Section A -Research paper

DEVELOPMENT OF A NEW EXPERIMENTAL MODEL OF PANCREATIC NECROSIS COMPLICATED BY SEPSIS

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ABSTRACT
Relevance. Acute destructive pancreatitis remains one of the far from solved problems of modern urgent surgery. High mortality after pancreatic necrosis, especially when a generalized form of the inflammatory process develops, taking into account the very high mortality rate, is considered today one of the most tragic situations in the outcome of the disease. That is why any experimental research in the field of studying the pathogenesis of pancreatic necrosis complicated by sepsis is considered relevant today. Material. In order to model the optimal variant of the course of pancreatic necrosis complicated by sepsis, we have studied 5 series of experiments on animals. Results. As our research and review of the issues discussed on this issue have shown, modeling acute pancreatic necrosis, which could be complicated by sepsis, remains far from being a solved problem of modern experimental surgery. When modeling acute pancreatic necrosis complicated by sepsis, along with the use of all pathogenetically significant factors, a separate link is required, which can radically affect the course of the overall reactivity of the body. Modeling of acute pancreatic necrosis complicated by sepsis is possible only under the condition of a combined approach of both local and general effects.

Keywords: pancreatic necrosis, pancreatogenic sepsis, experimental modeling, systemic inflammatory reaction syndrome, multiple organ dysfunction

INTRODUCTION
In the modern view of the mechanism of development of any variant of sepsis, the process associated with the development of a systemic inflammatory reaction syndrome is put forward in the first place. At the same time, the presence of a purulent focus in the body, and even more so in the presence of organ dysfunction of at least one vital organ, correspond to the full presentation of the verdict of surgical sepsis. It is based on these considerations that the main objectives of our study were to develop an experimental model of pancreatic necrosis complicated by sepsis, the trigger mechanism in which the general reaction of the body that takes place in clinical practice will play [1,17,19].

The clinical picture of the systemic inflammatory reaction syndrome is a fairly common severe complication in patients with pancreatic necrosis. This prompted clinicians to single

out a separate form from the group of abdominal sepsis as pancreatogenic sepsis. This step characterizes the peculiarity of the development of pancreatogenic sepsis, which, unlike abdominal sepsis, begins with an aseptic process. Only if an infectious agent is attached, pancreatogenic sepsis acquires a full-fledged picture corresponding to abdominal sepsis as such. [2,18,20]

The holding of the Chicago Conciliation Conference on Sepsis back in 1991 made it possible to continue to receive analytical information on the frequency of sepsis. At the conference, it was decided to divide all forms of generalization of inflammatory reactions of the body into systemic inflammatory reaction syndrome, sepsis syndrome, severe sepsis and septic shock. At the same time, according to the decision of this conference, it is the development of multiple organ dysfunction or multiple organ failure syndrome in patients with severe sepsis and septic shock that should be considered the leading initiators of the fatal outcomes of this disease. [3,21,23]

Studies on modeling pancreatitis and pancreonecrosis have been conducted for a long time. Meanwhile, experimental modeling of pancreatic necrosis in the standard version of its reproduction does not always allow to form the course of the disease in the form of a systemic inflammatory reaction syndrome or sepsis syndrome, as the starting phase of the development of pancreatogenic sepsis [8,9,11,12,13,16,22].

The most popular option for modeling pancreatitis and pancreonecrosis in experimental animals are methods with isolated ligation of the Virsung duct of the pancreas. The reproducibility of pancreatitis begins from 3 days after the intervention. However, any process occurring in animals with a similar experimental model does not characterize the development of pancreatogenic sepsis. In other words, there are no those characteristic clinical manifestations of generalization of infection that determine the symptom complex of any surgical sepsis (body temperature above 38 ° C or below 36 ° C, tachycardia over 90 beats/min, tachypnea over 20 breaths in 1 min, the number of leukocytes over 12x109/l or below 4x109 / l, or the number of their immature forms exceeds 10%). In the development of sepsis syndrome, the presence of a purulent focus of infection and possible bacteremia is characteristic. All of the above characterizes the main phases of sepsis development. It is this approach that should determine the course of pancreatogenic sepsis, which is close to clinical conditions. To create conditions that meet these conditions, we conducted a number of experimental simulations of pancreatogenic sepsis.

The aim of our study was to develop an optimal experimental model of acute infected pancreatic necrosis complicated by sepsis.

MATERIALS AND METHODS

At the first stage, it was necessary to determine the dose of the injected microbial agent. To do this, when modeling pancreatogenic sepsis, animals on the 3rd day after ligation of the Virsung duct, 1 ml of 0.9% sodium chloride solution containing a virulent microbial culture of Escherichia Coli at a dose of 20-30 million microbial bodies per 1 gram of animal weight was injected into the pancreas after repeated laparotomy. The choice of the dose of the injected microbial bodies is due to the fact that when injecting animals with relatively small doses (up to 20 million microbial bodies per 1 gram of animal weight), pancreatogenic sepsis did not develop in 5 out of 7 cases, the inflammatory process regressed. An inflammatory

process of a non-infectious nature was detected in the abdominal cavity, and an adhesive process formed at the injection site.

The animals had a picture of non-infected pancreatic necrosis. Only in 2 (28.6%) animals in this series of experiments, the presence of a limited purulent-inflammatory process in the form of an abscess of the abdominal cavity was found at the autopsy. We have not received generalization of the inflammatory reaction.

At the same time, in the second series of experiments (series 2), the dose of microbial bodies in the injected suspension was over 30 million microbial bodies per 1 gram of animal weight. In this series of experiments, 6 out of 7 animals died within 24 hours after injection. The autopsy revealed signs of the development of infectious and toxic shock (putrefactive stock in the abdominal cavity, hemorrhagic effusion in the abdominal cavity and pleural cavities, sharp fullness of internal organs, multiple foci of hemorrhage in the visceral and parietal leaves of the peritoneum), which, apparently, was associated with a massive intake of microbial bodies into the body. That is, despite the development of the inflammatory process, the latter was of a formal nature, since, in essence, we were dealing with the development of septic shock, the entrance gate of which was the abdominal cavity.

Under the condition of an increase in the amount of microbial bodies injected, a lightning-fast course of the inflammatory process occurs without any stages of pancreatogenic sepsis occurring in clinical practice. It should also be noted that the process of infected pancreatic necrosis simply does not have time to form, transferring the entire inflammatory reaction from local exposure to general, where the main culprit is the peritoneum, not the pancreas. The inflammatory reaction in the pancreas did not differ in any way from another zone of the abdominal cavity, and the lesion proceeded without the formation of a necrobiotic process, and even more so parapancreonecrosis.

Thus, at this stage of the conducted research, it can be assumed that such an unusual picture of the course of pancreatogenic sepsis is associated, on the one hand, with the use of a monoculture of pathogens (in this situation, there was no interspecific struggle of microorganisms). The created conditions allowed the entry of a massive number of microorganisms into the systemic circulation and the development of corresponding changes without an adequate response of the body. On the other hand, the use of monoculture in modeling pancreatogenic sepsis does not correspond to the clinical conditions of the disease, since in life practice, the presence of polyinfection is the prevailing condition in the occurrence of any purulent-inflammatory process. The latter is the main argument in favor of using as a microbial agent a suspension obtained from the auto-feces of the animals themselves.

To select the dose of the administered his feces of animals, we conducted a number of microbiological studies. It was revealed that the microbial contamination of animal auto-feces obtained from the rectal cavity was represented by a large number of different microorganisms, both aerobic and anaerobic. In the studied material, the total number of microbial bodies was equal to the value of 109-1010 CFU/ml. Gram-negative pathogens prevailed (72.4%) over gram-positive ones.

Among the gram-negative ones, Veillonella, Enterobacteriales, Klebsiella, and Proteus were prevalent. Rod-shaped pathogens were detected in 72% of cases and cocci in 28%. Of the

total microbial landscape of the animal's fecal mass, 68.6% were excited groups of obligate anaerobes, and 31.4% were facultative anaerobes.

When choosing the appropriate dose of the injected microbial suspension, we found that the level of acceptability of the reproduction of the purulent-inflammatory process (infection) is x104 CFU / ml, which corresponds to 20% concentration of the animal auto-feces solution. The maximum value of microbial invasion in the form of a critical level should be considered the concentration at the level of x105 CFU/ml.

The choice of the concentration of microbial suspension of animal auto-feces was confirmed by us in the following 3 and 4 series of experiments. When a solution with an amount exceeding x105 CFU / ml was administered, bacterial shock also developed. Whereas with the introduction of a microbial agent in the volume of x103 CFU / ml, the inflammatory process, as in the previous block of experiments, simply did not develop.

It should also be noted that pancreatic necrosis simply does not have time to develop in the variant that was necessary for the formation of pancreatogenic sepsis. In this connection, we decided to stimulate the necrobiotic process with 10% solutions of calcium chloride. Accordingly, the choice of the timing of the introduction of both 10% calcium chloride and animal auto-feces should determine the timing of the formation of the main process.

RESULTS AND DISCUSSION

As a result of our research, we have made the following conclusions:

- when large doses of microbial suspensions are injected into the pancreas, even if they are polymorphic, the formation of infected pancreatic necrosis and pancreatogenic sepsis does not occur;
- the introduction of massive doses of microbial suspension provokes the development of infectious-toxic (septic) shock, with a high percentage of deaths and a lightning-fast course of the pathological process, which does not allow the model to be used for experimental studies;
- septic shock, which occurs when large doses of animal auto-feces are administered, along with a high percentage of early mortality, proceeds without the formation of purulent pancreatic necrosis, and accordingly excludes the phases of formation of all links of pancreatogenic sepsis;
- the pancreas, when administered with large doses of animal auto-feces, acts as an entrance gate for microorganisms and the formation of a persistent source of infection (purulent pancreatic necrosis) does not occur;
- for the formation of pancreatogenic sepsis with such forms as severe sepsis and sepsis syndrome, preliminary changes in the macroorganism are required, which characterize the subsequent reaction of the animal organism, that is, changes in the reactivity of the macroorganism are required, provided that the virulence of the microorganism decreases.

It is known that the process of pancreatogenic sepsis is already formed in the absence of infection in the pancreas. However, this reaction can be interpreted only as a syndrome of a systemic inflammatory reaction of the body, the cause of which may be autolysis of the pancreas. Only if an infectious agent is attached, it is possible to form a classic infected pancreatic necrosis with the subsequent development of sepsis syndrome and severe sepsis. The proof of this judgment can be a series of experiments with a low concentration of

injected animal auto-feces. The resulting hyporergic reaction forms a picture of the conflict of the aggressive principle between the macroorganism and the microorganism.

Thus, reproduction of infected pancreatic necrosis and pancreatogenic sepsis is possible only if there are initial destructive changes in the focus of inflammation (necrobiotic) and a decrease in the response of the macroorganism (immunosuppression). Only if the above conditions are met, a low concentration of microbial suspension of animal auto-feces will increase the reproducibility of the required pathological process and experimental model. At the same time, the destructive process in the pancreas can, as is known, be modeled using a 10% solution of calcium chloride.

To change the reactivity of the macroorganism, we used antilympholine-Kr, which is an immunosuppressive drug. It is obtained from rabbit blood proteins immunized by human thymus lymphocytes. 1 dose of the drug corresponds to 40-60 mg of protein. Along with this, as already indicated above, the animal's auto-feces is a source of polymorphic pathogenic flora. The latter makes it possible to bring the conditions for the development of the pathological process closer to the clinical ones.

In order to confirm our judgments, we conducted a new series of experiments (series 5), in which the modeling of pancreatogenic sepsis was carried out by preliminary, two-day intraperitoneal administration of antilympholin-Kr at a dose of 0.03 mg per 100 grams of animal. On the 3rd day of the simulation, laparotomy was performed, the stomach, duodenum and pancreas were removed into the wound and the Virsung duct was ligated. After the formation of acute pancreatitis, which usually occurred on the 3rd day of the operation, the abdominal cavity was re-opened and, under aseptic conditions, 0.5 ml of 10% calcium chloride solution was injected into the pancreas in order to provoke a necrobiotic process. A day later, 0.5 ml of a 20% solution of animal auto-feces was injected into the pancreas through a laparotomy wound. In dynamics, starting from the first day after the injection of microbial fecal suspension, the development of pancreatogenic sepsis against the background of pancreatic necrosis was observed.

Over the next 7 days, the animals developed a progressive clinical picture of all forms of pancreatogenic sepsis with signs of a systemic inflammatory reaction syndrome (tachycardia, tachypnea, hyperthermia, leukocytosis). The results of blood seeding in 100% of cases revealed the presence of hemoculture already on the 3-4 day of modeling.

The pancreas was in a purulent-necrotic state at all times of the experiments. The purulent-destructive process easily spread to nearby tissues, liver gates, mesentery root. Such results of modeling pancreatogenic sepsis occurred in 11 (91.7%) of 12 rats in this series, 1 rat died on the 1st day of observation with pathomorphological signs of bacterial shock, which we identified at autopsy. In our proposed model of pancreatic necrosis complicated by sepsis, the initial signs of a systemic inflammatory reaction syndrome (in the form of respiratory failure, increased rectal body temperature, tachycardia, leukocytosis or leukopenia) are observed for 10-12 hours of the experiment.

Nowadays, it is difficult to find in its pathogenesis a more complex inflammatory disease of the abdominal organs than acute pancreatitis. Over the past 50 years, acute pancreatitis has been ranked third among acute surgical diseases of the abdominal cavity and accounts for about 12.5% of all urgent pathology. [4,17] At the same time, diagnostics and surgical tactics in pancreatic necrosis remain in our time, collectively, one of the far from solved problems in

urgent abdominal surgery. There is no doubt that this problem is related to the difficulties of forecasting and early diagnosis of destructive forms of acute pancreatitis.

Also, the urgency of the problem is due to the frequency of acute pancreatitis in the majority of patients (65-70%) at the working age. At the same time, in the case of pancreatic necrosis and the use of surgical methods of treatment, disability is observed in more than half of patients – from 62.8 to 75.3% of cases. All this gives the problem the same socio-economic significance [15].

The mechanism of pathogenesis of acute pancreatitis is multifaceted. And despite the fact that 80-90% of acute pancreatitis manifests itself in the form of mild inflammation with a low number of deaths [6], severe forms of this disease, with progressive systemic inflammatory reaction syndrome and pancreatic necrosis, are potentially fatal and form the basis of fatal outcomes [5,6,7]. At the same time, the basis of deaths in infected forms of pancreatic necrosis is formed by cases of sepsis and organ failure. For example, the general statistics of deaths in pancreatic necrosis is 3.9–26%, and in infected pancreatic necrosis – up to 85%, in the fulminant course of the disease - 100% [14].

Difficulties in choosing therapeutic and diagnostic tactics for acute pancreatitis are due to the multi-vector features of the course of this disease. The issues of choosing diagnostic methods and treatment methods for uncomplicated and complicated, severe and mild pancreatitis, so-called "edematous" pancreatitis and pancreonecrosis, complications of pancreatogenic toxemia and destructive complications, sterile and infected pancreonecrosis, early infection and late destructive complications are discussed.

At the same time, dissimilar and often opposite opinions are expressed on the same issue [7,10].

CONCLUSION

As our research and review of the issues discussed on this issue have shown, modeling of acute pancreatic necrosis, which could be complicated by sepsis, remains far from being a solved problem of modern experimental surgery.

When modeling acute pancreatic necrosis complicated by sepsis, along with the use of all pathogenetically significant factors, a separate link is required, which can radically affect the course of the overall reactivity of the body.

Modeling of acute pancreatic necrosis complicated by sepsis is possible only under the condition of a combined approach of both local and general effects.

1. The optimal model of pancreatic necrosis complicated by sepsis is its reproduction against the background of altered reactivity of experimental animals, by preliminary, two-day intraperitoneal administration of antilympholine-Kr at a dose of 0.03 mg per 100 grams of animal.
2. When modeling acute infected pancreatic necrosis complicated by sepsis, the structural picture of the microcirculatory bed completely changes. Capillaries were the leaders in increasing the diameter (by 2.82 times; $p<0.05$). The morphological picture of their transformation was characterized by the development of not only stasis and aggregation of cells, but also with the formation of microthrombs. Subsequently, there was an increase in the volumes of both postcapillary venules (by 2.25 times; $p<0.05$) and the venules themselves (by 2.54 times; $p<0.05$) against the background of an increase in the diameters of both

arterioles (by 1.45 times; $p<0.05$), and precapillary arterioles (1.72 times; $p<0.05$). This indicates the breakthrough of the angiogenic barrier, the complete destruction of the capillary system and the opening of the corresponding arteriol-venous shunts.

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