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Enzymatic Debridement in Necrotizing Pancreatitis

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Multiple organ failure and pancreatic necrosis are the factors that determine prognosis in acute pancreatitis attacks. We investigated the effects of collagenase on the debridement of experimental pancreatic necrosis. The study covered 4 groups; each group had 10 rats. Group I was the necrotizing pancreatitis group. Group II was the collagenase group with pancreatic loge by isotonic irrigation following necrotizing pancreatitis. Group III was the collagenase group with pancreatic loge following necrotizing pancreatitis. Group IV was the intraperitoneal collagenase group following necrotizing pancreatitis. The progress of the groups was compared hematologically and histopathologically. There was no difference among the groups regarding the levels of leukocyte, hemogram, and urea. The differences in AST levels between Group I and II; and differences in glucose, calcium, LDH, AST, and amylase between Group II and III; between Group II and IV; between Group I and III; and between Group I and IV were statistically significant ($P < 0.05$). There were statistically significant differences between Group II and III, and Group II and IV regarding edema, acinar necrosis, inflammatory cell infiltration, hemorrhage, and fat necrosis ($P < 0.05$). In conclusion, the collagenase preparation used in this experimental pancreatitis model was found to be effective in the debridement of pancreatic necrosis.

Key words: Acute pancreatitis – Necrose – Collagenase – Debridement

Acute pancreatitis (AP) is a nonbacterial inflammatory disease of the pancreas that can range from interstitial edema to pancreatic necrosis in its severest form. In about 20% of AP attacks necrosis can develop in the pancreas while the disease limits itself and regresses in a couple of days in many patients (80%).[1]

The definitions that are still widely in effect today regarding the classification of acute pancreatitis were determined in 1992 at the Atlanta Conference.[2] The

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conference aimed at achieving a common classification for AP and its complications. Within severe acute pancreatitis, of which necrotizing pancreatitis is a part, organ failure and local complications can be seen (necrosis, pseudocyst, and abscess). Multiple organ failure and pancreatic necrosis are the factors that determine the prognosis. Half of the mortalities are observed within a period of 1 or 2 weeks. Necrotizing pancreatitis makes up for the 10–20% of AP cases. Severe pancreatitis has a high mortality rate and functional diseases like diabetes are seen in one-third to one-fifth of the recovered patients.[3]

While the mortality rates are about 10% in the presence of sterile pancreatic necrosis, they go up over 30% in the existence of infected necrosis.[1] Regarding acute necrotizing pancreatitis, there is still no consensus on surgical indications and the time of surgical intervention, the surgical method to be used, and which patients need conservative treatment and which ones need surgical treatment. The goal in the surgical treatment of acute necrotizing pancreatitis is to isolate the necrotic tissue that might cause sepsis and multiple organ failure and to reduce the risk of mortality. The timing of necrosectomy as well as the way in which necrosectomy is performed is significant in necrotizing pancreatitis. The issue of the possibility that necrosectomy can be performed through minimally-invasive interventions instead of open surgery is still being discussed.[3]

We planned to investigate the activity of collagenase clostridiopeptidase A (EC 3.424.3), which has never been attempted before in the debridement of experimental pancreatic necrosis (but which has been used for enzymatic debridement), and the enzyme preparation containing the accompanying proteases (Sterile Novuxol®, Abbott, Uetersen, Germany). We aimed to evaluate the response of the disease to treatment through laboratory and histopathologic data, by using the enzyme preparation to treat necrotizing pancreatitis.

Material and Methods

The study was conducted at Necmettin Erbakan University, Meram Medical School's Experimental Animal and Research Laboratory, through the consent of the Board of Ethics (Number: 2011-058/ Date: May 30, 2011).

Animals

4–6 weeks old 40 Wistar albino rats weighing between 200 and 250 grams were used in the study. The rats were kept at standard lab conditions and were continuously fed by rat feed and water until the experiment's completion. All the rats were weighed on a digital scale, and each rat's weight was recorded just before the start of the study.

Surgical procedures

The rats were fasted 8 hours before the experiment, but their water intake was not limited. The rats were administered 40 mg/kg of ketamine (Ketalar, Pfizer Ltd Co, İstanbul, Turkey) and 10 mg/kg of xylazine (Rompun, Bayer, İstanbul, Turkey) peritoneally as anesthetics before the procedure. The rats' abdominal hair was shaved. Operative procedures were performed under sterile conditions by applying antisepsis with Poviodine 10% (Diagnokim Co, Istanbul, Turkey) solution on the abdominal skin of the animals. The rats' abdomens were entered through an abdominal midline incision of about 4 cm in all groups. The common biliopancreatic channel (CBC) was found in the animals with planned AP (Groups I, II, III, and IV). The duodenal wall was pierced from the antimesenteric field by a 24-gauge catheter (Introcan-w, Braun, inner diameter 7 mm) and was cannulized by the movement of the catheter towards the CBC. The choledoch was temporarily suspended at a close point to the hepatic hilus by 4/0 silk (Mersilk, Ethicon, Cincinnati, Ohio) in order to prevent reflux to the intrahepatic bile ducts. Further, micro aneurysm clips were placed at a point close to the CBC's duodenum in order to prevent the reflux of duodenal content to the CBC. Then, 5% Na-taurocholic acid (Sigma, St. Louis, Missouri) was infused to the CBC in 1 mL/kg amounts through a catheter for 2 minutes, not exceeding 30 mmHg pressure.[4] After the completion of the infusion, the silk at the proximal of the CBC and the clip at its distal were opened up. The entry point at the duodenum was closed by a single 6/0 polypropylene (Prolene, Ethicon) suture. Following the execution of the above-mentioned procedures to the groups, the abdominal wall and the skin were closed up with continuous 3/0 silk (Mersilk, Ethicon) sutures. The rats were fed by water and standard feed during the postoperative period. The experiment was concluded by the sacrifice of the rats by high doses of anesthetic material 7 days later.

Study protocol

The rats were divided into 4 groups of 10 rats.

Group I: Necrotizing pancreatitis was formed.

Group II: The pancreatic loge was irrigated by 4 mL of isotonic every 24 hours through relaparotomy, following the formation of necrotizing pancreatitis.

Group III: 4 mL of Sterile Novuxol (Abbott) was applied to the pancreas so as to cover it with a minimum of 2 mm thickness through relaparotomy every 24 hours following the formation of necrotizing pancreatitis.

Group IV: 4 mL of Sterile Novuxol (Abbott) was intraperitoneally administered to the pancreatic loge through relaparotomy every 24 hours following the formation of necrotizing pancreatitis.

Laboratory tests

Blood samples drawn from Group I, II, III, and IV were analyzed regarding leukocyte count (K/uL), hemogram (g/dL), glucose (mg/dL), urea (mg/dL), calcium (mg/dL), serum lactic dehydrogenase [(LDH), (u/dL)], aspartate aminotransferase [(AST), (u/dL)], and amylase (u/dL) levels. Blood samples drawn from Group I and IV were analyzed regarding hemoglobin (Hb), aspartate aminotransferase (AST), alanine aminotransferase (ALT) (u/dL), urea (mg/dL), creatinine, and albumin levels. The mean values and standard deviations of the measured parameters were calculated for each group.

Histopathologic study

Samples containing the main ductal structure from the similar anatomic localization of the pancreas were taken from Group I, II, III, and IV, while samples from the peritoneum and the liver were also taken from Group I and IV. The samples were fixed in 10% formalin and were subsequently stained in hematoxylin eosin through 5μ thick sections following routine follow-up procedures. They were studied through a light microscope at ×40 enlargement. The samples were studied by a pathologist who did not know about the groups. The samples taken from the pancreatic tissue were investigated regarding edema, acinar necrosis, inflammatory cell infiltration, hemorrhage, fat necrosis, and perivascular inflammation.[5] Liver and peritoneum samples were studied separately. Within the scope of the peritoneum study the levels of increase in connective tissue, muscular atrophy, inflammatory cell infiltration, eosinophilia, necrosis and vein proliferation were investigated, while hydropic degeneration, necrosis, nuclear pleomorphism, inflammatory cell infiltration, congestion, and fibrosis findings were investigated within the scope of hepatic study. The classification of the achieved values was done in the following way: normal (0), mild (1), moderate (2), and severe (3).

Statistical analysis

The statistical analysis of the obtained values was carried out by the SPSS statistics program. The mean ± standard deviation of the values was recorded. The Mann–Whitney *U* Test was used for the lab tests. The Kruskal–Wallis test with Bonferroni correction was used for the histopathologic evaluation. All the <0.05 *P*-values were regarded to be statistically significant.

Results

During the experiment, 4 rats from Group I, 3 from Group II, and 1 rat from Group III and 1 from Group IV were lost before the experiment was completed.

Macroscopic changes

In Group I, II, III, and IV, the pancreas and the surrounding fat tissues were extremely edematous. Hemorrhagic characterized acid and necrosis were observed on the pancreas, and this condition was distinctive in Group I and II.

Biochemical and hematological parameters

No difference was detected among the groups regarding leukocyte, hemogram, and urea. There was a statistically significant difference between Group I and II regarding the value of AST ($P < 0.05$). Between Group II and III, low amylase, glucose, calcium, LDH, and AST were significant ($P < 0.05$). Between Group II and IV, low amylase, glucose, calcium, LDH, and AST were significant ($P < 0.05$). Between Group I and III, amylase, glucose, calcium, LDH, and AST values were significantly different ($P < 0.05$). Between Group I and IV, amylase, glucose, calcium, LDH, and AST values were significantly different ($P < 0.05$). None of the parameters studied revealed a statistical difference between Group III and IV ($P > 0.05$; Table 1). We achieved better progress in the Sterile Novuxol (Abbott) group in comparison to the other groups regarding lab values.

Table 1 Laboratory values

Parameters	Group I	Group II	Group III	Group IV
Leukocyte (K/uL)	5017.5 ± 1239.4	5124.4 ± 1139.52	4388.3 ± 925.16	4448.2 ± 900.90
Hemogram (g/dL)	12.5 ± 1.76	12.9 ± 1.28	12.9 ± 0.96	12.9 ± 0.95
Glucose (mg/dL)	214.8 ± 31.22[c]	205.1 ± 47.18[b]	171.4 ± 61.62[b,c]	171.4 ± 60.56[b,c]
Urea (mg/dL)	39.2 ± 8.16	40 ± 6.20	40 ± 6.86	40 ± 7.10
Calcium (mg/dL)	8.5 ± 1.26[c]	8.9 ± 1.19[b]	9.9 ± 0.62[b,c]	9.9 ± 0.68[b,c]
LDH (u/dL)	959.7 ± 220.77[c]	813.2 ± 153.17[b]	718.5 ± 155.56[b,c]	718.7 ± 156.97[b,c]
AST (u/dL)	841.7 ± 181.8[a,c]	672.5 ± 167.16[a,b]	411.7 ± 378.94[a–c]	410.7 ± 358.33[a–c]
Amylase (u/dL)	4399.1± 754.93[c]	3831.5 ± 917.33[b]	1240.7 ± 1098.99[b,c]	1240.2± 1096.05[b,c]

Values are mean ± SD.

[a]Versus groups I and groups II ($P < 0.05$).

[b]Versus groups II and groups III–IV ($P < 0.05$).

[c]Versus groups I and groups III–IV ($P < 0.05$).

Within the scope of the comparison of biochemical and hematologic parameters between Group I and IV, there was no statistically significant result, except for the AST level ($P > 0.05$).

Histopathologic study

When Group I and II were compared, it was observed that there was no difference between the values studied. There was a significant difference in edema, acinar necrosis, inflammatory cell infiltration, hemorrhage, and fat necrosis between Group II and III ($P < 0.05$). There was a significant difference regarding edema, acinar necrosis, inflammatory cell infiltration, hemorrhage, and fat necrosis between Group II and IV ($P < 0.05$). We did not observe any differences between Group II and III–IV regarding the rate of perivascular inflammation. A significant progress was seen regarding the values of perivascular inflammation, edema, acinar necrosis, inflammatory cell infiltration, hemorrhage, and fat necrosis between Group I and IV (Table 2). All the values were found to be similar for Group III and IV in the histopathologic study ($P > 0.05$).

The histopathologic study revealed no statistically significant result between Group I and IV ($P > 0.05$). It was seen that Sterile Novuxol (Abbott) did not cause any histopathologic change in the hepatic and peritoneal structure.

Discussion

While most acute pancreatitis patients recover through medical treatment, about 20% of the cases are severe and end up in local and general complications.[6–8] Among these complications, pancreatic necrosis has a significant role. The questions of how, when, and through which method should the necrosis in pancreas be treated are still on the agenda.

It has been reported that the necrosis should be debrided (necrosectomy) after the necrosis in the pancreas exceeds its half value (50%) or when it gets infected before it reaches this rate.[9,10] It has also been emphasized that necrosectomy should be performed after about 4 weeks as opposed to the old views.[11,12] It has been shown that necrosis can be cleaned up through minimally-invasive methods

Table 2 Histopathologic study results

Parameters	Group I	Group II	Group III	Group IV
Edema	2.8 ± 0.42[a]	2.3 ± 0.67[b]	0.8 ± 0.79[a,b]	0.8 ± 0.96[a,b]
Acinar necrosis	2.4 ± 0.52[a]	2.4 ± 0.70[b]	0.7 ± 0.67[a,b]	0.7 ± 0.76[a,b]
İnflammatory cell infiltration	2.3 ± 0.67[a]	1.8 ± 0.63[b]	0.8 ± 0.63[a,b]	0.8 ± 0.50[a,b]
Hemorrhage	2.6 ± 0.70[a]	2.2 ± 0.79[b]	1.1 ± 0.88[a,b]	1.2 ± 0.84[a,b]
Fat necrosis	2.4 ± 0.52[a]	2.4 ± 0.70[b]	1.0 ± 0.67[a,b]	0.58[a,b]
Perivascular inflammation	2.5 ± 0.71[a]	1.8 ± 0.92[b]	0.8 ± 0.79[a]	0.8 ± 0.75[a]

Normal: 0, mild: 1, moderate: 2, severe: 3.

Values are mean ± SD.

[a]Versus groups I and groups III–IV ($P < 0.05$).

[b]Versus groups II and groups III–IV ($P < 0.05$).

rather than open surgery.[13] Further, the issue that necrosis can be treated by antibiotherapy alone, even if it is infected besides surgery, is still under discussion.[13]

While the above mentioned variations go on at a clinical level, we have been continuing our work also at an experimental level within a wide scope, ranging from the formation of acute pancreatitis to its prevention or treatment. However, to be inspired by clinical work in experimental pancreatitis studies, as is the case with many experimental studies, remains at a low level. We tried to treat the experimental animals with formed necrotizing pancreatitis through an approach that was never before attempted. We used an enzyme preparation containing collagenase + protease (Sterile Novuxol, Abbott), which has been used for wound healing, especially for the healing of surgical site infections with ischemic and necrotic tissues and for open wounds (like burns), for the debridement of the necrosis in the pancreas. To the best of our knowledge, this is the first time such an experiment has been conducted for acute pancreatitis. Collagenase is being used for the local treatment of infected and ischemic wounds for debridement. It enables the wound to be granulated as soon as possible by separating the connection of necrotic ischemic tissues by collagen to the main tissue through lysis.[14] Methods like surgical debridement, hydrostatic debridement, maggot therapy (debridement by maggot), and honey application instead of this enzymatic debridement are among the other options used in ischemic wound debridement.

The difference between the debridement of pancreatic necrosis and open wounds is the fact that the organ is indispensible and it refers to the necessity that the best result should be achieved through acinus and islet protective debridement as much as possible. Another characteristic is the difficulty of reaching the pancreas since it is retroperitoneally located inside the abdomen. But this refers to human anatomy and is not valid for the experimental rats in our model because rat pancreas is located on a mobile mesentery and is superficial.

In our study, the mentioned preparation was administered to Group III by everyday applications to the pancreatic loge and this enabled us both to observe how the impaired pancreas was affected on a daily basis and to administer the new preparation. The reason for having Group IV was to see what kind of a result would be achieved in comparison to Group III if the collagenase was administered to the peritoneal cavity instead of the loge. Indeed there is data on successful results achieved by administering allopurinol into the peritoneal cavity of experimental animals with formed acute necrotizing pancreatitis.[15] It is possible to object to this group when one considers that the effect of allopurinol is different from that of Sterile Novuxol (Abbott). But the other reason for forming this group was to investigate the effects of the toxicity of Sterile Novuxol (Abbott) on peritoneal and abdominal viscera. While the enzyme preparation used in this group had no negative effects on the visceral surfaces, it was seen that it affected pancreatic necrosis. The reason for this was probably the fact that the preparation administered to the peritoneum did not stay at one point but spread all over the abdomen by changing its place in the abdomen and debriding the ischemic tissues through bowel movements as well as the movements of the experimental animal. The relaparotomy procedures and anesthesia used within the framework of our method had such negative effects as impairing the animals' health and decreasing the pace of healing although they enabled us to see the pancreas. This negative effect can be eliminated by the help of a catheter located intraperitoneally. We believe that this model can be used in a similar study.

In this experimental study, which was also inspired by necrosectomy performed in the treatment of acute necrotizing pancreatitis in clinic, it was seen that the pancreatic debridement results of both direct application on the pancreas (Group III) and administration of Sterile Novuxol (Abbott) to the peritoneal cavity (Group IV) were similar. It was also observed that this preparation administered to the peritoneal distance (Group III and Group IV) was not locally toxic. Further studies can be conducted in order to investigate its long-term systemic effects. The association of collagenase and protease, however, is something familiar to the organism. Indeed, when it is used for local wounds the enzymes mix with blood even if in a small amount. Further, we have not come across any information referring to any systemic side effect of Sterile Novuxol on wound debridement.

In conclusion, Sterile Novuxol (Abbott, collagenase + protease) preparation used in this experimental pancreatitis model was found to be effective on the debridement of pancreatic necrosis. The possible results of the model in clinic can be uncovered, if this study, which we know is the first of its kind, can give way to further studies. If the necessary consents can be obtained and gain

functionality, the model may offer a fresh perspective on the treatment of necrotizing pancreatitis.

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