Clinics and Research in Hepatology and Gastroenterology (2015) 39, 145—150

Available online at ScienceDirect www.sciencedirect.com

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ORIGINAL ARTICLE

Trimetazidine significantly reduces cerulein-induced pancreatic apoptosis

Alpaslan Tanoglu [a,*], Yusuf Yazgan [a], Mustafa Kaplan [a], Ufuk Berber [b], Muammer Kara [a], Dilaver Demırel [b], Osman Metin Ipcioglu [c]

[a] *GATA Haydarpasa Training Hospital, Gastroenterology Department, 34668 Uskudar-Istanbul, Turkey*
[b] *GATA Haydarpasa Training Hospital, Pathology Department, 34668 Uskudar-Istanbul, Turkey*
[c] *GATA Haydarpasa Training Hospital, Biochemistry Department, 34668 Uskudar-Istanbul, Turkey*

Available online 4 July 2014

Summary

Objective: Acute pancreatitis continues to be associated with significant rates of mortality and morbidity, and therapeutic options are still very limited. We aimed to investigate the efficacy of trimetazidine on cerulein-induced pancreatic apoptosis and histopathological and biochemistrical consequences of acute pancreatitis.

Methods: Thirty-two Wistar albino rats were randomized into four groups (group 1: control group; group 2: acute pancreatitis group; group 3: acute pancreatitis and trimetazidine treatment group; group 4: placebo group). Acute edematous pancreatitis was induced by subcutaneous cerulein injection (20 μg/kg) four times at one-hour intervals. Trimetazidine was prepared in suspension form. In group 3, after gas anesthesia, trimetazidine was administrated to rats via a catheter. Serum interleukin (IL)-1β, tumor necrosis factor (TNF)-α, amylase, lipase and leukocyte levels, pancreatic apoptotic status and pancreatic Schoenberg scores were determined for all groups. Results are given as the mean ± SD. A value of $P < 0.05$ was accepted as statistically significant. SPSS for Windows v15.0 was used for statistical analyses.

Results: In the acute pancreatitis group IL-1β, amylase, lipase and leukocyte levels were elevated and pancreatic histopathological evaluation revealed a diagnosis of acute pancreatitis IL-1β amylase and lipase levels and pancreatic inflammation were decreased significantly in the trimetazidine group ($P < 0.01$). White blood cell counts and TNF-α concentrations for the trimetazidine group and the acute pancreatitis group were not significantly different. Trimetazidine significantly reduced apoptosis in pancreatic tissues and Schoenberg scores were also significantly reduced ($P < 0.05$).

Conclusion: In this study, we showed that trimetazidine treatment significantly decreases the levels of IL-1β, amylase and lipase reduces pancreatic apoptosis and ameliorates the

* Corresponding author. Tel.: +90 216 542 202 0; fax: +90 216 542 200 7.
E-mail addresses: alpaslantanoglu@yahoo.com (A. Tanoglu), yusufyazgan@gmail.com (Y. Yazgan), dr_mustafakaplan@yahoo.com (M. Kaplan), patologub@gmail.com (U. Berber), drmuammerkara70@hotmail.com (M. Kara), demireldilaver@yahoo.com (D. Demırel), osmetip@yahoo.com (O.M. Ipcioglu).

http://dx.doi.org/10.1016/j.clinre.2014.06.003

histopathological findings of cerulein-induced acute pancreatitis. Trimetazidine could be a new therapeutic option in the early treatment of acute pancreatitis.

Introduction

Acute pancreatitis is an inflammatory course of action of the pancreas, with participation of affected regional tissues or organ systems. Most cases are associated with alcoholism or gallstones, but the pathogenetic mechanisms are not entirely understood [1]. Fundamentally, activated trypsin causes the intra-acinar activation of proteolytic enzymes [2—4], which eventually leads to an autodigestive injury to the gland. Then microcirculatory injury, leukocyte chemoattraction, release of cytokines (tumor necrosis factor, interleukins 1, 6, and 8), oxidative stress and, in severe cases, systemic response occurs [5—7].

Despite increasing knowledge about the etiology and pathophysiology of acute pancreatitis, therapeutic options are still very limited. Hence, acute pancreatitis continues to be associated with significant rates of mortality and morbidity. Recently, pathogenesis-based experimental studies and new therapeutic alternatives in acute pancreatitis have gained substantial interest [8,9]. Trimetazidine (1-[2,3,4-trimethoxibenzyl]-piperazine HCl) is a piperazine derived anti-anginal drug. It has cytoprotective effects on ischemic cardiac tissue [10,11]. In the presence of ischemia, trimetazidine maintains cellular ATP levels and reduces intracellular acidosis in cardiac tissue. Trimetazidine also secures improvement of coronary microcirculation following reperfusion and also limits reactive oxygen species (ROS) related membrane damage and protects tissues from the harmful effects of free radicals with its antioxidant properties [12—14]. It has been suggested that ROS and nitric oxide mediated damage increase the release of pro-inflammatory mediators, such as C-reactive protein, TNF-α, IL-1 and IL-8 [14].

In this study, we aimed to determine the efficacy of trimetazidine on biochemical parameters aside from pancreatic tissue histopathology in an experimental rat model of cerulein-induced acute pancreatitis.

Materials and methods

Study design

Thirty-two male Wistar albino rats, weighing 250—350 g, were maintained in stainless steel cages in a room at a constant temperature of $22 \pm 1\,^{\circ}C$ with 12-hour light/dark cycles and fed a standard pellet diet and tap water *ad libitum*. Our experimental study was performed in according to the recommendations of the national guidelines for the care and handling of laboratory animals, and followed a protocol approved by the Animal Ethics Committee of Marmara University Medical Faculty. The study was done in Marmara University Medical Faculty Experimental Research Animal Laboratory Center.

Experimental acute edematous pancreatitis was induced through subcutaneous cerulein (Sigma, St. Louis, MO, USA) injection (20 μg/kg) [15]. Rats were divided into four groups, each containing eight animals. Group 1 was the control group: subcutaneous saline injection was performed four times at 1 h intervals, and no medication was applied. Group 2 was the cerulein-induced acute pancreatitis group. Acute pancreatitis was induced through subcutaneous 20 μg/kg cerulein injection four times at 1-h intervals. Group 3 was the acute pancreatitis + trimetazidine treatment group. Trimetazidine was administrated orally at a dose of 20 mg/kg, 12 h after the last injection of cerulein. (Trimetazidine was prepared in suspension form in the GATA Haydarpasa Training Hospital Biochemistry Laboratory. After gas anesthesia, trimetazidine was administrated to rats via a catheter). Group 4 was the placebo group. In group 4, oral saline administration (of the same volume as the trimetazidine suspension) was performed to rats as a placebo agent via a catheter. Animals were sacrificed through intraperitoneal injection of ketamine hydrochloride (40 mg/kg) and xylazine (5 mg/kg) 6 h after oral saline/trimetazidine administration.

Soon after blood specimens were obtained via cardiac puncture, the whole pancreas was extracted quickly and the rats were sacrificed with high dose anaesthesia. All blood samples were centrifuged at 3500 rpm for 10 min and the plasma was stored at $-80\,^{\circ}C$ until assayed. White blood cell count, amylase, lipase, IL-1β and TNF-α concentrations were measured. White blood cell analyses were performed within 2 h of collection of blood samples in a Cell-Dyn Sapphire (Abbott Diagnostics Division, Santa Clara, CA) automated analyzer. Plasma amylase (Scientific Research Special Hangzhou Eastbiopharm Co. Ltd. Hangzhou), lipase (Scientific Research Special Hangzhou Eastbiopharm Co. Ltd. Hangzhou), IL-1β (AssayPro, USA) and TNF-α (Bioscience, Austria) concentrations were measured in accordance with the manufacturer's instructions and guidelines using enzyme-linked immunosorbent assay (ELISA) kits. For histopathological analysis, pancreatic tissue samples were fixed in 10% buffered formaldehyde and processed for routine paraffin embedding. Tissue sections were stained with hematoxylin and eosin (H&E), and examined under a photomicroscope (Olympus BX 51; Tokyo, Japan) according to the Schoenberg grading system [16]. Apoptotic status of pancreatic tissues was determined with apoptosis kits (ApopTag® Plus Peroxidase, In Situ Apoptosis Detection Kit, Chemicon International, Temecula, CA 92590, USA) using the ''Terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick-end labeling''TUNEL method and apoptotic indices were assessed as previously described [17]. Histological assessments were performed by two experienced pathologists who were blinded to the treatment conditions.

Statistical analysis

Results are given as mean $\pm$ SD. Comparisons of means between groups were made using Student's *t*-test or