Effect of Anti-TNFα Therapy by Infliximab against Pancreatic Tissue Damage in Severe Acute Necrotizing Pancreatitis

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Abstract

Nowadays, it is difficult to find a more complicated inflammatory disease of the abdominal organs in its pathogenesis than acute pancreatitis. Usage of antimediator drugs and antimetabolites is the most promising direction in inflammatory pathological processes correction. The study of the possible uses of a new group of drugs – monoclonal antibodies to this trigger of inflammation- is also of great interest. Current study was aimed to study the effect of Infliximab on the lethality, volume and nature of the pancreatic lesion in severe necrotizing ductal pancreatic necrosis. The experiment was performed on 30 female Wistar rats of the same age weighing 200-250g. All manipulations were performed under general anesthesia by intraperitoneal injection of the zoletil at a dose of 60 mg/kg and chloral hydrate at a dose of 125 mg/kg. Model of severe acute necrotizing pancreatitis was performed by invented method with the injection of 0.5 ml of a buffer solution containing a bile acid salt - sodium taurocholate introductory. The animals were divided into following groups: Group A normal values (n = 6); Group B (n = 6), in a period of 24 h mortality study was conducted in acute destructive pancreatitis; Group C (n = 6), the group in which the simulation of acute severe necrotic pancreatitis was performed with the study of the volume of the pancreatic lesion for a period of 6 hours from the moment of modeling; Group D (n = 6) in this group, the effect of Infliximab at a dose of 60 mkg/kg on mortality in severe destructive pancreatitis was studied for a period of 24 hours from the moment of modeling; Group E (n = 6) in this group, the effect of infliximab at a dose of 120 mkg/kg on the volume of pancreatic lesion in severe destructive pancreatitis was studied for a period of 6 hours from the moment of modeling. During the assessment of pancreatic damage, volume of pancreatic lesion was found that in a period of 6 h after modeling was at 34.8%±1.2% level. Assessment of pancreatic damage in group E, with studying the protective effect of Infliximab at a dose of 60 mg/kg, showed that the total volume of the necrotic pancreatic lesion after a period of 6 h
from the moment of modeling of acute pancreatitis was 21.3%±1.4%. In the course of this study, it was revealed that the use of Infliximab at a dose of 60 mcg/kg leads to a pronounced positive effect on the pancreatic lesion, manifested by a decrease in mortality by 1 day in group D to 50%. Infliximab has a definite protective effect in acute pancreatitis, decreasing the volume of the injury and decreasing the mortality rate for 24 hours by half. Anti-TNF therapy with Infliximab can significantly reduce the volume of pancreatic lesions in severe forms of pancreatic necrosis, which contributes to a pronounced decrease in mortality for 1 day from the moment of reproduction of the pathology.

**Keywords:** Severe Acute Necrotizing Pancreatitis, Volume of Pancreatic Damage, Infliximab

### 1. Introduction

Nowadays, it is difficult to find a more complicated inflammatory disease of the abdominal organs in its pathogenesis than acute pancreatitis. This pathology is included in the group of diseases united by the features of medical care, called “acute abdomen”, consistently occupying the 2-3 place in this group along with acute cholecystitis. According to the World Health Organization, acute pancreatitis affects between 200 and 800 people per 1 million of the world's population. According to the State Statistics Committee, the incidence of acute pancreatitis in the Russian Federation ranges from 36-40 cases per 100,000 population. The prevalence of destructive forms is currently 15-20% of all cases, respectively, from 75 to 80% of the diseases that occur in the so-called abortive or edematous form. The development of pancreatic tissue destruction is a life-threatening complication with a mortality rate of more than 80% {Goodchild, 2019 #1;Group, 2019 #2;Li, #3}. An important role in the spreading of the necrotizing process in the pancreas plays ischemic damage, leading to activation of acute inflammation 5-7. One of the most important factors in the treatment of patients with acute pancreatitis is the diagnosis of the disease as soon as possible. Mild pancreatitis is easy to treat, but treatment for acute pancreatitis includes intensive care. Access to and observation of the pancreas is not possible without surgery, and imaging studies may not provide sufficient information to the physician 8. There are also inherited and chronic forms of the disease that can have irreversible effects throughout life. Patients often suffer from pain and malnutrition and are more likely to have a higher risk of pancreatic cancer 9.

Conservative treatment is an approach to treating some ailments such as low back pain, neck pain, and spinal diseases using non-surgical treatment options such as physiotherapy, medication, and injections 10. The development of conservative therapy methods requires research and creation of a methodological complex approaches in understanding the place and role of certain pharmacological targets 11-16.

One of the treatment methods is the use of synthetic antibodies that have an inhibitory effect on pancreatic enzymes. Therefore, anti-protein drugs are expected to prevent necrotic changes in the
pancreas and reduce mortality. Usage of antimiatory drugs and antimetabolites is the most promising directions in inflammatory pathological processes correction.

One of initial mediators of inflammation is the tumor necrosis factor alpha (TNF-alpha), the involvement of which in systemic inflammation in pancreatic necrosis has been recently proven. Norman et al. (1995) showed that tumor necrosis factor gene expression occurs locally during acute pancreatitis and that large amounts of TNF are produced within the pancreas with continuous levels higher than those in serum. The overall increase in TNF concentration in tissue and serum is directly related to the severity of pancreatic damage and inflammation, and they stated that intrusive macrophages play the largest role in this process. It was found that TNF-alpha concentration in blood furthered notably after acute pancreatitis was induced.

Today, the study of the possible use of a new group of drugs – monoclonal antibodies to this trigger of inflammation-is of great interest. A number of studies have shown the effectiveness of monoclonal antibodies to TNF-alpha in acute pancreatitis. However, in analysis of these research works, it was revealed that the investigation of this drug took place on “non-severe” models of acute pancreatitis with low mortality, which is most often represented in the foreign literature as mild necrotizing pancreatitis. Therefore, the question of extrapolating the results of these studies to clinical conditions is debatable.

The work aimed to study the effect of infliximab on the lethality and volume and nature of the pancreatic lesion in severe necrotizing ductal pancreatic necrosis.

2. Materials and Methods

The experiment was performed on 30 female white Wistar rats of the same age weighing 200-250g. All studies were performed in compliance with the rules of humane treatment of animals. For the study, we took rats without external signs of the disease, which passed the quarantine regime and were kept in standard conditions.

The animals were divided into following groups:

- group A normal values (6 animals).
- group B (6 animals) – a group in which a 24-hour mortality study was conducted in acute destructive pancreatitis.
- group C (6 animals) – the group in which the simulation of acute severe necrotic pancreatitis was performed with the study of the volume of the pancreatic lesion for a period of 6 hours from the moment of modeling.
- group D – (6 animals) in this group, the effect of infliximab at a dose of 60 mg/kg on mortality in severe destructive pancreatitis was studied for a period of 24 hours from the moment of modeling.
• group E – (6 animals) in this group, the effect of infliximab at a dose of 120 mkg/kg on the volume of pancreatic lesion in severe destructive pancreatitis was studied for a period of 6 hours from the moment of modeling.

All manipulations were performed under general anaesthesia by intraperitoneal injection of the zoletil at a dose of 60 mg/kg and chloral hydrate at a dose of 125 mg/kg.

The model was performed as follows. Under general anaesthesia, zoletil at a dose of 50 mg/kg together with chloral hydrate at a dose of 125 mg/kg intraperitoneally, after treatment of the surgical field with antibiotic solutions, the abdominal cavity was opened layer by layer. A loop of the duodenum was pulled into the wound. During transillumination, the main papilla of the duodenum was found.

![Image of cannulated common bile duct](image)

Figure 1. Cannulated common bile duct

After finding the ampoule and the outlet of the main papilla of the duodenum, 0.5-0.6 cm was retreated from it and a needle with a diameter of 32G was used to perforate the anterior wall of the duodenum. Cannulation of the common bile duct was performed with a 36G diameter catheter (Figure 1).

In order to reproduce severe destructive pancreatitis, insulating clips were applied to the cannula, distal to the place of duodenal duct junction, and to the common bile duct, so that the injected solution completely entered the pancreatic ducts.

Failure to comply with this condition threatens, on the one hand, the discharge of the solution into the duodenum, and on the other— an excessive amount of the solution entering the common bile duct,
which in a rat performs the function of the gallbladder, damping excessive 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 isolation clips were removed 1 minute after administration, 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, and the abdominal cavity was sutured layer by layer completely. Ignoring of rat pancreatic ducts system isolation leads to a sharp decrease in the volume of its lesion, which is reflected in a lower mortality rate.

After the macroscopic evaluation, the pancreas, liver, kidneys, and lungs were collected for histological examination. The organs were collected, weighed, and fixed in a 10% neutral formalin solution. After fixation, the tissue areas were washed in running water, dehydrated and poured into paraffin according to the standard procedure. Paraffin sections of the pancreas with a thickness of 5-7 mkm were prepared at standard intervals of all parts of the organ. The sections were stained with hematoxylin and eosin.

Histological processing was performed using the equipment of the company "Leica" (Germany). During microscopic examination, the finished micro-preparations were scanned using the computer system of archiving and image analysis “Mirax Desk”. Image analysis and morphometry were performed using the program "Pannoramic Viewer“ 1.15.4. Quantitative data was recorded in MS Excel.

The volume of the lesion of the pancreas was counting by calculating the ratio of the area of necrosis to the area of the unaffected part of the pancreas, on digitized computer images that were obtained using a digital microscope, scanning in the "total scan" mode and archiving images of the Mirax Desk and the program "Pannoramic Viewer“ 1.15.4. The ratio was expressed in percents.

Infliximab (MSD Remicade) was administered 1 hour before the acute pancreatitis modeling at a dose of 60 mkg/kg.

Statistical analysis of the obtained data was carried out using Microsoft Excel version 2007, calculating the average value (M) of indicators and the error of the average (m), and the confidence criterion (p). differences were considered statistically significant at values \( p \leq 0.05 \).

3. Results

One of the most accurate integral indicators for evaluating the effectiveness of a particular treatment method is the mortality rate at the study period. At the time of 24 hours of modeling of severe pancreatic necrosis according invented to the method developed by us, the mortality rate was 100%.

In group C, at the time of 6 hours from the modeling of acute pancreatitis, a large amount of hemorrhagic effusion localized in all parts of the abdominal cavity. The liver is with marks of venous
hyperemia with a smooth surface and sharpened edges of the lobes. There is a hyperemia of the spleen.

The greatest changes affect the pancreas, which is sharply edematous, 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, presence of diapedetic hemorrhage spots are noted.

Period 6 from the moment of modeling of acute destructive pancreatitis was characterized by typical large-focal hemorrhagic pancreatic necrosis changes. The zone of necrotic lesion in the duodenal part of the pancreas affects 50% of this part and is localized closer to the wall of the duodenum spreading along the duct.

The greatest destruction in the sizes noted in the gastro-splenic part of the gland in form of coagulation necrosis of acinar cells, fibrinoid necrosis of vascular walls and stromal elements, partial necrosis of acini and severe neutrophil infiltration centrolobular with a primary lesion (Figure 1 and Figure 2).

During assessment of pancreatic damage volume was found that in the period of 6 since modeling it was at 34.8%±1.2 level.

Figure 2. Intact rat pancreas. G+E, magnification X 90
Assessment of pancreatic damage in group D, with studying the protective effect of infliximab at a dose of 60 mkg/kg, showed that the total volume of necrotic pancreatic lesion for a period of 6 hours from the moment of modeling acute pancreatitis was 21.3%±1.4%. An increase of infliximab dose up to 120 mkg/kg did not lead to a significant reduction in the volume of pancreatic destruction, which was also not reflected in a decrease in mortality, which in this group was 50% (Table 1).

Table 1. Influence of infliximab in different doses on the volume of pancreatic destruction in severe acute necrotizing pancreatitis

<table>
<thead>
<tr>
<th>Group</th>
<th>Volume of pancreatic tissue damage (%)</th>
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<tbody>
<tr>
<td>group C (6h model of acute necrotizing pancreatitis, n=6)</td>
<td>34.8±1.2</td>
</tr>
<tr>
<td>group D (Infliximab 60 mkg/kg, n=6)</td>
<td>21.3±1.4 *</td>
</tr>
<tr>
<td>group E (Infliximab 120 mkg/kg, n=6)</td>
<td>19.3±1.2 **</td>
</tr>
</tbody>
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Note. * p <0.05 - significance of differences with control group  
** p ≥0.05 absence of significance of differences with group D
In the course of the work, it was revealed that the use of MAB infliximab at a dose of 60 mcg/kg leads to a pronounced positive effect on the pancreatic lesion, manifested by a decrease in mortality by 1 day in group D to 50%.

Positive protective effect of monoclonal antibodies to tumor necrosis factor in a dose of 60 mkg/kg 1 hour prior modeling of acute destructive pancreatitis is manifested in the reduction of interstitial edema microscopic examination, a decrease of centrolobular areas of necrosis and reduction zones and the degree of leukocyte infiltration Figure 3.

Figure 4. Centrolobular necrosis with a moderate lesion volume when corrected with infliximab at a dose of 60 mkg/kg in the simulation of acute pancreatitis for a period of 6 hours from the moment of simulation. G+E, magnification X 200

Discussion
Biologic drugs act on a molecule called TNF (Factor Necrosis Tumor) and are called TNF blockers or anti-TNF drugs, which are used to treat rheumatoid arthritis, pediatric arthritis, psoriatic arthritis, and ankylosing spondylitis. Remicade or Infliximab is used to reduce the pain and swelling of certain inflammatory diseases (such as rheumatoid arthritis, psoriasis) and certain intestinal diseases (Crohn's disease). Infliximab reduces inflammation by blocking interleukin-12 and interleukin-23 (a substance made by the body that causes inflammation). Infliximab does not cure the disease, but it does help reduce the symptoms of the disease. This drug can help reduce the number of plaques in plaque psoriasis, pain and swelling in arthritis, and heartburn and cramps in Crohn's disease.
The development of acute destructive pancreatitis is accompanied by the disruption of the pancreatic stroma. The parietal and visceral peritoneum are edematous and hyperemic in almost all parts, there is a pronounced edema of the intestinal mesentery. One of the most effective treatments for AP is the use of TNF-α. As the results of this study showed, TNF-α was significantly increased in pancreatic tissue in AP and anti-TNF-α treatment significantly improved pathological and biochemical findings.

One of the important features is the disorder of pancreatic exocrine secretion. During pancreatitis, significant amounts of amylase are secreted, indicating the release of the enzyme from damaged cells. Lipase levels increase and cytokine levels increase when pancreatic tissue is destroyed and oxidative tissue stress increases. Thus, infliximab has a pronounced protective effect in acute pancreatitis, reducing the volume of the lesion and reducing the mortality rate for a period of 24 hours by half. It becomes clear that further research of monoclonal antibodies against tumor necrosis factor alpha will form the basis for its use in clinical conditions in severe forms of necrotizing pancreatitis.

Alpha tumor necrosis factor (TNF) drugs, such as infliximab, may be an alternative treatment for patients who do not respond to treatment with corticosteroids or immunosuppressants. The researchers showed that by blocking TNF, inflammation could be minimized and pancreatic damage limited. TNF-α is responsible for local and systemic effects in pancreatitis and it is thought that inhibition of TNF-α can reduce local and systemic complications. Hughes et al. reported that TNF blockade in acute pancreatitis could reduce the complications associated with pancreatitis. Some researchers have reported that inhibition of TNF-α solution can reduce the severity of experimental pancreatitis and mortality, but does not affect pancreatic vacuolation, necrosis, and inflammatory cell infiltration. Oruc et al. pointed that infliximab has anti-inflammatory effects in the model of edematous and necrotic pancreatitis, and the possible reason for this difference may be due to differences in performance between different molecules used in the studies. Other researchers report that a TNF-receptor fusion protein etanercept could improve histological scores and biochemical parameters in the necrotizing Na-taurocholate pancreatitis model. In conclusion, Anti-TNF therapy with infliximab can significantly reduce the volume of pancreatic lesions in severe forms of pancreatic necrosis, which contributes to a pronounced decrease in mortality for 1 day from the moment of reproduction of the pathology.

The main findings of the current study are as follows:

1. Administration of infliximab at dose of 60 mkg/kg in model of severe acute necrotizing pancreatitis helps to reduce death rate at 24 hours period by 50%.
2. Use of infliximab at dose of 60 mkg/kg at period of 6 hours period after modeling of severe acute necrotizing pancreatitis leads to decreasing of pancreatic damage volume from 34.8%±1.2% of group C to 21.3%±1.4% of group D.
3. An increase of infliximab dose up to 120 mkg/kg in group E did not lead to a significant reduction in the volume of pancreatic destruction compared to group D.

References


