Effects of trimetazidine in acute pancreatitis induced by L-arginine

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Background: In acute pancreatitis, oxygen free radicals (OFRs) and cytokines have been shown to play a role in the failure of pancreatic microcirculation and the development of local tissue damage. We studied the effects of trimetazidine (TMZ), a potent antioxidant and anti-ischemic agent, on acute pancreatitis.

Methods: Rats were randomized into 3 groups: a control group (n = 15), a study group (n = 15) in which acute pancreatitis was induced with with L-arginine, and a treatment group (n = 15) in which pancreatitis was induced and treated with TMZ intraperitoneally. The rats were followed for 24 hours. At the 24th hour we determined serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), amylase, lactate dehydrogenase (LDH), interleukin 1-β (IL-1β), interleukin 6 (IL-6) and tumour necrosis factor-α (TNF-α), and the pancreatic tissues were analyzed histopathologically.

Results: The AST ($p < 0.001$), ALT ($p < 0.01$), amylase ($p < 0.001$), LDH ($p < 0.01$), TNF-α ($p < 0.01$), IL-1β ($p < 0.001$) and IL-6 ($p < 0.001$) levels, and pancreatic tissue edema ($p < 0.01$), hemorrhage ($p < 0.05$), acinar cell necrosis ($p < 0.001$) and level of perivascular inflammation ($p < 0.01$), were significantly lower in the treatment group than the study group.

Conclusion: Trimetazidine markedly decreases biochemical and histopathologic changes during the early stages of acute pancreatitis, thus preserving the pancreas histologically.

Contexte : Dans la pancréatite aiguë, les radicaux libres de l'oxygène et les cytokines contribuent à l'insuffisance de la microcirculation pancréatique et à l'endommagement des tissus localement. Nous avons étudié les effets de la trimétazidine (TMZ), un puissant agent antioxydant et anti-ischémique, sur la pancréatite aiguë.

Méthodes : Des rats ont été assignés aléatoirement à 1 de 3 groupes : un groupe témoin (n = 15), un groupe (n = 15) dans lequel la pancréatite aiguë a été induite au moyen de L-arginine et un groupe (n = 15) dans lequel la pancréatite a été induite, puis traitée par TMZ par voie intrapéritonéale. Les rats ont été suivis pendant 24 heures. À la 24e heure, nous avons mesuré les taux sériques d'aspartate aminotransférase (AST), d'alanine aminotransférase (ALT), d'amylase, de lacticodéshydrogénase (LDH), d'interleukine 1-β (IL-1β), d'interleukine 6 (IL-6) et de facteur de nécrose tumorale α (TNF-α), et les tissus pancréatiques ont été soumis à un examen histopathologique.

Résultats : Les taux d'AST ($p < 0{,}001$), d'ALT ($p < 0{,}01$), d'amylase ($p < 0{,}001$), de LDH ($p < 0{,}01$), de TNF-α ($p < 0{,}01$), d'IL-1β ($p < 0{,}001$) et d'IL-6 ($p < 0{,}001$), de même que l'œdème tissulaire ($p < 0{,}01$), les saignements ($p < 0{,}05$), la nécrose des cellules acineuses ($p < 0{,}001$) et le degré d'inflammation périvasculaire ($p < 0{,}01$) pancréatiques, étaient significativement moindres dans le groupe traité que dans le groupe non traité.

Conclusion : La trimétazidine atténue nettement les modifications biochimiques et histopathologiques qui accompagnent les premiers stades d'une pancréatite aiguë, ce qui permet de préserver le pancréas au plan histologique.

Acute pancreatitis is a disease with high morbidity and mortality. Various theories have been suggested regarding the physiopathology of acute pancreatitis, yet the underlying mechanism is not clearly understood. Oxygen free radicals (OFRs) and basic proinflammatory cytokines, such as tumour necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and interleukin-6 (IL-6), which play a role in acute pancreatitis and other systemic inflammatory

conditions, have been suggested to be responsible for the local tissue damage and multiple organ failure that occur during acute pancreatitis. The OFRs lead to membrane lipid peroxidation, changes in the main components of the cytoplasm and early activation of digestive enzymes of pancreatitis, and they initiate protein damage in acute pancreatitis.[1–4]

L-arginine is a semi-essential amino acid found in our diet. Numerous animal studies have reported on L-arginine–induced acute pancreatitis. In 1984, Mizunuma and colleagues[5] developed a new type of experimental necrotizing pancreatitis by intraperitoneally administering a high dose of L-arginine in rats. The model is highly reproducible and noninvasive and produces dose-dependent acinar necrosis; therefore, it is ideal for studying the pathogenesis of acute pancreatitis. In this study, we used L-arginine to create pancreatitis, similar to the study by Szaboles and colleagues.[6]

Trimetazidine (2,3,4-trimethoxybenzyl-piperazine dihydrochloride; TMZ), which does not have any direct hemodynamic effects, has an anti-ischemic effect at the cellular level.[7–9] This drug has an antioxidant effect by protecting the cell against harmful effects of OFRs that arise during ischemia and inflammation.[7–11] By decreasing the inhibition of OFR production and membrane lipid peroxidation, TMZ exerts a protective effect.[9–12] On the other hand, TMZ is a chemical agent that also inhibits neutrophil infiltration following ischemia/reperfusion.[9,13] The aim of this study was to investigate TMZ effects on acute pancreatitis.

Methods

This study was approved by the local ethics committee of the Firat University, Elazıg, Turkey. All animals received care in compliance with the Principle of Laboratory Animal Care formulated by the National Society for Medical Research and the Guide for Care and Use of Laboratory Animals prepared by the Institute of Laboratory Animal Resources.

Forty-five male Wistar albino rats, weighing 180–220 g, were used in this study. Rats were fed with standard pellets and tap water, and standard temperature and humidity conditions were maintained. All rats were deprived of food and water for 12 hours before the surgical procedure. The rats were randomized into 3 groups, each comprising 15 rats. The control group received 5 mL of 0.9% physiologic saline administered intraperitoneally. In the study group, acute pancreatitis was induced by 2 doses of 250 mg/100 g of L-arginine (Sigma Chemical) prepared with 20% 0.15 M NaCl and administered intraperitoneally at a 1 hour interval. In the treatment group, acute pancreatitis was induced in the same way as in the study group, but rats were then treated with 2 doses of 5 mg/kg/day of TMZ (Servier Laboratories) administered intraperitoneally 30 minutes and 12 hours after induction of pancreatitis.

The rats were followed for 24 hours and then anesthetized using ketamine HCl (Ketalar; JHP Pharmaceuticals) in a 50 mg/mL concentration and xylazine HCl (Rompun; Bayer) in a 20 mg/mL concentration administered intramuscularly in the right rear leg of the rats at a dose of 0.25 mL/100 g of body weight. We obtained blood samples to determine levels of amylase, TNF-α, IL-1β, IL-6, lactate dehydrogenase (LDH), alanine aminotransferase (ALT) and aspartate aminotransferase (AST). In addition, we performed a laparotomy to remove the pancreatic tissue. The pancreatic tissue samples were fixed in 10% neutral buffered formalin solution for histopathologic analysis, and the microscopic sections were stained with hematoxylin eosin. The histopathologic analysis was performed under light microscopy by the same pathologist (I.H.O), who was blinded to the study groups. We histopathologically evaluated edema, acinar cell necrosis, hemorrhage and the degree of inflammation in the pancreas. The scoring system used by Schmidt and colleagues[12] was used for histopathological evaluation (Table 1).

Serum amylase, LDH, AST and ALT levels in the rat blood samples were determined using commercial kits with an Olympus AU 600 autoanalyzer (Olympus Optical Co.), whereas serum IL-1β, IL-6 and TNF-α levels were determined using BioSource commercial kits (Medgenix, Biosource International) with an enzyme-linked immunosorbent assay (ELISA) method.

Table 1. Histopathologic scoring system

Diagnosis; score	Symptoms
Edema	
0	Absent
1	Focal expansion of interlobular septa
2	Diffuse expansion of interlobular septa
3	Diffuse expansion of interlobular septa, focal expansion of interacinar septa
4	Diffuse expansion of interlobular septa, diffuse expansion of interacinar septa
5	Diffuse expansion of interlobular septa, diffuse expansion of interacinar septa and increase in the distance between cells
Acinar cell necrosis	
0	Absent
1	1–4 necrotic cells
2	5–10 necrotic cells
3	11–16 necrotic cells
4	> 16 necrotic cells
Hemorrhage	
0	Absent
1	In 1 area
2	In 2 areas
3	In 3 areas
4	In more than 4 areas
Inflammation and perivascular infiltration	
0	0–1 interlobular or perivascular leukocyte
1	2–5 interlobular or perivascular leukocytes
2	6–11 interlobular or perivascular leukocytes
3	12–20 interlobular or perivascular leukocytes
4	> 20 leukocytes or widespread microabscesses

Statistical analysis

Results are reported as means (and standard deviations [SD]). We evaluated the data using 1-way analysis of variance (ANOVA), as there were more than 2 study groups. When the parameters were significant in the variance analysis, we used the Duncan test for paired comparison of the groups.

Results

Our biochemical, cytological and pathological results were significant. The biochemical parameters are compared in Table 2. The AST, ALT, LDH and amylase levels were significantly lower in the treatment group than in the study group ($p < 0.001$). The AST, ALT and amylase levels were lower in the treatment group than in the control group; however, the difference was not significant (AST $p = 0.65$, ALT $p = 0.32$ and amylase $p = 0.50$).

The levels of cytological parameters (IL-1β, IL–6 and TNF-α) were compared among the groups. The IL-1β, IL-6 and TNF-α levels were significantly lower in the treatment group than in the study group (Fig. 1A–C; $p < 0.001$).

The histopathologic evaluation of the groups demonstrated that edema, acinar cell necrosis, hemorrhage and the level of perivascular inflammation were significantly lower in the treatment group than in the study group (Fig. 1E–G; $p < 0.001$).

Figure 2 comprises histologic images of control, study and treatment groups.

Discussion

Acute pancreatitis is a disease with high morbidity and mortality; however, the physiopathology of acute pancreatitis is not fully understood. During the treatment of acute pancreatitis, vital signs are followed, intravascular volume is maintained, electrolyte balance is achieved, analgesics are provided

Table 2. Biochemical results

	Group; mean (SD)			
Parameter	Group 1, control	Group 2, study	Group 3, treatment	*p* value
AST, μkat/L	3.16 (0.64)	4.44 (0.72)	2.95 (0.28)	< 0.001
ALT, μkat/L	0.84 (0.16)	0.96 (0.18)	0.75 (0.12)	0.004
LDH, μkat/L	21.53 (5.18)	26.99 (5.44)	22.41 (4.49)	0.003
Amylase, μkat/L	25.94 (2.05)	31.92 (4.17)	23.29 (1.32)	< 0.001

ALT = alanine aminotransferase; AST = aspartate aminotransferase; LDH = lactate dehydrogenase; SD = standard deviation.

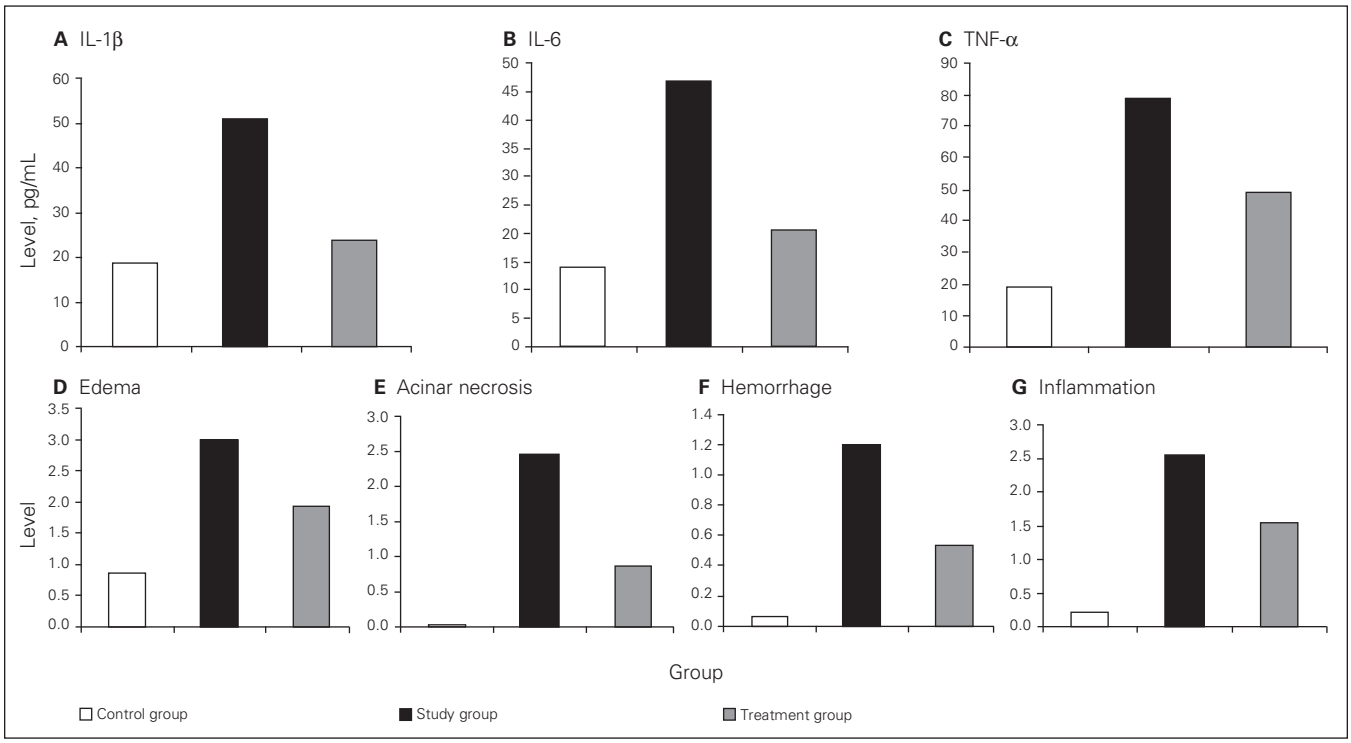

Fig. 1. Levels of (**A**) IL-1β, (**B**) IL-6, (C) TNF-α, (**D**) edema, (**E**) acinar cell necrosis, (**F**) hemorrhage and (**G**) perivascular inflammation.

and treatment for possible complications is scheduled. No clear benefit from the administration of medications, such as aprotinin, gabexate mesylate, glucagon and calcitonin, to treat acute pancreatitis has been demonstrated.[14,15] In theory, somatostatin is thought to be beneficial in acute pancreatitis since it suppresses the release of pancreatic enzymes; however, very little or no benefit has been demonstrated.[16–18] Narcotic analgesics should be used carefully when providing analgesia to patients with acute pancreatitis; pethidine is the preferred agent.[19]

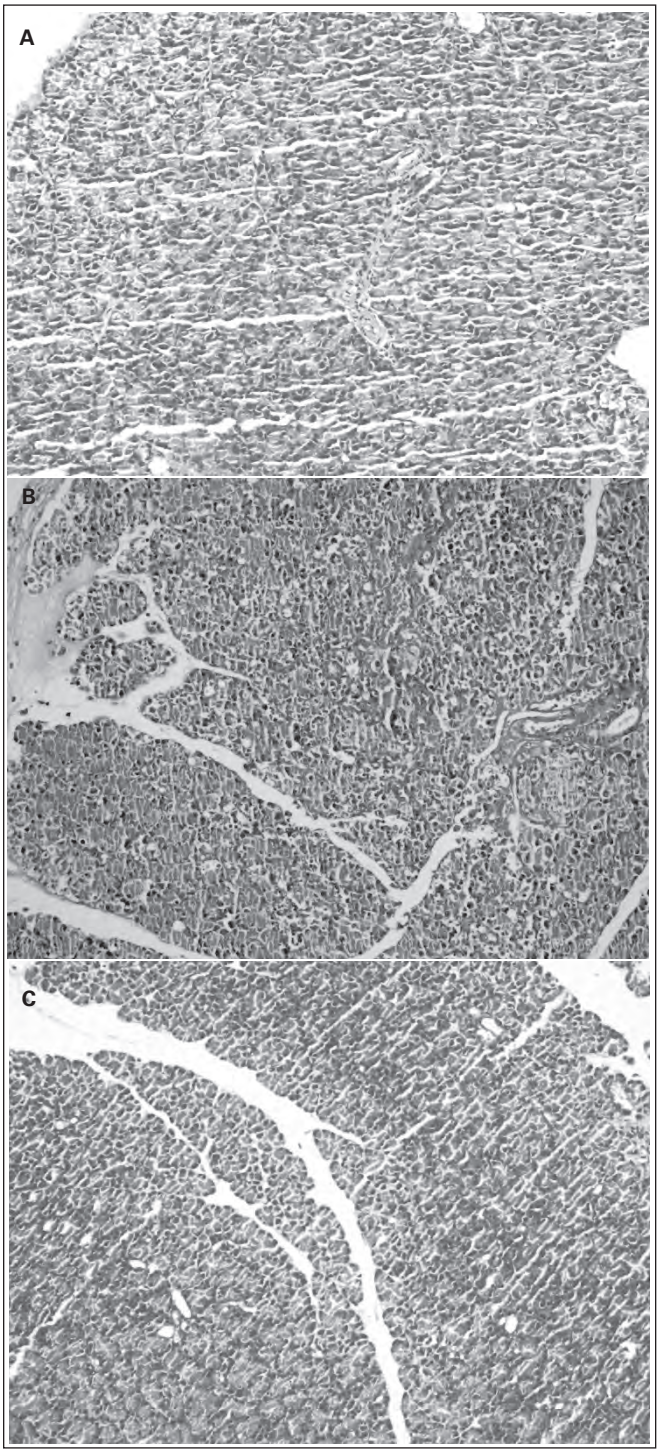

Fig. 2. Histological images of pancreatic tissue in the (**A**) control, (**B**) study and (**C**) treatment groups; hematoxylin and eosin stain, magnification ×200.

Following the extravasation of pancreatic secretions through the tissue spaces, proteolytic enzymes, especially activated trypsinogen, start the process of autodigestion of pancreatic tissue.[20] Tissue edema develops, along with microcirculation failure and ischemia at the cellular level. Circulatory failure leads to increased severity of the inflammation and accumulation of toxic mediators (OFRs) in the pancreas. Inflammatory infiltration becomes pronounced, and secretion of cytokines from monocytes and macrophages begins.[21–25]

Some studies have demonstrated that the presence of high doses of basic amino acids in rats causes pancreatic damage and leads to the development of acute pancreatitis.[26–28] A significant increase in the levels of IL-1β, IL-6 and TNF-α has been reported in acute pancreatitis induced by L-arginine. Following the intraperitoneal injection of L-arginine, IL-1β, IL-6 and TNF-α have been reported to be released from macrophages and monocytes, which are activated in stimulation of peritoneal macrophages or severe damage to the pancreas.[26–28]

In a study by Aubert and colleagues,[8] phenazine methosulfate (PMS) was administered to the perilymphatic region in a frog model to produce OFRs, resulting in a decrease in the secretion of afferent neurotransmitters. On the other hand, when PMS and TMZ were administered together, TMZ inhibited the effects of PMS, and the authors concluded that TMZ eliminated the effects of OFRs induced by ischemia on the cochleovestibular system.

In the present study, the serum levels of AST, ALT, amylase, LDH, IL-1β, IL-6 and TNF-α increased markedly following the intraperitoneal injection of L-arginine solution; however, the intraperitoneal administration of TMZ led to a significant reduction in these parameters ($p < 0.001$). The anti-ischemic and antioxidant effects of TMZ may explain our results. Pancreatic microcirculation damage developed, together with the induction of acute pancreatitis, ischemia and inflammation. With ischemia and inflammation, the production of OFRs increases; therefore, the development of ischemia and inflammation resulted in an increase in AST, ALT, LDH and amylase levels and an increase in proinflammatory cytokines (IL-1β, IL-6 and TNF-α). Trimetazidine, an anti-ischemic agent at the cellular level, does not have a direct hemodynamic effect. This property of TMZ may lead to a significant decrease in the levels of AST, ALT, amylase, LDH, IL-1β, IL–6 and TNF-α by decreasing the severity of cellular ischemia and inflammation. On the other hand, in the study group in which pancreatitis was induced by the administration of L-arginine, failure in the microcirculation led to a histopathologically significant increase in edema, acinar cell necrosis, hemorrhage and perivascular inflammation level, whereas

administration of TMZ in the treatment group demonstrated a significant decrease in these parameters compared with the study group ($p < 0.001$).

Conclusion

Trimetazidine is a powerful anti-ischemic and anti-inflammatory agent. It eliminates OFRs, which arise early in the course of acute pancreatitis, thereby significantly reducing the biochemical and histopathological changes and protecting the pancreas histologically.

Competing interests: None declared.

Contributors: A. Yenıcerıoglu and Z. Cetınkaya designed the study. B. Ustundag and I.H. Ozercan acquired the data. M. Gırgın, R. Ayten and B.H. Kanat analyzed the data. M. Gırgın, R. Ayten and B.H. Kanat wrote the article. A. Yenıcerıoglu, Z. Cetınkaya, B. Ustundag and I.H. Ozercan reviewed the article. All authors approved its publication.

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