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EXPERIMENTAL STUDY

Appreciation of trimetazidine treatment in experimental sepsis rat model

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Abstract: *Introduction:* Our aim was to determine the efficacy of trimetazidine on experimental sepsis rat model.
Material and methods: Sixty rats were randomized into three groups. In Group 1, sepsis was induced. In Group 2, sepsis was induced and as a therapeutic agent trimetazidine was given. In Group 3, rats were sham operated. Serum interleukin-1 beta (IL-1ß), tumor necrosis factor alfa (TNF-α), superoxide dismutase (SOD), glutathion peroxidase (GSH-Px) and malondialdehyde (MDA) levels were determined in all groups.
Results: In Group 2, serum GSH-Px and SOD levels were statistically significantly higher than in Group 1 ($p < 0.05$) and serum MDA levels were statistically significantly lower than in group 1 ($p < 0.05$). Trimetazidine also significantly decreased the levels of IL-1ß and TNF-a which are the proinflammatory cytokines ($p < 0.05$).
Conclusion: Trimetazidine treatment significantly improved inflammation, oxidative stress and membrane destruction in LPS-induced sepsis. As the proinflammatory cytokines are supposed to play a primary role in the pathogenesis of sepsis, we assumed that the trimetazidine treatment would give new insights into the treatment of sepsis *(Tab. 1, Fig. 5, Ref. 29)*. Text in PDF *www.elis.sk.*
Key words: sepsis, trimetazidine, inflammation, proinflammatory cytokines.

Introduction

Sepsis is considered the major cause of death among critically ill patients in the developed world (1). Up to now, many novel treatment agents have been employed against sepsis. Despite the increased attention and intervention which have been focused on the acute management of severe sepsis and septic shock, sepsis is still a highly fatal disease with an estimated hospital mortality rate approaching 30 % and new curative modalities are highly needed (2, 3).

Sepsis is a complex syndrome characterized by the fundamental signs of inflammation, vasodilatation, leukocyte gathering, increased microvascular permeability, occurring in tissues in response to microbial insult. These events manifest as a systemic inflammatory response syndrome (SIRS)/sepsis symptoms through release of proinflammatory cytokines (especially TNF-α and IL-1) and procoagulants (eg, fibrinogen and prothrombin), and adhesion molecules (eg, intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1)) from immune cells and/or damaged endothelium (4, 5). Sepsis can be named as malignant intravascular inflammation. Progression to sepsis, as well as uncontrolled, unregulated and self-sustaining inflammation are the main actors (6). Tissue ischemia, especially impaired microcirculation, cytopathic injury, apoptosis and immunosuppression are the major mechanisms causing the systemic effects of sepsis (7–9). So, in view of these mechanisms, a therapeutic agent with positive healing effects on these mechanisms may be the new curative agent for sepsis.

Trimetazidine (1-(2,3,4-trimethoxibenzyl)-piperazine HCl; TMZ) is a piperazine-derived antianginal drug. It has antioxidant, anti-ischemic, anti-inflammatory and healing effects on impaired microcirculation. It has been found to have cytoprotective effects on ischemic cardiac tissue (10, 11). During ischemic periods, TMZ maintains cellular ATP levels, and limits the intracellular acidosis in cardiac tissue. TMZ also limits the membrane damage induced by reactive oxygen species (ROS) and protects tissue from free radicals with its antioxidants effects (12–14). It has been suggested that ROS and nitric oxide-mediated damage enhances the release of proinflammatory mediators such as C-reactive protein, TNF-α, IL-1β and IL-8 (14).

In this study, we aimed at showing the efficacy of trimetazidine on clinical and laboratory markers of sepsis in rats with intraperitoneal LPS injection-induced sepsis. It was also our aim to determine the effects of trimetazidine on oxidative stress and membrane destruction in experimental sepsis model.

Materials and methods

Study design

Experimental design was approved by The Animal Ethics Committee of the Gulhane Military Medical Academy in December 2007 (Acceptance Number: 2007/95) and the study was performed in May 2008. The study was performed in Gulhane Military Medi-

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cal Academy, Experimental Medicine Research and Application Center, Ankara, Turkey. A total of 60 male Sprague Dawley rats of 250–350 g, were used in the study. Rats were held in stainless steel cages. The animal room was maintained on a 12-h light/dark cycle at 21–22 °C. Animals were fed standard pellet diet and *ad libitum* tap water.

Rats were divided into three equal groups. In animal sepsis model, the rats were injected with lipopolysaccharide (LPS) (Escherichia coli 0111:B4 Sigma, Deisenhofen, Germany), with a dose of 5 mg/kg intraperitoneally in Group 1(n = 20). In Group 2 (n = 20), the rats were administrated 20 mg/kg TMZ orally for seven days before the sepsis induction and after 7 days, injected with LPS with a dose of 5 mg/kg intraperitoneally for sepsis induction. In this group, trimetazidine was suspensioned for oral administration. After gas anesthesia, TMZ was administrated to rats via a catheter. The Group 3 was the sham group and rats were intraperitoneally injected with saline in the same volume as LPS (n = 20).

Induction of sepsis in rats was determined with increased white blood cell (WBC) levels, increased rectal fever and tachycardia proved with ECG. After sepsis induction, rats were kept in their cages and secured their feeding and water consumptions. After six hours, rats were sacrificed with a high-dose anesthesia, intraperitoneal injection of ketamine hydrochloride (40 mg/kg) and xylazine (5 mg/kg). Blood samples were collected by cardiac puncture. Serum samples were stored at –80 °C until analysis. Determination of serum TNF-α, IL-1ß levels, which are the main actors for inflammation in septic process, were made using an enzyme-linked immunoassay (ELISA; MWGt Lambda Scan 200, Winooski, VT, USA). As oxidative stress markers, serum malondialdehyde (MDA), superoxide dismutase (SOD), and glutathion peroxidase (GSH-Px) levels were determined. Serum MDA levels accepted as indirect marker of tissue cell membrane injury (15) and SOD (16) and GSH-Px (17) accepted as indirect markers of antioxidant capacity levels, were determined with previously designed methods.

Statistical analysis

Results are given as the mean ±SD. Comparisons of means between groups were made using the Student *t*-test or the one-way analysis of variance test for normally distributed data and the Mann–Whitney U test or the Kruskal Wallis test for non-parametric data. A value of $p<0.05$ was accepted as statistically significant. SPSS for Windows version 15.0 was used for statistical analyses.

Results

In Group 2, sepsis and trimetazidine treatment group, serum GSH-Px (2.09 ± 0.01 U/L and 1.42 ± 0.02 U/L, respectively) and SOD levels (1.78 ± 0.01 U/L and 1.12 ± 0.01 U/L, respectively) were statistically significantly higher than in Group 1, sepsis group ($p < 0.05$) (Tab. 1). On the other hand, in Group 2, serum MDA levels (2.21 ± 0.01 nmol/ml and 4.66 ± 0.04 nmol/ml, respectively) were statically significantly lower than in Group 1 ($p < 0.05$) (Tab. 1). In other words, trimetazidine administration to septic rats improved the oxidative stress and tissue membrane injury. Similarly, in sepsis and trimetazidine treatment group, serum IL-1ß (1195.2 ± 25.6 ng/L and 693.5 ± 41.1 ng/L, respectively) and TNF-αlevels (117.2 ± 3.7 ng/L and 91.5 ± 4.2 ng/L, respectively) were significantly lower than in Group 1 ($p < 0.05$). This means that, trimetazidine significantly reduced the levels of main proinflammatory markers, IL-1ßand TNF-α.

Tab. 1. Mean results of all groups and p values.

	Sepsis	Trimetazidine	Sham	p value
IL-1β (ng/L)	1195.2±25.6	693.5±41.1	167.9±9.9	<0.05
TNF-α (ng/L)	117.2±3.7	91.5±4.2	49.3±4.1	<0.05
MDA (nmol/ml)	4.66±0.04	2.21±0.01	0.98±0.01	<0.05
SOD (U/L)	1.12±0.01	1.78±0.01	2.65±0.01	<0.05
GSH-Px (U/L)	1.42±0.02	2.09±0.01	2.77±0.02	<0.05

Discussion

Deaths due to sepsis are a major concern of public health in developed countries. It is appraised that in the United States more than 750,000 septic cases occur once a year and even with optimal treatment, mortality is approximately 40 percent and can exceed 50 percent in some critically ill patients with worse clinical status (18–21). Until the present time, no worldwide approved pharmacologic agents for the treatment or prevention of sepsis are present.

Because of having extremely high mortality rates, many novel treatment agents have been employed against sepsis. Conventional treatments have focused on source control, antimicrobials, vasopressors, and fluid resuscitation; however, new treatment modalities including improving host response against infection and treating harmful effects of inflammation occurring in tissues. Several new interventions have been done recently and other original studies are being investigated (22–24). As mentioned above, uncontrolled, unregulated, and self-sustaining inflammation are the essential factors in sepsis (6). In septic process, impaired microcirculation due to tissue ischemia, cytopathic injury, apoptosis and immunosuppression are the major mechanisms which are causing systemic septic effects (7–9) From this point of view, a therapeutic agent which has positive healing effects on these mechanisms may be the new curative modality for sepsis.

Trimetazidine (TMZ) is a piperazine-derived antianginal drug which has cytoprotective effects on ischemic cardiac tissues (19, 20). It has antioxidant, anti-ischemic, anti-inflammatory, and healing effects on impaired microcirculation. Recent studies have focused on TMZ's antioxidants and anti-inflammatory effects. During ischemic periods, TMZ maintains cellular ATP levels, and limits the intracellular acidosis in cardiac tissue. Oxidative stress, specified as an excessive production of ROS, has been demonstrated to be involved in the pathophysiology of sepsis. In case of sepsis, ROS damage the cellular DNA and lead to cellular protein peroxidation especially lipid membrane peroxidation. Then these harmful effects progress to tissue damage. Lipid membrane peroxidation causes an ncrease in membrane permeability, while cellular integrity and vital cellular functions will later be damaged. Hence after these occurrences, the outcome is often the same, namely multiple organ dysfunction syndrome (MODS) and death (25). TMZ also limits the membrane damage induced by reactive oxygen species

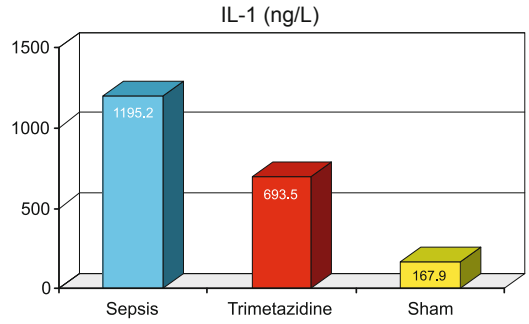

Fig. 1. Serum IL-1ß levels of all groups.

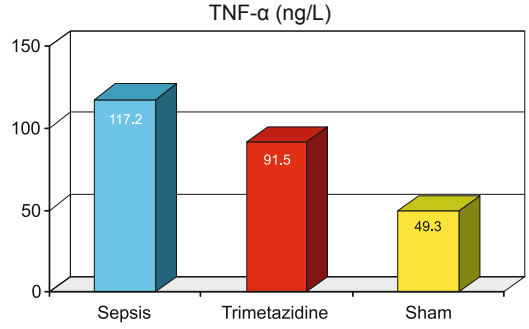

Fig. 2. Serum TNF-α levels of all groups.

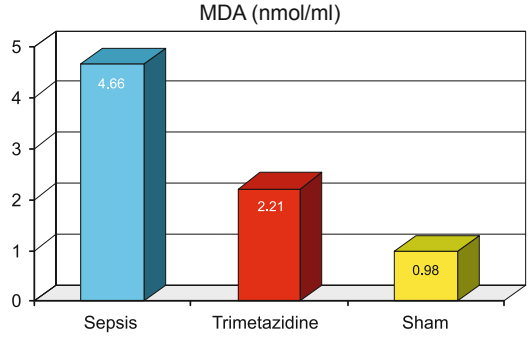

Fig. 3. Serum MDA levels of all groups.

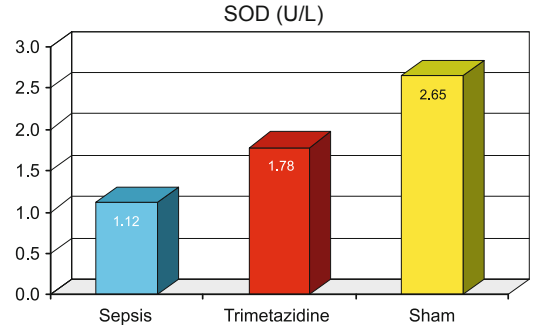

Fig. 4. Serum SOD levels of all groups.

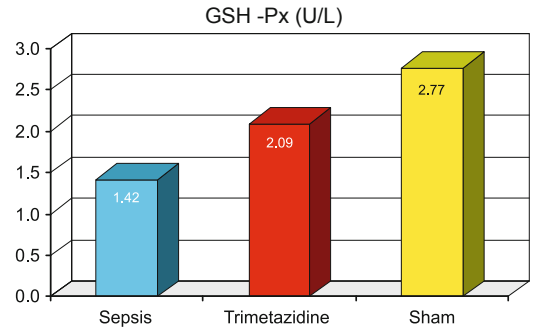

Fig. 5. Serum GSH-Px levels of all groups.

(ROS) and protects tissue from free radicals with its antioxidant effects (12–14). ROS and nitric oxide-mediated damage enhances the release of proinflammatory mediators such as C-reactive protein, TNF-α, IL-1 and IL-8 as suggested by many authors (14).

The progress in molecular mechanisms of sepsis has provided a new level of understanding in the complex clinical course of human sepsis. Previously, mediators such as cytokines (e.g. TNF, IL-1) were considered most important in the pathophysiology of sepsis (26). Nevertheless, in the mid 1990s it became evident that this view of the pathophysiological alterations in sepsis was extremely limited and in fact there is a very complex interaction between microbial pathogen, immunocompetent cells, their mediators, endothelial cells, and coagulation system. The application of this new knowledge in pathophysiology of sepsis both with optimized patient choice and guidance of clinical trials eventually yielded the satisfying progress in sepsis therapy (26).

In this study, it was our aim to determine and exhibit the effects of trimetazidine on clinical and laboratory markers of sepsis in rats with intraperitoneal LPS injection-induced sepsis. Trimetazidine's anti-oxidant, anti-ischemic and anti-inflammatory effects had a heartening role in using TMZ in our experimental sepsis study.

Kuralay et al designed a trimetazidine study on patients subject to percutaneous transluminal coronary angioplasty (PTCA). Ischemic reperfusion cycle in PTCA leads to systemic inflammation and extensive tissue injury by the production of reactive oxygen species including nitric oxide (NO) radicals (14). In patients treated with PTCA due to coronary artery disease, the effects of TMZ on several indirect markers of inflammatory response (TNF-α, CRP and NO products) were studied by Kuralay et al. In that study all parameters (TNF-α, CRP and NO products) were lower in pre-procedural oral TMZ-administrated group (14). In other words, TMZ suppressed the PTCA related inflammation. Similarly, in our experimental sepsis study, in Group 2, serum IL-1ß and TNF-α levels were statically significantly lower than in Group 1 ($p < 0.05$). Results of these different two studies support the theory that TMZ has suppressing effects on inflammation. Also our results made us think that TMZ may be used as a novel agent in sepsis treatment due to its exhibited anti-inflammatory effects. But further researches are needed to support this suggestion.

It is not logical that a unique therapeutic intervention may be useful for all patients with sepsis and there is most likely no magic gun for sepsis. The fact that organ failure which happens in sepsis is a result of a dysregulated host immune responses, rather than the direct result of bacterial toxicity per se, led many researchers to study immune modulation as a possible therapeutic agent. A lot

of clinical studies were accomplished, especially focusing on the pro-inflammatory cytokines such as TNF-α and IL-1 (27, 29). In this way, our results encouraged us to theorize that TMZ may be beneficial in sepsis treatment if it is carried out in particular clinical practice. On the other hand, our research was not a survey study and therefore TMZ effects on sepsis progress cannot be determined.

Kara et al. designed a different TMZ study. In that study, they administrated TMZ to anesthetized rats before using carbachol. They showed a notable decrease in infarcted area of cardiac tissues and diminished serum malondialdehyde (MDA) and lactate levels in TMZ-administrated rats (29). They related the lower MDA levels to TMZ's inhibitor effects on myeloperoxidase activity and neutrophil aggregation (29). Our study was an experimental sepsis study, but our results supported Kara et al.'s relationship between TMZ and MDA. Serum MDA levels, as an indirect marker of membrane injury in sepsis, were statically significantly lower in our sepsis+TMZ group and this finding strengthens the TMZ's inhibitor effects on myeloperoxidase activity and neutrophil aggregation.

In Tikhaze et al's TMZ study (12) they showed that chronic TMZ treatment in patients with coronary artery disease lowered serum malondialdehyde levels and lipid peroxidases and increased serum glutathion peroxidase activity. Similar to these results, TMZ group in our experimental sepsis study showed serum glutathion peroxidase and superoxide dismutase levels (accepted as indirect markers of tissue antioxidant capacity) to be statistically significantly higher than in group 1 ($p < 0.05$). In other words, TMZ administration in septic rats improved oxidative stress and tissue membrane injury.

Our study is a preliminary study which investigated the TMZ's effects on experimental sepsis model. In PubMed, there is no previously designed TMZ study which would be focusing on experimental sepsis. Also our research is an original article which investigates the TMZ's anti-oxidant, anti-ischemic and anti-inflammatory effects on LPS-induced experimental rat sepsis model. We assumed that the trimetazidine treatment would give new insights into the treatment of sepsis.

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