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Original article

Intermediate products of purine metabolism in an experimental model of pancreatic necrosis

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Abstract. *Background and aim:* Determine the level of purines in the blood plasma of experimental animals at three stages of induced pancreatic necrosis. Find out the potential of purines as predictors of the severity of pancreatitis. *Methods:* The experiment was carried out on white outbred rabbits. The pancreatic necrosis was modeled by introducing self-bile into the pancreatic parenchyma. The pancreas of rabbits, after isolation, was subjected to microscopic description. Blood was also taken from rabbits to determine the plasma levels of adenine, guanine, hypoxanthine, xanthine, and uric acid. *Results:* 12 hours after the administration of self-bile, the level of xanthine significantly increases and the concentration of uric acid in the blood plasma increases by 3 times. 24 hours after the introduction of self-bile, there is a slight decrease in the level of adenine, xanthine and uric acid, and the indicators of purine metabolism remain elevated. 48 hours after the introduction of self-bile, the levels of guanine, hypoxanthine and xanthine are reduced. *Conclusions:* The concentration indices of absolute and relative intermediate products of purine metabolism were increased at the initial stage of pancreatic necrosis. The activity of enzymes and metabolites of purine metabolism involved in the formation of reactive oxygen species and free radicals increased. The hypothesis that intermediate products of purine metabolism can be predictors of pancreatic necrosis was confirmed.

Key words: purines, purine metabolism intermediates, predictors of necrosis, pancreas, pancreatitis, pancreatic necrosis

Introduction

Acute pancreatitis is an acute inflammation of the pancreas that manifests itself as nausea, vomiting, abdominal pain, and fever (1). The incidence of acute pancreatitis continues to raise worldwide causing significant medical and social burdens (2, 3, 4, 5). The classification of acute pancreatitis was reconsidered in 2012. Acute pancreatitis has been divided into interstitial edematous pancreatitis (IEP) and necrotizing pancreatitis (NP) (6). The diagnosis of acute pancreatitis requires at least two of the following three criteria: 1. abdominal pain associated with the disease 2. biochemical signs of pancreatitis (serum amylase and / or lipase levels more than three times the upper limit of normal), and 3. characteristic abdominal imaging results (7). According to the WSES 2019 Guidelines for the Management of Severe Acute Pancreatitis (8), the following diagnostic laboratory parameters should be performed: Determine the level of amylase and lipase (9), the C-reactive protein level ≥ 150 mg / L can be used as a prognostic factor for severe acute pancreatitis

on the third day (10). Hematocrit> 44% is an independent risk factor for pancreatic necrosis. A low level of hematocrit indicates a low risk of pancreatic necrosis (PNec) among the patients with acute pancreatitis (11). Urea> 20 mg / dL acts as an independent predictor of mortality (12). Organ failure in acute pancreatitis can be predicted with high accuracy at the hospital using a combination of plasma interleukin 10 and serum calcium measurements (13). A lot of studies based on understanding the process of development of acute pancreatitis and its transition to the stage of pancreatic necrosis were conducted (PNec). According to Lipinski M. (2013), an increase in creatinine during the first 48 hours is closely associated with the development of necrotizing pancreatitis (14). In the studies of Rainio, the levels of biomarkers (trypsinogen 1, trypsinogen 2 and trypsinogen 3, trypsin 2 complex and α 1 -antitrypsin) and creatinine in plasma correlated with the severity of acute pancreatitis and the development of acute pancreatitis. Among the patients without acute pancreatitis on the admission to the hospital, SPINK1 (Serum serine peptidase inhibitor Kazal-Type 1) was an independent marker of later development of severe acute pancreatitis (15). The results of Shu W. team work (2020) show that an initially increased level of lactate in arterial blood is independently associated with adverse outcomes and death among patients with severe acute pancreatitis and may serve as an early indicator of high risk stratification (16). A decrease in high-density lipoprotein cholesterol in studies by Zhang Y. (2017) also proved to be an independent predictor of persistent organ failure, pancreatic necrosis and mortality in acute pancreatitis (17). The study of serum glycoprotein 2 as a marker for predicting the severity of acute pancreatitis has shown that serum GP2 levels are increased among patients with acute pancreatitis. This correlates positively with the severity of acute pancreatitis, suggesting its potential for predicting the severity of the disease (18). However, these indicators are inadequate; consequently, numerous biomarkers are still being studied as potential early predictors of the severity of acute pancreatitis. Currently, there are practically no laboratory tests to predict the severity of AP (19). Early identifying of patients at risk of acute pancreatitis is an important step in managing treatment and improving outcomes. Purines may be one of the potential biomarkers of acute pancreatitis. Purines as guanine, hypoxanthine, adenine, xanthine and uric acid are the subsequent metabolites of ATP and GTP. The intracellular pool of ATP breaks down into adenosine and subsequent metabolites in tissue that is under metabolic stress, for example, during hypoxia or ischemia. These purines are released from cells via transporters and, thus, are very early markers of metabolic stress that occurs before cell death (20, 21). Purine levels are studied in the process of searching for diagnostic and prognostic biochemical parameters in the diagnosis of neonatal hypoxic-ischemic encephalopathy (22), in ischemic tissues of bottlenose dolphins during diving (23), in studies of the effect of antiviral drugs on the mucous membrane in case of pneumonia (24). Serum purine Nucleoside Phosphorylase (NP) metabolites can determine pancreatic adenocarcinoma (25). Guanine-based purines play a key role in cell metabolism and in several models of neurodegenerative disorders such as Parkinson's and Alzheimer's disease (26).

The objective of this study is to determine the level of purines in the blood plasma of experimental animals at three stages of induced pancreatic necrosis and to establish the potential of purines as predictors for predicting the severity of pancreatitis.

Materials and methods

Animals

The experiment was carried out on 60 outbred rabbits of both sexes, weighing averagely 2.5 kg. The animals were taken out of the experiment by euthanasia under anesthesia (exsanguination). To reproduce the model of pancreatic necrosis intravenous anesthesia with solutions of xylazine 0.2 mg / kg + ketamine 0.5 mg / kg was used, the abdominal cavity was opened. Then bile was taken from the gallbladder with an insulin syringe. The resulting self-bile was injected in amount of 0.1 ml into the right and left lobes of the pancreas (27).

Withdrawal from the experiment was carried out in terms of 12 (n = 6) - group 1, 24 hours (n = 6) - group 2 and 48 (n = 6) hours - group 3, after the injection of

self-bile with material sampling for histological and biochemical research. Also included in the experiment was a group of sham-operated animals that underwent laparotomy followed by suturing of the abdominal cavity without additional manipulations. Blood sampling from rabbits was carried out 12 hours after the sham operation (n=6), 24 hours after the sham operation (n=6), and 48 hours after the sham operation (n=6). All results were compared with the results of the intact group of animals (n = 6).

The study was carried out in accordance with the requirements of the European Convention for the Protection of Vertebrate Animals used for Experiments and Other Scientific Purposes (Strasbourg, 1986), GLP OECD requirements, EAEU Good Laboratory Practice No. 81, Order No. 392 of the Ministry of Health and Social Development of the Republic of Kazakhstan dated May 25, 2015. The study was approved by the decision of the Bioethics Committee of the Karaganda Medical University on June 17, 2019, protocol No. 20.

Biochemical research

Catabolites of purine metabolism in blood plasma were determined by direct spectrophotometry according to the method of Oreshnikov et al. (28) on the basis of the Biomedicine Department of the Medical University of Karaganda. The resulting samples were pretreated according to the recommended procedures and subjected to spectrophotometric analysis using a PD-303UV digital UV-VIS spectrophotometer, manufactured by APEL, Japan.

0.3 ml of blood plasma was put to a standard glass tube. Then thermocoagulation was performed in a boiling water bath for 5 min. The most important condition to avoid defragmentation of the coagulant was active boiling. After cooling at room temperature for several minutes 3 ml of bidistilled water was poured into the test tube. After 30 min of incubation at 37 °C, the extinction of the extract was measured against a pure extractant (bidistilled water) in a cuvette with an optical path 10 mm length. In this case, it was assumed that the extinctions at waves of 246, 250, 261, 276, and 293 nm respectively reflect the concentration of guanine, hypoxanthine, adenine, xanthine and uric acid in plasma, blood and erythrocytes. The concentration of purine bases was expressed in extinction units (ext. Units), UA - in μmol / L.

$$C = E \times 1000$$

C - concentration of purine metabolites, ext. (μmol / l); 1000 - conversion of extinction to concentration coefficient. The activity of the xanthine oxidase enzyme was assessed by calculating the indices xanthine / hypoxanthine (stage 1), uric acid / xanthine (stage 2), and uric acid / hypoxanthine (both stages). The ratio of xanthine to guanine was calculated which is an indicator of the severity of hypoxia.

As an indicator of the purine metabolism intensity (PMI) the value representing the ratio of the concentration of hypoxanthine to the amount of products formed from it: xanthine and uric acid and determining the irreversibility of purine catabolism was calculated.

$$PMI = [HC] / [X] + [UA]$$

Statistical analyses

Differences in the compared groups are determined by the Kruskal-Wallis ANOVA by Ranks test for 3 or more unrelated groups. The data was analyzed at a significance level of p = 0.05. STATISTICA 8.0 StatSoft, Inc. software was used for calculations and graphic presentation of the results.

Results

The duration of the experiment: 12 hours after the injection of self-bile (group 1)

At the end of the experiment, 12 hours after the injection of self-bile into the blades of the pancreatic structures, a macroscopic picture of plethora of the capsule vessels as well as hemorrhagic foci at the injection sites were noted, a flabby-elastic texture of the tissues was noted, the size of the organ did not change significantly: length - 7.3 cm., Width - 1.8cm (Figure 1).

For the convenience of presenting the results obtained during the study of purine metabolism, the

studied indicators were combined into two groups. The concentrations of all determined purines and their metabolites: adenine, guanine, hypoxanthine, xanthine, and uric acid were attributed to the absolute indicators of purine metabolism. All ratios as xanthine / guanine, xanthine / hypoxanthine, uric acid / xanthine and uric acid / hypoxanthine constituted a group of relative indicators of purine metabolism.

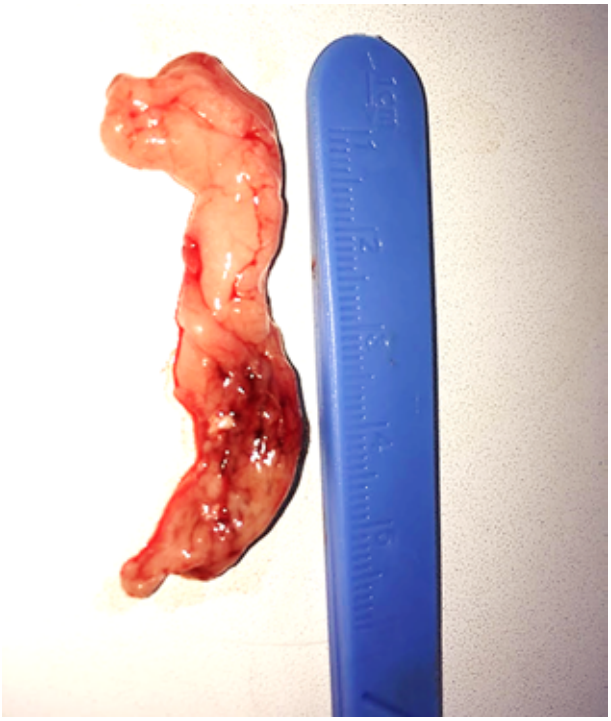


Figure 1. Preparation of rabbit pancreas (macroscopic image).

The results of the study of purine metabolites concentration in the blood plasma of experimental animals 12 hours after the self-bile injection (group 1) show a slight increase (within 10-20%) in the levels of adenine, guanine and hypoxanthine. The level of xanthine is increased in relation to the indicators of the control group by 63%, and the concentration of uric acid is significantly increased threefold (Table 1).

In the group of sham-operated rabbits, 12 hours after the operation, the following changes in the parameters of purine metabolism occurred in the blood plasma compared with those in the group of intact animals: the level of adenine decreased by two times, the levels of guanine and hypoxanthine increased slightly by 8% and by 9%, respectively, the concentration of xanthine remained at the level of the control group, the level of uric acid increased by 30%.

The analysis of the relative indicators of purine metabolism in the group showed an increase in the activity of the enzyme xanthine oxidase as all the coefficients reflecting this process are increased in relation to the figures of the control group. The increase in the UA / Hypoxanthine ratio by 130% is particularly noted. The indicator of the intensity of purine metabolism is increased three times, but the level of hypoxia is insignificant.

Analysis of the relative indicators of purine metabolism in the group of sham-operated rabbits 12

Table 1. Absolute and relative figures of purine metabolism intermediates in blood plasma, 12 hours after self-bile injection (group 1).

Absolute figures of purine metabolism in groups studied (Mediana (Q25; Q75))					
	Me (Q25;Q75)	**Me (Q25;Q75)**	**Me (Q25;Q75)**	**Me (Q25;Q75)**	**Me (Q25;Q75)**
Indicator	Adenine	Guanine	Hypoxanthine	Xanthine	Uric acid
12 hours	231 (220,5; 288)	206 (200; 263)	173 (167,75; 221)	137 (134,75; 167,75)	92 (89,75; 105,5)
12 hours SHAM	92,5 (91,25; 93,75)	200 (195; 205)	171,5 (162,75; 180,25)	84 (81,5; 86,5)	41,5 (37,75; 45,25)
Relative figures of purine metabolism intermediates in blood plasma in groups studied (Mediana (Q25; Q75))					
	Me (Q25;Q75)	**Me (Q25;Q75)**	**Me (Q25;Q75)**	**Me (Q25;Q75)**	**Me (Q25;Q75)**
Indicator	Xanth/Hypox	UA/Xanth	UA/Hypox	PMI	Xanth/Guan
12 hours	0,665 (0640; 0,674)	0664 (0,630; 0,670)	0,447 (0404; 0,449)	93,504 (91,234; 107,064)	0,593 (0,583; 0,611)
12 hours SHAM	0,489 (0,479; 0,500)	0,493 (0,463; 0,523)	0,242 (0,232; 0,251)	43,541 (39,747; 47,334)	0,420 (0,418; 0,423)

hours after the operation showed a slight increase in the activity of the xanthine oxidase enzyme. The indicator of the intensity of purine metabolism increased by 30%, but the level of hypoxia decreased by 10%.

24 hours after injection of self-bile (group 2)

At the end of the experiment, 24 hours after the injection of self-bile, a macroscopic picture of organ reduction was noted while the indicators were length - 5.5 cm, width - up to 1.0 cm. Among other things there were foci of merging hemorrhages, the vessels of the capsule were full-blooded in the injection area, the consistency of the parenchyma is flabby (Figure 2).

The results of a study of the purine metabolites concentration in the blood plasma of experimental animals, 24 hours after the injection of self-bile (group 2), show a slight decrease in the level of adenine, xanthine and uric acid in relation to group 1. But in relation to the indicators of the control group, the indicators remain increased. The levels of guanine and hypoxanthine continue to increase both in relation to the indicators of the control group and to the indicators of the first group (Table 2).

In the group of sham-operated rabbits, 24 hours after the operation, the following changes in the parameters of purine metabolism occurred in the blood plasma compared with those in the group of intact animals: the level of adenine decreased by 1.4 times, the guanine level increased by 23%, the concentration of hypoxanthine and xanthine remained at the level of the control group, the level of uric acid increased by 75%.

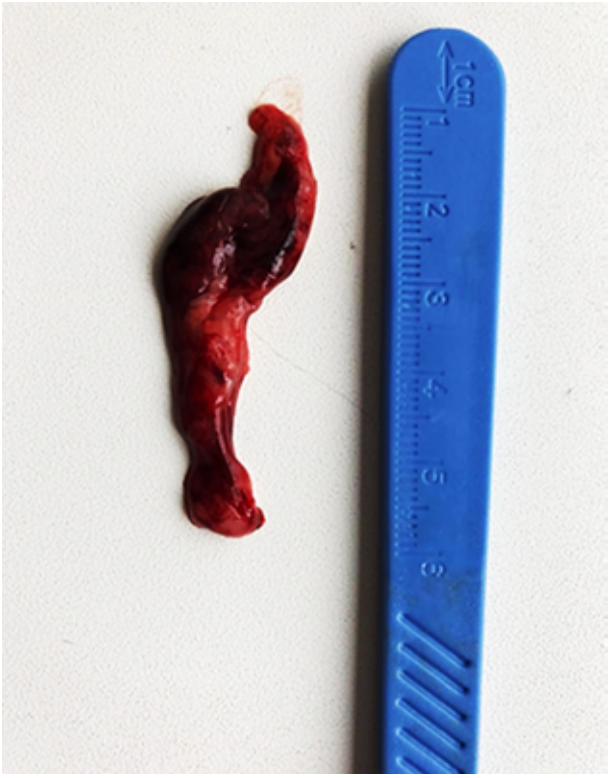


Figure 2. Preparation of rabbit pancreas 24 hours after injection of self-bile (macroscopic image).

Table 2. Absolute and relative figures of purine metabolism intermediates in blood plasma, 24 hours after the injection of self-bile (group 2).

Absolute figures of purine metabolism in groups studied (Mediana (Q25; Q75))					
	Me (Q25;Q75)	**Me (Q25;Q75)**	**Me (Q25;Q75)**	**Me (Q25;Q75)**	**Me (Q25;Q75)**
Indicator	Adenine	Guanine	Hypoxanthine	Xanthine	Uric Acid
24 hours	225,5 (169,25; 331,25)	215 (150; 300,25)	179 (113; 253,25)	105,5 (22,675; 170,25)	50,5 (33; 89)
24 hours SHAM	129 (121,5; 136,5)	227,5 (223,75; 231,25)	153,5 (142,25; 164,75)	86 (83,5; 88,5)	55 (47,5; 62,5)
Relative figures of purine metabolism intermediates in blood plasma in groups studied ((Mediana (Q25; Q75)					
	Me (Q25;Q75)	**Me (Q25;Q75)**	**Me (Q25;Q75)**	**Me (Q25;Q75)**	**Me (Q25;Q75)**
Indicator	Xanth/Hypox	UA/Xanth	UA/Hypox	PMI	Xanth/Guan
24 hours	0,463 (0,149; 0,599)	0,620 (0,495; 3,764)	0,221 (0,220; 0,313)	62,458 (54,739; 90,711)	0,404 (0,132; 0,581)
24 hours SHAM	0,561 (0,537; 0,587)	0,640 (0,537; 0,749)	0,359 (0,288; 0,440)	56,784 (49,361; 64,203)	0,378 (0,361; 0,395)

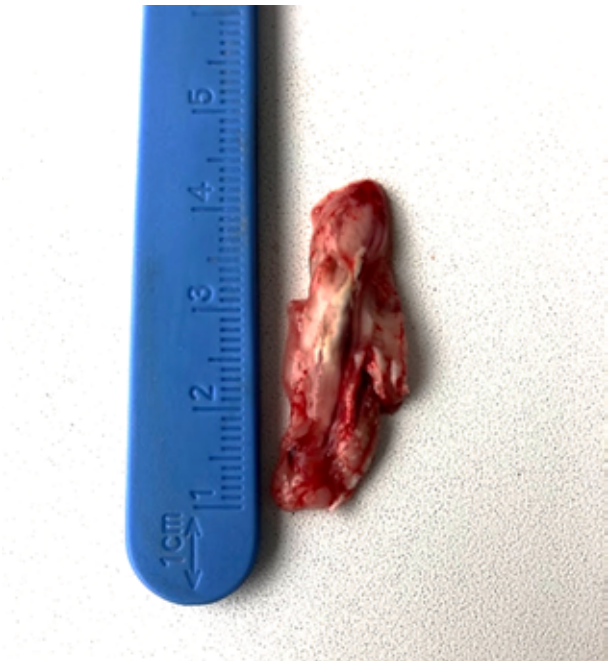


Figure 3. Preparation of rabbit pancreas 48 hours after injection of self-bile (macroscopic picture).

The analysis of the relative figures of purine metabolism 24 hours after the injection of self-bile showed a decrease in the activity of the xanthine oxidase enzyme and the intensity of purine metabolism as all the coefficients reflecting this process are reduced in relation to the indicators of group 1, but in relation to the indicators of the control group remain high. The indicators of hypoxia are decreased compared to those of the control group.

Analysis of the relative indicators of purine metabolism in the group of sham-operated rabbits 24 hours after the operation showed a slight increase in the activity of the xanthine oxidase enzyme. The indicator of the intensity of purine metabolism increased by 65%, but the level of hypoxia decreased by 25%.

48 hours after injection of self-bile

48 hours after the intraparenchymal injection of self-bile a visual evaluation of the macroscopic picture showed deformation and a significant decrease in the size of the organ: length - 3.7 cm, width - up to 1.0 and 0.8 cm. The amount of hemorrhage foci and pronounced vascular congestion were noted under the capsule of the pancreas. Consistency is flabby (Figure 3).

Analysis of the concentration indicators of purine metabolites in the blood plasma of experimental animals 48 hours after the injection of self-bile showed an increase in the concentration of adenine in relation to all groups. The levels of guanine, hypoxanthine and xanthine are decreased in relation to the indicators of all experimental groups, but exceed the control values. The uric acid level dropped almost to the values of the control group (Table 3).

In the group of sham-operated rabbits, 48 hours after the operation, the following changes in the parameters of purine metabolism occurred in the blood plasma compared with the parameters of the group of intact animals: the level of adenine decreased by 1.4 times, the guanine value decreased by 30%, the concentration of hypoxanthine and xanthine increased by 25% and 45% respectively, the level of uric acid increased by 73%.

Analysis of the relative figures of purine metabolism 48 hours after the injection of self-bile showed a dynamic decrease in the activity of the xanthine oxidase enzyme and the intensity of purine metabolism as all the coefficients reflecting this process are reduced in relation to the indicators of group 1 and group 2, but in relation to the indicators of the control group remain elevated. Indicators of hypoxia remain at the level of indicators of the control group.

Analysis of the relative indicators of purine metabolism in the group of sham-operated rabbits 48 hours after the operation showed an increase in the activity of the xanthine oxidase enzyme. The indicator of the intensity of purine metabolism increased by 70%, and the level of hypoxia increased by 1.8 times.

A one-way Kruskal – Wallis analysis of variance was carried out in order to conduct a detailed comparative analysis of changes in the concentration of purine metabolism catabolites of the groups studied in comparison with the reference values of the control group. The results of the comparison of adenine, guanine, xanthine, hypoxanthine and uric acid indicators are presented in Figure 4.

According to Figure 4 in the groups studied all indicators of purine metabolism in blood plasma

Table 3. Absolute and relative figures of purine metabolism intermediates in blood plasma, 48 hours after the injection of self-bile (group 3).

Absolute figures of purine metabolism in groups studied (Mediana (Q25; Q75))					
	Me (Q25;Q75)	**Me (Q25;Q75)**	**Me (Q25;Q75)**	**Me (Q25;Q75)**	**Me (Q25;Q75)**
Indicator	Adenine	Guanine	Hypoxanthine	Xanthine	Uric Acid
48 hours	252,5 (189,5; 296,75)	205 (163,75; 245,5)	150,5 (123; 184)	102 (79,75; 109,25)	40 (35,25; 53,75)
48 hours SHAM	130,5 (121,75; 139,25)	142,5 (128,75; 156,25)	196 (171; 221)	121 (111; 131)	55 (52,5; 57,5)
Relative figures of purine metabolism intermediates in blood plasma in groups studied ((Mediana (Q25; Q75))					
	Me (Q25;Q75)	**Me (Q25;Q75)**	**Me (Q25;Q75)**	**Me (Q25;Q75)**	**Me (Q25;Q75)**
Indicator	Xanth/Hypox	UA/Xanth	UA/Hypox	PMI	Xanth/Guan
48 hours	0,445 (0,407; 0,518)	0,476 (0,432; 0,519)	0,221 (0,171; 0,259)	42,427 (37,673; 55,976)	0,371 (0340; 0,4258)
48 hours SHAM	0,618 (0,593; 0,649)	0,454 (0,439; 0,473)	0,281 (0,260; 0,307)	56,618 (54,039; 59,186)	0,851 (0,711; 1,019)

changed most significantly 12 hours after the injection of self-bile. There is a statistically significant increase of adenine (p = 0.0248), guanine (p = 0.0131), hypoxanthine (p = 0.0446), xanthine (p = 0.0446). Statistically the concentration of uric acid in plasma (p = 0.0065) is significantly increased. 24 hours after the injection of self-bile, the change in the concentration of purine metabolism intermediates is noted, namely, the levels of guanine (p = 0.0099), hypoxanthine (p = 0.0025) increase sharply, the remaining parameters as adenine (p = 0.0099), xanthine (p = 0.0087) and uric acid (p = 0.0162) are decreased. All indicators of purine metabolism with the exception of adenine (p = 0.0137) decrease 48 hours after the injection of self-bile.

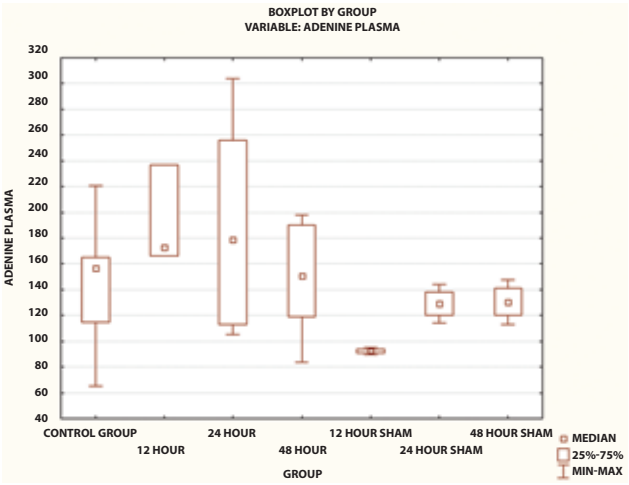
In the groups of sham-operated rabbits, the concentration of adenine (p = 0.0052) remains lower than in the group of intact animals 12, 24, and 48 hours after the operation to open the abdominal cavity. In terms of the concentration of guanine (p = 0.0030), hypoxanthine (p = 0.0932) and xanthine (p = 0.0278), slight fluctuations occur, which fit into the statistical error of 30%. The level of uric acid (p = 0.0026) is higher than in the group of intact animals in all three groups of sham-operated rabbits.

The results of comparative Kruskal-Wallis analysis of the studied groups in terms of the relative parameters of the blood plasma in the studied groups are presented in Figure 5.

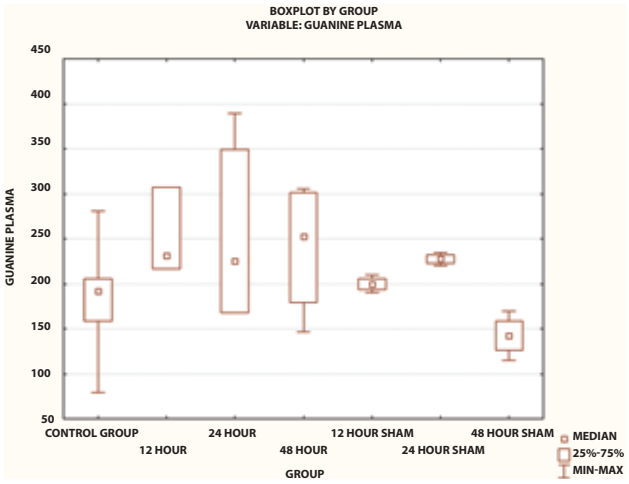
According to the data in Figure 5 the relative indicators of purine metabolism in blood plasma changed most significantly 12 hours after the injection of self-bile in groups studied. Increased activity of xanthine oxidase and increased indicators of the intensity of purine metabolism (p = 0.0070) are noted. There is a decrease in all relative indicators of purine metabolism in plasma in the groups studied within 24 hours and 48 hours after the administration of self-bile. The intensity of xanthine oxidase and indicators of the intensity of purine metabolism is decreased. There was no significant difference in three groups in terms of hypoxia.

Indicators of xanthine oxidase activity and indicators of the intensity of purine metabolism (p = 0.0010) increase in all three groups of sham-operated rabbits. The level of hypoxia (p = 0.002), which is reduced 12 and 24 hours after the sham operation, increases sharply 48 hours after the sham operation.

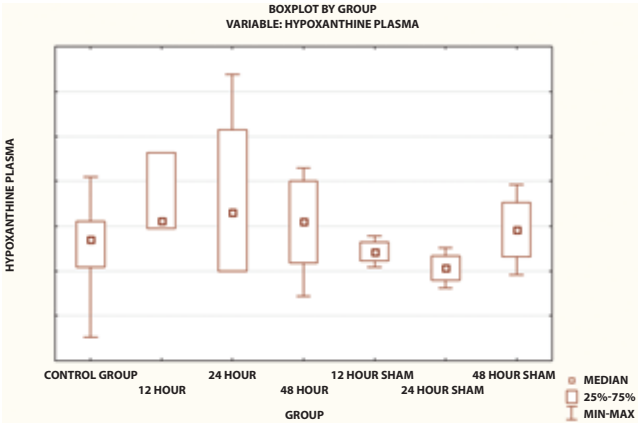
Thus, comparing purine metabolism indices in the group of sham-operated rabbits with the indices of the groups after the administration of self-bile showed that the operation itself does not have a statistically significant effect on the concentration of purine metabolism indices, except for the level of adenine. Furthermore, at 24 and 48 hours after surgery, the intensity of purine metabolism is directly opposite - with the introduction of self-bile, they decrease, and with a false operation, they increase.



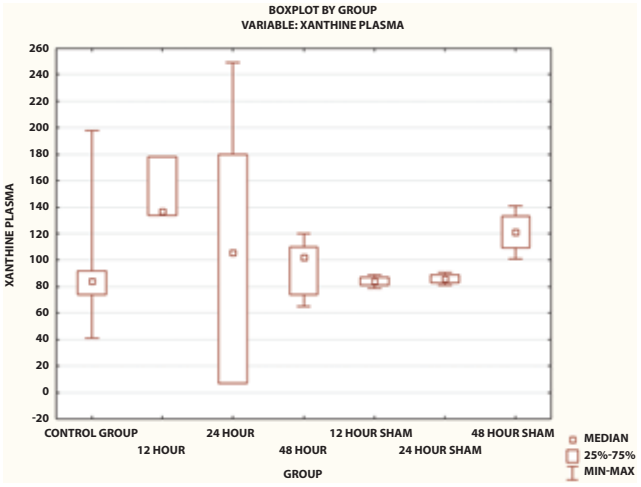
A - Adenine



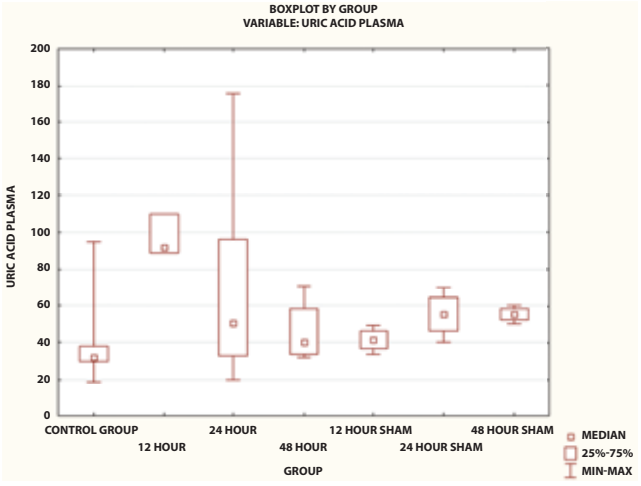
B - Guanine



C - Hypoxanthin



D - Xanthin

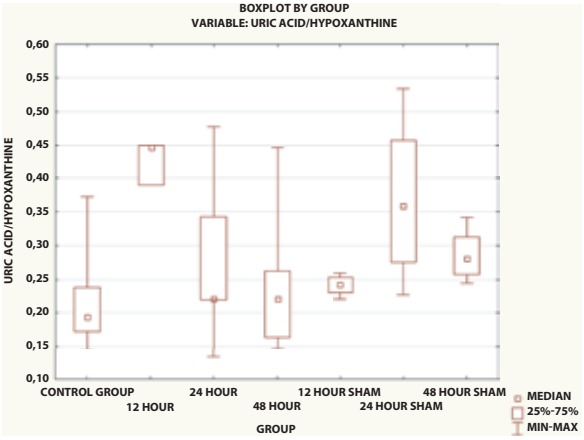


E - Uric Acid

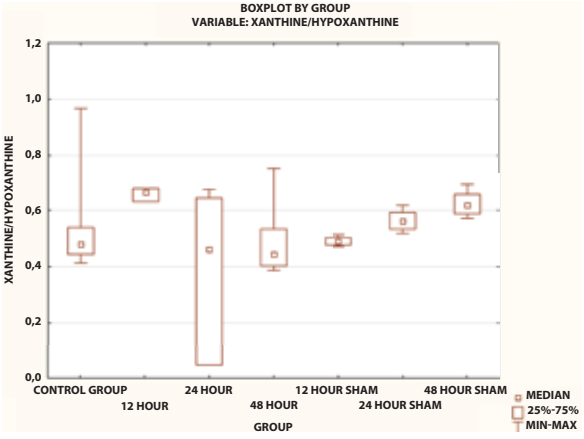
Figure 4. Comparative characteristics of the concentrations of purine metabolism intermediates in plasma in the groups studied (A – Adenine, B – Guanine, C – Hypoxanthin, D – Xanthin, E - Uric Acid).

Discussion

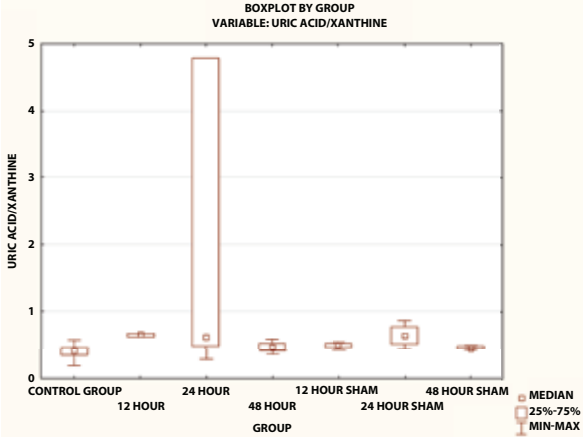
Acute pancreatitis is an inflammatory process that is accompanied by the inflammatory mediators' release and the subsequent activation of cytokines. Synthesis of secondary metabolites as thromboxanes, prostaglandins, nitric oxide, etc. occurs (29). Activation of leukocytes in the pancreas causes increased production of reactive oxygen species (ROS) in the later stages of pancreatitis. ROS directly attack lipids and proteins of the cell membrane and induce an increased thromboxane secretion. Thromboxanes cause platelet aggregation and have a vasoconstrictor effect, and as a result, the blood circulation is disrupted. ROS also induce increased production of leukotrienes which enhances the activation of leukocytes and the release of lysosomal enzymes that causes the development of tissue necrosis. Consequently, an increase in the inflammatory process and destruction of the tissues of the gland occur (30,31). Extracellular metabolites of purine metabolism are released into the bloodstream from cells through hydrolysis of nucleotides. The



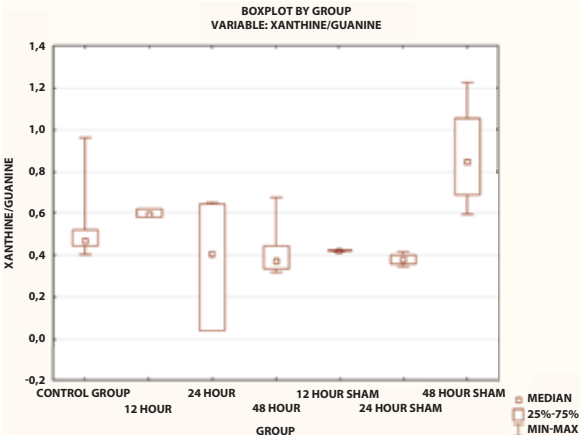
A - Uric Acid/ Hypoxanthin



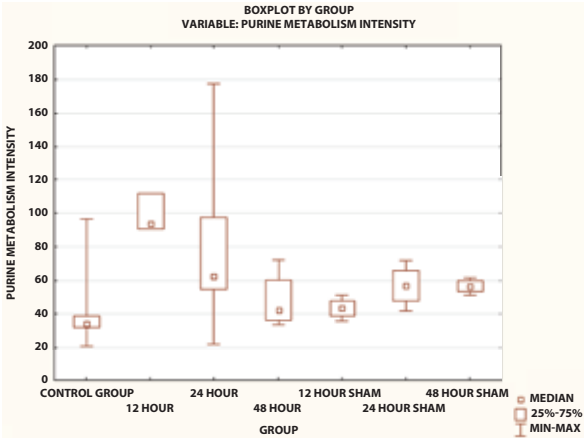
B – Xanthin/ Hypoxanthin



C - Uric Acid/ Xanthin



D – Xanthin/ Guanine



E - The of purine metabolism intensity

Figure 5. Comparative characteristics of the relative indicators of purine metabolism in plasma in the groups studied (A - Uric Acid/ Hypoxanthin, B – Xanthin/ Hypoxanthin, C - Uric Acid/ Xanthin, D – Xanthin/ Guanine, E - The of purine metabolism intensity).

release process is realized mainly as a result of cell lysis. The release of a large amount of purine metabolites into the extracellular environment occurs in case of an inflammatory process and an increase in the level of neutrophils and macrophages (32). Plethora of the capsule vessels and foci of hemorrhage in the area of injection of the self-bile were macroscopically noted in the pancreas, the size of the organ did not have significant changes in terms of 12 hours after completion of the experiment. The histological picture was characterized by the appearance of foci of necrobiotic changes (27). In the blood plasma, the concentration of all intermediates of purine metabolism is increased. The level of adenine, guanine, hypoxanthine, xanthine and uric acid are increased. There is an increase in the activity of the enzyme xanthine oxidase, the intensity of purine metabolism and the level of hypoxia. Hypoxanthine has a low molecular weight (136 dalton units) and can be easily transferred from affected tissues into the bloodstream by passive diffusion. An increase of hypoxanthine in plasma is a marker of hypoxia (33). Hypoxanthine is converted to uric acid in the liver. It has been suggested that disorder of purine catabolism in tissues except the liver is associated with the

formation of free radicals as well as with hyperuricemia (34). An increase in the concentration of hypoxanthine is a factor that has a negative effect on the vascular wall. Hypoxanthine provokes vasoconstriction and disrupts endothelial barriers (35). Uric acid is the final product of purine metabolism oxidation in humans. Xanthine oxidoreductase (XOR) catalyses the oxidative hydroxylation of hypoxanthine to xanthine and uric acid, accompanying the formation of reactive oxygen species (ROS). Uric acid is a powerful antioxidant that absorbs reactive oxygen species (ROS) (36). The xanthine oxidase enzyme plays an important role in the catabolism of purines. Its mechanism of action is the oxidation of xanthine and hypoxanthine with molecular oxygen. The high activity of xanthine oxidase formed from xanthine dehydrogenase increases the formation of purines in the blood serum. It stimulates the production of free radicals and superoxide anions which increase oxidative stress and weaken the nitric oxide system. The cell membrane is damaged and pro-inflammatory cytokines are released (37). The level of hypoxia that also increases of pancreatic necrosis development at the initial stage (12 hours after the injection of self-bile), induces an increase in the transformation of xanthine dehydrogenase into xanthine oxidase which leads to an increase of its activity followed by the oxidation of hypoxanthine into xanthine and xanthine into uric acid.

In terms of 24 hours after the end of the experiment, the pancreas decreased in size. Histologically, there was a structural disorder of the organ, diffuse lymphoid-leukocyte infiltration and necrobiotic changes in the parenchyma were noted (27). At this time of the experiment compensatory mechanisms were already activated and all indicators of purine metabolism in blood plasma were decreased except hypoxanthine and guanine. Guanine continues to be intensively deaminated by guanase with the formation of xanthine and the ammonia release. Indicators of xanthine oxidase activity are controversial but a decrease of purine metabolism intermediates indicates its high activity.

In terms of 48 hours after the end of the experiment, macroscopically, the pancreas was sharply decreased in size. Multiple foci of hemorrhage were observed, histologically extensive areas of necrosis of the exocrine apparatus and foci of necrosis of the parenchyma in the form of non-nuclear uniform outlines were observed (27). At this point of the experiment, the concentration of both absolute and relative indicators of purine metabolism intermediates was decreased to the level of indicators in the control group.

The concentration of potential substrates of purine metabolism and catabolic intermediates were decreased in proportion to the increase in the area of necrosis.

Conclusion

The indicators of the concentration of absolute and relative intermediates of purine metabolism were increased at the initial stage of pancreatic necrosis induced by intraparenchymal injection of self-bile. This data confirmed the proposed mechanism of the conversion of acute pancreatitis to the stage of pancreatic necrosis.

An increase in the activity of enzymes and metabolites of purine metabolism that are involved in the formation of reactive oxygen species and free radicals and their inactivation was noted. Not all indicators were unambiguous, and, nevertheless, the hypothesis that purine metabolism intermediates can be markers of the development of pancreatic necrosis in its early stages was confirmed. Based on the data obtained it is reasonable to assume that this issue requires further study of the blood plasma of patients with a diagnosis of acute pancreatitis and pancreatic necrosis.

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