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ORIGINAL ARTICLE

Test of antimicrobial activity of morpholine 2-(5-(3-fluorophenyl) -4-amino-1,2,4-triazol-3-ilthio) acetate (BKP-115) by experimental model of pancreatitis in rats

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The purpose of this research was to study the anti-inflammatory properties of morpholine 2-(5-(3-fluorophenyl) -4-amino-1,2,4-triazol-3-ylthio) acetate (BKP-115) in intramuscular and intragastric introduction on the model of experimental pancreatitis in rats. Determination of the anti-inflammatory activity of the substance morpholine 2- (5- (3-fluorophenyl)-4-amino-1,2,4-triazol-3-ylthio) acetate was performed on 68 male rats with a starting weight of 180-200 g, which were kept in standard vivarium conditions. Since the main chain of pathogenesis of AP is the activation and influence of proteolytic enzymes of the pancreas, the toxic-infectious model was chosen – the introduction of trypsin into the tissue of the pancreas. The introduction of trypsin in animals resulted in pancreatitis with endogenous intoxication, inflammation of the tissue of the pancreas and violation of the liver functional state. As a result, one animal died in the positive control group. Prophylactic i/g and i/v injection of the BKP-115 substance contributed to a significant reduction in the content of MAW (molecules of average weight) and CIM (circulating immune complexes) in animals from control and intact groups, indicated a decrease in the level of endogenous intoxication and detoxification properties of tested substance. The highest effectiveness of BKP-115 was registered after intragastric injection. Animals from this group had similar parameters with animals injected by "Contryven" and from intact control. Intramuscular injection of BKP-115 had therapeutic effect on experimental pancreatitis and endogenous intoxication, but it was less effective after intragastric injection and had weaker efficiency than "Contryven".

Key words: Experimental pancreatitis; Endogenous intoxication; 1,2,4-triazole derivatives

Introduction

In the comparative analysis of abdominal diseases, acute pancreatitis (AP) occupies a special place due to its nature, severity, certain features and continues to be a pressing problem in gastroenterology and surgery. According to various data, in Ukraine the incidence of AP is 5.2–6.7 cases per 10 thousand population. More than 55.5–70% of patients with AP are people of working age. About 75-90% of patients with AP have a fairly favorable clinical course, which responds well to conservative treatment. Whereas in 10–15%, and according to other authors in 25% of patients the disease has a severe and very severe course and requires comprehensive, long-term and costly treatment (Porchuk, 2006; Vasylev, 2011). The overall mortality in AP is 5–15%, in destructive forms – 15–75%, and in complicated destructive forms – 54–97% (Shlapak et al., 2008; Thandassery et al., 2013; Yang et al., 2014). Despite the significant progress made in recent decades, the problem of treating AP remains unresolved, and there are currently no specific treatments that can change the course of the disease. Unsatisfactory treatment results and high mortality in severe forms of AP are mainly due to the complexity of the mechanism of development of this disease.

AP is characterized by the ability to progress rapidly from mild to severe due to the formation of pro-inflammatory mediators and the development of systemic inflammatory response, activation of free radical oxidation and lipid peroxidation (LPO), microcirculation disorders (Papachristou et al., 2006; Pezzilli, 2009; Robles et al., 2013). It is considered that the systemic inflammatory response and oxidative stress cause further progressive damage to the parenchyma of the pancreas (PN), its necrosis and the development of multiple organ failure (MOF) (Yasuda et al., 2006). Increased levels of reactive oxygen species, activation of LPO are accompanied by depletion of stocks of endogenous antioxidants. These changes correlate with the severity of the patient's condition (Homeriki et al., 2004) and activity level of the glutathione-dependent enzyme system (Martinez et al., 2014). Pathomorphological processes occurring in AP both in the PN and surrounding tissues and in the organism as a whole, are accompanied by severe endotoxicosis, the development of multiple organ failure and purulent-necrotic complications at the appropriate stages of the process. Data of modern publications indicate that, along with a variety of causes, the pathogenetic

mechanisms of AP development are the same, in three phases (Ivanov et al., 2005; Zatevahin et al., 2007; Agapov & Gorskij, 2009):

1) Tissue damage by activated pancreatic enzymes;
2) Exudation with the development of aseptic parapancreatitis, which causes the severity of endogenous intoxication syndrome, multiple organ failure (MOF), immunodeficiency and the risk of developing infected pancreatic necrosis;
3) Actually, an inflammatory reaction.

According to modern ideas, the primary activator of the cascade of severe pathobiochemical reactions is trypsin, and the severity of pathological reactions is explained by the action of the integrated set of all enzyme systems of the pancreas (trypsin, chymotrypsin, lipase, phospholipase A, elastase, carboxypeptidase and carboxypeptidase, and collagenase) (Savel'ev et al., 2008). The prognosis in AP depends on both the extent of pancreatic lesions and the prevalence of purulent-necrotic process in the organ itself and surrounding tissues (Tolstoy, 2000). The important role of intensification of LPO processes and AOS insufficiency in the pathogenesis of AP development is proved, and causes the expediency of inclusion in the complex therapy of the disease preparations with pronounced antioxidant action, which are able to interrupt the cascade of free radical processes.
Today, a known fact is the high biological activity of 1,2,4-triazole derivatives, which causes increased attention to this heterocyclic system. Among the 1,2,4-triazole derivatives, a series of compounds owning antioxidant, hepatoprotective, immunomodulatory properties have been found (Parchenko, 2014), some of which are widely used in veterinary medicine (Knysh et al., 2020). Most derivatives of 1,2,4-triazole are low-toxic or practically non-toxic compounds. There is also known information about some 1,2,4-triazole derivatives that can be recommended for the treatment and prevention of pancreatitis (Knysh et al., 2020). The results of previous researches indicate that morpholine 2-(5-(3-fluorophenyl)-4-amino-1,2,4-triazol-3-ylthio) acetate (BKP-115) is a low-toxic compound, which according to the results of computer forecasting reveals antioxidant, anti-inflammatory properties.
Thus, the purpose of this research was to study the anti-inflammatory properties of morpholine 2-(5-(3-fluorophenyl)-4-amino-1,2,4-triazol-3-ylthio) acetate (BKP-115) in intramuscular and intragastric introduction on the model of experimental pancreatitis in rats.

Materials and Methods

All presented researches were carried out according to national "General ethical principles of experiments on animals" (Ukraine, 2001), that is coordinated with the consistent of the "European Convention for the protection of vertebrate animals used for experiments and other scientific purposes" (Strasbourg, France, 1985), as well as in accordance with the directive of the EU Council.
Determination of the anti-inflammatory activity of the substance morpholine 2-(5-(3-fluorophenyl)-4-amino-1,2,4-triazol-3-ylthio) acetate was performed on 68 male rats with a starting weight of 180–200 g, which were kept in standard vivarium conditions. Since the main chain of pathogenesis of AP is the activation and influence of proteolytic enzymes of the pancreas, the toxic-infectious model was chosen – the introduction of trypsin into the tissue of the pancreas, which is well worked out and has 100% reproducibility. The enzyme trypsin is a serine proteinase with esteroprotease properties. At local introduction in PN trypsin activates not only all zymogenic enzymes PN (elastase, carboxypeptidase, chymotrypsin, etc.), but also lysosomal enzymes and proteinases, which contributes to proteolytic violation of pancreatic tissue and leads to the development of hemorrhagic pancreatic necrosis.
In addition, trypsin activates the starting reactions of proteolytic cascade blood systems. In general, the introduction of trypsin causes the development of irreversible hemodynamic shock, hemorrhagic pancreatic necrosis, the formation of the syndrome of DIC blood and MOF with a possible fatal outcome. Test samples (TS) and reference sample (RS) were induced in preventive mode for 7 days before modeling pancreatitis.
Animals were anesthetized with sodium thiopental at a dose of 40 mg/kg intraperitoneally. The abdominal cavity was dissected and in PN along the common pancreatobiliary duct from its entry in the duodenum trypsin solution at a dose of 50 mg/kg was injected (Chan & Leung, 2007). The wound was sutured in layers. Investigated TS and RS were injected for 5 days. On the 6th day, the animals were degassed, serum was received, and the liver and pancreas were removed, and samples were subjected to morphological examination. The liver was weighed, calculated the relative weight of the organ (mass coefficient, MC) according to formula (1), expressed as a percentage:

$$MC_{organ} = \frac{m_{organ}}{M_{animals}} \times 100\%$$

Evaluation of the pharmacotherapeutic action of substances was performed on survival, relative liver weight, biochemical indicators, which were determined in serum and liver homogenates. The activity of aspartate and alanine transaminases, alkaline phosphatase, circulating immune complexes in the serum (CIM, Instructions for the assortment for the determination of CIM, "Granum" Company, Kharkiv), molecules of average weight (MAW), total and direct bilirubin (by the Endrashyk method) by means of diagnostic sets "Philisit", "Granum", "Pliva-Lachema" was determined. The pro/antioxidant status of animals and the influence of substances on it were assessed by the main indicators in the serum and biological substrate (liver tissue): defined the content of secondary products LPO – TBA-active products (TBA-R – products that react with thiobarbituric acid (TBA), the activity of superoxide dismutase (SOD) and catalase (CAT). In liver tissues – the content of TBA-R, reduced glutathione (VG) and the activity of SOD and CAT. The content of TBA-R was determined by colorimetric method, which is based on the ability of the sum of these compounds to form in an acidic environment colored trimethine complexes with TBA, which have a maximum absorption at a wavelength of 532 nm].
The determination of RG content was performed on the ability of low molecular weight thiol compounds to form during interaction with 5,5'-dithio-bis-2-nitrobenzoate colored compound – thio-2-nitrobenzoic acid, an aqueous solution which has a characteristic maximum absorption at a wavelength of 412 nm. CAT activity was determined by decreasing the hydrogen peroxide content in the incubation medium. The method for determining the activity of CAT is based on the ability of hydrogen peroxide to form stable colored extracts with ammonium salts, which have a maximum absorption at a wavelength of 410 nm. SOD was determined spectrophotometrically by the value of optical density at a wavelength of 347 nm for 3 min by the reaction of auto-oxidation of adrenaline in an alkaline environment. SOD activity was evaluated by the degree of inhibition of adrenaline autooxidation in experimental and control samples and expressed in %. Calculated by the formula:

$$SOD\ \% = (1- D_{experiment} / D_{control}) \times 100\%, \quad (1)$$

where $D_{experiment}$ and $D_{control}$ – the reaction rate of the autooxidation of adrenaline in the presence of experimental serum ($D_{experiment}$) or in its absence ($D_{control}$), in extinction units in 1 minute.

Immediately after the introduction of TS daily recorded possible manifestations of intoxication were: impaired motor activity, respiration, the presence and severity of seizures, ophthalmic, cardiovascular symptoms, salivation, piloerection. It was observed muscle tone, the state of excrement. The animals were allowed to eat in 2-3 hours, access to water was free. In case of death, the animals were dissected and a macroscopic examination of the internal organs was performed in order to exclude a manipulation error and establish the probable cause of death. The general physiological condition of the animals was assessed by the dynamics of body weight, which was recorded at the beginning of the experiment (before the introduction of TS), on 3, 7 and 14 days after the introduction of TS.

At the end of the observation period, the animals were subjected to autopsy and macroscopic examination – external examination, examination of the internal organs of the thoracic cavity (heart, thymus, lungs) and abdominal cavity (liver, spleen, testes), as well as kidneys and adrenal glands. Internal organs were weighed and the relative weight (MC,%) was calculated according to equation 1.

Results and Discussion

According to the received data, the introduction of trypsin led to an increase in the level of medium weight molecules (MAW), circulating immune complexes (CEC) and alkaline phosphatase (AP) activity (Table 1). At the same, a significant activation of LPO processes and a decrease in the power of antioxidant protection were registered. The level of TBA-R increased almost 3 times and the activity of SOD, but not catalase, decreased in rats on the background of pancreatitis (Table 2).

Table 1. The influence of morpholine 2- (5- (3-fluorophenyl) -4-amino-1,2,4-triazol-3-ylthio) acetate (BKP-115) and "Contryven" on biochemical markers of inflammation in serum blood of rats under experimental pancreatitis.

Indicators	Groups of animals					
	Intact Control	**Negative control (pseudooperated animals)**	**Positive control (trypsin)**	**BKP-115, 6 mg/kg (i/m) + trypsin**	**BKP-115, 6 mg/kg (in/g) + trypsin**	**"Contryven", 28330 Un/kg + trypsin**
MAW, (LSD criterion)	0.047 ± 0.003	0.051 ± 0.004	0.058 ± 0.003 p1=0.0548 p3=0.0000	0.056 ± 0.003 p4=0.0000	0.035 ± 0.002 p1=0.0368 p1/p0=0.00068 p2=0.0000 p3=0.0047	0.023 ± 0.003 p1=0.0001 p1/p0=0.0001 p2=0.0000
CIM, (LSD criterion)	0.079 ± 0.011	0.070 ± 0.005	0.116 ± 0.007 p1=0.0012 p1/p0=0.0006	0.088 ± 0.006 p2=0.0006 p3=0.0005 p4=0.0019	0.061 ± 0.004 p1=0.0436 p2=0.0000	0.054 ± 0.004 p1=0.0135 p2=0.0000
AP, μkat/l) (Mann-Whitney criterion)	5.61 ± 0.32	7.02 ± 0.54 p1=0.0649	6.42 ± 0.21 p1=0.0575	6.51 ± 0.32 p1=0.0187 p3=0.0086	6.17 ± 0.42	5.81 ± 0.20 p1/p0=0.0239

Here and then, intergroup comparisons were performed using *LSD* or *Mann-Whitney* criteria, $p<0.05$, p_1/p_{no} – the level of statistical significance relative to intact and negative controls, p_2 – the level of statistical significance relative to positive control, p_3 – the level of statistical significance for the "Contriven", p_4 – the level of statistical significance relative to the substance BKP-115 (in/g).

When injected into the pancreas of rats, trypsin activates PN enzymes, lysosomal enzymes and proteinases, trigger reactions of proteolytic cascade blood systems, which leads to disruption of the PN tissue and the development of hemorrhagic pancreatic necrosis. Chymotrypsin and elastase, like trypsin, cause edema, hemorrhage, and limited necrosis when isolated. At the same time, lipolytic enzymes cause intense necrosis PN and damage to surrounding tissues. Phospholipase A2 with its inherent pronounced cytolytic properties damages cell membranes, releasing lysolecithin, which has a cytotoxic action.

Activation of elastase leads to the destruction of the elastic components of blood vessels, contributing to the development of intrapancreatic hemorrhage.

This produces intermediate decay products of cell membrane structures and the intercellular matrix, which are toxic substrates. These components of cellular disorganization, incomplete decay of highly molecular protein compounds and are molecules of medium mass. The accumulation of MAW is a marker of endogenous intoxication. In the future, they increase the course of the pathological process, acquiring the role of secondary toxins, affecting the vital functions of all systems and organs. Despite the large number of works, the final pool of MAW has not been identified, however, it is well set up, that the composition of MAW includes components of peptide nature, as well as derivatives of oligoalcohols and glucuronic acid. Today, nonspecific signs of endotoxicosis are also considered to be an increase in the serum content of LPO products. It has been shown that the decay products of lipids (aldehydes, dialdehydes, epoxides) damage proteins, nucleic acids and various cell structures, and therefore are endopathogenic. Peroxide damage of protein substances leads to their degradation and formation of toxic fragments, including MAW and CIM (Martyshuk et al., 2016; Khariv et al., 2016; 2017; Gutyj et al., 2017; 2018; 2019; Brygadyrenko et al., 2019).

In animals of positive control, a significant increase in serum bilirubin was observed. The increase in total bilirubin is due to an increase in its indirect, toxic fraction, and direct (Table 2).

However, the most definitely increased direct bilirubin content, which indicates the deterioration of hepatocyte function due to the inflammatory process caused by the development of pancreatitis. It is known that lesions of the pancreas ultimately leads to dysfunction of the liver and other organs. During inflammatory processes in the liver, hepatocytes are shrunk, wide gaps are formed between them, and it enters the bloodstream through the bile. This movement of bile is facilitated by increased pressure in the bile

ducts, due to connective tissue edema and / or due to obstruction of the bile ducts and due to hemodynamic disorders that develop during endogenous intoxication. The above-indicated dynamics of bilirubin content is closely correlated with the results of liver transaminases. In our experiment, a statistically significant decrease in AlAt activity was observed, which also indicates a violation of intracellular hepatocyte metabolism. The activity of AsAt decreased tendentiously, but these changes had no statistical significance relative to the values of intact animals (Table 3).

Table 2. The influence of morpholine 2- (5- (3-fluorophenyl) -4-amino-1,2,4-triazol-3-ylthio) acetate (BKP-115) and "Contriven" on the indicators of LPO/AOS in the serum of rats under experimental pancreatitis.

Indicators	Groups of animals					
	Intact control	**Negative control (pseudooperated animals)**	**Positive control (trypsin)**	**BKP-115, 6 mg/kg (i/m) + trypsin**	**BKP-115, 6 mg/kg (in/g) + trypsin**	**"Contryven", 28330 Un/kg + trypsin**
TBA-R, μmol/l (Mann-Whitney criterion)	0.23 ± 0.01	0.69 ± 0.04 p1=0.0019	0.62 ± 0.02 p1=0.0001	1.04 ± 0.05 p1=0.0001 p1/p0=0.0089 p2=0.0063 p3=0.0484	1.03 ± 0.04 p1=0,0001 p1/p0=0.0010 p2=0.0076 p3=0.0255	1.19 ± 0.06 p1=0.0001 p1/p0=0.0018
SOD,% (Mann-Whitney criterion)	24.47 ± 0.42	24.27 ± 0.52	18.55 ± 0.51 p1=0.0000 p1/p0=0.0001	22.68 ± 0.47 p2=0.0000 p4=0.0004	25.58 ± 0.61 p2=0.0000	23.55 ± 0.74 p2=0.0000
Catalase, μmol/ (min * l) (LSD criterion)	17.71 ± 1.05	19.15 ± 1.04	19.44 ± 1.13	18.77 ± 0.76	17.39 ± 0.68	17.47 ± 0.86
K (LPO/AOS)	1.0	1.0	1.5	1.3	1.4	1.6

Thus, as a result of trypsin injection, pancreatitis is developing in animals, which is characterized by endogenous intoxication, the development of inflammation and is accompanied by violation of the functional state of the liver. As a result, one animal died in the positive control group (Table 4).
Prophylactic injection of the substance BKP-115 contributed to a statistically significant reduction in the content of MAW, CIM not only in comparison with the positive control, but also with the values of intact animals (Table 1), indicating the detoxifying properties of the test sample. The substance was most effective when introduction intragastrically. The values of indicators in animals of this group were at the level of animals injected the comparison preparation known inhibitor of proteolytic enzymes "Contryven" (Table 1). It should be noted that with the use of the test compound there was a statistically significant increase in serum levels of TBA-R, which was accompanied by a slight, except for a group of animals, which were injected the substance BKP-115 intragastrically, an increase in the activity of SOD (Table 2). During intravenous injection, the activity of SOD was increased to the level of intact animals and was statistically significantly higher than in the CC group (Table 2). However, the activity of AP in all investigated groups remained at the level of CC (Table 1).

The decrease in the level of endogenous intoxication with the use of the investigated means had a positive influence on the functional state of the pancreas and liver (Table 3). The content of all fractions of bilirubin decreased statistically significantly compared with the positive control, but the greatest effectiveness against this indicator was found against the background of intragastric injection of the substance BKP-115, which was not inferior in expressiveness to the preparation comparison "Contryven". During intravenous injection of the substance BKP-115 the content of total and indirect bilirubin remained at the level of values of animals from the group of CC, indicating a lower effectiveness of the means than when injected intravenously and the preparation comparison "Contryven" (Table 3). With the use of the substance BKP-115 both intravenously and i/g, but not the preparation comparison "Contryven", the activity of AlAt was also reduced compared with intact control, but the activity of AsAt remained at the physiological level (Table 3).

The determination of the relative weight of the liver showed no significant changes in the condition of pancreatitis, which is probably due to the short duration of the research (only 6 days, Table 3). However, imbalances were observed in the LPO/AOS system: in liver homogenates statistically significantly increased the content of TBA-R and decreased the activity of enzyme antioxidants – SOD and catalase (Table 5). It should be noted a vague but statistically significant increase in the pool of RG, which can be explained by the compensatory activation of antioxidants synthesized in the liver (Table 5).

Against the background of intragastric introduction of the substance BKP-115 and the comparison preparation, the relative weight of the liver also did not change. However, with the use of intramuscular injection of the substance BKP-115 a significant increase in relative mass was observed, which may indicate the functional stress of this organ (Table 5). A significantly smaller decrease in total and indirect bilirubin than in the groups of animals which introduced the substance BKP-115 intragastrically and Contryven is in favor of this assumption (Table 2). The restoration of the functional state of the liver is also evidenced by the preservation of the balance of pro/antioxidant processes: in the liver homogenates of the animals to which the test substance was injected, the content of secondary products of LPO was decreasing. However, the dynamics of antioxidant enzymes was not unambiguous. By intramuscular injection, the activity of both SOD and catalase was reduced and did not differ from the values of the positive control, but with intragastric injection, the activity of SOD approached the level of intact animals (Table 5). However, catalase activity

remained reduced. With the use of the comparison preparation "Contryven" there was an imbalance in pro/antioxidant processes – increased level of TBA-R were accompanied by a decrease in the activity of SOD and catalase (Table 4). It should be noted that in all research groups the content of reduced glutathione was higher than the value of intact animals. The increase in the RG pool in animals to which the test substance and the reference preparation were injected in the treatment-and-prophylactic regimen is probably explained by the activation of the enzyme pentose phosphate cycle – glucose-6-phosphate dehydrogenase, whose main

function is the reduction of NADP to NADPR, necessary for the transition of glutathione from the oxidized form to the reduced and the synthesis of macroenergetic compounds in liver tissue. Thus, the introduction of trypsin into the pancreas caused tissue damage, which led to the development of endogenous intoxication and inflammation, liver dysfunction and imbalance in the LPO/AOS system.

Table 3. The influence of morpholine 2-(5-(3-fluorophenyl) -4-amino-1,2,4-triazol-3-ylthio) acetate (BKP-15) and the "Contryven" on the biochemical indicators in serum blood of rats under of experimental pancreatitis.

Indicators	**Groups**					
	Intact control	**Negative control (pseudooperated animals)**	**Positive control (trypsin)**	**BKP-115, 6 mg/kg (i/m) + trypsin**	**BKP-115, 6 mg/kg (in/g) + trypsin**	**"Contryven", 28330 Un./kg + trypsin**
Total bilirubin, μmol/l (Mann-Whitney criterion)	3.07 ± 0.42	4.44 ± 0.41 p1=0.0417	7.66 ± 0.62 p1=0.0001 p1/p0=0.0006	4.55 ± 0.31 p1=0.0179 p2=0.0000 p3=0.0000 p4=0.0052	2.62 ± 0.51 p1по=0.0263 p2=0.0000	1.81 ± 0.19 p1=0.0092 p1/p0=0.0001 p2=0.0000
Direct bilirubin, μmol/l (Mann-Whitney criterion)	0.63 ± 0.11	1.07 ± 0.13 p1=0.0652	2.88 ± 0.13 p1=0.0001 p1/p0=0.0001	0,77 ± 0.06 P2=0.0000 p4=0.0155	1.78 ± 0.33 p1=0.0162 p2=0.0049 p3=0.0317	0.91 ± 0.09 p1=0.1805 p2=0.0001
Indirect bilirubin, μmol/l (Mann-Whitney criterion)	2.38 ± 0.43	3.35 ± 0.41	4.55 ± 0.51 p1=0.0218	3.65 ± 0.28 p1=0.0421 p3=0.0000 p4=0.0000	0.88 ± 0.25 p1=0.0067 p1/p0=0.0005 p2=0.0000	0.94 ± 0.17 p1=0.0071 p1/p0=0.0005 p2=0.0000
AlAt, μkat/l (LSD criterion)	0.49 ± 0.03	0.44 ± 0.03 p1=0.0111	0.45 ± 0.03 p1=0.0079	0.45 ± 0.02 p1=0.0115	0.43 ± 0.02 p1=0.0007 p3=0.0094	0.49 ± 0.03
AsAt mkkat/l (Mann-Whitney criterion)	0.77 ± 0.03	0.82 ± 0.03	0.77 ± 0.02 p1/p0=0.0127	0.75 ± 0.02	0.73 ± 0.02	0.74 ± 0.03
MC of the liver,% (Mann-Whitney criterion)	3.72 ± 0.13	3.49 ± 0.11	3.85 ± 0.12	4.22 ± 0.13 p1=0.0411 p2=0.0452 p3=0.0054 p4=0.0022	3.51 ± 0.07 p2=0.0238	3.66 ± 0.07 p2=0.0289

Table 4. The influence of morpholinium 2-(5-(3-fluorophenyl)-4-amino-1,2,4-triazol-3-ylthio) acetate (BKP-115) and "Contryven" on rat mortality

Groups	**Mortality (death/number of animals per group)**
Intact control	0/6
Negative control (pseudooperated animals)	0/6
Positive control (trypsin)	1/14
BKP-115, 6 mg/kg (i/m) + trypsin	1/14
BKP-115, 6 mg/kg (i/g) + trypsin	0/14
"Contryven", 28330 Un./kg (v/o) + trypsin	2/14

Table 5. The influence of the substance morpholine 2-(5-(3-fluorophenyl)-4-amino-1,2,4-triazol-3-ylthio) acetate BKP-115 and the comparison preparation "Contryven" on the biochemical indicators in the liver homogenate of rats under the condition of experimental pancreatitis.

Indicators	Groups of animals					
	Intact control	Negative control (pseudooperated animals)	Positive control (trypsin)	BKP-115, 6 mg/kg (i/m) + trypsin	BKP-115, 6 mg / kg (i/g) + trypsin	"Contryven", 28330 Un/kg + trypsin
TBA-R, μmol/g (LSD criterion)	56.92 ± 3.27	64.65 ± 2.92	88.94 ± 3.05 p1=0.0000 p1/p0=0.0001	57,55 ± 2,27 p2=0.0000 p3=0.0008	63.62 ± 4.79 p2=0.0000 p3=0.0108	80.75 ± 3.44 p1=0.0001 p1/p0=0.0112
SOD,% (LSD criterion)	25.95 ± 2.46	29.33 ± 3.57	20.75 ± 2.25 p1/p0=0.0342	19.12 ± 3.07 p1=0,.815 p1/p0=0.0128 p2=0.0579	26.68 ± 1.58	22.11 ± 2.56 p1/p0=0.0735
Catalase, mmol/l (min * l) (Mann-Whitney criterion)	2.77 ± 0.02	1.88 ± 0.12 p1=0.0652	1.86 ± 0.07 p1=0.0016	1.69 ± 0.06 p1=0.0002 p1/p0=0.0612 p3=0.0043 p4=0.0036	1.92 ± 0.03 P1=0.0002	1.86 ± 0.02 p1=0.0001
RG, μmol/g (LSD criterion)	2.07 ± 0.08	2.28 ± 0.13 p1=0.0579	2.49 ± 0.07 p1=0.0001 p1/p0=0.0557	2.63 ± 0.06 p1=0.0000 p1/p0=0.0062	2.59 ± 0.06 p1=0.0000 p1по=0.0117	2.48 ± 0.02 p1=0.0033

Prophylactic and therapeutic injection of the substance BKP-115 helped to reduce the level of endogenous intoxication, inflammation and dysfunction of the pancreas and liver. The antioxidant properties of BKP-115 substance have been established. In general, the effectiveness of the test substance when injected intravenously was not inferior to the comparison preparation "Contryven", and the ability to inhibit excess lipoperoxidation processes outweighed it. Substance BKP-115 when administered intravenously was significantly inferior to both intravenous injected and the comparison preparation "Contryven".

Conclusion

The introduction of trypsin in animals resulted in pancreatitis, characterized by endogenous intoxication, inflammation of pancreas tissue and violation of liver functional state. One animal died in positive control group. Prophylactic i/g and i/v injection of BKP-115 contributed to a significant reduction in the content of MAW and CIM in rats from positive control and intact group, resulted in decrease of the level of endogenous intoxication and detoxification properties of the tested substance.

The highest effectiveness of BKP-115 was registered after intragastric injection. Animals from this group had similar parameters with animals injected by "Contryven" and intact animals. Intramuscular injection of BKP-115 had therapeutic effect on experimental pancreatitis and endogenous intoxication, but was less effective after intragastric injection and had weaker efficiency than "Contryven".

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