) for injuries of the musculoskeletal system] http://www.mif-ua.com/archive/article/27704. [date access 26.07.2021] (in Russian).
10. Medvedev V.V. Klinicheskaya laboratornaya diagnostika: spravochnik dlya vrachey [Clinical laboratory diagnostics: a reference book for doctors]. St. Petersburg: Hippocrates. 2006, 360p.
11. Lifshits V.M., Sidelnikova V. I. Biokhimicheskiye analizy v klinike: spravochnik [Biochemical analyzes in the clinic: a reference book] ed. 2nd. Moscow: MIA. 2001, 303p. (in Russian).
12. Rybolovlev Yu.R., Rybolovlev R.S. Dozirovaniye veshchestv dlya mlekopitayushchikh po konstantam biologicheskoy aktivnosti [Dosing of substances for mammals according to the constants of biological activity] Reports of the USSR Academy of Sciences. 1979; 6:1513-1516 (in Russian).
13. Stefanova O.V. Doklinichni doslidzhennya likars'kykh zasobiv : metod. rek. [Preclinical studies of drugs: a method. Rivers]. Kyiv. 2001, 527p. (in Ukrainian).
14. European convention for the protection of vertebrate animals used for experimental and other scientific purposes. Council of European. Strasbourg. 1986; 123: 51.
15. Suchasni problemy bioetyky [Modern problems of bioethics] resp. ed. Yu.I. Kundiev. Kyiv: Akademperiodika. 2009, 278p. (in Ukrainian).
16. Podilchak M.K. Klinicheskaya enzimologiya [Clinical enzymology]. Determination of ceruloplasmin in serum according to Ravin. Kiev. 2003, 87p. (in Russian).
17. Emelyanova O. I., Gontar I. P., Rusanova O. A. et al. Diagnosticheskoye znacheniye pokazateley tseruloplazmina pri sistemnoy sklerodermii [Diagnostic value of ceruloplasmin indices in systemic scleroderma] Medical Immunology. 2019;21(2):351-356 (in Russian).
18. Ravin H.A. An improved colorimetric enzymatic assay of ceruloplasmin. J Lab Clin Med. 1961; 58: 161-8.
19. Burtis C., Ashwood E., Bruns D. Tietz textbook of clinical chemistry and molecular diagnostics. Elsevir Inc. 2006, 2412p.
20. Martin F., Linden T., Katschinski D. M. et al. Copper-dependent activation of hypoxia-inducible factor (HIF)-1: implications for ceruloplasmin regulation. Blood. 2005;105(12):4613-4619.
21. Glantz S. Mediko-biologicheskaya statistika [Biomedical statistics]: trans. from English Moscow: Practice. 1998, 459p. (in Russian).
22. Sokolov A.V., Ageeva K.V., Kostevich V.A. et al. Vzaimodeystviye tseruloplazmina s serprotsidinami [Interaction of ceruloplasmin with serprocidins]. Biochemistry. 2018;75:1544-1552. (In Russian).
23. Gorudko I.V., Grigorieva D.V., Shamova E.V. et al. Hypohalous acidmodified human serum albumin induces neutrophil NADPH-oxidase activation, degranulation and shape change. Free Radic. Biol. Med. 2019;68:326-334.
24. Sokolov A.V., Ageeva K.V., Cherkalina O.S. et al. Identification and properties of complexes formed by myeloperoxidase with lipoproteins and ceruloplasmin. Chemistry and Physics of Lipids. 2017;163:347-355.
25. Sokolov A.V., Kostevich V.A., Gorbunov N.V. et al. Svyaz' mezhdu aktivnoy miyeloperoksidazoy i khlorirovannym tseruloplazminom v plazme krovi patsiyentov s serdechno-sosudistymi zabolevaniyami [A link between active myeloperoxidase and chlorinated ceruloplasmin in blood plasma of patients with cardiovascular diseases]. Medical Immunology. 2018; 20(5):699-710. doi:10.15789/1563-0625-2018-5-699-710. (In Russian).
26. Bunenkov N.S., Komok V.V., Sokolov A.V. et al. Novyye vozmozhnosti otsenki intraoperatsionnogo ishemicheski-reperfuzionnogo povrezhdeniya miokarda pri operatsiyakh revaskulyarizatsii v usloviyakh iskusstvennogo krovoobrashcheniya i na rabotayushchem serdtse [New methods of intraoperative evaluation of myocardial ischemic-reperfusion injury during on- and off-pump coronary artery bypass grafting]. Clinical and Experimental Surgery Petrovsky Journal. 2017;16(2);40-48. (In Russian).
27. Thorburn T., Aali M., Kostek L. et al. Anti-inflammatory effects of a novel iron chelator, DIBI, in experimental sepsis. Clin. Hemorheol. Microcirc. 2017;67(3-4):241-250. doi: 10.3233/CH-179205.

Initiative research work of the Department of Medical and Bioorganic Chemistry of Kharkiv National Medical University "Chemical-pharmaceutical substantiation of the creation of biologically active compounds, conjugates and drug compositions with anti-inflammatory and analgesic activities"

ORCID and contributionship
Ganna O. Syrova: 0000-0001-8849-9755 [A-F]
Olena V. Savelieva: 0000-0002-3115-9626 [A,C-F]
Tetyana S. Tishakova: 0000-0002-0257-7757 [C-F]
Larysa V. Lukianova : 0000-0001-6108-1711 [A,C-F]

Conflict of interest:
The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Larysa V. Lukianova
Kharkiv National Medical University,
4 Nauky Avenue, 61022 Kharkiv, Ukraine
e-mail: lv.lukianova@knmu.edu.ua

Received: 27.05.2021
Accepted: 04.05.2022

A - Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis, **D** – Writing the article, **E** – Critical review, **F** – Final approval of the article