Transpl Int (1999) 12: 108–112

ORIGINAL ARTICLE

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Cytoprotective effect of trimetazidine on 60 minutes of intestinal ischemia-reperfusion injury in rats

Received: 7 May 1998
Received after revision: 25 September 1998
Accepted: 12 October 1998

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Abstract Trimetazidine (TMZ), a potent antioxidant agent, has been used to protect the myocardium, liver and kidney from ischemia reperfusion (IR) injury. We investigated the effect of TMZ, a cellular anti-ischemic agent and a free radical scavenger, on 60 min of warm intestinal IR injury in rats. Sprague-Dawley rats were divided into three groups: a sham-operated group (no IR injury, $n = 8$), an ischemic control group (control, $n = 8$), and a TMZ-treated group (3 mg/kg, $n = 8$). Malondialdehyde (MDA) levels, myeloperoxidase (MPO) activity, and mucosal damage were investigated after 120 min of reperfusion. MDA levels and MPO activity were more elevated and histopathological damage more severe in the control group than in the sham group ($P < 0.05$). MDA levels and MPO activity were lower and there was less histopathological damage in the TMZ group than in the control group ($P < 0.05$). Accumulation of lipid peroxidation products and neutrophils in mucosal tissues were significantly inhibited by TMZ treatment. We conclude that pretreatment of rats with TMZ before intestinal ischemia attenuates but does not prevent, histological damage.

Key words Trimetazidine · Reperfusion injury · Intestinal ischemia · Malondialdehyde · Myeloperoxidase

Introduction

Free oxygen radical generation has been implicated as a major mediator in intestinal ischemia-reperfusion (IR) injury [4, 5, 16, 18, 24]. Lipid peroxidation and protein oxidation caused by free oxygen radicals result in structural and functional cell damage. Established sources of oxygen radical production are the hypoxanthine/xanthine oxidase system and neutrophil granulocytes. Most of the free radicals are produced as a result of the accumulation of hypoxanthine and the formation of xanthine oxidase during the ischemic period following reperfusion. Superoxide radicals produced in the xanthine oxidase reaction trigger neutrophil infiltration into ischemic tissues. The importance of neutrophils in the development of IR injury in various organs such as the liver and intestine has been shown [4, 12, 16, 21, 24]. Activated polymorphonuclear leukocytes (PMN) release substances such as oxygen free radicals, arachidonic acid products, and proteolytic enzymes such as elastase. Intestinal mucosal damage can be prevented by monoclonal antibody blocking leukocyte adherence to endothelial cells [11, 24].

Free radical scavengers such as superoxide dismutase, catalase, and allopurinol have reduced the severity of intestinal IR injury [5, 14, 18, 24]. Trimetazidine (TMZ), a potent antioxidant agent, has been used to protect IR injury of the myocardium, liver, and kidney [7, 17, 20, 22]. However, there is no report on the use of TMZ in intestinal IR injury.

In this study, we have investigated the effect of TMZ, a cellular anti-ischemic agent and a free radical scavenger, on 60 min of warm intestinal IR injury in rats.

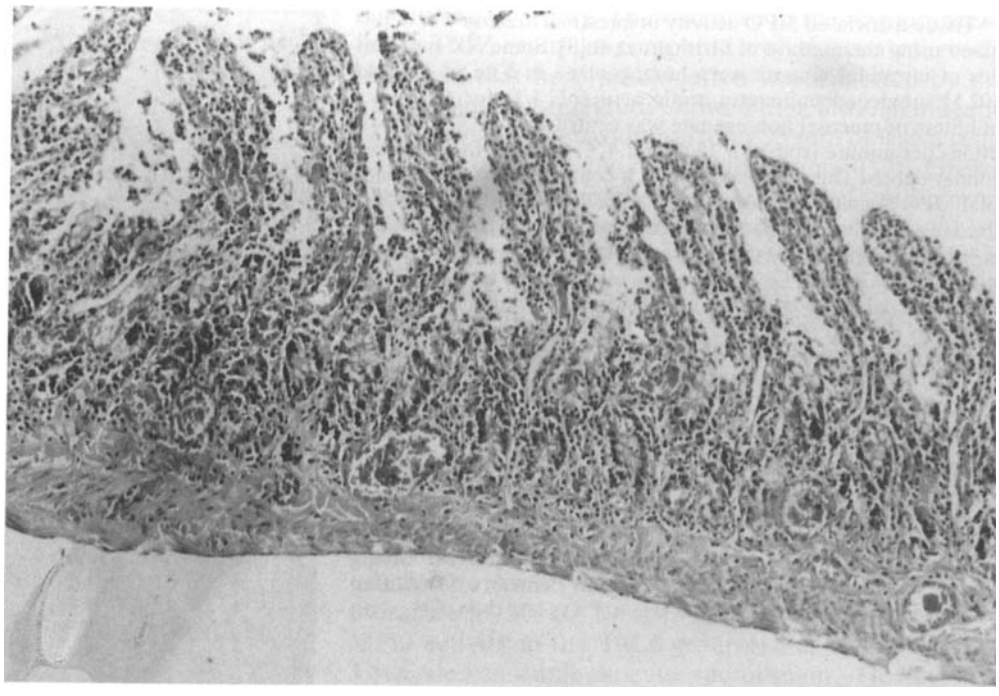

Fig. 1 Severe mucosal damage after IR injury in the control group (grade 4, HE × 100)

Materials and methods

This study was carried out on male Sprague-Dawley rats weighing 220–250 g. Rats were kept under standardized conditions for food, water, light, and temperature. All animals were fed standard rat chow and water ad libitum and were given only water for 12 h before surgery.

After an overnight fast, the rats were anesthetized with an intramuscular injection of 50 mg/kg ketamine hydrochloride (Ketalar, Parke-Davis, Eczacbaşı, Turkey) and 10 mg/kg xylazine (Rompun, Bayer, Leverkusen, Germany), and the right internal jugular vein was catheterized using 24-gauge catheters. After skin shaving and preparation of the abdominal wall with 10% povidone-iodine solution, a midline laparotomy was performed. The small bowel was exteriorized and the ligament of Treitz cut to expose the superior mesenteric artery (SMA). The SMA was dissected. After heparinization (80 IU), an atraumatic microvascular clamp (Aesculap BH 21) was then placed across the SMA just after its origin from the aorta for occlusion, avoiding occlusion of the superior mesenteric vein. Mesenteric ischemia was confirmed when the mesenteric pulsations were lost and the intestines became pale. The bowel was returned to the abdominal cavity, and the incision was closed with interrupted atraumatic 4/0 silk sutures. After 60 min of ischemia, a relaparotomy was performed and the microvascular clamp on the SMA was removed for 120 min of reperfusion. Mesenteric reperfusion was confirmed with the restoration of pulsation and color. The bowel was then returned to the abdominal cavity once more, and the incision was closed with 4/0 silk sutures. The bowel was left in the abdomen during IR. The sham operation involved the same technique and exposure without clipping of the SMA. At the end of 120 min, the animals were sacrificed.

Ileal tissue samples were obtained and stored at −78 °C after immersion in liquid nitrogen for later determinations of tissue malondialdehyde (MDA) and myeloperoxidase (MPO) levels. A 5-cm ileal segment was fixed in 10% formaldehyde for histopathological examination. Throughout the study, 10 ml/kg Ringer's lactate was infused from the internal jugular catheter.

Experimental design

Three groups of animals were used in this study. Group 1, the sham-operated group (sham, $n = 8$), underwent laparotomy without IR injury. Group 2, the ischemic control group (control, $n = 8$), underwent laparotomy plus 60 min of ischemia and 120 min of reperfusion; they received only intravenous Ringer's lactate during IR injury. Group 3, the trimetazidine-treated group (TMZ, $n = 8$), received 3 mg/kg TMZ (Vastarel, Servier, France) intravenously 10 min before the induction of ischemia.

Histopathological evaluation

The tissue specimens were fixed in 10% formaldehyde. Samples of intestine were embedded in paraffin, sectioned, and stained with hematoxylin-eosin (HE). They were then submitted for histopathological evaluation, which was performed in a blinded fashion by two pathologists. Mucosal lesions were graded on a scale from 0 to 5 as described by Chiu et al [3]. Grade 0 indicated normal mucosal villi; grade 1, the development of subepithelial Gruenhagen's space, usually at the apex of the villus, often with capillary congestion; grade 2, extension of the subepithelial space with moderate lifting of epithelial layer from the lamina propria; grade 3, massive epithelial lifting with a few denuded villi; grade 4, denuded villi with exposed dilated capillaries; and grade 5, digestion and disintegration of lamina propria, hemorrhage, and ulceration.

MDA and MPO determinations

The levels of MDA were determined in intestinal mucosa homogenized in the ratio of 1:10 (weight: volume) in 1.15% cold KCl solution following the thiobarbituric acid method, and the results were obtained in nmol/g tissue weight [13].

Tissue-associated MPO activity in intestinal mucosa was determined using the method of Grisham et al [8]. Some 300-mg samples of intestinal mucosa were homogenized in 5 ml of ice-cold 0.02 M ethylenediaminetetra-acetic acid (pH 4.7) for 60 s. Five milliliters of mucosal homogenate was centrifuged at 20000 revolutions per minute (rpm) for 15 min at 4 °C to pellet the insoluble cellular debris. The supernatant, which contained less than 5% of total MPO activity, was discarded. The pellet was then rehomogenized in an equivalent volume of 0.05 M potassium phosphate buffer (pH 6.0) containing 0.5% hexadecyltrimethylammonium bromide (HETAB). MPO activity was assessed by measuring the H_2O_2-dependent oxidation of O-dianisidin. One unit of enzyme activity was defined as the amount of MPO present that caused a change in absorbance of 1.0/min at 410 nm and 37 °C [6].

Statistics

The results were evaluated using a one-way analysis of variance, and the differences among the groups were analyzed with the Tukey HSD test, except for histopathological values. For the latter, differences among the groups were evaluated with the Mann-Whitney U-test. All values were expressed as mean ± SD. Differences were considered significant when P was less than 0.05.

Results

In the sham group, the results of the histopathological examinations of the small intestinal epithelium and villi were normal. The specimens from this group were classified as grade 0–1 according to the Chiu classification. In the control group, grade 3–5 histopathological damage was detected (Fig. 1). In addition, PMN infiltration was detected in 50% of the specimens. In the TMZ group, grade 1–2 damage was detected (Fig. 2). In this group, two specimens were detected as grade 5. However, PMN infiltration was not seen.

Mucosal injury scores were 0.5 ± 0.53, 4.25 ± 0.70, and 2.25 ± 1.75 in the sham, control, and TMZ groups, respectively (Fig. 3). More intense histopathological damage was observed in the control group than in the sham group ($P < 0.01$). The histopathological injury in the TMZ group was significantly lower than in the control group ($P < 0.05$). Pretreatment with TMZ attenuated, but did not prevent, intestinal IR injury.

The mean MDA levels were 16.42 ± 2.44, 30.62 ± 8.46, and 17.66 ± 3.01 nmol/g tissue in the sham, control, and TMZ groups, respectively (Fig. 4). The difference between the sham and TMZ groups was not significant, but the MDA levels in the control group were significantly elevated in comparison to those in the sham and TMZ groups ($P < 0.05$).

MPO levels were 0.74 ± 0.09, 4.94 ± 1.91, and 0.57 ± 0.23 U/g tissue in the sham, control, and TMZ groups, respectively (Fig. 5). In the control group, the MPO level was significantly higher than in the other groups ($P < 0.05$). Pretreatment with TMZ attenuated MPO levels after intestinal IR injury.

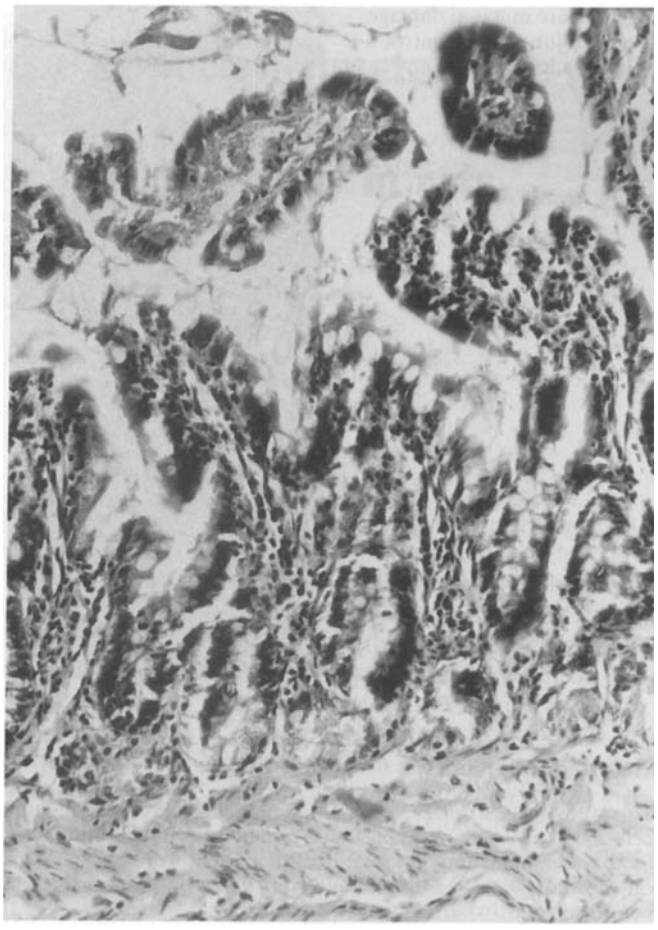

Fig. 2 Mild mucosal damage after IR injury in the TMZ group (grade 2, HE × 200)

Discussion

This study demonstrated that 120 min of reperfusion following 60 min of ischemia of the rat intestine caused severe mucosal damage. The pretreatment of rats with TMZ before intestinal ischemia attenuated, but did not prevent, histological damage from IR by inhibiting lipid peroxidation and neutrophil infiltration in the mucosal tissue.

TMZ (1-(2,3,4-trimethoxy,benzyl) piperazine dihydrochloride) is an anti-ischemic agent known to improve exercise tolerance and cardiac function in patients with ischemic heart disease [2]. It has been shown that pretreatment with TMZ is effective in reducing the size of the infarct that developes in the blood-perfused rabbit model of myocardial ischemia [15]. Various experimental studies have shown that TMZ preserves the intracellular concentration of adenosine triphosphate and inhibits the extracellular leakage of potassium during cellular ischemia. TMZ reduces intracellular accumulation of sodium and calcium and inhibits platelet ad-

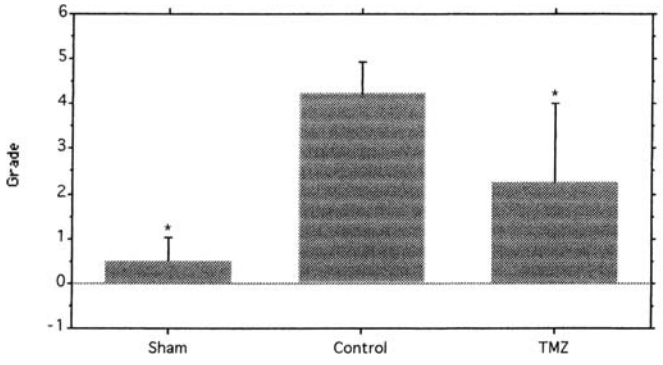

Fig. 3 Histopathological grades of the groups $^*P < 0.05$ vs controls

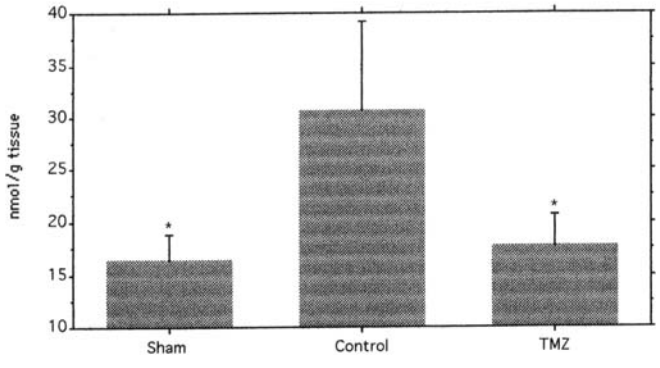

Fig. 4 MDA levels in the experimental groups $^*P < 0.05$ vs controls

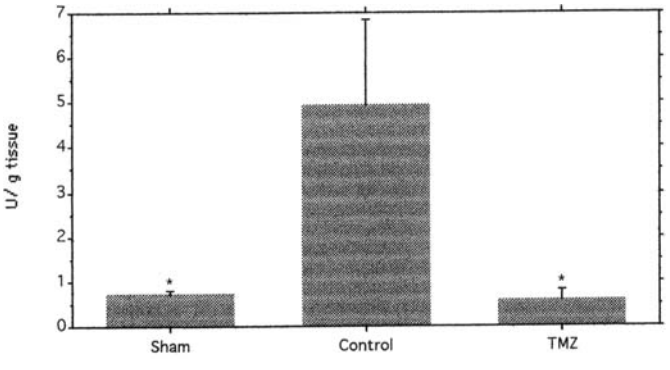

Fig. 5 MPO levels in the experimental groups $^*P < 0.05$ vs controls

hesion-aggregation, neutrophil infiltration, and the generation or activity of oxygen-derived free radicals [1, 9, 22]. The potent antioxidant effect of TMZ has been demonstrated in myocardial, renal, and hepatic IR injury [7, 17, 20, 22].

A significant increase in MDA levels has been observed in various organs including the liver, kidney, and intestine during IR injury [7, 16, 19, 23, 24]. In this study, the elevated levels of MDA in the control group supported the notion that lipid peroxidation processes occur during IR injury. The decrease in the level of MDA in the TMZ group, as compared with the control group, indicated that lipid peroxidation was inhibited by this agent. Our previous study showed that TMZ significantly reduced lipid peroxidation after 75 min of warm renal ischemia followed by reperfusion injury [17]. Grekas et al. have also described the inhibition of lipid peroxidation by TMZ in renal IR injury [7].

We used MPO activity as an index of PMN accumulation in the intestine in this study. It is directly proportional to the numbers of neutrophils seen in histological sections of intestine tissue, and is therefore regarded as a reliable index of neutrophil infiltration [4, 5, 8, 24, 25]. It has been reported that MPO activity in intestinal tissue increases threefold after 60 min of intestinal ischemia, and eight to ninefold after 60 min of reperfusion [12]. In this study, MPO activity in the mucosal tissue was higher in the control group, reflecting neutrophil infiltration after reperfusion, than in the sham group. Our results are in accordance with those in the literature [5, 12, 16, 19, 25]. The significant decrease in MPO activity in the TMZ group led us to believe that TMZ decreased leukocyte recruitment. However, it does not demonstrate an inhibition of PMN activation in postischemic tissue. The reperfusion-induced recruitment of leukocytes can be largely prevented by pretreatment with superoxide dismutase or allopurinol [8, 25]. Previous studies have demonstrated that resident leukocytes may play a pivotal role in the pathogenesis of intestinal IR injury [10, 11].

Histological damage that occurs after intestinal IR is characterized by shortening of the villus length, loss of villus epithelium, necrosis, and invasion of inflammatory cells [12, 16, 19, 23]. In the present study, we observed that 60 min of intestinal ischemia following 120 min of reperfusion produced severe mucosal injury and PMN infiltration in the rats. Pretreatment with TMZ attenuated the histological damage and PMN infiltration. This improvement might be due to the inhibition of neutrophil accumulation.

We conclude that pretreatment of rats with TMZ before intestinal ischemia attenuates, but does not prevent, histological damage from IR by inhibiting lipid peroxidation and neutrophil accumulation in the mucosal tissue.

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