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Research Article

Combined anti-mediator therapy for severe destructive forms of acute necrotizing pancreatitis in rats

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Abstract

Introduction: Inflammatory mediators play a major role in pathogenesis of acute pancreatitis with TNF (tumor necrosis factor) as the most important one. Development of effective combined therapy could help to decrease tissue damage, improve results and, finally, diminish the mortality rate in this severe pathology.

Materials and methods: All the studies were performed on 120 female white Wistar rats, weighing 250±25g. Acute pancreatitis reproduced by an intracanalicular injection of bile salts compound.

Results and discussion: The data obtained in the course of the study on the pronounced pancreatoprotective effect of infliximab are explained by its key role in the onset of the systemic inflammatory response, and, therefore, with the blockade of tumor necrosis factor alpha in the early stages, there is no pronounced secondary damage to the pancreas, which is reflected in a significant decrease in edema from 4.87±0.03 in the model up to 2.75±0.04, and as a consequence, an improvement in the blood supply of the acinar tissue from 182.38±15.92 PU up to 287.92±14.64 PU, which is expressed in a decrease in the zones of necrosis and in a decrease in mortality and, finally, efficiency coefficient from 13480.000 to 4283.348. A selective blocker of cysteinyl leukotrienes has a less pronounced protective reaction against damage to pancreatocytes, but to a much greater extent than octreotide. That is expressed by changes in the efficiency coefficient to the level of 8621.18 in montelukast group and 12767.30 in octreotide group, respectively. On the other hand, the effect of the use of infliximab does not surpass that of montelukast, and their combined use has a pronounced additive effect, which is proved by the efficiency coefficient at the level of 2390.33. This reaction is explained by the fact that TNF alpha-mediated pathway of activation of leukotriene biosynthesis is the main, but not the only one.

Conclusion: The combined anti-mediator therapy provides a great opportunity to improve the standard therapy of acute pancreatitis.

Keywords

acute pancreatitis, anti-mediator therapy, leukotrienes, TNF.

Introduction

It is difficult to find a more complicated inflammatory disease of the abdominal organs than acute pancreatitis in terms of its pathogenesis. This pathology is included in a group of diseases with the similar peculiarities of medical care, called "acute abdomen", which consistently ranks 2–3 in this group, along with acute cholecystitis. According to the World Health Organization, acute pancreatitis affects 200 to 800 people per million of the world population. The incidence of acute pancreatitis in the Russian Federation ranges from 36 to 40 cases per 100,000 population. The prevalence of destructive forms is currently 15–20% of all cases, respectively, so from 75 to 80% of the cases occur in the so-called abortive or edematous form. The development of destruction of pancreatic tissue is a formidable complication with a mortality rate of up to 80% (Goodchild et al. 2019; Leppaniemi et al. 2019; Li et al. 2019; PanWessex Study et al. 2019).

The starting point in the formation of a modern view of the pathogenesis of acute pancreatitis was the Chicago Consensus Conference, which classified it as a disease proceeding according to the mechanisms of systemic inflammatory response (SIRS) but differing from the rest by the mechanisms of the initial phases.

Based on this theory, many pharmacological targets have been identified.

Drugs from the group of selective and non-selective blockers of cyclooxygenase (COX) have been used to treat inflammatory diseases for a long period of time, and therefore an attempt to use pharmaceuticals in this group has become logical. In the course of studies, ambiguous effects of their influence on the course of acute pancreatitis were negative in some cases (Foitzik et al. 2003; Mennecier et al. 2007). The unsatisfactory results of the use of COX blockers prompted researchers to study in depth other metabolites of arachidonic acid. Certain experimental efficacy was determined in the study of the receptor blocker to tromboxan seratrodast (Hirano and Hirano 1999).

Two of the few drugs, recommended for using in the national clinical guidelines of the last iteration for the treatment of destructive forms of acute pancreatitis, are somatostatin and its analogue octreotide. However, in the draft of a next edition of the national clinical guidelines, this drug is absent, which, in all likelihood, is due to its low (statistically proven) efficacy.

Correction of damaged endothelial cells could provide us another approach in pharmacological correction (Pokrovskii et al. 2009; Danilenko and Pokrovskiy 2014; Denisuk et al. 2015; Denysiuk et al. 2015).

In the course of numerous studies, the participation of other eicosanoids in the implementation of pathological processes in the pancreas and extrapancreatic tissues in acute pancreatitis – leukotrienes – was established. Determination of leukotrienes as one of the key factors of aggression in pancreatic necrosis made it possible to substantiate the experimental use of a sufficiently large pool of antileukotriene drugs, which are mostly used for the pharmacotherapy of bronchial asthma (Dahinden et al. 1988; van Ooijen et al. 1988; Hotter et al. 1995; Wang and Li 2003; Gureev et al. 2015; Li et al. 2018).

Despite encouraging experimental studies, antileukotriene therapy for acute destructive pancreatitis has not yet found its reflection in clinical use. This is possibly due to the fact that all eicosanoids, and leukotrienes in particular, are secondary mediators of systemic inflammation, and the blockade of the action of one secondary factor, even though one of the most significant ones, cannot completely block the negative effects of others and, as a consequence, yielding moderate results use in a clinical setting.

In this connection, the blockade of primary factors, or starting inflammatory mediators, or in other words, the first generation inflammatory mediators, is of the greatest interest. One of these starting mediators of inflammation is tumor necrosis factor alpha (TNF-alpha), the involvement of which in systemic inflammation in pancreatic necrosis has been repeatedly proven.

The study of the possible use of a new group of drugs – monoclonal antibodies to this inflammatory trigger – is also of great interest.

A number of studies have shown the effectiveness of monoclonal antibodies to TNF-alpha in acute pancreatitis. However, when analyzing these studies, it was revealed that the study of this drug was carried out on "mild" models of acute pancreatitis with low mortality, which is most often reflected in the foreign literature as mild necrotizing pancreatitis. In this connection, the issue of extrapolating the results of these studies to clinical conditions is controversial.

Therefore, it was relevant to study the effectiveness of anti-mediator therapy for acute pancreatitis in moderate and severe cases.

Aim of the study: to increase the efficiency of pharmacological correction of pathological changes in the pancreas in experimental pancreatic necrosis.

Materials and methods

The experiment was performed at the Research Institute of Pharmacology of Living Systems of Belgorod State National Research University according to *The Rules of Laboratory Practice*, approved by Order No.199н of the Ministry of Health of the Russian Federation of 01.04.2016, and in strict compliance with *The European Convention for the Protection of Vertebrate Animals Used for Experiments or for Other Scientific Purposes* (Directive2010/63/EU). The experimental studies were approved by the Bioethical Commission of Belgorod State National Research University. Vivisection was performed in compliance with the ethical principles of treating laboratory animals outlined in *The European Convention for the Protection of Vertebral Animals Used for Experimental and Other Scientific Purposes. CETS No.123*. The study was approved by the Ethics Committee of Belgorod State National research University (No. 124 14.11.2017).

Experimental animals

All studies were performed on 120 female white Wistar rats, weighing 250±25 g, without any external signs of the disease, which had passed the quarantine period for 10–14 days. During the experiment, the rats were kept in a standard experimental biologically clean room; the air temperature was 22–24 °C, light pattern – 12 h/12 h light/dark cycle; all animals received granulated food and filtered water.

Simulation of severe acute necrotizing pancreatitis in rats

The most physiological models, based on the etiopathogenesis of more than 80% of all cases of acute pancreatitis, are canalicular-hypertensive.

The complexity of the simulation of acute pancreatitis in rats by the introduction of various solutions is due to the small size of the structures that have to be cannulated. In this connection, we used a Leica microscope for visualization.

The simulation was performed as follows: under general anesthesia, zoletil at a dose of 50 mg/kg together with chloral hydrate at a dose of 125 mg/kg was administered intraperitoneally; after the treatment and fencing off the operating area, the abdominal cavity was opened layer by layer. A loop of the duodenum was brought out into the wound. During transilumination, the main duodenal papilla was identified. Finding the papilla opening is a big problem.

After finding the ampulla and the orifice of the main duodenal papilla, a 32G needle was used to perforate the anterior duodenal wall, 0.5–0.6 cm away from the papilla. The common bile duct was cannulated with a 36G cannula.

In order to simulate severe destructive pancreatitis, isolating clips were applied to the cannula, distal to the duodenal duct junction, and to the common bile duct, so that the injected solution would completely enter the pancreatic ducts.

Failure to comply with this condition threatens, on the one hand, with the discharge of the solution into the duodenum, and on the other, with an excess amount of solution entering the common bile duct, which performs the function of the gallbladder in the rat, dampening excess pressure and accumulating a significant amount of bile.

After isolation, 0.5 ml of a buffer solution containing a bile acid salt – sodium taurocholate –

was injected into the pancreatic ducts. The insulating clips were removed 1 minute after injection, allowing the solution to penetrate deeper into the pancreatic parenchyma. At the next stage, the clips were removed, the cannula was removed from the ducts, the abdominal cavity was sutured tightly layer by layer.

The most accurate integral indicator for assessing the effectiveness of a particular treatment method is the mortality rate over the study period. For the model of acute destructive pancreatitis used in the study, all deaths occurred within 12–24 hours. When choosing a pathology model to assess the effectiveness of the treatment, it is necessary not only to be guided by the etiology and pathogenicity of the model. It is necessary to adapt the model to the goals and objectives of the study. In this case, none of the animals in the model group survived 24 hours. After the death of the animals, an autopsy was performed, and the material was taken for morphological examination in order to establish the cause of death.

Pancreatic edema study

The early stages of acute pancreatitis are characterized by the development of the so-called vitreous edema of the pancreas. In order to measure the size of this edema, we used the calculation of the Wet/Dry ratio, which is more suitable than the Evans blue extraction for assessing the size of edema of the pancreatic tissues at the early stages, characterizing the disruption of the permeability of the vascular wall for low molecular weight compounds and liquid.

Studying the Wet/Dry ratio is especially sensitive to small details during material collection, sample preparation, and drying.

Despite the fact that the ratio of the ex vito preparation and the dried one was investigated, we tried to minimize the error by carefully isolating the pancreatic tissue and separating it from the parapancreatic tissues, paying special attention to the tissue array, mainly with fiber in the region of the intestinal mesentery root. After removing the preparation of the pancreas, the latter was soaked in order to remove peritoneal exudate, which is usually in abundance, weighed and placed in a Petri dish. Drying took place at 56 °C until the weight stabilized, but at least 2 days. Then re-weighting was carried out.

We measured the Wet/Dry ratio as the ratio of the weight of the pancreas immediately after collection to the weight of the pancreas after drying.

Pancreatic perfusion study

To assess microcirculation, we used equipment manufactured by Biopac systems: a polygraph with an MP150 module for recording volumetric blood flow by means of laser Doppler flowmetry and an invasive needle probe TSD144.

The data were recorded and processed using the AcqKnowledge 4.2.0, the microcirculation values were expressed in perfusion units (PU).

The study of microcirculation in the rat pancreas presents a certain problem due to the different perfusion rates in tissue areas and zones that are unequally distant from the central blood flow. Microcirculation was examined during surgery: after a midline laparotomy, the stomach with the pancreas was taken out into the operating area, and in order to minimize the possibility of recording the central blood flow, a point was selected which was located 1.5 cm from the third segmental branch of the splenic artery, with special attention paid to maintaining the serous

membrane in a moist condition (Emelyanov et al. 2007). Attention was also paid to maintaining the ambient temperature at 36 °C throughout the study, which reduced the likelihood of recording the erroneous data resulting from a peripheral vascular spasm due to the low temperature. In order to avoid the registration of erroneous data caused by micromovements of the hands, a sensor positioning system was used which allowed simultaneously standardizing a degree of pressure of the sensor on the tissue, which determines a decrease in reliability associated with the compression of the vessels of the microvasculature.

While processing by AcqKnowledge 4.2.0 GLP, the min value (minimum) was taken into account, which made it possible to avoid taking into account the recorded micromovements of tissues relative to the sensor, caused by tremor of the animal, respiratory movements, and also to reduce the possibility of recording data from the central blood flow.

Morphological study

After an autopsy, the macroscopic picture was assessed by determining the severity of pancreatogenic peritonitis, namely the presence or absence of exudate in the abdominal cavity and its nature, the presence, size and type of necrotic changes in the pancreas and parapancreatic tissue.

To assess the macroscopic picture of lung damage, thoracotomy was performed, followed by assessing the presence and nature of pleural effusion, the intensity and nature of macroscopic changes in the lungs, liver, and kidneys.

After macroscopic assessment, the material was collected from the pancreas, liver, kidneys, and lungs for the purpose of histological examination. The organs were collected, weighed, and fixed in a 10% solution of neutral formalin. After fixation, the tissue sections were washed in running water, dehydrated and embedded in paraffin according to the standard technique. Paraffin sections of the pancreas, 5–7 microns thick, were prepared at standard intervals from all the parts of the organ. The sections were stained with hematoxylin and eosin.

Histological processing was performed using Leica equipment (Germany).

Determination of the pancreatic lesion volume

The volume of the lesion in the pancreas was calculated by computing the ratio of the necrotic area to the area of the unaffected part of the pancreas, on digitized computer images, which had been obtained using a digital microscope. The ratio was expressed as a percentage.

Study design. The mode of administration and doses of pharmacological agents

All the studies were conducted in compliance with the ethical standards of handling laboratory animals under general anesthesia.

General anesthesia was performed by intraperitoneal administration of zoletil at a dose of 60 mg/kg and chloral hydrate at a dose of 125 mg/kg.

A model of acute pancreatitis was simulated by intracanalicular injection of 0.5 ml of a buffer solution containing sodium taurocholate.

Compounds under study

Infliximab, Remicade (MSD), was administered intraperitoneally 1 hour before simulating acute pancreatitis at doses of 10, 60 and 120 mg/kg.

Montelukast, Singular (MSD), was administered intragastrically via a tube at doses of 0.6 mg/kg, 0.9 mg/kg, 1.8 mg/kg twice: 25 hours and 1 hour before simulation.

Octreotide (Pharmsynthez CJSC) was administered at a dose of 17.14 μg/kg 2 hours before the laparotomy, and then at a dose of 17.14 μg/kg 3 times a day.

Doses and modes of administration of the studied substances are based on the interspecies scaling coefficients in experimental studies.

Evaluation of the effectiveness of the studied drugs on the model of acute pancreatitis was carried out by determining the daily lethality rate, a degree of pancreatic edema starting at the 6th hour after the moment of acute pancreatitis simulation, expressed in a Wet/Dry ratio and the volume of pancreatic lesions in percentage terms.

All the experimental animals were divided into the following groups:

A. Infliximab intraperitoneally 1 hour before simulating acute pancreatitis at a dose of 30 mg/kg once.
B. Infliximab intraperitoneally 1 hour before simulating acute pancreatitis at a dose of 60 mg/kg once.
C. Infliximab intraperitoneally 1 hour before simulating acute pancreatitis at a dose of 120 mg/kg once.
D. Montelukast intragastrically at a dose of 0.6 mg/kg, twice: 25 hours and 1 hour before simulation.
E. Montelukast intragastrically at a dose of 0.9 mg/kg, twice: 25 hours and 1 hour before simulation.
F. Montelukast intragastrically at a dose of 1.8 mg/kg, twice: 25 hours and 1 hour before simulation.
G. Infliximab intraperitoneally 1 hour before simulating acute pancreatitis at a dose of 60 mg/kg once and montelukast intragastrically at a dose of 0.9 mg/kg, twice: 25 hours and 1 hour before simulation.
H. Octreotide intraperitoneally at a dose of 17.14 μg/kg 2 hours before laparotomy, and then at a dose 17.14 μg/kg 3 times a day.

General anesthesia was performed by intraperitoneal injection of zoletil at a dose of 60 mg/kg and chloral hydrate at a dose of 125 mg/kg.

Acute pancreatitis was simulated by intracanalicular injection of 0.5 ml of a buffer solution containing sodium taurocholate.

The mortality rate was assessed 24 hours after the simulation. The animals were removed from the experiment at the point 6 hours after the start of simulation.

A degree of pancreatic edema was assessed by the method of determining the Wet/Dry ratio of 6 hours after the start of simulation.

Statistical data processing

Descriptive statistics was applied to all the data: data were checked for normality of distribution. The distribution type was determined by the Shapiro-Wilk test. In the case of a normal distribution, the mean (M) and standard error of the mean (m) were calculated. The outstanding values in the animals at each time point were identified using the statistical Grubbs test. If for any value, Z exceeded the critical value for a given number of measurements N, this experiment was excluded from further calculations. In cases of abnormal distribution, the median (Me) and quartile range (QR) were calculated. Intergroup differences were analyzed by parametric (Student's t-test) or nonparametric (Mann-Whitney test) methods, depending on a type of distribution. Differences were determined at the 0.05 level of significance. A statistical analysis was performed using Statistica 10.0 software.

Results and discussion

Study of effectiveness of the pancreatoprotective action of infliximab in acute destructive pancreatitis

When simulating acute pancreatitis, the mortality rate was 100% 24 hours into the simulation.

The study of morphological parameters, the Wet/Dry ratio, was carried out for a period of 6 hours from the moment of acute pancreatitis simulation.

So, 6 hours after the moment of simulating acute pancreatitis, during the opening of the abdominal cavity, there is a large amount of hemorrhagic exudate localized in all areas. The development of acute destructive pancreatitis is accompanied by the destruction of the pancreatic stroma. The parietal and visceral peritoneum are edematous and hyperemic in almost all sections, with a pronounced edema of the mesentery of the intestine.

The liver is not enlarged, but with traces of venous congestion, smooth surface and sharp-edged lobes. The spleen is full of blood.

The greatest changes affect the pancreas, which is sharply edematous and with areas of hemorrhagic pancreatic necrosis. The retroperitoneal tissue is edematous. In the chest there is a hemorrhagic effusion with hyperemia of the parietal and visceral pleura; in the basal parts of the lungs there are punctuate hemorrhages.

Pancreatic edema

During the study, it was found that in the intact Wet/Dry group, the coefficient was 1.72±0.04.

One hour after the moment of simulation in group B, there was an increase in the Wet/Dry ratio to the level of 3.63±0.08($p<0.05$), which indicates a sharp increase in pancreatic edema, which is visible. Three 3 hours after the moment of acute pancreatitis simulation, the Wet/Dry ratio increased to the level of 4.28±0.04 ($p<0.05$) and continued to increase to the level of 4.87±0.07 ($p<0.05$) by the point of 6 hours after the simulation.

In the course of the study, it was revealed that the rate of volumetric perfusion expressed in perfusion units in the intact animals after 2-day fasting was at the level of 418.09±39.11 PU.

When simulating severe acute destructive intracanalicular pancreatic necrosis, the blood flow velocity in the microvasculature of the pancreas decreased to the level of 182.38±15.92 PU ($p<0.05$).

Pancreatic perfusion

One of the most accurate and reliable research methods that made it possible not only to verify the presence of necrotic lesions of the pancreas, but also to predict its appearance in the future development of interstitial changes is the blood flow velocity in the microvasculature, and the most common way to measure it at present is the method of laser Doppler flowmetry.

When simulating severe acute destructive pancreatitis, 6 hours after the moment of simulation, the perfusion rate dropped to the level of 182.38±15.92 PU ($p<0.05$), which further led to the development and progression of necrotic lesions.

Morphology

Six hours after the moment of simulating acute destructive pancreatitis, there were typical changes in the type of macrofocal hemorrhagic pancreatic necrosis. The zone of necrotic lesion in the duodenal part of the pancreas was at least half when determining the ad oculus and was localized mainly closer to the wall of the duodenum, spreading along the duct.

The largest lesion in size was observed in the gastro-splenic part of the gland, manifested by coagulation necrosis of acinar cells, fibrinoid necrosis of the vascular walls and stromal elements, partial necrosis of the acini and severe neutrophilic infiltration with a predominant centrilobular lesion.

When determining the total volume of the lesion of the pancreas, it was found that 6 hours into the simulation, it was 34.8%±1.2%.

Doses selection study

Determination of the Wet/Dry ratio along with perfusion in the study groups served as screening methods to establish the effective dosage of the drug; further, a certain dosage was used.

The use of monoclonal antibodies to the tumor necrosis factor infliximab at a dose of 30 mg/kg one hour before the simulation of acute biliary pancreatitis led to a decrease in the Wet/Dry to a level of 3.21±0.04 ($p<0.05$) by the first hour after simulation. By the 3rd hour into the simulation, the level of edema expressed as the Wet/Dry ratio decreased to 3.97±0.09 ($p<0.05$), and by the 6th hour – to 4.39±0.06 ($p<0.05$).

An increase in the infliximab dosage to a level of 60 mg/kg one hour before the simulation of acute biliary pancreatitis led to a pronounced decrease in the Wet/Dry ratio to a level of 2.52±0.05 ($p<0.05$) by the 1st hour, 2.64±0.03 ($p<0.05$) and 2.75±0.04 ($p<0.05$) by the 3rd and 6th hours, respectively.

The most protective effect of monoclonal antibodies to tumor necrosis factor infliximab against pancreatic edema in acute ductal necrotizing pancreatitis is expressed at a dose of 120 mg/kg. One hour into the simulation, the edema of the pancreas was expressed 1.53 times less than in the control group, and 3 hours and 6 hours into the simulation – it was 1.73 and 1.85 times less, respectively. When compared with a dosage of 60 mg/kg, a decrease in edema was less pronounced when compared with the control group – 1.44; 1.62; and 1.77 times, respectively.

Due to the slight differences between group D and group E, it was decided to continue to use a dosage of 60 mg/kg.

For clarity, the data of pancreatic edema in acute destructive pancreatitis are presented in the table below (Table 1).

Table 1. Infliximab Effect on the Degree of Pancreatic Edema Measured in Wet/Dry Index in Destructive Experimental Pancreatitis (M±m; n=12), U

Group	Hours from the moment of simulation		
	1	3	6
Acute Destructive Pancreatitis Model	3.63±0.05	4.28±0.04	4.87±0.03
Correction with infliximab, 30 mg/kg	3.21±0.04 *	3.97±0.09*	4.39±0.06*
Correction with infliximab, 60 mg/kg	2.52±0.05*	2.64±0.03*	2.75±0.04*
Correction with infliximab, 120 mg/kg	2.37±0.03*	2.46±0.04*	2.62±0.03*

Note: * $p<0.05$ – in comparison with the control group.

Morphology

In the course of the study, it was revealed that damage to the pancreas when using MAB infliximab at a dose of 60 mg/kg led to a pronounced positive protective effect, which was reflected in a decrease in mortality by the 1st day to 50%.

The positive protective effect from the action of monoclonal antibodies to tumor necrosis factor at a dose of 60 mg/kg 1 hour before the simulation of acute destructive pancreatitis is manifested in a decrease in interstitial edema during a microscopic examination, a decrease in the zones of centrilobular necrosis and a decrease in zones and the degree of infiltration by polymorphonuclear leukocytes.

When determining the total volume of pancreatic necrosis zones against the background of the use of infliximab at a dose of 60 mg/kg, it was found that the use of monoclonal antibodies to tumor necrosis factor in acute destructive pancreatitis by the 6th hour after the moment of the simulation leads to a decrease in the lesion area from 34.8%±1.2% in the control group to 21.3%±1.4% ($p<0.05$) in the group using infliximab.

Thus, infliximab has a pronounced protective effect in acute pancreatitis, reducing the volume of the lesion and halving the lethality by the 24th hour. Moreover, the most effective dosage is a dose of 120 mg/kg; however, due to statistically insignificant differences between the studied groups D and E, the dosage of 60 mg/kg is considered effective.

Study of the effectiveness of the pancreatoprotective action of montelukast in acute destructive pancreatitis

The use of montelukast, a selective blocker of cell receptors for cysteinyl leukotrienes (Singular MSD), intragastrically at a dose of 0.6 mg/kg twice: first 25 hours before the simulation and again 60 minutes before the simulation – led to a decrease in the Wet/Dry ratio to the level of 3.21±0.04 ($p<0.05$).

By the 3rd hour after the start of the simulation, with the action of montelukast, a blocker of cell receptors for cysteinyl leukotrienes (Singular MSD), intragastrically at a dose of 0.6 mg/kg twice: first 25 hours before the simulation and again 60 minutes before the simulation, there was a decrease in the Wet/Dry ratio to the level of 4.12±0.05 ($p<0.05$).

Pancreatic edema

The period of 6 hours after the start of acute pancreatitis simulation was characterized by a We/Dry ratio of 4.63±0.03 ($p<0.05$).

When montelukast was used in study group G at a dose of 0.9 mg/kg intragastrically according to the scheme described above, there was a decrease in the Wet/Dry ratio to the level of 2.93±0.04 ($p<0.05$) by the 1st hour after the start of the simulation.

By the 3rd hour after the start of acute pancreatitis simulation, pancreatic edema increased to the level of 3.25±0.05 ($p<0.05$); however, it was significantly lower than in the control group and the group with the study of the drug at a dose of 0.6 mg/kg, but, higher than in the group using infliximab at a dose of 60 mg/kg.

By the 6th hour after the start of the simulation, the degree of pancreatic edema measured by the method of determining the Wet/Dry ratio was 3.87±0.04 ($p<0.05$), which was lower than in the control group B, but significantly higher than in the studied group D by this time point (Table 2).

In the study group H with the use of montelukast at a dose of 1.8 mg/kg, the degree of pancreatic edema, when simulating acute pancreatitis бин the 1st hour was at the level of 2.89±0.05 ($p<0.05$).

Three hours into the simulation, there was an increase in the Wet/Dry ratio and, consequently, in the degree of pancreatic edema in acute destructive pancreatitis to the level of 3.2±0.03 ($p <0.05$).

Six hours after the simulation of acute destructive pancreatitis, pancreatic edema with using montelukast at a dose of 1.8 mg/kg continued to increase, despite a twofold increase in the dosage from 0.9 mg/kg and amounted to 3.74±0.05 ($p\geq0.05$).

Table 2. Study of the Effectiveness of the Effect of Montelukast on the Severity of Edema When Simulating Acute Destructive Pancreatitis (M±m; n=12), U

Group	Hours after the start of simulation		
	1	3	6
Acute Destructive Pancreatitis Model	3.63±0.05	4.28±0.04	4.87±0.03
Correction with montelukast, 0.6 mg/kg	3.32±0.05*	4.12±0.05*	4.63±0.03*
Correction with montelukast, 0.9 mg/kg	2.93±0.04*	3.25±0.05*	3.87±0.04*
Correction with montelukast, 1.8 mg/kg	2.89±0.05*	3.22±0.03*	3.74±0.05*

Note: * $p<0.05$ – in comparison with the control group.

Perfusion

In the study of the effect of montelukast, a blocker of cysteinyl leukotriene receptors, at a dose of 0.9 mg/kg, twice: first 25 hours before the simulation and again 60 minutes before the simulation of severe acute pancreatic necrosis – on the rate of volumetric perfusion in the microvasculature pancreas,the perfusion rate was found to restore to the level of 243.65±12.47 PU ($p<0.05$), which is displayed in Table 3.

Table 3. Effect of the Combination of Infliximab at a Dose of 60 mg/kg and Montelukast at a Dose of 0.9 mg/kg on the Perfusion of Pancreatic Tissue in Acute Destructive Pancreatitis in Comparison With Effect of Octreotide at a Dose of 17.14 μg/kg 2 Hours Before Simulation, and Then 17.14 μg/kg 3 Times a Day (M±m; n=12), PE

Group	6 hours after the start of simulation
Intact group	418.09±39.11
Acute Destructive Pancreatitis Model	182.38±15.92*
Correction with infliximab, 60 mg/kg	287.92±14.64 *,**
Correction with montelukast, 0.9 mg/kg	243.65±12.47 *,**
Correction with infliximab, 60 mg/kg + montelukast, 0.9 mg/kg	327.47±18.34*,**
Correction with octreotide at a dose of 17.14 μg/kg 2 hours before simulation, and then 17.14 μg/kg 3 times a day	191.43±18.51 *,***

Note: * $p<0.05$ – in comparison with the intact group; ** $p<0.05$ – in comparison with the control group (model); *** $p>0.05$ – in comparison with the control group (model).

Morphology

The use of montelukast, a selective blocker of cysteinyl leukotrienes, at a dose of 0.9 mg/kg twice: first 25 hours before the simulation and again 60 minutes before the simulation of acute destructive pancreatitis, reduced daily mortality by 16.6%. The research data on the effectiveness of the effect of montelukast on the severity of edema in simulating acute destructive pancreatitis are presented in Table 2.

A histological examination of micropreparations at the point of 6 hours after the start of simulation of acute pancreatitis revealed that the following changes in the pancreas were observed: edema of the stroma and parapancreatic tissue with polymorphonuclear leukocyte infiltration moderately expressed in the duodenal part and weakly expressed in the gastro-splenic parts. In the acinar tissue, single zones of necrosis are observed with a greater severity of destructive changes located on the border of the duodenal and gastro-splenic parts of the gland. As in the case of the control group, necrosis is of a centrilobular nature. Diffuse plethora of venous vessels is observed.

When studying the total volume of necrosis as a percentage when using montelukast, it was found that the introduction of this drug at a dose of 0.9 mg/kg twice: first 25 hours before the simulation and again 60 minutes before the simulation leads to a decrease in the degree of destructive lesion of the pancreas gland from 34.8%±1.2% ($p<0.05$) in the control group to 28.15±2.3% ($p<0.05$).

A study of the effectiveness of the pancreatoprotective action of a combination of infliximab at a dose of 60 mg/kg and montelukast at a dose of 0.9 mg/kg in acute destructive pancreatitis in comparison with octreotide

The use of a combination of montelukast, a selective blocker of cell receptors for cysteinyl leukotrienes (Singular MSD), intragastrically at a dose of 0.9 mg/kg twice: first 25 hours before the simulation and again 60 minutes before the simulation, and monoclonal antibodies to tumor necrosis factor Infliximab drug Remicade (MSD) intraperitoneally 1 hour before simulating acute pancreatitis at a dose of 60 mg/kg led to a pronounced decrease in mortality by the 24th hour into the simulation by 66.6%.

In the study of lethality in group J when using octreotide at a dose of 17.14 μg/kg (n=12) 2 hours before the simulation, and then 17.14 μg/kg 3 times a day to correct pathological changes when simulating acute destructive pancreatitis, there were no changes in mortality when compared to the control group – all the animals died, thus in this group before the end of the 1st day, the mortality rate was 100%.

When analyzing the results obtained, group H was characterized by lower severity of almost all the markers.

Pancreatic edema

The study of the Wet/Dry index by the 6th after the start of the simulation showed its decrease from 4.87±0.03 in the control group to 2.12±0.03 in group I treated with the combination of infliximab and montelukast, which is significantly lower than in group J treated with octreotide – 4.63±0.06, which is shown in Table 4.

Perfusion

When determining the effect of a combination of infliximab at a dose of 60 mg/kg and montelukast at a dose of 0.9 mg/kg twice compared with the effect of octreotide at a dose of 17.14 μg/kg 2 hours before the simulation, and then 17.14 μg/kg 3 times a day on the rate of volumetric blood flow in the microcirculatory bed of the pancreas when simulating a severe destructive lesion, it was found that in group I, perfusion was restored to the level

Table 4. Effect of the Combination of Infliximab at a Dose of 60 mg/kg and Montelukast at a Dose of 0.9 mg/kg on the Change in the Wet/Dry Index in Acute Destructive Pancreatitis in Comparison With Effect of Octreotide at a Dose of 17.14 μg/kg 2 Hours Before Simulation, and Then 17.14 μg/kg 3 Times a Day (M±m; n=12)

Group	6 hours after the start of simulation
Intact group	1.72±0.04
Acute Destructive Pancreatitis Model	4.87±0.03
Correction with infliximab, 60 mg/kg + montelukast, 0.9 mg/kg	2.12±0.03*
Correction with octreotide at a dose of 17.14 μg/kg 2 hours before simulation, and then 17.14 μg/kg 3 times a day	4.63±0.06**

Note: * p<0.05 – in comparison with the control group (model); ** p>0.05 – in comparison with the control group (model).

of 327.47±18.34 IU, which is 1.79 times higher than in the group of acute pancreatitis model at the study period and 1.71 (191.43±18.51 PU) times higher than in the group using octreotide as monotherapy at the test dosage. The dynamics of volumetric perfusion in the microvasculature of the pancreas in the studied groups is clearly shown in Table 3.

Morphology

During the pathomorphological study, a pronounced positive dynamics was observed, consisting in a significant decrease in stromal edema, manifested in a decrease in the dispersion of pancreatic lobules.

In most cases, necrosis was of a piecemeal centrilobular nature without pronounced infiltration by polymorphonuclear leukocytes. Parapancreatic tissue is moderately edematous without infiltrative elements. A characteristic feature of the group G was practically no intravascular ulceration with extensive thrombosis and plasmorrhage, which occurred in the control group. Such phenomena were occasional and did not significantly affect the emerging picture of pancreatic lesions.

The study of the pancreatoprotective ability of octreotide at a dose of 17.14 μg/kg (n=12) 2 hours before the simulation, and then 17.14 μg/kg 3 times a day with pathomorphological examination did not reveal any significant changes compared to the control group. Necroses were massive and tended to merge with extensive damage to the parapancreatic tissue, with pronounced infiltrative changes.

Edema of the pancreas was of so-called. glassy character, leading to significant dispersion of the lobules.

When studying the total volume of necrosis as a percentage while using montelukast in combination with infliximab according to the dosing mechanism described above, it was found that within 6 hours from the start of the simulation, the area of necrosis decreased to 14.7±1.6% from 34.8%±1.2% in the control group, whereas in the octreotide group, the area of necrosis was 34.1±1.3% (Table 5). The introduction of this drug at a dose of 0.9 mg/kg twice:first 25 hours before the simulation and again 60 minutes before the simulation led to a decrease in the degree of destructive lesion to the pancreas from 34.8%±1.2% in the control group to 28.15±2.3%.

Table 5. The Effect of the Combination of Infliximab at a Dose of 60 mg/kg and Montelukast at a Dose of 0.9 mg/kg on the Area of Necrosis When Simulating Acute Pancreatitis by the 6th hour After the Start of Simulation (M±m; n=12), %

Group	6 hours after stimulation
Model of Acute Destructive Pancreatitis	34.8±1.2
Correction with infliximab, 60 mg/kg	21.3±1.4*
Correction with montelukast, 0.9 mg/kg	28.1±2.3*
Correction with infliximab, 60 mg/kg + montelukast, 0.9 mg/kg	14.7±1.6*
Correction with octreotide at a dose of 17.14 μg/kg 2 hours before simulation, and then 17.14 μg/kg 3 times a day	34.1±1.3**

Note: * p<0.05 – in comparison with the control group (model); ** p>0.05 – in comparison with the control group (model).

Conclusion

Despite the achievements of recent years in the treatment of inflammatory diseases and the introduction of new generations of drugs, such as monoclonal antibodies to various cytokines, there have been neither significant changes in the therapy of acute pancreatitis, nor significant changes in the structure of mortality in acute pancreatitis. At a certain stage in the late 20th century and early 21st century, a certain contribution to the improvement of treatment results was made by the introduction of minimally invasive technologies and methods of treatment of gallstone disease and purulent-necrotic complications of acute destructive pancreatitis.

With the development of the SIRS theory, it became clear that it was not possible to be restricted to purely surgical methods in solving the problem of high mortality and a large number of complications of this pathology, and revealing triggers and mediators of systemic inflammation, and even more so the key ones underlying the disease, opened the way for determining these cytokines and their points application as pharmacological targets. It became clear that it would not be possible to improve treatment results without the use of anti-mediators.

An adequate assessment of the effectiveness of the action of one or another pharmacological drug at the stage of preclinical laboratory trialas is inconceivable without the correct informed choice of the model of the studied pathology, comparable to the goals and objectives of the study. An incorrect assessment and, as a consequence, the choice of a model, lead to distortion of the research results. In experimental pancreatology, this problem is of particular importance, since for a long time the method of the so-called cryodestruction, which consists in a rapid cooling of the selected area of the pancreas with an applicator cooled in liquid nitrogen. An interesting fact is that

inflammation does not occur in this case, and therefore an attempt to simulate acute destructive pancreatitis in this way is essentially evaporation, or the so-called vaporization, only by using low temperatures.

In the course of the analysis, it is necessary to touch upon metabolic models that do not lead to the development of severe necrotic lesions, and, accordingly, can be considered as an object of study of the effectiveness of anti-mediator drugs. This does not refer to severe alcoholic pancreatic necrosis, the standardization of which is extremely difficult for the main experimental animal, namely, the rat.

The search for the most adequate model of acute pancreatitis for the goals and objectives of our experimental study led us to the group of canalicular-hypertensive models of acute pancreatitis.

Next in line, but not in importance, we had to select us the most accurate markers and indicators of the course of the process. The most commonly used marker in emergency abdominal surgery is the level of amylasemia; however, since the time of the first Chicago Consensus Conference, it became clear that the level of fermentation cannot be an evaluative criterion and is used by clinicians as a screening criterion. If we analyze the experience of using different scoring systems, we can come to the conclusion that starting with the criterion of severity of pancreatitis according to the Ranson score and ending with complex systems, such as APACHE II and APACHE III, the attitude to them has changed from enthusiastic to very skeptical one. However, the comparability of pancreatic lesions and the number of negative outcomes remained unchanged both in the systems and in the results of postmortem examinations.

Based on the results of the logical analysis, we obtained 4 assessment criteria that make up a single set of interrelated elements, namely: a degree of edema, a level of pancreatic perfusion, the total volume of the pancreas lesion 6 hours after the start of the simulation of severe acute destructive pancreatitis and daily mortality as the resulting marker.

Mortality in the selected model one day after the start of the simulation was at the level of 100%, which indicates a peracute form of lesion often called fulminant. It is this form of the course of the disease that is a vivid representative of the systemic inflammatory response syndrome.

Destruction of the permeability of the vessels of the pancreas manifests in an increase in the degree of edema, which is reflected in an increase in the degree of Wet/Dry index from 1.72±0.04 in the intact group to 4.87±0.07 ($p<0.05$) after the start of simulating acute pancreatitis, which is 2.83 times higher than in the intact group.

The emerging severe interstitial edema leads to a sharp drop in the perfusion of the pancreatic tissue, and therefore, as another assessment criterion, we chose the volumetric perfusion rate measured by laser Doppler flowmetry, which decreased from 418.09±39.11 PU in the intact group to the level of 182.38±15.92 PU ($p<0.05$) 6 hours after the start of the simulation. A decrease in perfusion, in our opinion, is a direct reflection of an increase in edema.

The study of blood flow velocity suggests that the volumetric perfusion value of 182.38 PU is critical and determines the so-called secondary lesion, which is etiopathogenetically associated with the primary affect and, in the case of pancreatic necrosis, cannot be separated from it.

In connection with the above, to assess the degree of damage to the stromal and parenchymal elements of the pancreas, we introduced such an indicator as the total volume of the lesion, which is defined as the ratio of the "intact" gland to the volume of edematous-infiltrative-necrotic lesion. Six 6 hours after the start of the simulation, the volume of the total lesion of the pancreas was 34.8%±1.2%, which confirms a severe course of the necrotic process, and mainly centrilobular lesion testifies to the ductal genesis of pancreatic necrosis.

The basis for the formation of modern views and the development of new approaches to the treatment of inflammation was laid in 1991 during a joint conference of the American College of Pulmonologists and the Society for Critical Care Medicine of the United States. It was then that the concept was adopted for the first time, which determined further strategies in the development of methods for treating inflammatory diseases, namely, the systemic inflammatory response syndrome (Bone et al. 1992a; Bone et al. 1992b).

Subsequent studies in this area resulted in the provisions of the Chicago Consensus Conference, which defined pancreatic necrosis as a disease proceeding through the mechanisms of systemic inflammatory response syndrome (SIRS), but different from the others by the mechanisms of the initial phases (Hirota et al. 2004; Bhatia 2009).

The starting point in the implementation of SIRS is considered to be the entry of various triggers into tissues, which can be exo- and endotoxins, massive trauma and acute pancreatitis. These are three main etiological causes that mediate the release of inflammatory mediators.

The primary mediators of SIRS are tumor necrosis factor and interleukins 1, 6 and interleukin 8, as they are the mediators, which first entering the local bloodstream, and then the systemic one, mediate the production of pro- and anti-inflammatory mediators of the secondary generation, with a significant part of the secondary mediators being eicosanoids - the result of enzymatic conversion of eicosapentaenoic or arachidonic acid.

Despite the fact that the participation of TNF as a starting metabolite of SIRS and septic shock has been proven in many studies, at the stage of clinical trials, the effect of exposure was sharply reduced due to the prescription of the drug, unlike experimental models, after the onset of the disease (Abraham et al. 1998).

Based on the foregoing, the next step towards determining the role of CIS-LT in the pathogenesis of acute pancreatitis and the development of new methods of treatment was to study the course of the pathological process in conditions of blockade of receptors to CIS-

LT and the development of a new method of therapy for acute pancreatitis.

The central idea in our study was the use of anti-mediator drugs in order to correct pathological changes in severe damage to the pancreas. This approach is not exclusive; however, we have not discovered any studies of fulminant forms of pancreatonecrosis. We also aimed at enhancing the anti-mediator effect of the preparation of monoclonal antibodies to the tumor necrosis factor, since this mediator, although it is central, is far from being the only one in the formation of the inflammatory response.

When developing the research strategy, we pursued the idea of creating ideal conditions for the drug to realize its action potential. In this connection, in the course of our study, we investigated the pancreatoprotective activity of infliximab, which was injected intraperitoneally 1 hour before the simulation of acute pancreatitis at range of doses of 10, 60, and 120 mg/kg in different groups.

With the use of infliximab at a dose of 60 mg/kg, the mortality was at the level of 50%; with montelukast, an antileukotriene drug, at a dose of 0.9 mg/kg, the mortality was at the level of 0.83%, while in the model of acute pancreatitis mortality was 100% after 24 hours of the experiment. Octreotide was found to be incapable in the studied dose of 17.14 μg/kg (n=12) 2 hours before the simulation, and then 17.14 μg/kg 3 times a day to have any effect on mortality by the 24th hours, resulting in 100% mortality, while when administering the combination of infliximab and montelukast in the studied dose range of 60 mg/kg and 0.9 mg/kg, respectively, the mortality was 16.6%.

When comparing the effect of different doses of the drug on the development of pancreatic edema, it was found that a dosage of 120 mg/kg had the greatest effect; however, this dosage has slightly pronounced statistical differences from a dosage of 60 mg/kg.

In comparison with infliximab, montelukast, a selective receptor blocker for cysteinyl leukotrienes, administered intragastrically through a tube at doses of 0.6 mg/kg, 0.9 mg/kg, 1.8 mg/kg twice: first 25 hours before the simulation and again 60 minutes before the simulation had a less pronounced effect on the degree of pancreatic edema than infliximab, reducing the degree of edema to the levels of 4.63±0.03; 3.87±0.04, and 3.87±0.04.

However, the most intense effect is exerted by the combination of infliximab and montelukast in the studied dose range, which is especially noticeable when comparing the results with the reference drug octreotide 4.63±0.06, and is clearly shown in the diagram in Fig. 1.

The analysis shows a more pronounced anti-edematous effect of infliximab even in comparison with montelukast, a selective blocker of leukotriene receptors, in the studied dosages, which in itself is quite an interesting fact, though cysteinyl leukotrienes are a determining factor in the formation of edema in the focus of pancreatic inflammation, leading to increased permeability.

In our opinion, this effect is due to the fact that cysteinyl leukotrienes belong to the category of secondary factors and the direct realization of their pathological action is under the control of primary agents, such as tumor necrosis factor and interleukin-6.

Of importance is the revealed fact that the anti-edematous effect is enhanced with the combined use of monoclonal antibodies to the tumor necrosis factor infliximab at a dose of 60 mg/kg and montelukast, a selective blocker of cysteinyl leukotriene receptors, at a dose of 0.9 mg/kg. In our opinion, this effect is due to the fact that there are more mechanisms to control the formation of active

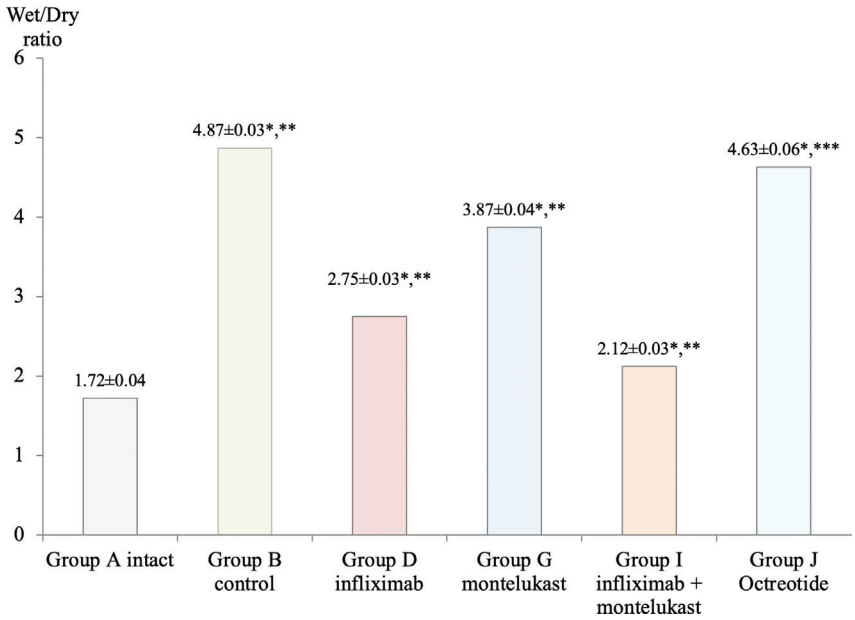

Figure 1. The effect of the combination of infliximab at a dose of 60 mg/kg and montelukast at a dose of 0.9 mg/kg on the change in the Wet/Dry ratio in acute destructive pancreatitis 6 hours after the start of simulation (M±m; n=12), Units. **Note:** * $p<0.05$ – in comparison with the intact group; ** $p<0.05$ – in comparison with the control group (model); *** $p>0.05$ – in comparison with the control group (model)

metabolites of arachidonic acid than those that are under the influence of tumor necrosis factor.

One could have assumed that we are dealing with an incomplete blockade of the action of tumor necrosis factor, but this assumption is not confirmed with an increase in the investigated dosage of infliximab. When using supramaximal dosages of monoclonal antibodies to tumor necrosis factor at a dose of 120 mg/kg, there were no significant changes in the degree of edema, examined by determining the Wet/Dry index. So in group D using a concentration of 60 mg/kg, this indicator was at the level of 2.75±0.04 ($p<0.05$), and with a two-fold increase in the concentration to 120 mg/kg, this indicator decreased to 2.75±0.04 ($p<0.05$).

However, even when using a combination of drugs, the Wet/Dry ratio does not reach the initial level of 1.72±0.04, which indicates the presence of an operative possibility to be corrected by other methods or drugs.

The next evaluative criterion to assess the effectiveness of the action of one factor or another, operative or conservative pharmacological action is an indicator that directly depends on the previous one – the rate of volumetric perfusion in the microvasculature pancreas.

This parameter is subject to significant fluctuations, both in the course of physiological and in the course of pathological changes occurring in the pancreas. During digestion, the perfusion rate can reach 1500 PU, which is comparable to the blood flow rate in the segmental arteries. The level of baseline perfusion was determined after 2 days of starvation of the animal – 418.09±39.11 PU (Fig. 2).

Back in the 1980s, the so-called interlobular edema was believed to play a role in the formation of secondary necrotic changes in the parenchyma of the organ, in connection with which a new method of treatment of the so-called interlobular drainage.

The forming dense interstitial edema acutely compresses the vascular elements of the acinar tissue, which leads to organ ischemia.

In the course of the study, we found that 6 hours after the start of the simulation, the volumetric perfusion rate determined by the laser Doppler flowmetry method falls by more than two times compared to the initial rate and reaches 182.38±15.92 PU ($p<0.05$).

Of interest is a higher perfusion rate in the case of using infliximab at a dose of 60 mg/kg in comparison with the perfusion rate in the group using montelukast, a blocker of cysteinyl leukotriene. Thus, the perfusion rate in group G was 243.65±12.47 PU ($p<0.05$), while in group D it was higher – at the level of 243.65±12.47 PU ($p<0.05$).

In our opinion, this dynamics is the result of less pronounced edema in group D, resulting from less compression of the lobular and acinar vessels and a higher rate of perfusion.

A higher blood flow velocity in the microvasculature of the pancreas was also naturally expected in the model of severe acute destructive pancreatitis 6 hours after the start of the simulation in the study of the effectiveness of the pancreatoprotective effect of the combination of monoclonal antibodies to tumor necrosis factor infliximab and montelukast, a cysteinyl leukotriene blocker, in the studied dose range.

Thus, perfusion in this group was at the level of 327.47±18.34 PU ($p<0.05$). As in the case of studying a degree of pancreatic edema by the method of determining the Wet/Dry , we noted there was some ground for using both conservative and other methods of exposure, since the level of perfusion did not reach the initial values of 418.09±39.11 PU.

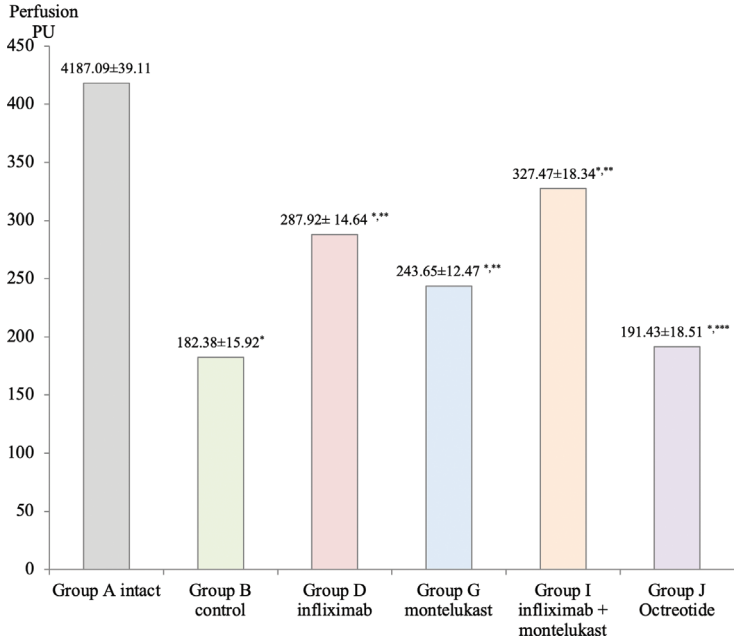

Figure 2. The effect of the combination of infliximab at a dose of 60 mg/kg and montelukast at a dose of 0.9 mg/kg on the perfusion rate in the microvasculature of the pancreas in acute destructive pancreatitis 6 hours after the start of simulation (M±m; n=12), %.
Note: * $p<0.05$ – in comparison with the intact group; ** $p<0.05$ – in comparison with the control group (model); *** $p>0.05$ – in comparison with the control group (model)

However, surgical methods of treatment at this stage proved not so much their ineffectiveness, but due to the aggressiveness of their impact (meaning interlobular drainage) in the acute phase led to a high percentage of both early complications in the form of bleeding and to long-term complications in the form of infecting the originally sterile foci of destruction of pancreatic tissue. The trend to decrease in the invasiveness of manipulations, the refusal from massive necrectomy at the early stages of the development of the pathological process, as well as the prevention of infection of pancreatic foci naturally led to a revision of views on the importance of such surgical interventions.

In the light of the above, the relevance of pharmacological correction of pathological changes in the pancreas at the early stages of the formation of severe necrotic lesions is of particular importance.

The control drug included in the list of drugs for pharmacological support in severe acute destructive pancreatitis, namely octreoted, had no significant effect on the blood flow rate in the microvasculature of the pancreas. Perfusion in this group (group J) was at the level of 191.43±18.51 ($p<0.05$).

We explain this fact by the lack of the possibility of implementing pharmacotherapeutic effects in such a narrow time frame of 6 hours. With a prolonged course of pancreatitis, octreotide can have an effect on the synthesis of new zymogenic granules, but cannot affect the amount of those already synthesized. The prescription of this drug for premedication will lead to a change in the structure of the acinar cell itself and, as a consequence, the failure to extrapolate the research results to other biological objects, in particular to humans.

Pancreatic edema and subsequent tissue ischemia during the formation of severe pancreatitis will inevitably affect the changes in the next efficiency criterion we are investigating – a degree of total lesion.

A method that makes it possible to assess a degree of lesion to the pancreas and thereby to determine the prognosis and effectiveness of treatment is a method of determining the areas of necrotic and infiltrative lesions on pancreas micropreparations pre-stained with hematoxillin-eosin.

Thus, the effect of infliximab at a dose of 60 mg/kg one hour before the simulation was characterized by a decrease in the areas of necrosis compared with the model of acute destructive pancreatitis to 21.3±1.4% from 34.8±1.2%, while the use of montelukast at a dose of 0.9 mg/kg reduced the lesion volume to 28.15±2.3%. The combination of infliximab and montelukast at doses of 60 mg/kg and 0.9 mg/kg, respectively, has the greatest protective effect, reducing the volume of destructive lesion to the pancreas by more than two times to 14.7±1.6%, whereas the drug recommended for clinical use – octreotide – showed no pancreatoprotective activity, and the area of necrosis, respectively, was 34.1±1.3%, which is shown in the diagram in Fig. 3.

When assessing the results of multivariate vector analysis, it is necessary to take into account the direction of the dynamics of the studied indicators in the course of the development of the pathological process. If the degree of edema, the volume of necrotic lesion and mortality increase during the development of acute destructive pancreatitis, then the rate of volumetric perfusion decreases sharply, in other words, the first three indicators are highly correlated, whereas the fourth one is inversely correlated. When calculating the areas of the diagrams, it is necessary to convert

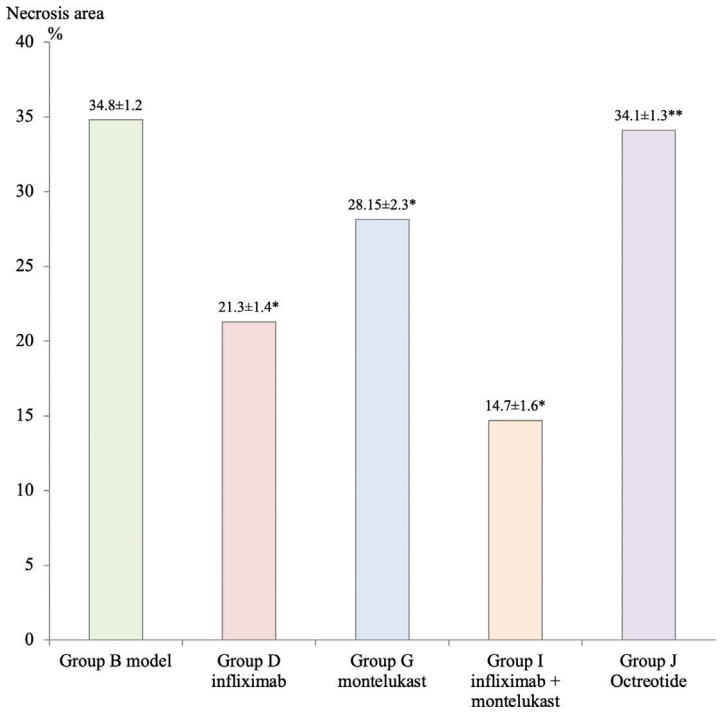

Figure 3. The effect of the combination of infliximab at a dose of 60 mg/kg and montelukast at a dose of 0.9 mg/kg on the area of necrosis in acute destructive pancreatitis 6 hours after the start of simulation (M±m; n=12), %. **Note:** *$p<0.05$ – in comparison with the control group (model); ** $p<0.05$ – in comparison with the control group (model)

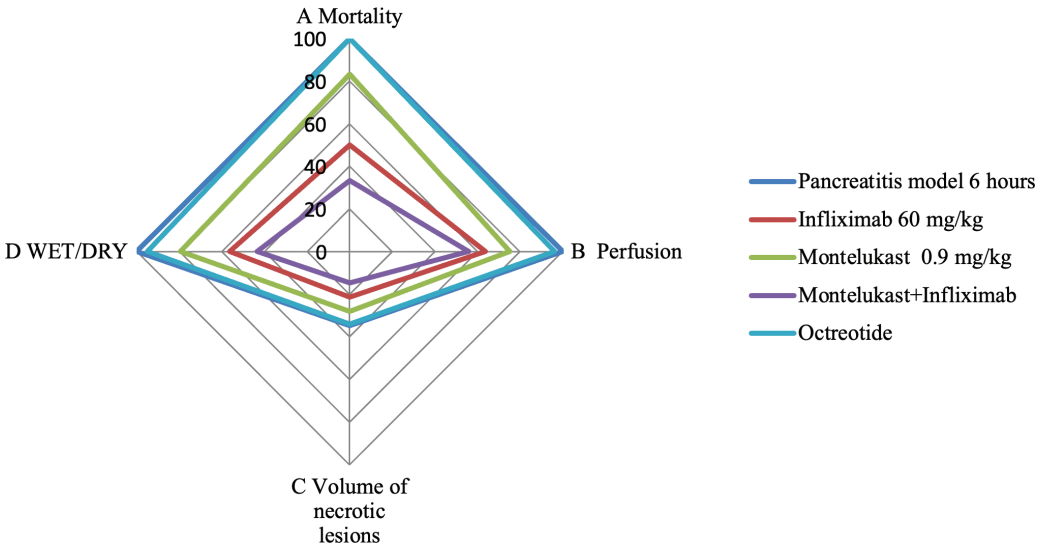

Figure 4. Radar plot showing daily mortality, perfusion of pancreatic tissue, edema and total volume of necrotic lesions of the pancreas in the study groups in comparison with the use of octreotide.

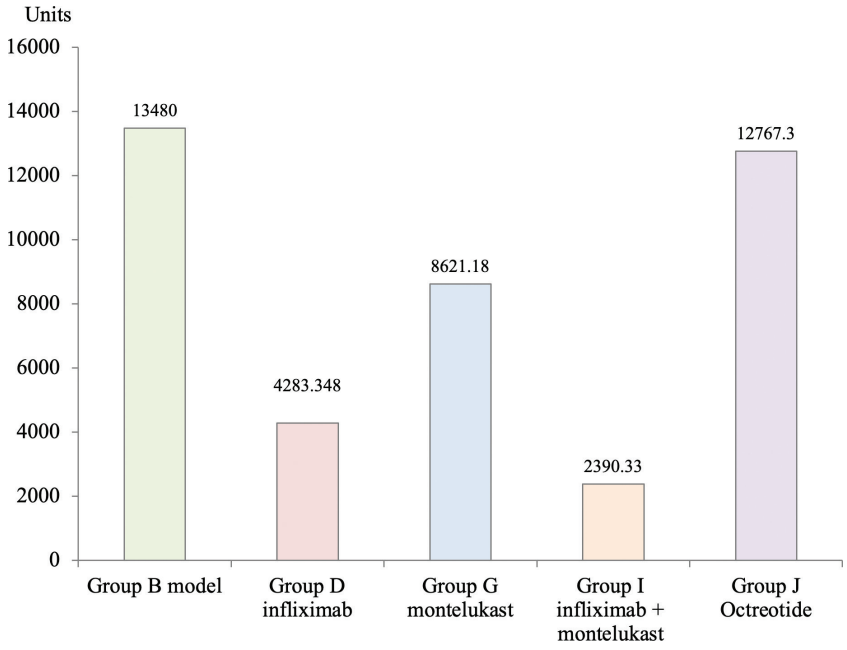

Figure 5. Efficiency coefficient of pathological changes correction in acute experimental pancreatitis with a combination of infliximab at a dose of 60 mg/kg and montelukast at a dose of 0.9 mg/kg 6 hours after the start of simulation

all the indicators to a single format, therefore, we recalculated the indicators along the 0B axis and the volumetric perfusion rate in the model of acute pancreatitis 6 hours after the start of the simulation was taken as 100% (Fig. 4).

To assess the effectiveness of the impact, we developed an efficiency factor that is inversely correlated to a degree of this impact and that is the area of the radar plot when conducting a multivariate vector analysis, i.e. the higher this coefficient, the lower the efficiency of correction of pathological changes occurring against the background of simulating acute severe destructive lesion to the pancreatic tissue. This ratio is shown in Fig. 5.

The analysis shows the minimum indicators of this coefficient in the group with a combination of infliximab at a dose of 60 mg/kg and montelukast at a dose of 0.9 mg/kg, where it was 2390.33 conventional units; the next most effective impact on the course of the pathological process in acute destructive pancreatitis was the use of infliximab, a preparation of monoclonal antibodies to the tumor necrosis factor, at a dose of 60 mg/kg, where this coefficient was 4283.348 conventional units. Monotherapy with montelukast, a blocker of cysteinyl leukotriene, at a dose of 0.9 mg/kg led to an increase in efficacy up to 8621.18 conventional units.

The use of octreotide did not lead to a significant decrease in the value of the coefficient determining the effectiveness of the pancreatoprotective effect, which was at the level of 12767.3 conventional units.

Conclusion

The data obtained in the course of the study on the pronounced pancreatoprotective effect of infliximab is explained by its key role in the onset of the systemic inflammatory response, and therefore, with the blockade of tumor necrosis factor alpha in the early stages, there is no pronounced secondary lesion to the pancreas, which is reflected in a significant decrease in edema, and as a consequence, in an improvement in the blood supply to the acinar tissue, which is expressed in a decrease in the zones of necrosis, resulting in a decrease in mortality. A selective blocker of receptors for cysteinyl leukotrienes has a less pronounced protective reaction against damage to the pancreatocyte, but to a much greater extent than octreotide. The less pronounced effect of montelukast in comparison with infliximab is explained by the fact that leukotrienes are secondary cytokines in relation to the starting cytokines and triggers, such as TNF can recruit other pathways of damage, not only lipoxygenase pathways. On the other hand, the effect of the use of infliximab does not exceed that of montelukast, and their combined use has a pronounced additive effect. This reaction is explained by the fact that TNF alpha-mediated pathway of activation of leukotriene biosynthesis is the main one, but far from being the only one. At the same time, the lack of effect from the use of octreotide can be explained by the fact that it affects the synthesis of new pancreatic enzymes, while the primary aggression affects the synthesis of enzymes to a small extent, involving the zymogen granules already present in the apical part in the colocalization process.

Thus, this fact does not close the window of opportunity for the use of octreotide in clinical practice, but rather specifies in more detail its prophylactic focus on the prevention of enzymemia at later stages by suppressing the development of the colocalization phenomenon in the long term.

Conflict of interest

The authors declare no conflict of interests.

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