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Preventive Effect of Kaptopril in Intestinal Ischemia Reperfusion Injury: Experimental Study

Cengiz F[1], Doğan SM[1], Vardar E[2], Bozkaya G[3], Bayol U[3], Erkan N[1], Ünalp HR[4] and Yetişir F[5]*

[1]İzmir Bozyaka Teaching Research Hospital, General Surgery Department, Turkey
[2]İzmir Bozyaka Teaching Research Hospital, General Pathology Department, Turkey
[3]İzmir Bozyaka Teaching Research Hospital, General Biochemistry Department, Turkey
[4]İzmir Atatürk Teaching Research Hospital, General Surgery Department, Turkey
[5]Ankara Atatürk Teaching Research Hospital, General Surgery Department, Turkey

Abstract

Aim: Ischemic Reperfusion (I/R) causelocal and systemic damage with wide array of inflammatory responses. Angiotensin II has pro-inflammatory actions. The aim of this study was to investigate the protective effect of Angiotensin Converting Enzyme (ACE) inhibitor captopril on intestinal I/R injury in rats.

Method: Animals were randomly assigned to two experimental groups: saline and kaptopril. All the rats received 1 ml/kg saline intraperitonealy 30 min before the surgical procedure. Kaptopril group also received 100 mg/kg kaptopril at the same time intraperitonealy. The Superior Mesenteric Artery (SMA) of all rats were isolated and clamped for 45 minutes. Blood samples were collected to analyze Lipid Peroxides (LPO) and serum Tumor necrosis Factor (TNF)-α level and 2 cm full-thickness samples of ileum were removed for analysis.

Result: According to histological scores pre-administration of kaptopril significantly attenuated the intestinal damage induced by I/R.Serum TNF- α level increase at the hours 0 and 2 was higher in the saline group compared with the kaptopril group. At 12th and 24th hours there was no difference between each group. Intestinal LPO level at all measured times are significantly higher in saline group.

Conclusion: Kaptopril may be potent drug to protect the intestines against I/R injury by its anti-inflammatory and anti-oxidative effects. Further studies are required to investigate not only protective but also the therapeutic effects of kaptopril in II/R injury.

Keywords: Intestinal ischemia; Reperfusion injury; Kaptopril

Introduction

In surgical and trauma patients intestinal ischemia–reperfusion injury (IIRI) is an important factor associated with high morbidity and mortality [1]. In the situations that cause interruption of blood flow to the gut as in strangulated hernias, abdominal aortic aneurysm surgery, neonatal necrotizing enterocolitis, cardiopulmonary bypass, intestinal trans-plantation and septic and hypovolemic shock IRI of the intestine can occur [2-4].

Tissue ischemia can be described as a situation of inadequate oxygen delivery to cover metabolic demands. Interruption of blood supply results in ischemic injury which rapidly damages metabolically active tissues. Restoration of blood flow to the ischemic tissue initiates a cascade of events that may lead to additional cell injury known as reperfusion injury. Frequently this reperfusion damage exceeds the original ischemic insult [5]. Ischemic injury is mainly due to oxygen-deprived cell death but reperfusion produces a wide array of inflammatory responses that heightens local damage and leads to systemic insult as well [6]. Ischemia / reperfusion (I/R) induces the release of cytokines, oxygen free radicals and proinflammatory mediators that activate leukocytes and endothelial cells leading to inflammotory injury and cell apoptosis [7,8]. Acute inflammatory response that is characterised by increased adherence, emigration of lecocytes and vascular permeability in post capillary venules was seen in microscopic studies of tissues exposed to I/R [9]. I/R also affects the secondary organs, including liver [10], heart [11], kidney [12], and lung [13], and even causes multiple organ failure. Therefore seeking agents to attenuate I/R induced multiple organ injury is required.

Angiotensin II (Ang II) is the principal effector molecule of the renin-angiotensin system and an important regulator of blood pressure, fluid homeostasis, and kidney function [11]. Recent observations have shown pro-inflammatory actions of Ang II such as, increased expression of adhesion molecules [14] and chemokine [15] and generation of Reactive Oxygen Species (ROS) [16], ultimately causing vascular injury and organ damage. Several lines of evidence have implicated that inhibition of Ang II may protect against ischemia reperfusion (I/R)-induced tissue injury in the heart, brain, and liver [13,17,18]. Also, mechanisms of AII induced leukocyte-endothelial interactions in the colon and decreased I/R induced leukocyte adhesion by AT1 receptor inhibition was demonstrated [12].

The aim of this study was to investigate the protective effect of Angiotensin Converting Enzyme (ACE) inhibitor captopril on II/R injury in rats.

Animals and Experiment Design

Sixtyfour8-10 week's old Sprague Dawley rats, 180-200 g in weight were supplied by the Animal research center of Ege University Medical

***Corresponding author:** Fahri Yetisir, Atatürk Research and Training Hospital, General Surgery Department, Mustafa Kemal, Çankaya, Ankara, Turkey, Tel: 90-536-2974888; E-mail: drfahriyetisir@hotmail.com

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School, İzmir, Turkey. The animals were maintained under standard conditions and fed rodent chow and water. All surgical procedures and care administered to the animals were in accordance with Animal Ethics Committee approval (Ege University medical school, İzmir, Turkey). Animals were randomly assigned to two experimental groups: saline and kaptopril. All the rats received 1 ml/kg saline intraperitonealy 30 min before the surgical procedure. The dosage of captopril was calculated according to previous experiences reported in the literature [19,20]. Kaptopril group also received 100 mg/kg kaptopril at the same time intraperitonealy. Under ketamine anesthesia, a midline laparotomy was made after the skin was shaved and sterilized with 10% povidone –iodine solution. The SMA was isolated and the small intestine was subjected to warm ischemia by clamping the SMA with non-traumatic vessel clamps for 45 minutes. Blood pressures were not monitored. Eight rats from each group were sacrificed 0, 2, 12, and 24 h after reperfusion, respectively. Blood samples were collected and 2 cm full-thickness samples of small intestine were removed from the terminal ileum for further analysis. The blood samples were spun at 1000×g for 10 min, and the sera were decanted and stored at -80°C.

Morphological Analysis

Specimens were fixed in 10% formalin and embedded in parafin. Prepared sections were stained with hematoxylin-eosin and blindly examined under light microscopy. Park/Chiu scoring system was used in a blinded manner using a light microscope [21]. Histological assessment for evaluating intestinal damage was done with histological criterias including crypt loss, infiltration of polymorphonuclear cells and lymphocytes, mucosal bleeding, erosion, villous fusion and stunting, disruption of the brush border, reduction in the number of goblet cells, dilatation of lymphatic and capillaries, sub mucosal edema. Each variable was scored from 0 (normal) to 3 (maximal damage) to give a maximum possible score of 27 for each intestinal sample [22].

Measurement of Lipid Peroxides (LPO)

The amount of LPO in the intestines was calculated by measuring malondialdehyde (MDA). Tissue samples were weighted and 25 mg tissue was put in 1.5 mL centrifuge tube with 250 mL RIPA buffer. It was sonicated for 15 seconds at 40V over ice. After sonication, the tubes were centrifuged at 1600 g for ten minutes and the supernatant was stored at -80°C until the analysis. The concentrations of Thio barbituric Acid Reactive Substances (TBARS) were measured in the intestinal mucosa using a commercially available kit (TBARS Assay Kit, Cayman Chemical Company, Ann Arbor, MI, USA). The MDA-Thiobarbituric Acid adduct formed by the reaction of MDA and Thiobarbituric Acid under high temperature and acidic conditions was measured colorimetrically at 530-540 nm. The intra-assay and inter-assay coefficient of variations were 5.5% and 5.9%, respectively.

Measurement of Serum Tumor Necrosis Factor (TNF)-α

Blood samples were spun at 1000 g for 10 min and the serum was decanted and stored at minus 80°C.TNF-α was determined with ELISA method by a commercially available kit according to the manufacturer's instructions. (Rat TNF-a, Bender MedSystems, Vienna, Austria). The intra-assay and inter-assay coefficient of variations were below 5% and 10%, respectively.

Statistical Analysis

Results are expressed as means ± standard deviation (SD), and a Student's t-test was used to evaluate statistical significance. P<0.05 was considered significant.

Results

Captopril attenuates intestinal damage caused by II/R

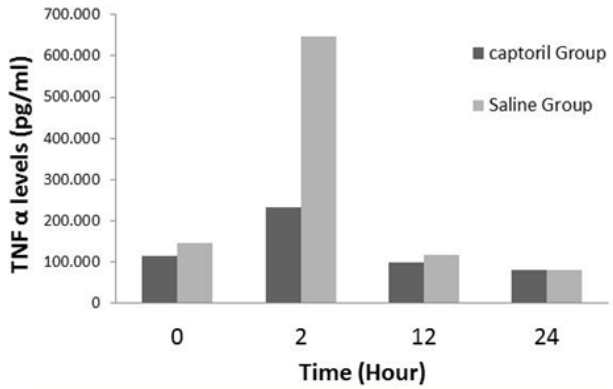
Pre-administration of kaptopril significantly attenuated the intestinal damage induced by I/R, evidenced by the significant reduction of histological scores at the indicated time-points compared with the saline group. At the beginning of reperfusion (0 hour) infiltration of polymorphonuclear cells and lymphocytes (p=0.003), erosion (p=0.013) and total score was significantly lower in the kaptopril group (kaptopril 16 ± 1, SF 19 ±1.4 p=0.000). Two hours after reperfusion, infiltration of polymorphonuclear cells and lymphocytes (p=0.003), villus fusion and stunting (p=0.013), submucosal edema (p=0.013) and total score was significantly lower in the kaptopril group (captopril 8.2 ± 1.2, SF 22.2 ± 1.4 p=0.000). At the twelveth hour of reperfusion, infiltration of polymorphonuclear cells and lymphocytes (p=0.026), mucosal bleeding (p=0.026), dilatation of lymphatics and capillaries (p=0.000) and total score was significantly lower in the kaptopril group (kaptopril 9.7 ± 0.8, SF 13.3 ± 1.5 p=0.000). Although, crypt loss, mucosal bleeding and villous fusion and stunting were lower in kaptopril group, these were not statistically significant.

Captopril reduces serum TNF- α and intestinal LPO level

Serum TNF- α level, a main inflammatory mediator involved in I/R injury, increase at the hours 0 and 2 was higher in the saline group compared with the kaptopril group. At 12th and 24th hours there was no difference between each group (Figure 1). Intestinal LPO level at all measured times are significantly higher in saline group (Figure 2).

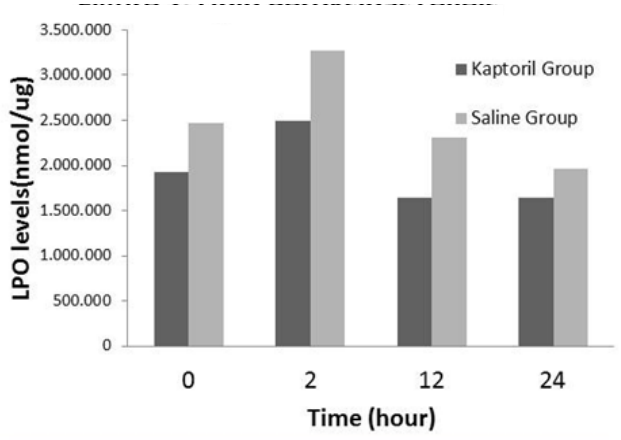
Discussion

II/R can cause organ dysfunction and multiple organ failure by initiating severe pathophysiological events. II/R induced tissue injury is caused by the inflammatory response involving inflammatory mediators, aggregation of platelets, neutrophil recruitment, and neutrophil-endothelium interactions [23]. II/R induced by SMA



	captoril Group n:32	Saline Groupn:32	p
0 hour)	11.6125±1.63745	14.6750±.52304	.003
2 hour)	23.3625±6.02351	64.7250±4.57251	.000
12 hour	10.0375±1.81418	11.7000±1.61068	.126
24 hour	8.0125±.32266	8.0375±.33780	.668

Figure 1: Serum TNF-α levels.



Mean ± SD	Kaptoril Group n:32	Saline Group n:32	p
0 hour	192.7500± 15.85425	246.6250±18.87506	.001
2 hour	249.1250±15.57872	326.7500±23.12543	.000
12 hour	164.8750±13.53765	231.0000±12.32883	.000
24hour	164.1250±11.65501	196.3750±8.61788	.000

Figure 2: Lipid peroksidas levels.

occlusion cause systemic inflammatory response syndrome by the release of destructive proinflammatory cytokines like oxygen free radicals and TNF- α into circulation [24]. Up-regulation of ACE in colon and AngII in the plasma [12], pro-inflammatory actions of Ang II such as, increased expression of adhesion molecules [14] and chemokine [15] and generation of Reactive Oxygen Species (ROS) [16], ultimately causing vascular injury and organ damage has been shown. Also it has been shown that the gastrointestinal mucosa is particularly vulnerable to even mild hypovolemia [25]. Suggesting Ang II is relatively more important in the gastrointestinal system in critical situations, which help to ensure adequate allocation of blood supply to certain organs, such as the heart and brain, Yilmaz et al. [26] have reported that the increase in Ang II in the splanchnic circulation is more prominent than that in the systemic circulation during major aortic surgery and hypovolemia, Moreover, extraordinarily high concentrations of Ang II receptors have been found in the splanchnic vasculature [27]. Zhao et al. [28] have reported that intestinal I/R induced obvious histologic injury to the intestinal mucosa with denuded villi, disintegration of the lamina propria, exposed capillaries, and obvious neutrophil and macrophage infiltration.

In our study the results showed that Pre-administration of kaptopril significantly attenuated the intestinal damage induced by II/R, evidenced by the significant reduction of histological scores at the indicated time points. The most prominent finding is the reduction of infiltration of polymorphonuclear cells and lymphocytes and lover total scores. Although, crypt loss, mucosal bleeding and villous fusion and stunting were lower in kaptopril group, these were not statistically significant.

In our study we have some limitation. While the design of a work if we added sham group, additional 32 rats will be needed. We didn't, it is our one major limitation. Our second limitation is that blood pressure was not measured.

The results of this study showed that administration of kaptopril significantly reduced the serum level of TNF-α at the hour 0 and 2. The serum level of TNF- α 12 and 24 hours after reperfusion was also reduced in the kaptopril group but this was not statistcally significant. This may be related with the half life of kaptopril.

Cell membrane phospholipids are susceptible to peroxidation during I/R injury, generating LPO, which is an important pathway in the diagnosis and patho- physiology of I/R injury [29]. This study also showed that increased production of LPO in intestinal tissues caused by II/R injury was inhibited by kaptopril indicating its ant oxidative activity. These results suggest that kaptopril may be potent drug to protect the intestines against I/R injury by its anti-inflammatory and ant oxidative effects. However the beneficial effect of kaptopril on the survival cannot be evaluated since all the rats were sacrificed at the end of the experimental period. Further studies are required to investigate not only protective but also the therapeutic effects of kaptopril in II/R injury.

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