Int Surg 2017;**102:**542–551
DOI: 10.9738/INTSURG-D-17-00122.1

Trimetazidine Increases Cell Survival and Inhibits the Activation of Inflammatory Response in Sodium Taurocholate–Induced Acute Pancreatitis

Sevil Işık[1], Neriman Şengül[2], Fatma Töre[3], Cemalettin Aydın[4], Açelya Aslan[5], Gülberk Uçar[5], Tülin Fırat[6], Aysel Kükner[6], Recep Bayram[7], Ali Eba Demirbağ[8], Taner Oruğ[9]

[1]*Department of General Surgery, Ordu Medical Park Hospital, Ordu, Turkey*

[2]*General Surgery Department, Medical Faculty, Abant İzzet Baysal University, Bolu, Turkey*

[3]*Physiology Department, Medical Faculty, Biruni University, Istanbul, Turkey*

[4]*General Surgery Department, Medical Faculty, Inönü University, Malatya, Turkey*

[5]*Biochemistery Department, Pharmacy Faculty, Hacettepe University, Ankara, Turkey*

[6]*Histology-Embryology Department, Medical Faculty, Abant İzzet Baysal University, Bolu, Turkey*

[7]*Pharmachology Department, Medical Faculty, Abant İzzet Baysal University, Bolu, Turkey*

[8]*Department of Gastroenterological Surgery, Türkiye Yüksek İhtisas Post-Graduate Training and Research Hospital, Ankara, Turkey*

[9]*General Surgery Department, Medical Faculty, Bahçeşehir University, Istanbul, Turkey*

Objective: To evaluate the therapeutic effects of trimetazidine (TMZ) in an experimental acute pancreatitis (AP) model induced with sodium taurocholate (STC).

Summary of Background Data: At present, AP is considered a disease with no specific treatment. Preventing mitochondrial dysfunction in acinar cells may be an option for

Corresponding author: Sevil Işık, General Surgery Department, Ordu Medical Park Hospital, Gaffar Okan Cad No. 39, 52100 Altınordu, Ordu, Turkey.
Tel.: +90 505 272 23 32; E-mail: isiksevil@hotmail.com

specific treatment of AP. TMZ is an anti-ischemic drug with anti-inflammatory, antioxidant, and mitochondrial modulatory effects.

Methods: Rats were divided into 4 groups. AP was induced in the AP (n = 7) and AP + TMZ (n = 7) groups by an injection of 4% sodium taurocholate to the pancreatic duct. The sham (n = 6) and drug (n = 6) groups were designated as control groups. The AP + TMZ and drug groups were administered TMZ. Samples were taken at 72 hours, and histopathologic changes as well as biochemical parameters were analyzed.

Results: Serum amylase, tissue myeloperoxidase activity, malondialdehyde levels, serum cytokine levels, and mast cell degranulation rates were elevated after induction of AP, whereas tissue antioxidant enzyme activities and cell viability rates [determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay] decreased. These parameters were found to be different in the AP group compared with those in all other groups ($P < 0.05$). A significant improvement of all parameters was achieved with the TMZ treatment of AP. Histologically, significant differences were found between the AP and AP + TMZ groups in terms of leukocyte infiltration, necrosis, and apoptotic cell counts.

Conclusions: In this study, we demonstrated that TMZ treatment protected the mitochondrial function and prevented the activation of the inflammatory cascade in the sodium taurocholate–induced AP model.

Key words: Acute pancreatitis – Mitochondria – Trimetazidine – Mast cell – Oxidative stress – Experimental

In the pathophysiology of acute pancreatitis, cell damage and inflammation occur as a cascade, increasing each other's effect. It is believed that acinar cells are central to this process.[1] The process is thought to begin with acinar cell necrosis, and the extent of necrosis is correlated with the severity of pancreatitis. It is known that the dispersal of cellular contents through necrosis increases the severity of inflammation. Mediators released from activated inflammatory cells, which are drawn to the area, also increase cell death.[2] On the other hand, in cases of increased apoptosis, which is a form of cell death that causes less inflammatory reaction, the severity of pancreatitis is lower.[3] The functionality of mitochondria in a damaged cell determines the form of death of acinar cells.[4] Opening of the mitochondrial permeability transition pore, and the resulting loss of mitochondrial membrane potential, penetration of calcium into mitochondria, ATP depletion, and inactivation of energy-dependent apoptosis are consecutive events in the pathophysiology of acute pancreatitis. Therefore, stabilization of mitochondrial permeability transition pores may prevent the dysfunction of mitochondria and uncontrolled cell death.[5,6]

Trimetazidine (TMZ) has been in clinical use as a cardiac anti-ischemic drug for a long time. It has been shown that, in addition to its anti-ischemic effects on cardiac muscles, and its metabolic effects, such as the inhibition of long-chain 3-ketoacyl-CoA thiolase activation, a decrease in fatty acid oxidation, and stimulation of glucose oxidation,[7] TMZ can also modulate the mitochondrial permeability transition. It has been reported that the drug prevents the opening of mitochondrial permeability transition pores induced by calcium.[8,9] It basically prevents the penetration of calcium into mitochondria, which in turn prevents swelling. In many experimental studies, it has been reported that TMZ can reduce ischemia/reperfusion not only in the cardiac muscle but in many other organs as well.[10] There have been incremental efforts to treat acute pancreatitis by blocking the penetration of calcium into mitochondria.[11] The aim of this study was to determine whether TMZ could alleviate the severity of acute pancreatitis, because of its protective effect on mitochondria and its weakening effect on the inflammatory response.

Materials and Methods

Animal experiments

The study was performed in accordance with the Guide for the Care and Use of Laboratory Animals

Table 1 Pancreas scoring criteria (modified Schmidt score)

	Grade 0	Grade 1	Grade 2	Grade 3
Edema	None	Interlobular	Intralobular	Including the periphery of acini
Inflammation	None	<20% (1–10 leukocytes in an area of ×40)	20%-50% (11–20 leukocytes in an area of ×40)	>50% (>20 leukocytes in an area of ×40)
Necrosis	None	<5%	5%–20%	>20%
Hemorrhage	None	1–2 sites	3–5 sites	>20%

and was approved by the Experimental Animals Ethics Committee of Abant İzzet Baysal University. A total of 26 Sprague-Dawley male rats, with an average weight of 210 g (180–320 g), were used in the study. The rats had access to water and a standard feed *ad libitum*.

Experimental design

The rats were randomly separated into 4 groups: acute pancreatitis (AP), AP treated with TMZ (AP + TMZ), sham (S), and drug (D) groups. The rats were anesthetized with intramuscular ketamine (50 mg/kg; Ketalar, Pfizer Inc, Kent, UK) and xylazine (10 mg/kg; Rompun, Bayer AG, Ontario, Canada). The rats in the AP and AP + TMZ groups had a midline laparotomy, and their common pancreatic duct was cannulated transduodenally via a 26-gauge Angiocath catheter (Hayat Medical, Istanvul, Turkey). To prevent hepatobiliary reflux, a mini–bulldog clamp was placed on the common bile duct at the hilum of the liver. Sodium taurocholate (4%; Sigma Aldrich Chemie GmbH, Munich, Germany) was infused into the common pancreatic duct by retrograde injection (0.1 mL per 100 g body weight per 1 minute). The catheter was removed at the end of the procedure, leaving 2 mL of normal saline in the abdominal cavity. After that, the abdomen and skin were closed. Thirty minutues after the induction of AP, the AP + TMZ and D groups were administered TMZ (10 mg/kg; Vastarel, Servier, Paris, France) through an orogastric tube. The AP and S groups were administered the same volume of normal saline.

AP rats (n= 7)

After the induction of pancreatitis, 1 mL of normal saline was administered orogastrically at 24-hour intervals until the end of the study.

AP + TMZ rats (n = 7)

After the induction of pancreatitis, 1 mL of TMZ at a 10 mg/kg dose was administered through an orogastric tube. Dosage was repeated every 24 hours.

S rats (n = 6)

The rats were anesthetized and subjected to laparotomy and hilar clamping using the same protocol as described above. Once the abdomen was closed, the same volume of normal saline was administered orogastrically at 24-hour intervals.

D rats (n = 6)

These rats received 10 mg/kg TMZ alone through the orogastric route on a daily basis.

After the operation, all animals were housed and had free access to food and tap water. At 72 hours of the study, relaparotomy was performed on the rats under anesthesia. The pancreas was immediately excised, and blood samples were collected. Tissue and serum samples were stored at –80°C until the time of examination.

Histopathologic examination

Pancreas tissue samples were fixed with 10% paraformaldehyde, embedded in paraffin blocks, and sectioned. Sections were stained with hematoxylin-eosin. Tissue samples were analyzed using criteria included in the method, which is a modification of the Schmidt scoring system[12] (Table 1), in terms of an edema, leukocyte infiltration, acinar cell necrosis, and a hemorrhage, and they were scored blinded to the groups.

Terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) staining was performed to detect the apoptosis of acinar cells. In tissue sections for every subject, TUNEL-positive cells were counted at 10 different sites with an eyepiece graticule under ×40 objective magnification (total area of 1 mm^2).

To examine mast cells, sections from all groups were stained with acidified toluidine blue (pH 2). Granulated and degranulated mast cells were

counted at 10 microscopic sites under ×10 objective magnification.

Cell viability assay

The assay was performed with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and repeated 3 times following the protocol. Tissues collected from the head and neck of the pancreases were weighed and placed into 1 mL of Dulbecco modified Eagle medium. The assay plate was read using an ELISA reader, and absorbances at 570 nm were recorded. The percentage of viability was calculated relative to the results obtained for the control group.

Determination of serum α-amylase activity

The serum α-amylase activity was determined by a simple, direct, and automation-ready procedure using a QuantiChrom α-amylase assay kit (BioAssay Systems, Hayward, California) and was expressed in $U.L^{-1}$.

Determination of tissue lipid peroxidation

Lipid peroxidation in tissue was determined by the spectrophotometric method of Ohkawa *et al*[13] based on the reaction of malondialdehyde (MDA) with thiobarbituric acid. The results were expressed in nmol.mg $protein^{-1}$.

Determination of tissue myeloperoxidase activity

The tissue myeloperoxidase (MPO) activity, which has been used to quantitatively determine the extent of polymorphonuclear leukocyte infiltration, was determined according to the method of Inci *et al*[14] and expressed in U.mg $protein^{-1}$.

Determination of tissue reduced glutathione levels

Reduced glutathione (GSH) levels were determined in pancreatic tissue according to the method of Sanchez-Alvarez *et al*[15] and expressed in nmol.mg $protein^{-1}$.

Determination of tissue antioxidant enzyme activities

The tissue catalase (CAT) activity was determined according to the method of Ueda *et al*[16] and expressed in $nmol.mg^{-1}$. The glutathione reductase activity was determined according to the method of Carlberg and Mannervrik[17] and expressed in $nmol.mg^{-1}$. Glutathione peroxidase (GPx) and superoxide dismutase (SOD) activities were determined using GPx and SOD assay kits (Cayman Chemical, Ann Arbor, Michigan), respectively, and expressed in U.mg $protein^{-1}$.

Determination of plasma cytokine levels

Serum samples were analyzed for cytokines using a cytokine rat 20-plex panel kit, according to the manufacturer's instructions (Invitrogen, Carlsbad, California). This kit contains analyte-specific components for the measurement of interleukin-1β (IL-1β), IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, and tumor necrosis factor-α (TNF-α). The measurement was performed using an xMAP Luminex 100 reader system (Austin, Texas), and cytokine levels were expressed in $pg.mL^{-1}$.

Statistical analysis

The data were analyzed with SPSS 16.0.0 (Chicago, Illinois) and are expressed as mean ± SD. For nonparametric data, the Kruskal-Wallis test was used for measured variables, whereas a χ^2 test was used for cross tables. The pair that caused the difference between groups identified as different by the Kruskal-Wallis test was determined using a post hoc multiple comparison test. Intergroup differences of histologic data were analyzed by the Mann-Whitney nonparametric *U* test. In all tests, $P < 0.05$ was accepted as statistically significant.

Results

The amylase levels were elevated after induction of acute pancreatitis, and the levels in the AP group were found to be different from the levels in all other groups ($P < 0.05$). In the AP + TMZ treatment group, however, the level was similar to those in the S and D groups (Fig. 1a).

MDA, which is a product of lipid peroxidation and an oxidative stress indicator of cell membrane injury, was elevated in acute pancreatitis. The MDA level in the AP group was found to be different from those in all other groups ($P = 0.000$). There was no difference between the AP + TMZ group and S S and D groups (Fig. 1b).

MPO activity, which is an indicator of neutrophil infiltration in tissue, increased in the pancreatitis group, and the activity in the AP group was found to be statistically different from those in the other groups ($P < 0.05$). No difference was observed

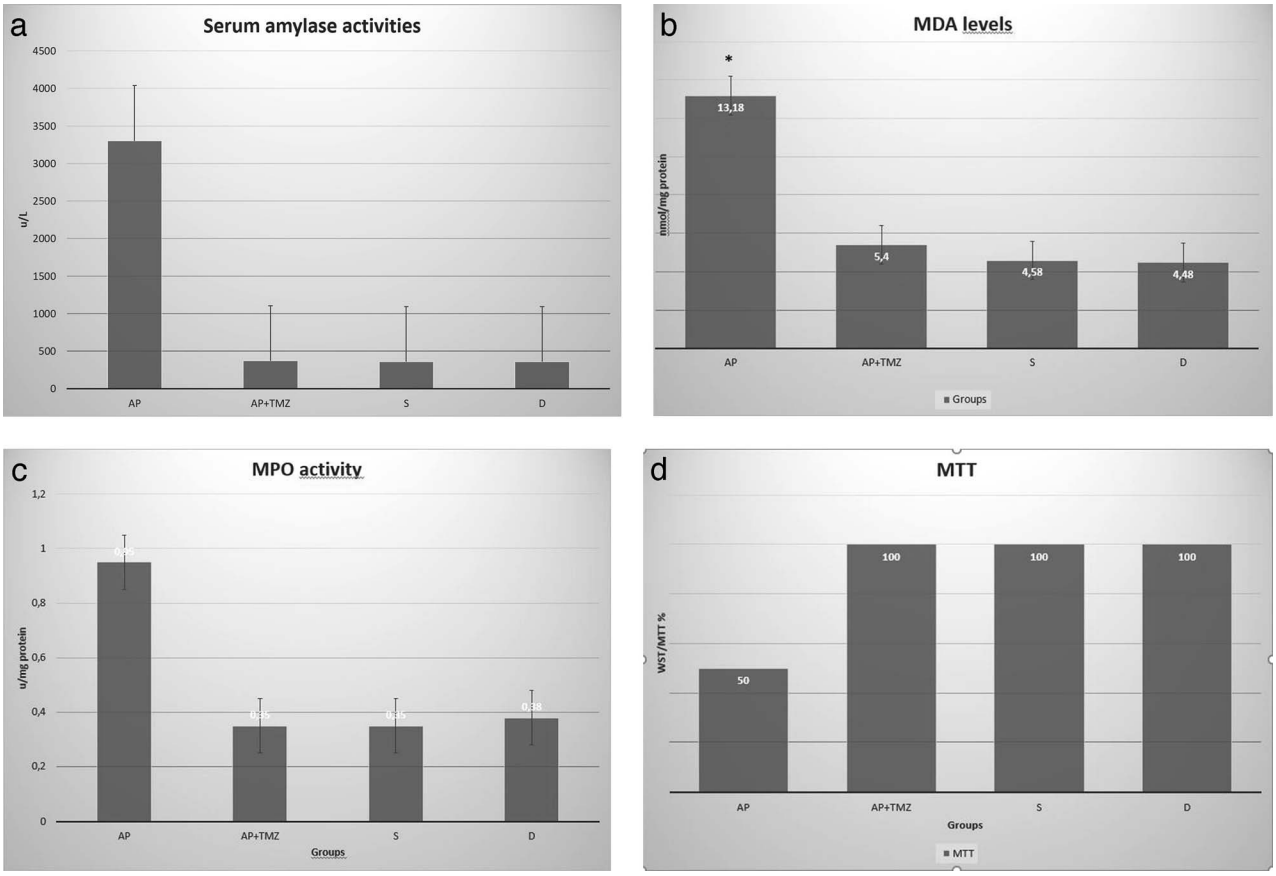

Fig. 1 (a) Serum amylase activities of the groups. The AP group was found to be statistically different from the other ones. (b) Tissue MDA levels of the groups. *$P < 0.05$, AP group was found to be statistically different from the other ones. Malondialdehyde level was elevated together with acute pancreatitis, and it declined with TMZ treatment. (c) Tissue MPO activities of the groups. *$P < 0.05$, tissue MPO activity in the AP group was found to be statistically different than the other ones. (d) Cell viability rate with MTT. Although cell viability rate was calculated as 100% with this method in groups S, D, and AP + TMZ, it was defined as 50% in the AP group.

between the rats in the AP + TMZ group and those in the S and D groups (Fig. 1c).

MTT is actively absorbed by cells and reduced to formazan, as a result of the reaction depending on mitochondrial function. The cell viability rate, which was calculated to be 100% in the S, D, and AP + TMZ groups, was only 50% in the AP group (Fig. 1d).

The nonenzymatic antioxidant GSH and enzymatic antioxidants SOD, and catalase, and an indirect marker of the tissue antioxidant capacity, GPx, as well as glutathione reductase activity, decreased with the induction of pancreatitis, and their levels in the AP group were found to be different from those in the other groups ($P < 0.05$). However, there were no statistical differences between the AP + TMZ group and the S and D groups (Table 2).

IL-1β and TNF-α are proinflammatory cytokines, and the expression of both cytokines was significantly elevated in the AP group compared with that in the other groups. The levels of IL-1β and TNF-α in the AP + TMZ group were not different from those in the S and D groups (Fig. 2a).

The levels of the other cytokines (IL-2, IL-4, IL-6, IL-8, IL-10, and IL-12) were elevated upon the induction of acute pancreatitis and in the AP group were statistically different from those in all other groups ($P < 0.05$). In the AP + TMZ treatment group, all cytokine levels declined, and there were no differences compared with their levels in the S and D groups (Fig. 2b–2d).

No differences were found in the mast cell counts among the groups. However, the number of mast cells that were degranulated upon acute pancreatitis

Table 2 Tissue antioxidant enzyme activities and reduced glutathione levels[a]

	AP (n = 7)	AP + TMZ (n = 7)	S (n = 6)	D (n = 6)	*P* value
SOD, U/mg protein	10.88 ± 4.08*	27.82 ± 3.4	28.51 ± 1.39	27.58 ± 1.48	0.002
CAT, nmol/mg	3.57 ± 1.03*	7.55 ± 0.60	7.22 ± 0.15	7.01 ± 0.17	0.000
GR, nmol/mg	17.52 ± 5.33*	33.33 ± 2.78	33.40 ± 2.20	32.11 ± 2.04	0.002
GPx, U/mg protein	0.47 ± 0.39*	1.43 ± 0.12	1.45 ± 0.20	1.60 ± 0.14	0.001
GSH, nmol/mg protein	37.85 ± 11.66*	75.85 ± 3.69	70.75 ± 3.32	74.750 ± 1.80	0.000

CAT, catalase; GR, glutathione reductase.

[a]Data are presented as means ± SD.

*$P < 0.05$, AP group was found to be different from all the other groups.

was reduced by 50% as a result of TMZ treatment (Fig. 3).

Based on the histopathologic analysis, the pancreatic sections from the S and D groups maintained a normal architecture. Histologically, significant differences were found between the AP and the AP + TMZ groups in terms of leukocyte infiltration, necrosis, and apoptotic cell counts. The AP group was different from the AP + TMZ, S, and D groups in terms of inflammation and necrosis ($P < 0.05$), whereas no significant differences were found between the AP and AP + TMZ groups in terms of the edema and hemorrhage ($P > 0.05$). The histopathologic data are shown in Table 3.

The mean apoptotic cell counts were 7.3 ± 6.7 per mm^2 and 2.7 ± 3.8 per mm^2 in the AP and AP + TMZ groups, respectively ($P < 0.05$).

Discussion

In our study, we demonstrated that TMZ treatment increased the functionality of mitochondria and prevented the activation of the inflammation cascade in a sodium taurocholate–induced pancreatitis model. It was observed that the decline in SOD, catalase, GPx, and glutathione reductase levels caused by the induction of pancreatitis was improved by TMZ treatment, potentially indicating that TMZ protects the mitochondrial function of acinar cells. As found by the MTT method, which is based on mitochondrial function, indirectly indicating the number of living cells, cell survival was decreased by pancreatitis induction; however, the percentage of surviving cells was calculated to be at the same level after TMZ treatment as that in the S and D groups. To our knowledge, there has been no report in the literature on the protection of mitochondria with TMZ in experimental acute pancreatitis. We performed this study in a sodium taurocholate–induced severe pancreatitis model representing biliary pancreatitis, one of the most common types of pancreatitis in humans. In the literature, there are 2 reports on caerulein-induced and L-arginine–induced acute experimental pancreatitis treated with TMZ.[18,19] In these studies, however, the abovementioned parameters were not analyzed. In our study, the MDA levels, which are the indicators of cellular membrane damage, regressed upon TMZ treatment to the levels found in the nonpancreatitis groups. He *et al*[20] have shown that increased MDA levels and decreased SOD and GPx levels in human umblical vein endothelial cells injured with H_2O_2 were improved by TMZ treatment. Similarly, Dehina *et al*[10] have shown in their study that TMZ decreased the production of mitochondrial reactive oxygen species in myocardial cells under ischemic conditions, whereas Zaouali *et al*[21] have concluded that the drug increased the survival of steatotic liver cells by reducing the stress caused by cold ischemia/reperfusion on mitochondria and the endoplasmic reticulum.

Table 3 Histopathologic data of the groups

Group	Edema	Hemorrhage	Inflammation	Necrosis
AP	2.28[a]	2[a]	2.14[a,b]	1.28[a,b]
AP + TMZ	1.7[a]	1.28[a]	1.28[a,b]	0.57[a,b]
S	0.83	0.16	0	0
D	0.83	0.16	0.16	0

Mean values of the groups according to histopathologic grade.

[a]$P < 0.05$, AP and AP + TMZ groups were found to be different from S and D groups.

[b]$P < 0.05$, AP group was found to be different from AP + TMZ group.

The reduction in microvascular polymorphonuclear leukocyte infiltration in pancreatic tissue and the reduction of the local inflammatory response are indicated by a decrease in MPO activity. In our study, the MPO activity in the AP + TMZ group was observed to be at the same level as that in the nonpancreatitis groups. Declines in the polymor-

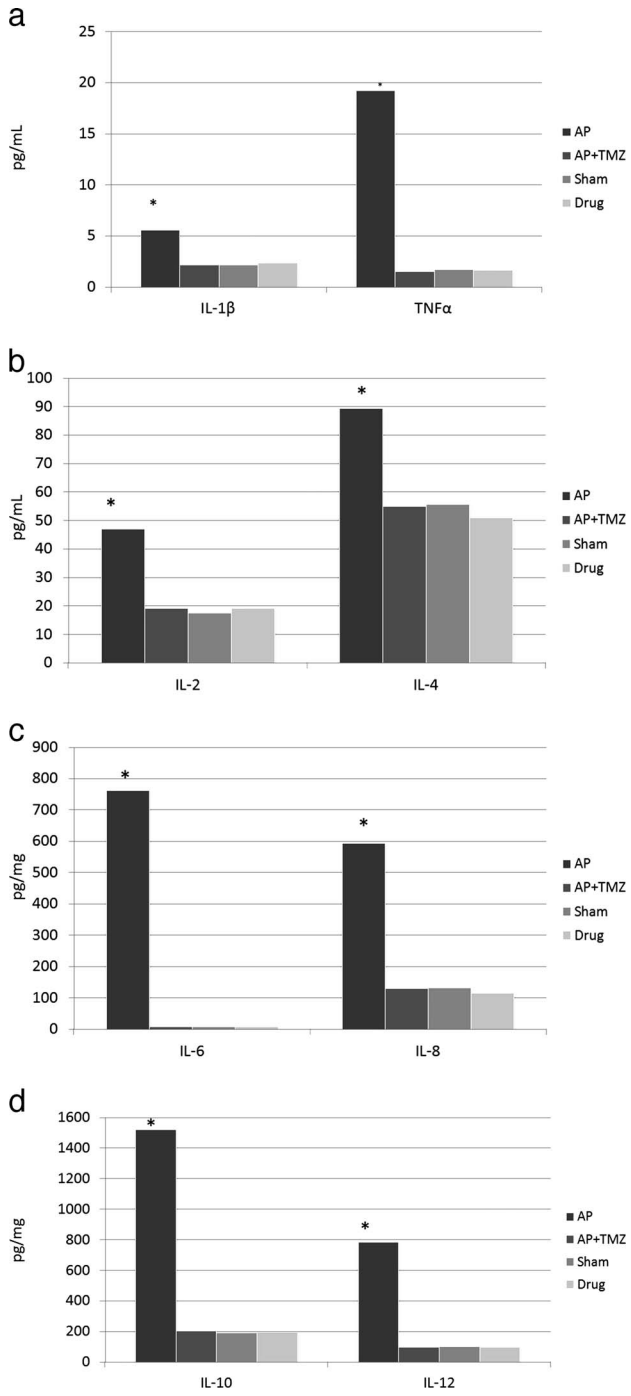

Fig. 2 Serum cytokine levels of study groups. (a) IL-1β and TNF-α. (b) IL-2 and IL-4. (c) IL-6 and IL-8. (d) IL-10 and IL-12. *All cytokine levels in the AP group were found to be statistically different from the other ones.

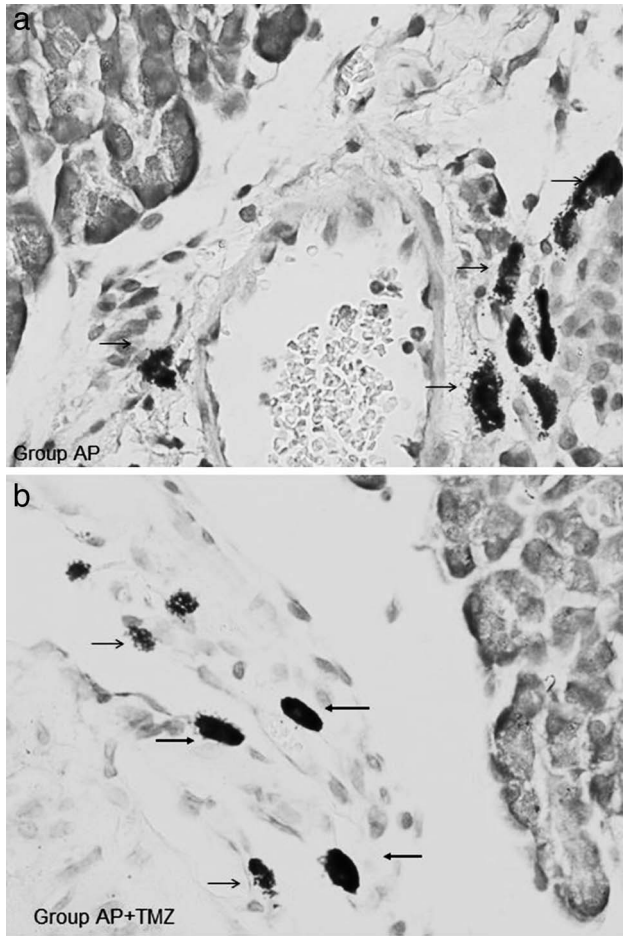

Fig. 3 Mast cells stained with toluidine blue. (a) All mast cells were degranulated (→) with acute pancreatitis. (b)The number of mast cells that were degranulated (→) with acute pancreatitis was reduced by 50% as a result of TMZ treatment. →, granulated mast cells.

phonuclear leukocyte transmigration and generation of proinflammatory cytokines lead to a decrease in acinar cell injury and a weakening of systemic inflammatory response syndrome.[22] Tanoğlu *et al*[23] have shown that TMZ treatment significantly improved the inflammation, oxidative stress, and membrane destruction in a lipopolysaccharide-induced sepsis model. It has also been established that TMZ inhibits the macrophage-mediated septic myocardial dysfunction, but it does not affect the biologic activity.[24] Tritto *et al*[25] have reported that experimentally, TMZ decreased neutrophil-mediated cardiac ischemia. In our study we showed that the MPO activity and cytokine release increased by acute pancreatitis were inhibited by TMZ treatment.

Cytokine levels, which are indicators of an increased inflammatory response, were elevated upon the induction of acute pancreatitis. In the AP + TMZ treatment group, all cytokine levels declined, and there were no differences with those in the

control group. One of the most interesting findings of this study was the elevation of IL-10, which is considered an anti-inflammatory cytokine, in acute pancreatitis, whereas it remained at normal levels in the TMZ treatment group. TNF-α and IL-1β are considered to be primary cytokines in acute pancreatitis, because these cytokines initiate and amplify the inflammatory cascade.[26] Our finding (regarding IL-10) can be explained by the fact that because TNF-α and IL-1β were not stimulated by TMZ treatment, the release of all other cytokines was not stimulated either. On the other hand, Wang *et al*[27] have indicated that the IL-10 level elevated upon the induction of pancreatitis. Pan *et al*[28] have reported that IL-10 levels were elevated at 24 hours of pancreatitis induction in the severe acute pancreatitis model. Furthermore, unlike our study, the IL-10 level was higher after treatment with ulinastatin. There is no consensus regarding the role of IL-10 in inflammation. Its anti-inflammatory benefit could not be fully proven in the clinic. In our study, similar to that by Fisic *et al*,[29] the IL-10 level was more likely associated with the severity of inflammation.

It is not yet known whether TMZ treatment is effective for mast cells. In our study, it was demonstrated that the number of mast cells that were degranulated upon acute pancreatitis was reduced by 50% as a result of TMZ treatment. Lopez-Font *et al*[30] have indicated that oxygen free radicals and inflammatory mediators, released as a result of pancreatic acinar cell injury and induced by stress, activated and degranulated mast cells within minutes after the induction of pancreatitis. The critical importance of calcium release–activated channels for the proliferation of mast cells and T cells, as well as for the production and release of cytokines, is well known.[31] Thus, it has been shown that the production and release of cytokines can be inhibited through the blockade of these channels. Vinokurov *et al*[32] have shown in THP1 human phagocytes that TMZ was able to reduce the inflammatory response by blocking the release of proinflammatory cytokines. In our study, the decreased degranulation of mast cells as a result of TMZ treatment can be speculatively explained by its reducing effect on the release of TNF-α from inflammatory cells, as well as by its effect on mast cells, similar to its regulating effect on calcium release–activated channels in THP1 cells.

We performed the assessment on the third day after the induction of pancreatitis because Kudari *et al*[33] have shown that histopathologic changes were prominent at 72 hours. The TMZ dose used in our experiments was determined based on the results of a study by Elimadi *et al*,[34] which established the optimal dose to protect mitochondria against the deleterious effects of ischemia/reperfusion in the liver. Based on histopathologic assessment, statistically significant differences were detected between the AP and AP + TMZ groups, and we observed less severity in the TMZ treatment group in terms of leukocyte infiltration, acinar cell necrosis, and apoptotic cell count ($P < 0.05$). Yenicerioglu *et al*[19] have observed a histopathologic improvement at 24 hours of TMZ treatment in an L-arginine–induced pancreatitis model, whereas Tanoğlu *et al*[18] have observed the same at 18 hours in a caerulein pancreatitis model. The apoptotic cell counts were reduced in the AP + TMZ group. Thus, we can conclude that the severity of pancreatitis was diminished in the group treated with TMZ. Our results were similar to those reported by Tanoğlu *et al*.[18] It has been reported that reactive oxygen species have a dual effect in pancreatitis; on the one hand, they increase apoptosis, and on the other hand, they increase inflammation by stimulating neutrophils.[35] It has been shown that during acute ischemia, TMZ treatment increases the ATP production in the heart and reduces the reactive oxygen species production in the mitochondrial respiratory chain and futile oxygen consumption.[9] In our study, the decrease in the apoptotic cell count can be explained by decreasing reactive oxygen species levels upon TMZ treatment.

In conclusion, in the sodium taurocholate–induced severe acute pancreatitis model, TMZ treatment not only increased cell survival, which is critical, but also inhibited the activation of the inflammatory response which facilitates the process. Thus, TMZ can be considered a promising agent for specific treatment of acute pancreatitis.

Acknowledgments

Financial support for this experimental study (drugs, equipment, etc) was provided by the authors. The authors have nothing to disclose. The authors have no conflict of interest.

References

1. Gukovsky I, Pandol SJ, Gukovskaya AS. Organellar dysfunction in the pathogenesis of pancreatitis. *Antioxid Redox Signal* 2011;**15**(10):2699–2710

2. Gukovskaya AS, Vaquero E, Zaninovic V, Gorelick FS, Lusis AJ, Brennan ML *et al*. Neutrophils and NADPH oxidase mediate intrapancreatic trypsin activation in murine experimental acute pancreatitis. *Gastroenterology* 2002;**122**(4):974–984
3. Kaiser AM, Saluja AK, Sengupta A, Saluja M, Steer ML. Relationship between severity, necrosis, and apoptosis in five models of experimental acute pancreatitis. *Am J Physiol* 1995; **269**(5, pt 1):1295–1304
4. Criddle DN, Gerasimenko JV, Baumgartner HK, Jaffar M, Voronina S, Sutton R *et al*. Calcium signalling and pancreatic cell death: apoptosis or necrosis? *Cell Death Differ* 2007;**14**(7): 1285–1294
5. Mukherjee R, Mareninova OA, Odinokova IV, Huang W, Murphy J, Chvanov M *et al*. Mechanism of mitochondrial permeability transition pore induction and damage in the pancreas: inhibition prevents acute pancreatitis by protecting production of ATP. *Gut* 2016;**65**(8):1333–1346
6. Gerasimenko JV, Gryshchenko O, Ferdek PE, Stapleton E, Hébert TO, Bychkova S *et al*. Ca^{2+} release-activated Ca^{2+} channel blockade as a potential tool in antipancreatitis therapy. *Proc Natl Acad Sci U S A* 2013;**110**(32):13186–13191
7. Kantor PF, Lucien A, Kozak R Lopaschuk GD. Trimetazidine shifts cardiac energy metabolism from fatty acid oxidation to glucose oxidation by inhibiting mitochondrial long chain 3 ketoacyl coenzyme A thiolase. *Circ Res* 2000;**86**(5):580–588
8. Argaud L, Gomez L, Gateau-Roesch O, Couture-Lepetit E, Loufouat J, Robert D *et al*. Trimetazidine inhibits mitochondrial permeability transition pore opening and prevents lethal ischemia-reperfusion injury. *J Mol Cell Cardiol* 2005;**39**(6):893–899
9. Monteiro P, Duarte AI, Gonçalves LM, Moreno A, Providência LA. Protective effect of trimetazidine on myocardial mitochondrial function in an ex-vivo model of global myocardial ischemia. *Eur J Pharmacol* 2004;**503**(1–3):123–128
10. Dehina L, Vaillant F, Tabib A, Bui-Xuan B, Chevalier P, Dizerens N *et al*. Trimetazidine demonstrated cardioprotective effects through mitochondrial pathway in a model of acute coronary ischemia. *Naunyn Schmiedebergs Arch Pharmacol* 2013; **386**(3):205–215
11. Criddle DN. Reactive oxygen species, Ca^{2+} stores and acute pancreatitis; a step closer to therapy? *Cell Calcium* 2016;**60**(3): 180–189
12. Liu ZH, Peng JS, Li CJ, Xiang J, Song H, Wu XB *et al*. A simple taurocholate-induced model of severe acute pancreatitis in rats. *World J Gastroenterol* 2009;**15**(45):5732–5739
13. Okhawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 1979;**95**(2):351–358
14. Inci I, Inci D, Dutly A, Boehler A, Weder W. Melatonin attenuates posttransplant lung ischemia-reperfusion injury. *Ann Thoracic Surg* 2002;**73**(1):220–225
15. Sanchez-Alvarez R, Almeida A, Medina JM. Oxidative stress in preterm rat brain is due to mitochondrial dysfunction. *Pediatr Res* 2002;**51**(1):34–39
16. Ueda M, Mozaffar S, Tanaka A. Catalase from *Candida boidinii* 2201. *Methods Enzymol* 1990:**188**:463–465
17. Carlberg I, Mannervik B. Glutathione reductase. *Methods Enzymol* 1985;**113**:484–490
18. Tanoğlu A, Yazgan Y, Kaplan M, Berber U, Kara M, Demirel D *et al*. Trimetazidine significantly reduces cerulein–induced pancreatic apoptosis. *Clin Res Hepatol Gastroenterol* 2015;**39**(1): 145–150
19. Yenicerioglu A, Cetinkaya Z, Girgin M, Ustundag B, Ozercan IH, Ayten R *et al*. Effects of trimetazidine in acute pancreatitis induced by L-arginine. *Can J Surg* 2013;**56**(3):175–179
20. He S, Yan F, Zhan J, Yan J, Bin Yuan, Chen S, Xie Y. Protective effects of trimetazidine against vascular endothelial cell injury induced by oxidation. *J Geriatr Cardiol* 2008;**5**(4):248–251
21. Zaouali MA, Boncompagni E, Reiter RJ, Bejaoui M, Freitas I, Pantazi E *et al*. AMPK involvement in endoplasmic reticulum stress and autophagy modulation after fatty liver graft preservation: a role for melatonin and trimetazidine cocktail. *J Pineal Res* 2013;**55**(1):65–78
22. Raraty MG, Murphy JA, Mcloughlin E, Smith D, Criddle D, Sutton R. Mechanisms of acinar cell injury in acute pancreatitis. *Scand J Surg* 2005;**94**(2):89–96
23. Tanoglu A, Yamanel L, Inal V, Ocal R, Comert B, Bilgi C. Appreciation of trimetazidine treatment in experimental sepsis rat model. *Bratisl LekListy* 2015;**116**(2):124–127
24. Chen J, Lai J, Yang L, Ruan G, Chaugai S, Ning Q *et al*. Trimetazidine prevents macrophage-mediated septic myocardial dysfunction via activation of the histone deacetylase sirtuin 1. *Br J Pharmacol* 2016;**173**(3):545–561
25. Tritto I, Wang P, Kuppusamy P, Giraldez R, Zweier JL, Ambrosio G. The anti-anginal drug trimetazidine reduces neutrophil-mediated cardiac reperfusion injury. *J Cardiovasc Pharmacol* 2005;**46**(1):89–98
26. Escobar J, Pereda J, Arduini A, Sandoval J, Sabater L, Aparisi P *et al*. Oxidative stress and pro-inflammatory cytokines in acute pancreatitis: a key role for protein phosphatases. *Curr Pharm Des* 2009;**15**(2):3027–3042
27. Wang XY, Tang QQ, Zhang JL, Fang MY, Li YX. Effect of SB203580 on pathologic change of pancreatic tissue and expression of TNF-α and IL-1β in rats with severe acute pancreatitis. *Eur Rev Med Pharmacol Sci* 2014;**18**(3):338–343
28. Pan Y, Fang H, Lu F, Pan M, Chen F, Xiong P *et al*. Ulinastatin ameliorates tissue damage of severe acute pancreatitis through modulating regulatory T cells. *J Inflamm (Lond)* 2017; **14**(7):7–17
29. Fisic E, Poropat G, Bilic-Zulle L, Licul V, Milic S, Stimac D. The role of IL-6, 8, and 10, sTNFr, CRP, and pancreatic elastase in the prediction of systemic complications in patients with acute pancreatitis. *Gastroenterol Res Pract* 2013;**2013**:1–6
30. Lopez-Font I, Gea-Sorlí S, de-Madaria E, Gutiérrez LM, Pérez-Mateo M, Closa D. Pancreatic and pulmonary mast cells activation during experimental acute pancreatitis. *World J Gastroenterol* 2010;**16**(27):3411–3417

31. Rice LV, Bax HJ, Russell LJ, Barrett VJ, Walton SE, Deakin AM *et al*. Characterization of selective Calcium-Release Activated Calcium channel blockers in mast cells and T-cells from human, rat, mouse and guinea-pig preparations. *Eur J Pharmacol* 2013;**704**(1–3):49–57
32. Vinokurov MG, Astashkin EI, Yurinskaya MM, Glezer MG, Sobolev KE, Grachev SV. Trimetazidine blocks store-operated Ca^{2+} channels in HL-60 and THP-1 cell lines and inhibits the secretion of tumor necrosis factor. *Dokl Biol Sci U S A* 2011; **441**(3):417–420
33. Kudari A, Wig JD, Vaiphei K, Kochhar R, Majumdar S, Gupta R *et al*. Histopathological sequential changes in sodium taurocholate-induced acute pancreatitis. *JOP* 2007;**8**(5):564–572
34. Elimadi A, Settaf A, Morin D, Sapena R, Lamchouri F, Cherrah Y *et al*. Trimetazidine counteracts the hepatic injury associated with ischemia-reperfusion by preserving mitochondrial function. *J Pharmacol Exp Ther* 1998;**286**(1):23–28
35. Booth DM, Murphy JA, Mukherjee R, Awais M, Neoptolemos JP, Gerasimenko O *et al*. Reactive oxygen species induced by bile acid induce apoptosis and protect against necrosis in pancreatic acinar cells. *Gastroenterology* 2011; **140**(7):2116–2125