**ANNOTATION**
**of the dissertation work**
**ABRAMOV GRIGORY ALEXANDROVICH**
**on the topic: "The influence of artificial local hypothermia on the dynamics of morphological changes and biochemical parameters of blood in an experimental model of pancreatic necrosis"**
**for the degree of Doctor of Philosophy (PhD)**
**in the specialty 6D110100 – «Medicine»**

Scientific consultants:
MD, professor K.T. Shakeyev
MD, professor M.M. Tusupbekova
Candidate of Medical Sciences, associate professor O.A. Ponamareva
MD, PhD J. Alberton

**Relevance of the problem.**

Despite the introduction of highly informative research methods in clinical practice for acute pancreatitis (AP) and pancreatic necrosis (PN), the use of effective methods of drug therapy including protease inhibitors, the frequency of unsatisfactory treatment results for this category of patients indicates that this problem has not been solved [Karakayali F. Y., 2014].

Even the use of minimally invasive laparoscopic surgical treatment methods did not reduce the frequency of fatal outcomes. Complex conservative therapy for acute forms of pancreatitis and pancreatic necrosis does not always stop destructive processes.

Analysis of literature data shows that the incidence of acute pancreatitis remains at the same level and varies from 20 to 80 patients per 100 thousand people per year. In recent years, there has been a worldwide trend towards an increase in the number of patients with acute pancreatitis, which ranks third among acute surgical diseases, second only to appendicitis and cholecystitis [Kurti F. et al., 2015; Munigala S., Yadav D., 2016]. According to various authors, destructive forms of acute pancreatitis are observed in 20-44% of patients, causing organ failure with a mortality rate of up to 30-47% [Borodin N. A. et al., 2015; Imaeva A. K., Mustafin T. I., Sharifgaliev I. A., 2014]. In the analysis of acute surgical diseases of the abdominal organs from 2002 to 2016 in the districts of the Central Kazakhstan region, the number of patients with acute pancreatitis is in 1st place and is 29.3%. At the same time, postoperative mortality up to 6 hours is 18.8%, from 6 to 24 hours 26.9%, after 24 hours 33.6%. [Buleshov M.A., 2017].

In many ways, the tactics for choosing conservative and surgical treatment of various forms of the disease remain debatable, individual therapeutic measures require a correct assessment of their effectiveness. The problem of mortality from destructive forms of acute pancreatitis is of a social nature, since the main contingent of these patients are patients of working age [Roberts S. E. et al., 2017 .; Vinnik Yu. S. et al., 2009].

Dissatisfaction with the results of AP treatment sets the task of searching for new drugs and treatment methods that can more effectively stop the progression of the destructive process in the pancreas. To date, there is no consensus on the effectiveness of somatostatin and its analogues in the treatment of acute pancreatitis. These drugs have

not found wide application, since they inhibit visceral blood flow and intestinal motility, and do not reduce the incidence of deaths in acute pancreatitis and pancreatic necrosis. Thus, somatostatin and its analogues were tested on several experimental models of AP, with contradictory, sometimes negative, and sometimes positive results. Moreover, in the conducted RCTs, which included 302 patients with severe acute pancreatitis, there was no significant difference in mortality, the frequency of complications from AP. All this indicates the fact that the use of artificial local hypothermia in severe forms of pancreatitis requires revision and new experimental and clinical justifications.

One of the methods of treating acute pancreatitis and pancreatic necrosis is artificial local hypothermia (ALH). Based on clinical and experimental experience, it has been proven that hypothermia of the pancreas inhibits the production of enzymes by the exocrine apparatus, which is a factor in interrupting the pathological process. This method has been well studied by V.I. Shaposhnikov et al., on the canalicular-hypertensive model of destructive pancreatitis in dogs, described by V.S. Saveliev. Hypothermia of the pancreas was produced by irrigation with ethyl chloride or by applying melting ice in animals with destructive pancreatitis of a day old. The thickness of the gland, the level of serum alpha-amylase, tyrspin-inhibiting activity, the level of cationic proteins of neutrophilic leukocytes before and after hypothermia were determined. After the completion of cooling, the temperature of the pancreas was measured. After measuring the pancreatic temperature, the animal was euthanized [Shaposhnikov V.I., 2013]. However, this work did not study the dynamics of the course of experimental pancreatitis. In the work of de Oliveira C. et al., a cerulein model of acute pancreatitis was reproduced in rats, then they underwent transgastric hypothermia. According to the results, in rats with acute pancreatitis, transgastric local hypothermia of the pancreas reduces the incidence of pancreatic necrosis, apoptosis, inflammation and markers of pancreatitis severity, and also increases survival [de Oliveira C. et al., 2019].

Thus, local hypothermia, as a targeted treatment method, leads to an interruption of the chain of the pathological process and a change in the clinical and anatomical picture of the disease, which can be recorded by morphological tests or laboratory test results. This work is devoted to the study of the effect of IPG on the course of experimental pancreatic necrosis, by assessing the dynamics of the morphological picture and changes in biochemical blood parameters. It differs from previously conducted works in that hypothermia is imposed by an independently developed method, intraoperatively immediately after the introduction of autobile into the parenchyma of the gland (the author's model of pancreatic necrosis). The dynamics of the course of experimental pancreatic necrosis under the influence of IPG is monitored during the first 2 days.

**The aim of the study** is to investigate the effect of artificial local hypothermia on the course of pancreatic necrosis in an experiment.

**Study objectives:**

1. To develop an experimental model of pancreatic necrosis in laboratory animals (rabbits) to study the effect of local hypothermia on the development of necrotic pancreatitis.

2. To conduct a comparative analysis of cooling agents in a bench experiment on biological tissues.

3. To develop a technique for the intraoperative use of artificial local hypothermia in laboratory animals.

4. To determine the effect of artificial local hypothermia on the course of experimental pancreatic necrosis based on morphological studies of pancreatic tissue.

**Scientific novelty**

A model of pancreatic necrosis in rabbits has been developed (certificate of entering information into the state register of rights to objects protected by copyright No. 7190 dated 23.12.2019 "Modeling pancreatic necrosis in an experiment");

The optimal cooling agent for applying intraoperative artificial local hypothermia has been determined (certificate of entering information into the state register of rights to objects protected by copyright No. 14050 dated 23.12.2020 "Selection of the most effective cooling agent by determining temperature field indicators in a bench experiment");

A technique for conducting intraoperative local hypothermia in an experiment has been developed (certificate of entering information into the state register of rights to objects protected by copyright No. 14953 dated 02/08/2021 "Methodology for conducting intraoperative artificial local hypothermia for pancreatic necrosis in an animal experiment");

The original model of pancreatic necrosis showed the effect of artificial local hypothermia on reducing the level of extracellular DNA in blood plasma.

**Practical significance of the work**

The developed model of pancreatic necrosis can be used in various experimental studies to study the main pathogenetic mechanisms of the disease and its complications.

The results of the study allow us to recommend a cold accumulator (carboxymethyl cellulose) as an effective and affordable cooling agent.

Based on the results of the study, it can be concluded that artificial local hypothermia can be used to abort the pancreatic necrosis process in an experiment.

**Main provisions submitted for defense**

1. The developed model of pancreatic necrosis by introducing autobile into the parenchyma of the pancreas is simple to implement, is performed with less surgical aggression on other tissues of the abdominal cavity, is well reproduced with obtaining a standard result.

2. The most optimal cold agent for assessing the therapeutic effect of hypothermia on the course of experimental pancreatic necrosis is the cold accumulator carboxymethylcellulose, due to its property to maintain a low temperature at the site of application for more than 15 minutes.

3. The technique of intraoperative application of artificial local hypothermia by immersing a cooling agent in the abdominal cavity with dynamic intra-abdominal thermometry is simple and effective, eliminates infection of the abdominal cavity due to the use of sterile containers, and the cooling procedure itself is completely atraumatic.

4. The imposition of artificial local hypothermia after the introduction of bile into the pancreatic parenchyma leads to the abortion of necrotic processes in the pancreas, regardless of the completion time of the experiment.

5. The use of artificial local hypothermia has a positive effect on the preservation of the regenerative potential of pancreatic tissues, assessed based on the absence of an increase in the level of extracellular DNA in the blood plasma of experimental animals.

**Implementation of research results**

The method of intraoperative artificial local hypothermia, an experimental model of pancreatic necrosis in rabbits was implemented at the Department of Biomedicine and in the shared-use laboratory of the Research Center of the Karaganda Medical University. The implementation of the results is confirmed by implementation acts.

**Work approval**

The main provisions of the study were presented at the scientific and practical conference of young scientists "PhD Scientific Day", dedicated to the 100th anniversary of the birth of B.A. Atchabarov (Karaganda, 2019); at a meeting of the Bioethics Committee (minutes No. 20, dated 17.06.2019).

**Publications**

8 scientific papers have been published on the topic of the dissertation, including: 2 publications in international scientific journals included in the Scopus information database on the date of publication; 3 publications in scientific journals of Kazakhstan recommended at the time of publication by the Committee for Control in the Sphere of Education and Science of the Ministry of Education and Science of the Republic of Kazakhstan; 3 certificates of entry of information into the state register of rights to objects protected by copyright.

**Materials and methods**

To achieve the purpose and objectives of the dissertation study, 63 laboratory animals were taken. The rabbits were divided into 4 groups. The first group included intact animals (n=9), which were necessary for intermediate control and equipment calibration. In the second group, pancreatic necrosis was modeled (n=18). The third group included animals with modeled pancreatic necrosis and artificial local hypothermia (n=18). The fourth group (Sham operation) consisted of rabbits that underwent abdominal dissection and suturing (n=18).

Depending on the time after the introduction of autobile into the pancreatic parenchyma, the groups were divided into subgroups (A, B, C). Pancreatic necrosis group: 12 hours - 6 rabbits (A), 24 hours - 6 rabbits (B), 48 hours - 6 rabbits (C); group of pancreatic necrosis with hypothermia: 12 hours – 6 rabbits (A), 24 hours – 6 rabbits (B), 48 hours – 6 rabbits (C); group sham: 12 hours – 6 rabbits (A), 24 hours – 6 rabbits (B), 48 hours – 6 rabbits (C).

In all groups and subgroups, blood was collected for biochemical analysis from the ear vein. Also, at the time of withdrawal from the experiment, the pancreas was collected for histological examination.

A bench experiment was conducted to select the most effective cooling agent for the purpose of creating artificial local hypothermia.

**Conclusions:**

1) The proposed experimental model of pancreatic necrosis, reproduced by introducing animal autobile into the pancreatic parenchyma, allows studying the pathogenesis and morphological substrate of the process at its different stages and is easily reproducible.

2) Cold accumulator (carboxymethylcellulose) is the most effective and accessible material for the imposition of artificial local hypothermia without the use of hardware. It has a long phase of temperature plateau assessed as a state of hypothermia, the temperature remains at +26.5 ° C most of the time, the effect of local cooling lasts more than 15 minutes.

3) The technique of intraoperative imposition of artificial local hypothermia by immersing a cooling agent in the abdominal cavity with dynamic intra-abdominal thermometry is simple and effective, eliminates infection of the abdominal cavity due to the use of sterile containers, and the cooling procedure itself is completely atraumatic.

4) Morphologically, it was determined that the use of artificial local hypothermia leads to an abortive course of pancreatic necrosis, if it is applied immediately after the introduction of autobile into the parenchyma of the pancreas of an experimental animal. A decrease in effusion was noted after 48 hours from the introduction of autobile from 8.55 ± 0.45 ml to 2.65 ± 0.35 ml ($p = 0.0039$), regression of gland edema from 1.47 ± 0.33 cm to 1.25 ± 0.55 cm ($p = 0.0036$). Wrinkling of the gland tissue becomes significantly less pronounced, changing from 3.35 ± 0.86 cm to 5.45 ± 0.31 cm ($p = 0.0039$). The effect of IPG on the preservation of the histological structure of the tissue of both the exocrine part and the endocrine component was revealed: 48 hours after the introduction of autobile without the use of cold, the prevalence of karyolysis (83.33%) and total necrosis (83.33%) is noted, with the application of IPG, shrinkage of the nucleus (66.66%) is noted, in 33.33% of cases the nucleus remained unchanged, in 50% of cases the tissue remains intact and in 50% of cases single foci of necrosis of the acini are noted. When assessing the volume of necrosis of the acini of the pancreatic tissue, reliable differences are noted according to the criterion of the presence of total necrosis at 48 ($p = 0.007$) from the beginning of the experiment between the groups with the application of artificial local hypothermia and without it.

5) The dynamics of changes in the activity of amylase and lipase in the blood serum of experimental animals in the group with modeling pancreatic necrosis decreases to 154.5 ± 22.7 U / l ($p = 0.005$), when applying IPG - the average concentration of amylase remains quite high - 401.7 ± 107.6 U / l ($p = 0.005$), which indicates a cytolytic syndrome without a pronounced necrotic component. The dynamics of changes in the level of acid-soluble protein fraction in the plasma and erythrocytes of the blood of animals in both experimental groups are similar to each other, which indicates a commensurate damaging effect on the organ produced by the injection of autobile. The DNA level in the erythrocytes of the animals' blood at 48 hours in the experimental pancreatic necrosis group with the imposition of ILH is 7 times lower compared to the group with pancreatic necrosis without the imposition of ILH, which indicates the preservation of the

regenerative potential of the pancreatic cells ($p = 0.013$). The dynamics of changes in the concentration of all purine metabolism metabolites in erythrocytes and blood plasma in the pancreatic necrosis group and with the imposition of hypothermia has a uniform character: a slightly increased level (by 1.2 times) from 12 o'clock decreases by 2.2 times by 48 hours of the experiment, which indicates a pronounced slowdown in metabolism as a reaction to hypothermia.

**Performer: Abramov G.A.**