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# Changes in the biochemical parameters of blood and the morphological structure of the pancreas in rats with acute pancreatitis and their correction using corvitin

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*Acute pancreatitis (AP) is an inflammation of the pancreas characterized by a severe course and a high mortality rate. The pathogenesis of AP is still not fully understood, so there is currently a lack of treatment. Corvitin is a water-soluble form of quercetin that retains all the properties of quercetin and has powerful antioxidant, anti-inflammatory and anti-apoptotic properties. The aim of our study was to evaluate the effect of corvitin on biochemical blood parameters and morphological features of the pancreas in rats with AP caused by intraperitoneal administration of L-arginine (200 mg/100 g). This model of AP is non-invasive, highly reproducible and causes selective, dose-dependent necrosis of acinar cells and is ideal both for studying the pathomechanisms of AP and for observing and influencing changes in the course of the disease. The legality of using this model is confirmed by the morphostructural changes in the pancreas that are characteristic of AP. In rats with AP, an increase in the blood concentration of α-amylase (twice), alanine aminotransferase (ALT) (three times), aspartate aminotransferase (AST) (one and a half times) compared to control values was observed already on the first day of the pathology development. On the second day of AP, the level of glucose and urea in the blood of rats increased by 34 and 22%, respectively, while the creatinine content did not change. Under the influence of corvitin (50 mg/kg), the levels of α-amylase, ALT and glucose decreased already after a day, while the content of AST and urea increased and remained so until the 8th day of observation. The most positive dynamics of morphological changes in the pancreas of rats was observed when corvitin was used on the first day of AP induction.*
*Keywords: L-arginine; acute pancreatitis; corvitin; blood; α-amylase; alanine aminotransferase; aspartate aminotransferase; glucose; urea; creatinine.*

## INTRODUCTION

Acute pancreatitis (AP) is a severe disease of the pancreas (PA), which is accompanied by damage to its glandular cells, tissue inflammation, and leukocyte infiltration with a high mortality rate due to the lack of specific treatment methods. The pathogenesis of this disease is still poorly understood, so there is currently a lack of effective treatment [1]. The basis of AP is a decrease in blood flow in the gland itself and adjacent organs, damage to acinar cells of the pancreas, hypersecretion of pancreatic juice and difficulty in its outflow [2-4]. The study of the pathophysiological mechanisms of AP makes it difficult to obtain clinical data from patients in the most dangerous early stages of the disease - in the first 48 hours. AP remains a serious disease also due to the concomitants complications that accompany it, primarily multi-organ and systemic dysfunctions [5]. In patients with this disease, large volumes of fluid can be sequestered not only in the retroperitoneal space but also intraintestinally and intrapleurally. Demarcation of necrotic tissue and resorption of exudate is carried out

very slowly. Absorption of toxic products from necrotized tissues and digestive disorders lead to a severe course of the disease, characterized by high temperature, anemia, exhaustion, hypoproteinemia, and electrolyte imbalance. Recovery and the treatment of AP are slow, with signs of hypofunction of the pancreas and post-necrotic pancreatopathy, which is manifested by dyspeptic disorders. Relapses, which occur quite often, are more difficult to treat than the initial period of the disease. Death in AP can occur as a result of complications, such as uremia, gastrointestinal hemorrhage, diabetic coma, pulmonary embolism, rupture of post-necrotic cysts, etc. Complete recovery is possible only with mild forms of the disease. Analysis of literary data allows us to draw two important conclusions. Firstly, there is currently no specific treatment for severe pancreatitis. Secondly, insufficient treatment can be complicated by the rapid progression of the pathological process. This means that in the early period of AP, the transition from a mild form of the disease with interstitial-edematous lesions of the pancreas to an extremely severe one with extensive necrosis of the gland often occurs very quickly. Bassi [6] emphasizes the rapid development of pathomorphological changes in the pancreas and points to a 12-hour «window» of opportunities for therapeutic influence. According to literature data, the use of quercetin alleviates symptoms of AP and prevents the occurrence of these dangerous disease complications [7]. Corvitin, as a watersoluble analog of quercetin, retains all the properties of the latter, so it has powerful antioxidant properties that help normalize the functions of the vascular endothelium and restore blood flow in organs [2, 8]. Quercetin regulates blood viscosity, fibrinogen content, and erythrocyte aggregation [9], exhibits strong antioxidant, anti-inflammatory and anti-hyperglycemic properties, can reduce platelet aggregation and capillary permeability [10], and powerful antiapoptotic agent [11]. It also causes NO-mediated vasodilation while inhibiting the synthesis and release of endothelin-1 from endotheliocytes [12]. Corvitin is a non-toxic compound with no harmful side effects. Therefore, its use is safe for patients with pancreatitis. This was the basis for choosing the drug corvitin for the correction of pathological disorders that occur at AP both in the pancreas and in nearby organs.

The aim of this study was to investigate changes in blood biochemical parameters in rats, such as the concentration of α-amylase, glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine and urea as well as features of the morphological structure of pancreas in the early stages of AP caused by L-arginine and the possibility of their correction by corvitin.

## METHODS

The experiments were carried out according to the current international requirements and norms of bioethics in experiments with animals (Strasbourg, 1986, Law of Ukraine dated February 21, 2006. No. 3447-IV) and following the decision of the Biological Ethics Committee of the Scientific Center «Institute of Biology and Medicine» of Taras Shevchenko National University of Kyiv (protocol No. 3 dated April 9, 2009). The research used the following materials: L-arginine (“Sigma-Aldrich”, USA), corvitin (Borshchagivskyi chemical and pharmaceutical plant, Ukraine), sodium thiopental (“Kyivmedpreparat”, Ukraine). The research was carried out in chronic experiments on mature male Wistar rats (obtained from the Institute of Pharmacology and Toxicology, Academy of Medical Sciences of Ukraine). The weight of rats was in the range of 180-250 g, age – 10 weeks. During the experimental period, the animals were housed under standard conditions such as a humidity of 55-60%, controlled temperature of 22 ± 2°C, 12-h light/dark cycle, and free access to tap water and commercial food. After a week of acclimatization, the rats were divided into 17 groups, of 8 animals in each: 1 group (I) intact animals; 4 groups (II) of negative control (NC/AP)

animals with AP in which blood samples for biochemical analysis were taken on the 2nd, 4th, 7th, and 8th day of pathologies; 6 groups of positive control rats with AP who were treated with physiological solution at different stages of pathology: after 6 h (III) (PC0/AP), after 1 day (IV) (PC1/AP) in which blood samples for biochemical analysis were taken on the 2nd, on the 4th, on the 7th day of the AP, and in the second case on the 2nd, on the 4th and on the 8th day of AP; 6 groups experimental rats with AP in which corvitin treatment was carried out after 6 h (V) (KOR0/AP) and after 1 day (VI) (KOR1/AP) of the AP and blood samples for biochemical analysis were taken in each of these cases on the 2nd, on the 4th, on the 7th day, and on the 2nd, on the 4th, on the 8th day of the disease, respectively.

AP was induced by intraperitoneal injection of L-arginine ("Sigma-Aldrich", USA) at a dose of 200 mg/100 g twice with an interval of 1 h. Corvitin (Kyiv BHFZ) was administered at a dose of 50 mg/kg. Biochemical indicators, in particular, α-amylase (units/l), glucose (mmol/l), ALT (units/l), AST (units/l), urea (mmol/l), creatinine (μmol/l) were measured in blood serum. The activity of the enzymes and other biochemical parameters was determined by enzymatic kinetic chemistry using commercial kits in a Roche/Hitachi medullary analytical system according to the manufacturer's protocols ("Roche", Germany). Rats were euthanized at the end of experiments by cervical dislocation.

For histological studies, the removed pancreas was fixed in a 10% solution of buffered formalin (72 h). Subsequently, the pancreas samples were dehydrated in a series of ethanol baths, clarified in xylene, and sealed in paraplast. Microtome sections (5 μm) were stained with hematoxylin and eosin, after which they were enclosed in a slide under a covered glass. Histological studies were performed using an Olympus BX51 light microscope. Changes detected under the microscope were documented using an Olympus DP-Soft 3.2 imaging system (Japan).

Statistical analysis. The obtained results were expressed as M ± SED or as a percentage of the difference. Data were analyzed by the methods of variational statistics using the software Statistica 8.0 for Windows ("StatSoft", USA). The normality of the distribution of digital arrays was checked using the Shapiro-Wilk W-test. In the case of normality of the distribution, the Student's t-test was used to estimate the reliability of the difference between the control and experimental groups. Differences were considered significant at $P < 0.05$.

## RESULTS AND DISCUSSION

Since during AP on the first day after AP simulation, the concentration of pancreatic enzymes in the blood increases (amylase activity increases to several thousand Wolgemuth units), the determination of their content is of great importance in the diagnosis of AP [13]. Measurement of the α-amylase level in the blood is a standard test in the clinical diagnosis of AP [14].

In our experiments, the level of α-amylase in the blood of intact rats was 1245.2 ± 48.1 units/l, in the NC group (day 2) it was 1272.25 ± 49.0 units/l (Table 1). In rats with AP (PC0/AP group), the content of this enzyme in the blood increased almost twice on the 1st day of AP – to 2256.9 ± 122.6 units/; ($P < 0.001$ relatives to the groups of intact rats and NC, 2 days).

Histological studies of the rat pancreas in the PK0/AP group revealed edema of the connective tissue stroma, which spread to the acinar tissue of the gland from the periphery to the center. Also, destructive changes of acinar cells were observed in the acinar tissue of the pancreas, which was accompanied by the formation of foci of necrosis and inflammatory leucocyte-lympho-macrophage infiltration. The lumens of arteries, veins, and capillaries were often dilated and filled with blood. Often, the enlarged openings of the pancreatic ducts were filled with acidophilic protein secretion. The formation of immature sclerotic tissue was observed in the

**Table 1. Biochemical parameters of blood in rats with L-arginine-induced AP. Corvitin was administered 6 h after disease induction. Blood samples were taken after one day of the corvitin administration (2nd day) (n = 8).**

| Animal group | α-amylase, u/l | Glucose, mmol/l | ALT, u/l | AST, u/l | Urea, mmol/l | Creatinine, μmol/l |
|---|---|---|---|---|---|---|
| Intact (I) | 1245.2±48.1 | 5.7±0.1 | 47.1±2.6 | 154.1±4.0 | 6.1±0.2 | 27.8±2.0 |
| NC/AP (II) | 1272.25±49.0 | 5.5±0.4 | 42.7±2.1 | 157.8±3.3 | 5.7±0.4 | 25.5±1.6 |
| PC0/AP (III) | 2256.9±122.6 $P_{I,II - III} < 0.001$ | 7.1±0.3 $P_{I,II -III} < 0.01$ | 121.1±3.6 $P_{I,II-III} < 0.001$ | 227.5±19.4 $P_{II- III} < 0.001$ | 5.9±0.4 | 24.1±1.0 |
| COR0/AP (V) | 2262.3±82.9 $P_{I,II — V} < 0.001$ | 5.7±0.2 $P_{III—V} < 0.01$ | 116.7±8.4 $P_{II—V} < 0.001$ | 386.25±53.6 $P_{II—V} < 0.001$ $P_{III — V} < 0.05$ | 9.7±0.9 $P_{II,III—V} < 0.001$ | 30.4±3.2 |

peripheral areas of the pancreas along with foci of edema, necrosis, and inflammatory leukocyte-lympho-macrophagal infiltration of the stroma and acinar tissue of the pancreas. Not all the fabric of the pancreas underwent destructive changes. In some parts of the organ, preservation of the characteristic pancreas histostructure was observed, where isolated pancreatic islets formed from insulocytes located around dilated and full-blooded capillaries were found among the exocrine tissue. Dystrophic changes of acinar cells were also detected in such areas of the pancreas, which were manifested by a decrease in the number of zymogen secretory granules in their apical cytoplasm, marked hyperchromia of the nucleus, often with marginalization of chromatin, as well as expansion of intercellular spaces. Apoptosis of pancreatic cells was also observed which was determined by the appearance of dense apoptotic bodies isolated from pancreatic acinus.

The blood concentration of α-amylase in rats of the COR0/AP group (day 2) did not change compared to the PC0/AP group (day 2). After 3 days (4th day) of AP development, the blood content of α-amylase in rats with AP increased by 48% relative to NC group (4th day). The use of corvitin contributed to the normalization of the α-amylase blood level (Table 2).

After 6 days (7th day) of the development of AP under the influence of L-arginine, the blood level of α-amylase in rats was 27% higher compared to the NC group on 7 days ($P < 0.001$): 1625.9 ± 62.9 u/l versus 1281.5 ± 37.8 u/l (Table

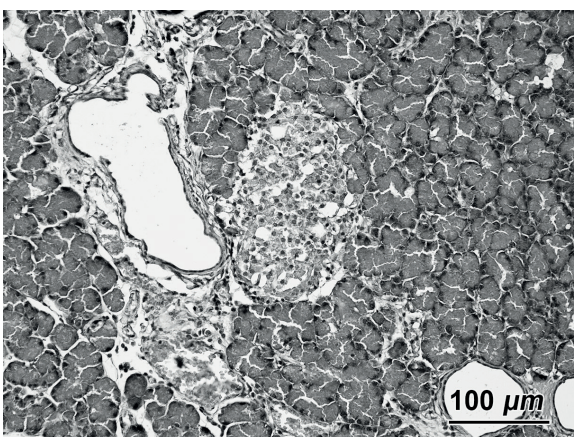


Fig. 1. Histological characteristics of the rat pancreas of the NC group. Hematoxylin and eosin

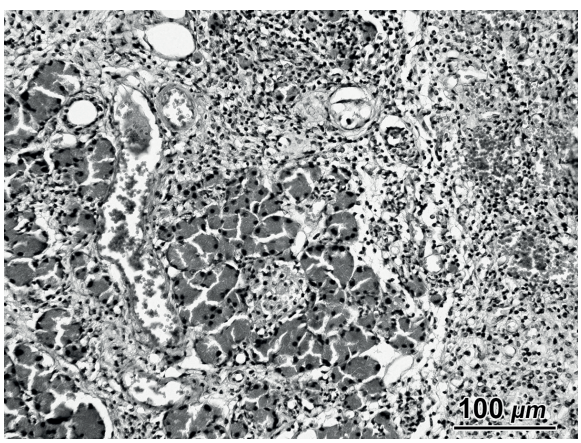


Fig. 2. Histological changes in the rat pancreas of the PC1/FP group. Hematoxylin and eosin

**Table 2. Effect of corvitin on biochemical blood parameters of rats with L-arginine-induced AP. The compound was administered 6 h after disease induction, blood samples were taken 3 days later (4th day) (n = 8)**

| Animal group | α-amylase, od/l | Glucose, mmol/l | ALT, u/l | AST, u/l | Urea, mmol/l | Creatinine, μmol/l |
|---|---|---|---|---|---|---|
| Intact (I) | 1245.2±48.1 | 5.7±0.1 | 47.1±2.6 | 154.1±4.0 | 6.1±0.2 | 27.8±2.0 |
| NC/AP (II) | 1252.4±49.0 | 5.6±0.2 | 46.8±0.9 | 153.3±2.3 | 5.8±0.4 | 27.8±1.2 |
| PC0/AP (III) | 1775.5±118.7 $P_{II-III} < 0.01$ | 6.4±0.6 | 45.4±2.3 | 166.3±16.4 | 7.1±0.4 $P_{II-III} < 0.05$ | 30.6±3.8 |
| COR0/AP (V) | 1210.9±66.8 $P_{III-V} < 0.001$ | 4.5±0.1 $P_{II-V} < 0.001$ $P_{III-V} < 0.01$ | 45.0±1.8 | 159.1±9.8 | 7.2±0.7 $P_{II-V} < 0.05$ | 30.5±1.8 |

3). In the blood of rats treated with corvitin, the content of α-amylase after 6 days of AP was even slightly lower than that of both NC, day 7 and PC0/AP, day 7, groups.

Histological studies of the rat pancreas in COR 0/GP group revealed foci of inflammatory mononuclear infiltration and the formation of sclerotic tissue in the areas of necrosis of the pancreas. In the newly formed sclerotic tissue, small areas of acinar tissue and individual acini, excretory pancreatic ducts, as well as arteries, veins and capillaries with unevenly dilated lumens, which were often filled with erythrocytes, were often found embedded in it.

When the treatment with corvitin was started one day after AP induction, the results are somewhat different. In particular, the levels of α-amylase in the blood of rats with AP on the second day after modeling the pathology were almost twice as high as in the animals of the NC group on the 2nd day ($P < 0.001$), while in the rats in the COR1/AP group on the 2nd day, this indicator was only 31% higher compared to NC on the 2nd day ($P < 0.01$; Table 4).

After 3 days from the moment of AP modeling, the blood concentration of α-amylase in rats of the PC1/AP group, on the 4th day, was 29% higher than the NC group (1616.9 ± 87.2 u/l vs 1252.4 ± 49.0 u/l), $P < 0.01$. After treatment with corvitin, this indicator decreased to 1361.0 ± 55.0 u/l, by 9% compared to NC, $P < 0.05$ (Table 5).

After 7 days of AP simulation, the level of α-amylase in the blood of rats continued to decrease, but it was still 16% higher compared to the NC group (1440.1 ± 46.8 u/l vs 1243.0 ± 40.4 u/l; $P < 0.01$) (Table 6). In rats treated with corvitin, the studied indicator became 22% lower than that for the NC group animals.

In rats of the COR1/AP group, foci of

**Table 3. Biochemical indicators of blood in rats with with L-arginine-induced AP. Administration of corvitin was started 6 h after disease simulation. Blood samples were taken 6 days (7th day) after AP induction (n = 8)**

| Animal group | α-amylase, u/l | Glucose, mmol/l | ALT, u/l | AST, u/l | Urea, mmol/l | Creatinine, μmol/l |
|---|---|---|---|---|---|---|
| Intact (I) | 1245.2±48.1 | 5.7±0.1 | 47.1±2.6 | 154.1±4.0 | 6.1±0.2 | 27.8±2.0 |
| NC/AP (II) | 1281.5±37.8 | 5.6±0.2 | 41.0±1.9 | 157.6±8.1 | 6.4±0.5 | 25.9±1.2 |
| PC0/AP (III) | 1625.9±62.9 $P_{II-III} < 0.001$ | 6.0±0.5 | 40.3±2.2 | 175.4±12.6 | 7.0±0.5 | 28.5±2.4 |
| COR0/AP (V) | 1107.0±63.0 $P_{II-V} < 0.05$ $P_{III-V} < 0.001$ | 4.6±0.2 $P_{II-V} < 0.01$ $P_{III-V} < 0.05$ | 92.1±4.8 $P_{II-V} < 0.001$ $P_{III-V} < 0.001$ | 160.5±11.0 | 13.4±1.3 $P_{II-V} < 0.001$ $P_{III-V} < 0.01$ | 28.75±1.6 |

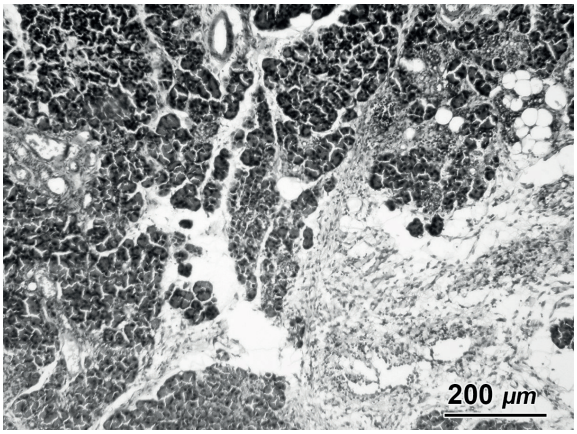


Fig. 3. Histological changes in the rat pancreas of the COR0/AP group. Hematoxylin and eosin

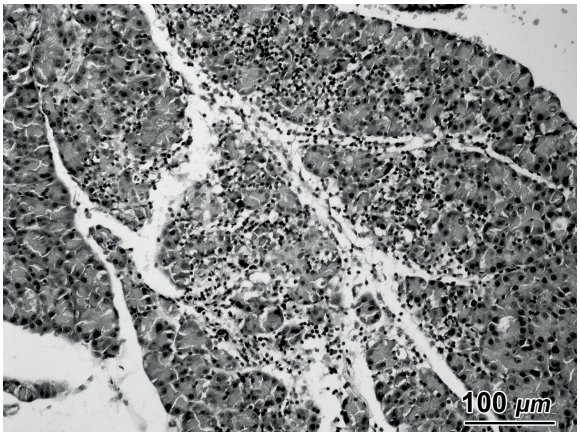


Fig. 4. Histological changes in the rat pancreas of the COR1/AP group. Hematoxylin and eosin

necrosis and inflammatory leukocyte-lympho-macrophage infiltration were observed against the background of edema of acinar tissue and connective tissue stroma. The formation of young sclerotic tissue with the remains of pancreatic acini walled-in it, pancreatic ducts and blood vessels with expanded and often empty lumens were also found (Fig. 4).

Comparative evaluation of morphological changes in the pancreas of rats from different experimental groups revealed more positive dynamics in the COR0/AP group. This was manifested by a noticeable improvement in blood microcirculation in the pancreas, stabilization of the destructive (necrotic) processes in the gland, as well as a decrease in acute inflammation, and the development of sclerotic foci in places of pancreatic necrosis.

Therefore, the level of α-amylase in the blood of rats with AP increases almost twice already in the first day from the moment of modeling the pathology compared to the normal control and remains increased, although it gradually decreases during the eight days of observation. Corvitin normalizes the content of α-amylase in the blood of rats with AP after three days of the development of the pathology, and the most effective was the administration of the drug one day after the AP simulation.

AP is often accompanied by disorders of carbohydrate metabolism, which is manifested by hyperglycemia and glucosuria. Hyperglycemia is observed in almost 60%, and glucosuria in 20% of cases, which indicates the progression of the pathological process [14]. In our experiments, the blood concentration of glucose in the intact

**Table 4. Biochemical indicators of blood in rats with L-arginine-induced AP. Administration of corvitin was started 1 day after induction of AP. Blood samples were collected after 1 day (2nd day) (n = 8)**

| Animal group | α-amylase, u/l | Glucose, mmol/l | ALT, u/l | AST, u/l | Urea, mmol/l | Creatinine, μmol/l |
|---|---|---|---|---|---|---|
| Intact (I) | 1245.2±48.1 | 5.7±0.1 | 47.1±2.6 | 154.1±4.0 | 6.1±0.2 | 27.8±2.0 |
| NC/AP (II) | 1272.25±49.0 | 5.5±0.4 | 42.7±3.1 | 157.8±3.3 | 5.7±0.4 | 25.5±1.6 |
| PC1/AP (IV) | 2311.75±201.4 | 7.2±0.5 | 130.8±14.9 | 193.9±16.3 | 6.9±0.4 | 25.6±1.0 |
| | $P_{I, II - IV} < 0.001$ | $P_{I,II - IV} < 0.01$ | $P_{II- IV} < 0.001$ | $P_{I, II - IV} < 0.05$ | $P_{I,II - IV} < 0.05$ | |
| COR1/AP (VI) | 1670.25±92.0 | 5.4±0.4 | | 320.3±43.3 | 14.0±1.5 | 30.5±1.9 |
| | $P_{II — VI} < 0.01$ | $P_{IV – VI} < 0.05$ | 149.25±21.0 | $P_{II—VI} < 0.001$ | $P_{II — VI} < 0.001$ | $P_{II—VI} < 0.05$ |
| | $P_{IV – VI} < 0.05$ | | $P_{II—VI} < 0.001$ | $P_{IV – VI} < 0.05$ | $P_{IV – VI} < 0.01$ | $P_{IV–VI} < 0.05$ |

rats with AP significantly increases compared to the groups of intact rats and negative control, and the maximum increase (by 34%) is observed 1 day after pathology modeling. The use of corvitin causes a decrease in blood sugar to normal within

(Table 6). Thus, on the 2nd day of AP modeling in rats, AST activity in the blood increased 1.5 times and after the administration of corvitin, this parameter doubled. However, on the 4th day of AP modeling, it significantly decreased, reaching control level. Similar changes were observed when corvitin was administered on the second day of the AP development, with the difference that in rats with AP, a repeated increase in AST activity was noted on the 8th day of observation.

Important indicators of the severity of AP are the level of creatinine and urea in the blood, which indicate the degree of metabolic disorder that occurs [16].

The blood concentration of urea in the intact rats was 6.1 ± 0.2 mmol/l, and in rats of the NC group, on the 2nd day of induced AP, it was 5.7 ± 0.4 mmol/l. After 1 day of AP modeling, the concentration of urea in the blood of rats did not differ from the control values (Table 1). The use of corvitin caused an increase in the concentration of urea in the blood of rats by 70% compared to control animals, reaching 9.7 ± 0.9 mmol/l ($P < 0.01$). 3 days after the induction of pathology, the blood content of urea in rats increased by 22% compared to the NC group on the 4th day ($P < 0.05$), and in rats with AP treated with corvitin, this indicator exceeded the control values by 24% ($P < 0,05$; Table 2). After 6 days of observation, the blood level of urea in rats with AP did not differ from the control ones, while in animals that were injected with corvitin, this parameter was 109% higher than the control values ($P < 0.001$; Table 3).

When treating rats with corvitin on the second day after the induction of AP (PC1/AP group on the 2nd day), the concentration of blood urea was 21% higher than in NC group, on the 2nd day ($P < 0.05$). Under the same conditions, in the COR1/AP group, on the 2nd day, the blood level of urea was 145.6% higher than in the NC group, on the 2nd day ($P < 0.001$; Table 4). 3 days and 7 days after AP induction, statistically significant changes in this indicator relative to the control were not observed in both experimental groups of rats (Tables 5; 6). Thus, in rats with AP, an increase in blood urea concentration up to 21% was observed on the 2nd and on 4th day. The use of corvitin 6 hours after the simulation of the disease caused an increase in this indicator in animals with AP throughout the entire period of observation with a maximum on the 7th day (doubled). On the other hand, the administration of this compound after 1 day led to a 2.5-fold increase in the studied indicator only on the 2nd day of the AP development and did not cause changes until the end of the experiment.

The blood level of creatinine in the intact rats was 27.8 ± 2.0 μmol/l, and in rats of the NC group, on the 2nd day, it was 25.5 ± 1.6 μmol/l. It should be noted that during all observation periods of AP modeling, the blood concentration of creatinine in rats with pathology did not change statistically significantly (Tables 1-6). This parameter did not change during the experiment and in rats with AP, which were treated with corvitin 6 h after the disease simulation.

If treatment with corvitin was started from the 2nd day, the creatinine content in the blood of rats receiving the drug increased compared to the control values: after 1 day - by 20%, after 3 days - by 33% and after 7 days - by 16% ($P < 0.05$ regarding NC in the relevant time periods). Therefore, in rats with the L-arginine model of AP, the concentration of creatinine in the blood does not change. This indicator remained unchanged during the experiment in rats with AP, which were treated with corvitin 6 h after the disease simulation. If corvitin was started to be used a day after the induction of AP, a negligible increase in the blood creatinine content in the rats was observed during the experiment.

An acinar cell is a functional unit of the exocrine pancreas. It synthesizes, stores and secretes digestive enzymes. Under normal physiological conditions, these enzymes are activated only after reaching the duodenum. Premature activation of these enzymes in the acinar cells of the pancreas leads to the occurrence of AP. Although significant

advances have been made recently in our understanding of the pathogenesis of this disease, available treatment options are still limited to traditional non-specific and palliative interventions. Based on current research into the physiology and pathophysiology of the disease, new therapeutic strategies have been proposed including administration of systemic antibiotics, antioxidants, cytokine antagonists, and, later, inhibition of the renin-angiotensin system. Despite these promising developments in treatment strategies, most of these potential therapies are still in the experimental or clinical trial phase. Further research is needed to prove the effectiveness of these new treatments.

For a better understanding of blood biochemical and clinical indicators in AP the processes that occur in the body with the onset of the disease should be mentioned. It was shown that this stage of AP is accompanied by a significant decrease in the volume of circulating blood due to huge pathological losses and sequestration of fluid. This can occur without a visible decrease in blood pressure but with distinct disturbances of microcirculation in the splanchnic zone, especially in the pancreas. For example, blood flow in the pancreas can decrease from 40% to 60% [2, 17]. Ischemia is probably the cause of further damage to the acinar apparatus with subsequent intracellular activation of digestive enzymes by lysosomal hydrolases and rapid growth of the pathological process with the occurrence of necrosis. Another consequence of hypoperfusion of the splanchnic zone is intestinal damage with destroying of its barrier function and subsequent infectious complications [18]. At first, vessels narrow in the pancreas and other organs, then they expand, the permeability of the vascular wall increases, and blood flow slows down. Further, there is an exit of the liquid part of the blood and formed elements from the lumen of the vessels to the adjacent tissues, which leads to swelling of the organ, hemorrhages in the tissue of the gland, and retroperitoneal tissue. Disorders of local blood flow promote thrombus formation. Under the conditions of blood vessel thrombosis, as a result of disruption of tissue metabolism and the direct effect of enzymes on cells, foci of necrosis are formed in the pancreatic parenchyma. Lipases are released from the destroyed cells, under the influence of which fat necrosis of the pancreas, omentum, retroperitoneal tissue, etc. occurs. General changes in the body are primarily caused by enzyme and then tissue (from necrosis foci) intoxication. The influence of vasoactive substances on the vascular bed leads to circulatory disorders at all levels: tissue, organ and systemic, which is the cause of dystrophic, necrobiotic and necrotic changes. Exudation into tissues and cavities causes pronounced disturbances of water-electrolyte, carbohydrate, protein and fat metabolism [19]. The pathogenesis of pancreatic tissue inflammation in AP remains mostly unknown. Recent studies show that 5-lipoxygenase (5-LOX) is an important mediator in the modulation of cell death pathways in human diseases [20] and facilitates the development of organ dysfunction [21]. In this study, we aimed to evaluate the effect of the 5-LOX inhibitor corvitin on the course of AP, in particular, on biochemical blood markers and on the state of pancreatic tissue. Amylase is a hydrolase and is produced in the pancreas, salivary glands, and in small amounts in other tissues. With AP, the blood level of amylase increases rapidly during the first 6 hours from the onset of the disease and remains elevated for 3-7 days. The half-life of this compound is 10-12 h. It is excreted mainly through the kidneys. The amylase test should preferably be carried out from 48 to 72 h after the first manifestations of AP because during this period the blood concentration of this enzyme is the highest. Spontaneous normalization of the blood amylase level occurs after 4-5 days [22]. High blood amylase activity, which is detected within 4 days of AP, indicates a severe course of the disease, although there is no complete parallelism between amylasemia and the severity of AP.

The increase in the activity of ALT and AST in the serum is largely associated with

the leakage of these enzymes from the cytosol of liver and muscle cells into the bloodstream [23], which indicates the destabilization of the membranes of these cells and the function of the organ as a whole. The presence of an elevated level and a change in the ratio of ALT and AST in the blood can be the result of the membrane structures destabilization in various tissues of the body. Monitoring the dynamics of changes in the activity of ALT and AST during the development of AP, it should be noted that in the process of self-rehabilitation, they quickly approached normal values. When using corvitin for the treatment of AP at the initial stage of its development, the activity of ALT characteristic of liver tissues increased significantly. At this moment, the activity of the AST enzyme, which is characteristic of the heart muscle, increased even more significantly, remaining elevated until the 3rd day of AP.

The latter indicates an unfavorable combination of factors accompanying this disease and corvitin on the condition of neighboring tissues, in particular the liver and heart, and possibly other muscles. At the same time, the use of corvitin to a certain extent smoothed out significant changes in the activity of these enzymes. An increased concentration of urea in the blood can occur, in particular, with rapid tissue breakdown, impaired excretion of urea by the kidneys in renal failure, non-gaseous acidosis, or hypothyroidism. An increased concentration of urea in the blood can occur, in particular, with rapid tissue breakdown, impaired excretion of urea by the kidneys in renal failure, non-gaseous acidosis, or hypothyroidism. Amino acids are the main source of ammonia for urea biosynthesis in liver tissue. Analyzing the obtained data on the blood level of urea during the development of AP, it should be noted that the use of a large dose of L-arginine did not cause a significant increase in the content of this metabolite on the first day, which was instead registered after corvitin application. This may be the result of a certain competition for the ways of excretion in the renal tissue, especially if we take into account the fact of renal failure that develops in AP. This is proven by data on the blood level of urea in an experiment using corvitin. It should be noted that applied L-arginine activated urea biosynthesis in the liver only on the third day. Creatine and creatinine are effective metabolites of energy metabolism in a number of body tissues, but they are synthesized with the participation of glycine, ornithine and arginine in the liver. In addition, creatinine clearance is a reliable criterion of renal filtration. It has been observed that quercetin can modulate mitochondrial biogenesis and mitochondrial membrane potential, oxygen respiration and ATP anabolism, intramitochondrial redox status, and apoptosis [26]. In the case of AP in the acinar cells of the pancreas, dysfunction of mitochondria is observed as a result of depolarization of their inner membrane. This leads to a disorder of energy metabolism, in particular to a decrease in ATP synthesis. Weber, et al. [27] showed that quercetin completely restored the level of ATP at cholecystokinin-induced mitochondrial dysfunction in isolated rat pancreatic acinar cells. It is known that amylase intensively breaks down polymeric carbohydrates into free sugars, in particular glucose, the blood level of which is clearly controlled with the direct participation of certain pancreatic hormones. Therefore, we were interested in investigating the dynamics of changes in the blood level of glucose and its correlation with the detected changes in amylase activity in AP. In our experiments, we observed an increase in the blood sugar content of rats with simulated AP during the first 3 days of the disease. Corvitin reduced this indicator to the norm, or even to a level below normal. An increase in the sugar level was not observed in the later period of AP (4 days). However, corvitin continued to act as a factor in reducing blood sugar to a level lower than normal during this period of time. We observed such effects of corvitin earlier in our experiments [2]. It is likely that the cause of this phenomenon is an increase in the consumption of glucose by cells under the influence of this compound, in which corvitin, like

its analog quercetin, increases energy metabolism.

## CONCLUSIONS

1. The blood level of α-amylase in rats with the L-arginine model of acute pancreatitis increased on the first day of pathology induction, almost twice as compared to the control, and remained elevated, although it gradually decreased during 8 days of observation. Corvitin normalized the blood content of α-amylase after three days of the pathology induction. Administration of the compound 1 day after AP induction was more effective, which indicates the membrane-stabilizing effect of corvitin on pancreatic cells.

2. The blood concentration of glucose in rats with AP significantly increased compared to control ones, and the largest increase (by 34%) was observed 1 day after the pathology was induced. The administration of corvitin led to a decrease of this parameter to the norm after one day. Later, this indicator even decreased relative to the control, facilitating the stabilization of the blood glucose level and more effective assimilation of this substrate by body tissues.

3. ALT activity tripled already on the 2nd day of AP simulation. The blood levels of ALT decreased, approaching the norm in the later periods of the AP development. If corvitin treatment was started immediately (after 6 h from the moment of modeling), it did not affect the activity of this enzyme, and sometimes even slightly increased it. When corvitin was administered to rats with AP after one day, the drug did not cause a similar effect, ALT activity in the rats was normal.

4. AST activity on the 2nd day of AP simulation increased by 1.5 times, and after the administration of corvitin increased by 2 times. However, on the 4th day, this indicator significantly decreased, almost reaching the control value. Similar changes were observed when corvitin was administered on the 2nd day of the AP induction with the difference that in rats with AP, a repeated increase in AST activity was observed on the 8th day of observation.

5. In rats on the 2nd and 4th days of the AP simulation, an increase in the urea blood concentration was 22%. Under the influence of corvitin, which was administered after 6 hours of AP, in rats with pathology, the concentration of urea in the blood significantly increased during the entire observation period. The maximum level of this compound was observed on the 7th day (doubled) after induction of the pathology. Corvitin, administered 1 day after AP simulation, caused a 2.5-fold increase in the studied indicator only on the 2nd day of AP, while on other days, until the end of the experiment, such changes were absent.

6. L-arginine, which takes a direct part in the liver biosynthesis of creatinine and was used for the reproduction of the AP, did not change the level of this metabolite in the blood of rats. In animals with AP, who were treated with corvitin 6 hours after the disease was induced, the concentration of creatinine in the blood remained unchanged during the experiment.

7. The most positive dynamics of morphological changes was observed in the pancreas of rats who were treated with corvitin on the 1st day of AP simulation. This was manifested by a significant improvement in pancreatic blood microcirculation and destructive processes stabilization, as well as a decrease in acute inflammation and the development of sclerotic areas in places of necrosis.

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## ЗМІНИ БІОХІМІЧНИХ ПОКАЗНИКІВ КРОВІ ТА МОРФОЛОГІЧНОЇ СТРУКТУРИ ПІДШЛУНКОВОЇ ЗАЛОЗИ У ЩУРІВ З ГОСТРИМ ПАНКРЕАТИТОМ ПРИ ЗАСТОСУВАННІ КОРВІТИНУ

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Гострий панкреатит (ГП) – запалення підшлункової залози, що характеризується тяжким перебігом та високим рівнем летальності. Патогенез ГП досконало не вивчений, тому засобів лікування наразі бракує. Корвітин – водорозчинна форма кверцетину, що зберігає всі властивості кверцетину і має потужні антиоксидантні, протизапальні та антиапоптотичні властивості. Метою нашої роботи було оцінити ефект корвітину на біохімічні параметри крові і морфологічні особливості підшлункової залози у щурів із ГП, викликаним інтраперитоніальним введенням L-аргініну (200 мг/100 г). Ця модель ГП є неінвазивною, високовідтворюваною та викликає вибірковий, дозозалежний некроз ацинарних клітин. Вона ідеально підходить як для вивчення патомеханізмів ГП, так і для спостереження та впливу на зміни при перебігу хвороби. Правомірність її застосування підтверджена виявленими нами, характерними для ГП, вираженими морфоструктурними змінами в підшлунковій залозі. У хворих щурів підвищувалася концентрація в крові таких сполук, як α-амілаза (вдвічі), аланінамінотрансфераза (АЛТ) (втричі), аспартатамінотрансфераза (АСТ; у 1,5 раза) порівняно з контрольними значеннями вже за 1-шу добу розвитку патології. На 2-гу добу ГП вміст глюкози та сечовини в крові щурів підвищився на 34 та 22% відповідно. Вміст креатиніну крові в цей період не змінювався. Застосування корвітину (50 мг/кг) спричиняло зниження вмісту α-амілази, АЛТ і глюкози вже через добу, а вміст АСТ і сечовини підвищувався і залишався таким до 8-ї доби спостереження. Найбільш позитивну динаміку морфологічних змін підшлункової залози спостерігали у щурів при застосуванні корвітину в 1-шу добу моделювання ГП.

Ключові слова: гострий панкреатит; L-аргінін; корвітин; кров; α-амілаза; аланін амінотрансфераза; аспартатамінотрансфераза; глюкоза; сечовина; креатинін.

## REFERENCES

1. Mederos MA, Reber HA, Girgis MD. Acute pancreatitis: A Review. JAMA. 2021;26;325(4):382-90.
2. Vovkun TV, Yanchuk PI, Shtanova LYa, Shalamai AS. Tissue blood flow in the digestive organs of rats with acute pancreatitis after corvitin administration. Fiziol Zh. 2015;59(1):53-9.
3. Wilden A, Glaubitz J, Otto O, Biedenweg D, et al. Mobilization of CD11b$^{+}$/Ly6c$^{hi}$ monocytes causes multi organ dysfunction syndrome in acute pancreatitis. Front Immunol. 2022;13: 991295. Published online 2022.
4. Singh P, Garg PK. Pathophysiological mechanisms in acute pancreatitis: Current understanding. Ind J Gastroenterol. 2016;35(3):153-66.
5. Garg PK, Singh VP. Organ failure due to systemic injury in acute pancreatitis. Gastroenterology. 2019; 156(7):2008-23.
6. Bassi C, Pederzoli P. Antibiotics in necrotizing pancreatitis / acute pancreatitis: diagnosis and therapy. 1994; 93-8.
7. Sheng B, Zhao L, Zang X, Zhen J, Liu Y, Bian W, Chen W. Quercetin inhibits caerulein-induced acute pancreatitis through regulating miR-216b by targeting MAP2K6 and NEAT1. Inflammopharmacology. 2021;29(2):549-59.
8. Vovkun TV, Yanchuk PI, Shtanova LY, Veselskyy SP, Baranovskyy VA. The influence of corvitin on secretory processes and blood flow in the rat gastric mucosa. Fiziol Zh. 2013;59 (1):40-6.
9. Pawlikowska-Pawlega B, Gruszecki W I, Misiak L E. The study of the quercetin action on human erythrocyte membranes. Biochem Pharmacol. 2003;66(4):605-12.
10. Dengyu Yang, Tiancheng Wang, Miao Long, and Peng Li. Quercetin: Its main pharmacological activity and potential application in clinical medicine. Oxid Med Cell Longev. 2020; 2020: 8825387.
11. Yu-Min Zhang, Zhen-Ye Zhang, and Ru-Xing Wang. Protective mechanisms of quercetin against myocardial ischemia reperfusion injury. Front Physiol. 2020;11: 956.
12. Loke WM, Hodgson JM, Proudfoot JM, McKinley AJ, Puddey IB, Croft KD. Pure dietary flavonoids quercetin and (-)- epicatechin augment nitric oxide products and reduce endothelin-1 acutely in healthy men. Am J Clin Nutr. 2008;88(4):1018-25.
13. Vissers RJ, Abu-Laban RB, McHugh DF. Amylase and lipase in the emergency department evaluation of acute pancreatitis. J Emerg Med. 1999;17:1027-37.
14. Abdelzaher WY, Ahmed SM, Welson NN, Marraiki N, Batiha GE, Kamel MY.Vinpocetine ameliorates L-arginine induced acute pancreatitis via Sirt1/Nrf2/TNF pathway and inhibition of oxidative stress, inflammation, and apoptosis. Biomed Pharmacother. 2021;133:110976.
15. Liu W, Du JJ, Li ZH, Zhang XY, Zuo HD. Liver injury associated with acute pancreatitis: The current status of clinical evaluation and involved mechanisms. World J Clin Cases. 2021;9(34): 10418-29.
16. Vannier E, Dupont-Lucas C, Lagarde B, Menahem B,

Chaigneau T, Piquet MA, Dupont B. Development of a score for predicting severe acute pancreatitis at admission. Pancreas. 2022;51(2):128-34.
17. Dembinski A, Warzecha Z, Konturek S J, Ceranowicz P, Dembinski M, Pawlik WW, Kusnierz-CabalaB, Naskalski JW. Extract of grapefruit-seed reduces acute pancreatitis induced by ishemia/reperfusion in rats; possible implication of tissue antioxidants. J Physiol Pharmacol. 2004;55(4):811-21.
18. Flint RS, Windsor JA. The role of the intestine in the pathophysiology and management of severe acute pancreatitis. HPB (Oxford). 2003;5(2):69-85.
19. Mizushima T, Ochi K, Koide N. Metabolic disorders of patients with acute pancreatitis: carbohydrate, lipid and protein metabolic disorders. Nihon Rinsho. 2004;62(11):1989-92.
20. Werz O. 5-lipoxygenase: cellular biology and molecular pharmacology. Curr Drug Targets Inflamm. Allergy. 2002;1(1):23-44.
21. Li L, Liu Y-R,Gao S, Li J-F, Li S-S, Zhang D-D,Liu S, Li B, Zheng S-J, Duan Z-P,Qi M, Chen Y. Inhibition of 5-lipoxygenase pathway attenuates acute liver failure by inhibiting macrophage activation. J Immunol Res. 2014: 697560.
22. Frank B, Gottlieb K. Amylase normal, lipase elevated: is it pancreatitis? A case series and review of the literature. Am J Gastroenterol. 1999;94:463-9.
23. Li X, Cao Y, Liu Zh, Chen H, Mao H. The relationship between liver injury and serum levels of C-reactive protein and procalcitonin in patients with acute pancreatitis. J Coll Phys Surg Pak. 2019;29(3):287-9.
24. Dai M, Fan Y, Pan P, Tan Y. Blood urea nitrogen as a prognostic marker in severe acute pancreatitis. Dis Markers. .2022:7785497.
25. Zhou H, Mei X, He X, Lan T, Guo Sh. Severity stratification and prognostic prediction of patients with acute pancreatitis at early phase: A Retrospective study. Medicine (Baltimore). 2019;98(16):e15275.
26. Qiu L, Luo Y, Chen X. Quercetin attenuates mitochondrial dysfunction and biogenesis via upregulated AMPK / SIRT1 signaling pathway in OA rats. Biomed Pharmacother. 2018;103:1585-91.
27. Weber H, Jonas L, Wakileh M, Krüger B. Beneficial effect of the bioflavonoid quercetin on cholecystokinin-induced mitochondrial dysfunction in isolated rat pancreatic acinar cells. Can J Physiol Pharmacol. 2014;92(3):215-25.

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