Trimetazidine has Promising Preventive Effects on Experimental Chronic Pancreatitis Rat Model

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ABSTRACT

Background: *It was aimed to evaluate the preventive efficacy of trimetazidine in an experimental chronic pancreatitis rat model.*

Methods: *Chronic pancreatitis model was accomplished with caerulein and alcohol administration. In the study, 40 female Sprague Dawley rats were randomized into 5 groups containing 8 animals in each. Group 1 (chronic pancreatitis); group 2 (chronic pancreatitis + low-dose trimetazidine group); group 3 (chronic pancreatitis + high-dose trimetazidine group); group 4 (placebo group (chronic pancreatitis + saline)); group 5 (sham group). 24 hours after the last injection, all animals were sacrificed. Tumor necrosis factor-alpha, transforming growth factor-β, malondialdehyde, and glutathione peroxidase levels were tested in blood samples. Histopathologic examinations were conducted by a senior pathologist who was unaware of the group allocations.*

Results: *Results of biochemical parameters of the trimetazidine groups (groups 2 and 3) were significantly favorable compared with the chronic pancreatitis group (group 1) ($P < .05$). The difference between the low-dose- and the high-dose trimetazidine group (group 3) was significant in terms of blood tests ($P < .05$). The difference between the low-dose trimetazidine group and the chronic pancreatitis group was not significant in terms of histopathologic scores ($P > .05$); however, the difference was significant between the high-dose trimetazidine group and the chronic pancreatitis group ($P < .05$).*

Conclusions: *To the best of our knowledge, this current research is the first study that evaluates trimetazidine's efficacy in the chronic pancreatitis rat model. Trimetazidine has affirmative preventive properties in the chronic pancreatitis course.*

Keywords: *Caerulein, chronic pancreatitis, ethanol, trimetazidine*

INTRODUCTION

Chronic pancreatitis (CP) is a heterogeneous group of diseases characterized by long-lasting pancreatic inflammation resulting in fibrosis and gradual loss of pancreatic function.[1] The mechanisms and pathways of CP are complex, but recurrent acute pancreatitis attacks are the most basic pathology.[2] Alcohol is also a very important risk factor for exocrine pancreatic insufficiency, and a history of alcohol use is found in about half of all patients.[1,2] The prevalence and incidence may vary between countries. In Western populations, hospitalization rates have increased significantly in recent years due to CP. There is no specific treatment method known to prevent the development of CP.[2] The basic principles of treatment are to eliminate the factors causing it, alleviate the symptoms, improve pancreatic function, prevent complications, and improve the patient's quality of life.[1-3]

A number of hypotheses exist regarding the etiopathogenesis of CP. First, a necrosis–fibrosis series hypothesis suggests that CP develops with severe attacks of acute pancreatitis.[3] As a result of numerous and repetitive attacks, the damaged area is tried to be healed through inflammatory responses resulting in fibrous tissue formation in the necrotic pancreatic parenchyma.[4,5] Secondly, there is another hypothesis named "sentinel acute pancreatitis event." According to this hypothesis, a single attack of acute pancreatitis generates an inflammatory response and activates pancreatic stellate cells. Then, ongoing stress or damage causes fibrosis through activated immune system cells.[6] The third hypothesis is the effects of environmental factors such as alcohol and tobacco use and their toxic properties of metabolites on acinar cells. Moreover, oxidative stress is also an inevitable process of CP.[7-9]

Trimetazidine (TMZ) selectively blocks the long-chain 3-ketoacyl coenzyme A thiolase enzyme. This mentioned enzyme is the last one in fatty acid beta-oxidation. Inhibition of this reaction causes a change in the energy substrate

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with increased glucose oxidation.[10,11] Trimetazidine has been shown to retain mitochondrial functions during ischemia by inhibiting Na/K-ATPase activity, reducing acylcarnitine levels, and preventing intracellular calcium accumulation. The cytoprotective effect of TMZ can be explained by this mechanism.[12]

The binding points of TMZ in the mitochondria have been determined, and TMZ has been shown to correct the impaired functions due to ischemia. Moreover, TMZ has anti-inflammatory, anti-oxidative, and anti-apoptotic features.[10,13] Chronic pancreatitis is a chronic inflammatory process. Oxidative stress and pancreatic cell apoptosis are the physiopathological aspects of CP.[1,2] Thus, we decided to use TMZ as a preventive agent in the course of CP.

In this current research, we aimed to determine the preventive efficacy of TMZ in an experimental chronic rat pancreatitis model.

MATERIALS AND METHODS

After the approval of the Local Ethics Committee for Animal Experiments of the University of Health Sciences, Turkey (2018-08/01), the study was accomplished in the Laboratory of Experimental Animals of the University of Health Sciences during September-October 2019. The ethical standards of the European Convention for the Protection of Vertebrate Animals used for experimental and other scientific purposes were met carefully in this current research.

Study Design (Study Groups and CP Model)

In the experimental CP protocol, 40 female Sprague Dawley rats, weights ranging from 200 g to 250 g were used. Rats were obtained from the Laboratory of Experimental Animals of University of Health Sciences. All animals were housed in proper cages at room temperature with a 12-hour day–night cycle.

Chronic pancreatitis model was carried out by injecting caerulein (Sigma, St. Louis, MO, USA) with a dose of IP 50 mcg/kg. Injections were performed 3 days per week, twice a day at 1-hour intervals. Moreover, IP 1.5 mL 33% ethanol was administrated 5 days per week, twice a day (at 8:00 AM and 5:00 PM). All injections for the experimental model were lasted for 6 weeks.

Forty rats were randomized into 5 study groups:

Group 1: Chronic pancreatitis group (IP caerulein injections + IP ethanol injections for 6 weeks).

Group 2: 5 mg/kg TMZ (low-dose) group (experimental CP model was carried out and a prepared TMZ suspension was given by orogastric gavage. Trimetazidine was given once a day for 5 weeks beginning from the second week).

Group 3: 10 mg/kg TMZ (high-dose) group experimental CP model was carried out and a prepared TMZ suspension was given by orogastric gavage. Trimetazidine was given once a day for 5 weeks beginning from the second week).

Group 4: The placebo group (when creating CP as described in group 1, saline was injected IP at a dose of 1 cm^3 once daily for 5 weeks, 5 days per week beginning from the second week).

Group 5: The sham group (1 cm^3 of saline was injected IP and no other medication was administered IP for 6 weeks, 5 days per week).

All rats were sacrificed 24 hours after the last injections. Under general anesthesia, the rats were sacrificed by taking blood samples from the right atrium. The study was terminated after the pancreatic tissue samples were obtained. All blood samples were centrifuged at 3500 rpm for 10 minutes and stored at –80°C until biochemical analysis.

Biochemical Analysis

Tumor Necrosis Factor (TNF)-α, Transforming Growth Factor-β (TGF-β), Malondialdehyde (MDA), and Glutathione Peroxidase (GPx) Levels Tumor necrosis factor (TNF)-α, transforming growth factor-β (TGF-β), malondialdehyde (MDA), and glutathione peroxidase (GPx) levels had been measured.

Rat sandwich enzyme-linked immunosorbent assay (ELISA) kits [Bioassay Technology Laboratory (BT Lab)] were used to measure biochemical parameters. Measurements were accomplished by following the instructions in the research ELISA kit.

Histopathological Evaluation

After scarification, rat pancreatic tissues were carefully obtained for histopathological inspection. Pancreatic tissues samples were paraffinized after being fixed in 10% formaldehyde solution. Histopathological evaluation was done by a senior pathologist who was blind for the rat groups under a Nikon Eclipse, E600 microscope

after hematoxylin–eosin staining. A modified pathological scoring system was used for evaluation in which inflammation, atrophy, and fibrosis were scored from 0 to 3.[14]

Statistical Analysis

The statistical analysis was performed using the PSPP (Free Software Foundation) computer program and Statistical Package for the Social Sciences software (version 20.0, SSPS Inc., Chicago, Ill, USA). Descriptive analysis was performed (frequency distributions, percentage, mean, standard deviation), and the Kolmogorov–Smirnov test was used to test the normality of data distribution. The results were evaluated with 95% CIs and $P < .05$ was accepted as statistically significant. The one-way analysis of variance was performed to determine whether there was a difference between the groups in terms of GPx, MDA, TNF-α, and TGF-β. Duncan's multiple range test was used to determine the significant group differences that were found in variance analyses. The chi-square test was used to determine the variation of histopathologic scores by groups. In addition, correspondence analysis was used to better show the relationship between histopathologic scores and groups.

RESULTS

Biochemical Results

Results of biochemical parameters are shown in Table 1. In view of all biochemical tests, the difference between both high-dose (group 3) TMZ group and CP group and between the high-dose group and placebo group (group 4) was statistically significant ($P < .05$). The difference between the high-dose TMZ group and the placebo group was significant in terms of all biochemical parameters ($P < .05$) (Table 1). Significant difference was found between G1 and G2 versus G3 versus G4.

Histopathological Analysis

When the study groups were compared in terms of total histopathologic scores, the statistical difference between them was significant ($P < .05$). As the result of the chi-square analysis performed, the histopathological scores differed from group to group. Here, a correspondence analysis was applied to determine which histopathological scores were more common in which group. The graphic obtained as a result of the correspondence analysis is given below (Figure 1). According to this, there were more "none-zero" (0) histopathological scores in the sham group, more severe (+++) histopathological scores in the chronic pancreatitis group, more moderate (++) histopathological scores in the low-dose TMZ group, and more mild (+) histopathologic scores in the placebo and high-dose TMZ groups.

The difference between the low-dose TMZ group and the placebo group was not statistically significant ($P > .05$). On the other hand, the difference between the high-dose TMZ and the placebo group was significant ($P < .05$). Histopathological appearances are shown in Figure 2.

DISCUSSION

In CP, exocrine and endocrine dysfunction occurs as a result of inflammation and fibrosis in the pancreas.[15] The most severe complication in the long term is the development of pancreatic cancer.[16] Chronic pancreatitis is one of the most critical matters of morbidity in Western countries.[15-17] Increase in alcohol consumption and smoking and growing incidence of CP is also noteworthy.[17]

To the best of our knowledge, this is the first research that evaluates TMZ's preventive properties in the chronic pancreatitis rat model. In this current research, we aimed to evaluate the efficacy of the active substance TMZ

Table 1. Comparison of Groups in Terms of Biochemical Parameters

	Group 1 (CP)	Group 2 (LD)	Group 3 (HD)	Group 4 (P)	Group 5 (S)	*P*
GPx (ng/mL)	6.51 ± 2.3^{1a}	12.01 ± 1.8	14.20 ± 0.8	9.88 ± 1.5^{2b}	16.76 ± 2.2	.008*
TNF-α (ng/mL)	90.63 ± 8.3^{3c}	66.60 ± 3.1	63.35 ± 3.6	82.33 ± 3.3^{4d}	44.32 ± 6.2	.006*
MDA (nmol/mL)	4.39 ± 0.8^{5e}	2.62 ± 0.6	1.97 ± 0.3	3.93 ± 0.8^{6f}	1.21 ± 0.7	.01*
TGF-β (ng/L)	426.2 ± 82.9^{7g}	304.7 ± 20.6	248.96 ± 18.9	374.8 ± 16.2^{8h}	227.4 ± 4.3	.0006*

*Significant difference was found between G1 and G2 versus G3 versus G4.
G1 versus G3: [1] $P = .04$, [3] $P = 0.1$, [5] $P = .04$, [7] $P = .0009$.
G1 versus G4: [2] $P = .01$, [4] $P = .02$, [6] $P = .04$, [8] $P = .005$.
G2 versus G3: [a] $P = .04$, [c] $P = .002$, [e] $P = .03$, [g] $P = .002$.
G2 versus G4: [b] $P = .04$, [d] $P = .01$, [f] $P = .04$, [h] $P = .006$.
CP, chronic pancreatitis group; GPx, glutathione peroxidase; HD, high-dose trimetazidine group; MDA, malondialdehyde; LD, low-dose trimetazidine group; P, placebo group; S, sham group; TGF-β, tranforming growth factor-β levels; TNF-α, tumor necrosis factor-α.

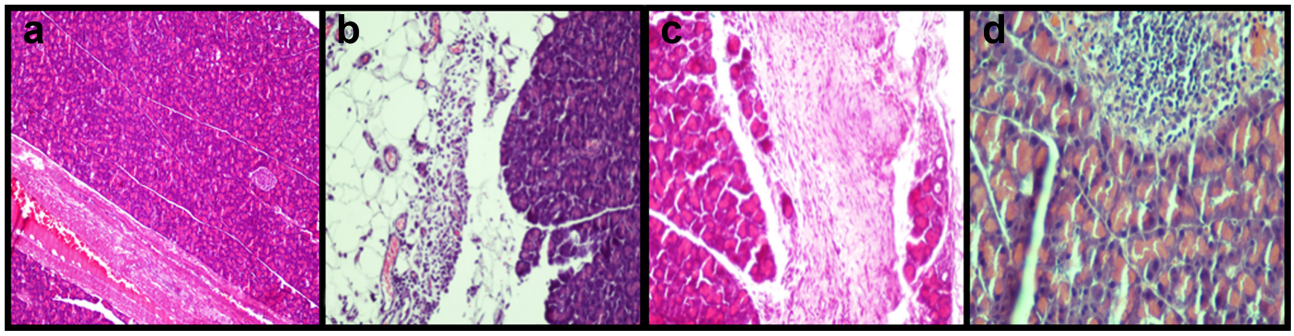

Figure 1. (a) Normal pancreatic tissue and its acinar structure, Hex200, (b) inflammation in chronic pancreatitis group, HEx200, (c) atrophy and fibrosis in chronic pancreatitis group, HEx200, (d) mild inflammation in the high dose trimetazidine group, HEx200.

as a therapeutic agent, which we thought might have positive effects on inflammation, inflammatory oxidative stress, and microcirculation disorders arising in the pathogenesis of pancreatitis in our chronic pancreatitis model.[10-13] Trimetazidine does not have a direct hemodynamic effect. Trimetazidine has cytoprotective and antiischemic efficacy in cardiac tissue and is therefore used as an antianginal drug. Trimetazidine restricts membrane damage caused by reactive oxygen species and protects the tissue from the effect of free radicals due to its antioxidant features.[18]

Previously, we performed this current experimental in another rat CP model (serial IP injections of caerulein and ethanol) and used pentoxifylline as a therapeutic agent.[19] In that experimental study, we showed that pentoxifylline, which has anti-inflammatory and antioxidant effects, has protective efficacy in the experimental CP model.[19] In the experimental mouse model of Perides et al[20] 24% of the calories in the diet came from ethanol, and 75 mg caerulein injections were administered at a dose of 50 µg/kg with 7-hour intervals, 3 days per week, and the animals were sacrificed

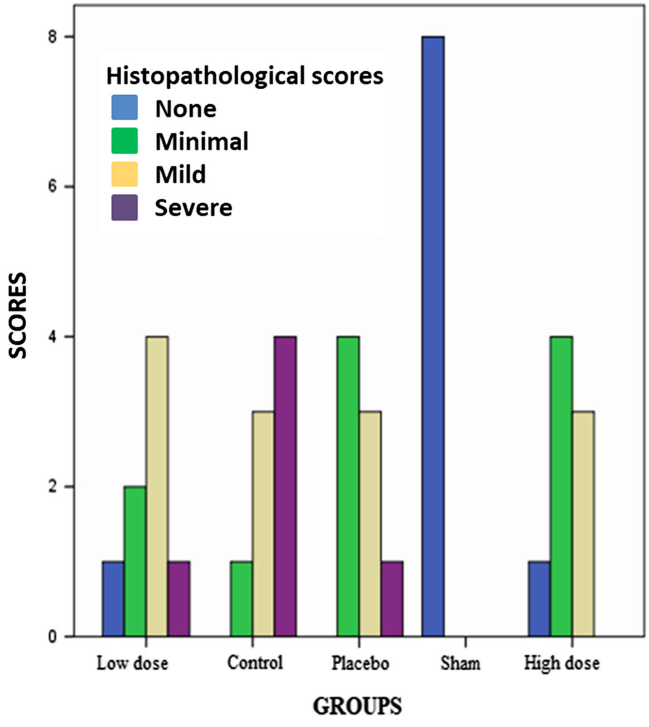

Figure 2. Distribution chart of histopathological scores by groups.

at 1, 3, and 5 weeks. The necrosis formed, alpha-1 collagen, and TGF-β levels were measured. In the study, a prominent increase in TGF-β levels and fibrosis were detected.[20] Therefore, in our study, we created CP by using caerulein and alcohol together.

Although CP and acute pancreatitis are two different situations, recurrent acute pancreatitis episodes and ongoing inflammation are known to result in CP. Tanoglu et al[21] performed a rat study investigating the use of TMZ in an acute pancreatitis model in rats induced by caerulein. In that study, a significant decrease in IL-1β, amylase, lipase, and leukocyte levels was detected in the TMZ group, and also histopathologic improvement was observed.[21] Similarly, we found favorable results in terms of both oxidative stress markers and histopathologic scores in the group given TMZ. In this current study, TNF-α and TGF-β levels, which are among the cytokines that play an important role in the development of pancreatitis, were also evaluated and cytokine levels were measured lower in the group given TMZ. At the same time, the effectiveness of TMZ in the chronic fibrotic process was also examined.

In another study evaluating the use of TMZ in the model of L-arginine-induced acute pancreatitis by Yeniçerioğlu et al[22] pancreatic tissue edema, hemorrhage, acinar cell necrosis, and perivascular cell infiltration, and aspartate transaminase, alanine transaminase, lactate dehydrogenase, amylase, TNF-α, IL-1β, and interleukin-6 levels were significantly reduced. In our study, alcohol, which has a pivotal role in the development of pancreatitis, was also used, and an improvement in terms of both oxidative stress markers and histopathologic scores was found in the TMZ group.

In conclusion, we believe that oral administration of TMZ, which has a direct hemodynamic effect, and antiischemic, anti-oxidant, and anti-inflammatory properties without a known toxic dose, may be an effective treatment option to prevent CP and to minimize complications. In order to use TMZ to prevent CP, to be a new modality of treatment, and to be used in routine practice in humans, more extensive experimental and clinical studies with different doses are needed.

Ethics Committee Approval: The study was approved by the medical ethics committee of niversity of Health Sciences (2018-08/01).

Informed Consent: N/A.

Peer-review: Externally peer-reviewed.

Author Contributions: Consept – M.K.; Design – A.K.; Supervision – A.T.; Resources – M.Y.; Materials – Y.Ö.T.; Data Collection and/or Processing – Z.Ç.; Analysis and/or Interpretation – Z.K.; Literature Search – M.K.; Writing Manuscript – A.K.; Critical Review – A.T.

Declaration of Interests: The authors declare that they have no competing interest.

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