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Brief communication (Original)

Angiotensin-converting enzyme inhibitor captopril ameliorates renal damage in a rat model of thermal injury

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Background: Burn is a posttraumatic inflammatory condition accompanied by both local and distant effects leading to intense inflammation, tissue damage, and infection. Acute renal failure is a well-known complication of severe burns and is an important factor leading to an increase in mortality.

Objective: To determine the effect of captopril treatment on renal damage in a rat model of thermal injury by evaluating oxidant–antioxidant system parameters, sialic acid levels, glutathione-S-transferase (GST), and tissue factor (TF) activities.

Methods: Under ether anesthesia, the shaved dorsum of the rats was exposed to a 90□C water bath for 10 seconds to induce burn injury. Captopril (1 mg/kg) or saline was administered intraperitoneally immediately after, and at 24 hours after burn injury. Rats were decapitated at 48 hours following the burn injury and trunk blood was collected to assay blood urea nitrogen (BUN) and creatinine concentrations. To evaluate the presence of oxidant injury, kidney tissue samples were taken to determine malondialdehyde (MDA), glutathione (GSH), sialic acid levels, and the activities of superoxide dismutase (SOD), catalase, GST, and TF. In the sham group the same protocol was applied except that the dorsum was dipped in a 25□C water bath for 10 seconds.

Results: Severe skin scald injury (30% of total body surface area) caused significant decreases in GSH level, SOD and catalase activities, and significant increases in TF and GST activities, and sialic acid levels. Treatment of rats with captopril (1mg/kg) significantly elevated the reduced GSH levels, SOD and catalase activities, while it decreased MDA, sialic acid levels, GST and TF activities.

Conclusions: The present study showed for the first time that, captopril scavenging of reactive free radicals, normalizing the activities of TF and GST seems to be a promising agent for restoring renal damage following thermal trauma.

Keywords: Burn, kidney, captopril, catalase, glutathione, lipid peroxidation, sialic acid, superoxide dismutase, tissue factor

Burn is a posttraumatic inflammatory disease accompanied by both local and distant effects leading to intense inflammation, tissue damage, and infection [1]. Accordingly thermal burns and related injuries are a major cause of death and disability, especially in subjects under the age of 40. Even in developed countries, more than 2 million individuals annually are burned seriously and require medical treatment [2]. Activation of toxic inflammatory mediators, oxidants and proteases may further damage skin and capillary endothelial cells and potentiate ischemic tissue necrosis [1]. Acute renal failure is a well-known complication of severe burns and is an important factor leading to an increase in mortality. The incidence of acute renal failure in severely burned patients ranges from 1.3% to 38% and this complication has always been associated with high mortality rates (73% to 100%). The pathophysiological mechanism may be related to filtration failure or tubular dysfunction [3].

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At the molecular level, both complement activation and intravascular stimulation of neutrophils results in the production of cytotoxic reactive oxygen species (ROS) [4]. Under a normal physiological conditions, free radicals and reactive nonradical species derived from radicals exist in biological cells and tissues at low, but measurable, concentrations [5]. Their concentrations are determined by the balance between rate of production and rate of clearance by various antioxidant compounds and enzymes. An intense affiliation has been demonstrated between the quantity of lipid peroxidation and the degree of burn complications, such as remote organ damage and shock in skin and plasma [6]. A good indicator of oxidative injury and an end product of lipid peroxidation is the formation of malondialdehyde (MDA). Several studies demonstrated that burn and ischemia reperfusion injury are associated with elevated levels of MDA in different organ and tissues [7, 8].

Burn and septic injuries induce profound changes in coagulation status. Blood coagulation is essential to maintain hemostasis in organisms with a vascular network. Formation of a fibrin-rich clot at a site of vessel injury is a highly complex process that is orchestrated by the coagulation protease cascade. Defects in the regulation of clot formation lead to either hemorrhage or thrombosis. Tissue factor (TF, Factor III, thromboplastin), the primary cellular initiator of blood coagulation, is a transmembrane receptor that is expressed in a tissue-specific manner [9]. Moreover, various tissues and body fluids have been known to have TF activity [10-12].

Since the first administration of the well-known angiotensin-converting enzyme (ACE) inhibitor captopril for the treatment of essential hypertension, it has been recognized that captopril has a positive influence on the cardiovascular system, on both a molecular and a clinical level. Over the past 25 years, the indications for ACE inhibitors have spread into many fields. During this period, interesting effects of ACE inhibitors in noncardiac fields have been reported [13]. Captopril has an additional action; scavenging of the oxygen-derived radicals and preventing reperfusion cardiac injury [14]. Although the oxygen-derived radical scavenging property of captopril has been shown before, to our knowledge our study is first evaluate the effects of captopril in a rat model of thermal injury investigating the levels of MDA, glutathione (GSH), and sialic acid, and the activities of catalase, superoxide dismutase (SOD), and glutathione-s-transferase (GST). Moreover, to our knowledge, our study is first to evaluate the effect of captopril on TF activity. Burn and septic injuries induce profound changes in coagulation status. Therefore in the present study, the protective effect of captopril treatment on burn-induced remote organ injury was investigated.

Materials and methods

Animals

Wistar albino rats of both sexes weighing 200 to 250 g were obtained from Marmara University School of Medicine Animal House. The rats were kept at a constant temperature (22 ± 1°C) under a 12 h:12 h light and dark cycle. They were fed with standard rat chow, and were fasted for 12 h before the experiments, but were allowed free access to water. Each group consisted of 8 rats. Equal numbers of male and female rats were used in each group. All experimental protocols were approved by the Marmara University School of Medicine Animal Care and Use Committee.

Thermal injury and experimental design

Under ether anesthesia, dorsum of the rats was shaved, exposed to a 90□C water bath for 10 seconds, which resulted in a partial-thickness second-degree skin burn. This procedure has been shown to result in a burn involving 30% of the total body surface area [15]. Sham control rats, which served as controls, were anesthetized, shaved, and exposed to a 25°C water bath for 10 seconds. After sham or burn, all the animals were then resuscitated with physiological saline solution (10 ml/kg subcutaneously on the hind limb) immediately after and at 24 hours after the burn injury. In the saline-treated burn group, saline was administered intraperitoneally, whereas captopril (1 mg/kg in saline) was administered in the captopril-treated burn group immediately after burn injury and this treatment was repeated at 24 hours following burn injury. In all groups rats were decapitated 48 hours following the burn injury. After decapitation, trunk blood was collected and serum samples were stored at -70°C. To evaluate the presence of oxidant injury in the distant organ, kidney tissue samples were taken and stored at –70°C.

Biochemical analysis

Blood urea nitrogen (BUN) and creatinine concentrations were studied to assess the renal functions [16]. Creatinine levels were determined using

DICT-500-QuantiChrom Creatinine Assay Kit (BioAssay Systems, Hayward, USA) according to the instructions provided by the manufacturer. Renal tissue samples were homogenized with ice-cold 150 mM KCl for the determination of sialic acid, MDA, GSH levels and SOD, catalase, GST and TF activities.

Determination of sialic acid

Sialic acid levels were measured in serum using Warren's thiobarbituric acid assay [17]. Samples were incubated with 0.1 N H_2SO_4 at 80□C for 1 hour and sialic acid in the hydrolysate was determined. In this calorimetric assay sialic acids are oxidized with sodium periodate in concentrated phosphoric acid. The periodate oxidation product is coupled with thiobarbituric acid and the resulting chromophore is extracted into cyclohexanone.

Determination of lipid peroxidation

MDA an end product of lipid peroxidation content in serum was determined as thiobarbituric acid reactive substances (TBARS) according to the method of Yagi [18]. Lipid peroxidation was expressed in terms of MDA equivalents using an extinction coefficient of $1.56 \quad 10^5\ M^{-1}\ cm^{-1}$ and results are expressed as nmol MDA/mg protein (mg P).

Determination of superoxide dismutase activity

SOD activities were assayed by its ability to increase the effect of riboflavin-sensitized photooxidation of ortho-dianisidine [19]. The activity of superoxide is generated by illuminating the reaction mixture containing ortho-dianisidine and riboflavin by light from a fluorescent lamp. The oxidation of ortho-dianisidine, as sensitized by riboflavin, is enhanced by SOD. The increase is linearly dependent on SOD concentration. The absorbance of the colored product is evaluated by spectrophotometry at 460 nm.

Determination of glutathione

GSH concentration was determined according to the method of Beutler et al. [20] using metaphosphoric acid for protein precipitation and 5,5′-dithiobis-(2-nitrobenzoic acid) as a chromogen. GSH levels were calculated using an extinction coefficient of $1.36 \times 10^4\ M^{-1}\ cm^{-1}$. Results are expressed in □g GSH/mg P.

Determination of catalase activity

Catalase activity was measured spectrophotometrically at 240 nm according to the method of Aebi [21]. The principle of this method was based on the hydrolyzation of H_2O_2 and decreasing absorbance at 240 nm. The conversion of H_2O_2 into H_2O and 1/2 O_2 in 1 minute under standard condition was considered to be the enzyme reaction velocity.

Determination of proteins

Total protein levels were determined according to the method of Lowry [22].

Determination of tissue factor activity

TF activities of tissue homogenates were evaluated according to Quick's one-stage method using pooled plasma collected from healthy subjects. TF activity was performed by mixing 0.1 ml tissue homogenate with 0.1 ml of 0.02 M $CaCl_2$, with the clotting reaction being started on addition of 0.1 ml of plasma. All reagents were brought to the reaction temperature (37°C) before admixture. Because the clotting time is inversely proportional to the TF activity, the lengthening of the clotting time is a manifestation of decreased TF activity [23].

Statistics

Statistical analysis was conducted using GraphPad Prism 3.0 (GraphPad Software, San Diego, USA). All data were expressed as means ± SD. Groups of data were compared with a Kruskal–Wallis test followed by Dunn's multiple comparison tests. Values of $p < 0.05$ were regarded as significant.

Results

Serum creatinine and BUN levels

BUN levels in the saline-treated burn group (19.20 ± 0.82 mg/dl) group increased significantly when compared with the sham group (15.44 ± 0.83 mg/dl; $p < 0.05$). Captopril treatment reversed the elevations in BUN levels significantly ($p < 0.05$) (16.60 ± 1.05 mg/dl).

Serum creatinine also increased significantly in the saline treated burn group (0.65 ± 0.024) when compared with the sham group (0.36 ± 0.019 mg/dl; $p < 0.05$), while captopril was observed to prevent this increase (0.36 ± 0.022 mg/dl; $p < 0.05$). The details are shown in **Table 1**.

Table 1. BUN and serum creatinine values

	Control	Burn	
		Saline-treated	Captopril-treated
BUN (mg/dl)	15.44 ± 0.83	19.20 ± 0.82*	16.60 ± 1.05**
Creatinine (mg/dl)	0.36 ± 0.019	0.65 ± 0.024*	0.36 ± 0.022**

*$p < 0.05$ compared with control group, **$p < 0.05$ compared with saline treated burn group

Kidney GSH levels

The levels of GSH in the kidney tissue decreased (1.07 ± 0.13 μg GSH/mg P) following burn injury, which were statistically different than that of the sham group (1.53 ± 0.3 μg GSH/mg P, $p < 0.05$). Captopril treatment significantly ($p < 0.05$) elevated the GSH levels (1.47 ± 0.35; μg GSH/mg P) (**Figure 1**).

Kidney MDA levels

Although statistically not significant the kidney MDA was found to be higher in the saline-treated burn (1.11 ± 0.18 nmol MDA/mg P) group than in the sham group (0.76 ± 0.088 nmol MDA/mg P). Treatment with captopril significantly decreased the elevations in MDA levels ($p < 0.001$) (0.48 ± 0.14 nmol MDA/mg P (**Figure 2**).

Kidney catalase activities

Kidney catalase activity showed a tendency to decrease in the saline-treated burn group (19.84 ± 8.22 U/mg P) compared with the sham group (23.38 ± 3.33 U/mg P). Captopril treatment increased the catalase activities in kidney significantly (33.65 ± 5.22 U/mg P) when compared with the saline-treated burn group ($p < 0.05$) (**Figure 3**).

Kidney glutathione-S-transferase activities

GST activities in kidney increased significantly in the saline-treated burn (0.15 ±0.039 U/mg P) group when compared with the sham group (0.088 ± 0.018 U/mg P). Captopril treatment further increased GST activity significantly compared with the saline treated burn group (0.17 ± 0.03 U/mg P) (**Figure 4**).

Kidney superoxide dismutase activities

SOD activities in kidney decreased significantly in the saline-treated burn group (1.03 ± 0.09 U/mg P) when compared with the sham group (1.54 ± 0.26 U/mg P). On the other hand captopril treatment increased SOD activities significantly (1.26 ± 0.105 U/mg P) compared with the saline-treated burn group (**Figure 5**).

Kidney sialic acid levels

Sialic acid levels in kidney increased significantly in the saline treated burn group (0.046 ± 0.007 mg/μg P) when compared with the sham group (0.031 ± 0.007 mg/μg P). Captopril treatment decreased sialic acid levels significantly (0.029 ±0.004 mg/μg P) compared with the saline-treated burn group (**Figure 6**).

Tissue factor activity of kidney

TF activity of kidney increased significantly in the saline treated burn group (26.23 ± 0.49 s) when compared with the sham group (32.21 ± 1.41 s). Captopril treatment decreased TF activity significantly (33.69 ± 2.93 s) compared with the saline treated burn group (**Figure 7**).

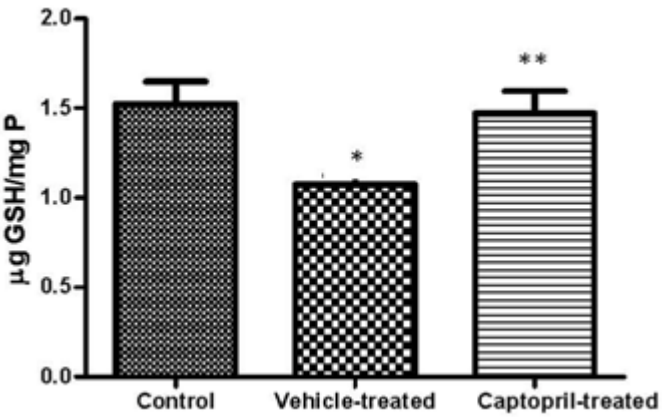

Figure 1. Kidney GSH levels of the control, vehicle-treated, and captopril treated burn groups.
*$p < 0.05$ significantly different from the control group, **$p < 0.05$ significantly different from the vehicle-treated burn group

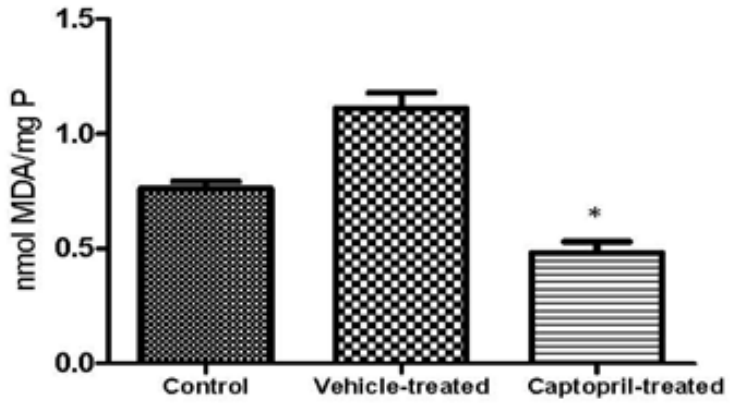

Figure 2. Kidney MDA levels of the control, vehicle-treated, and captopril treated burn groups.
*$p < 0.05$ significantly different from the vehicle-treated burn group

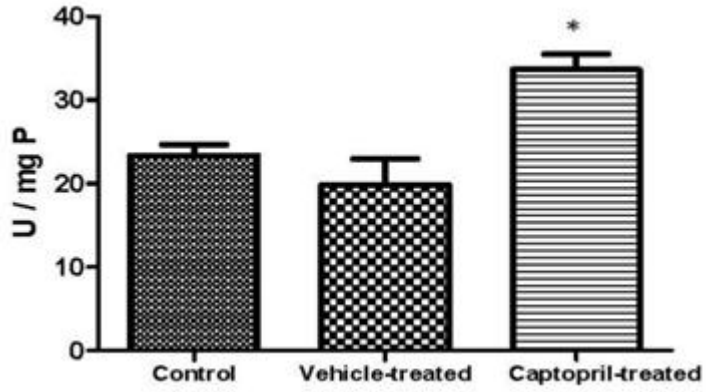

Figure 3. Kidney catalase activities of the control, vehicle-treated, and captopril treated burn groups.
*$p < 0.05$ significantly different from the vehicle-treated burn group

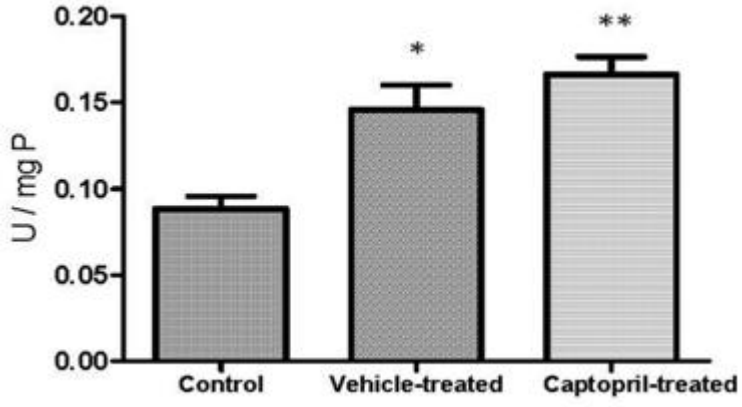

Figure 4. Kidney GST activities of the control, vehicle-treated, and captopril treated burn groups.
*$p < 0.05$ significantly different from the control group, **$p < 0.05$ significantly different from the vehicle-treated burn group

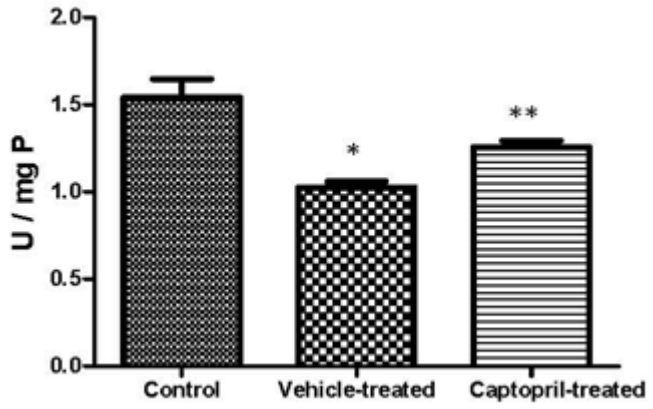

Figure 5. Kidney SOD activities of the control, vehicle-treated, and captopril-treated burn groups.
*$p < 0.05$ significantly different from the control group, **$p < 0.05$ significantly different from the vehicle-treated burn group

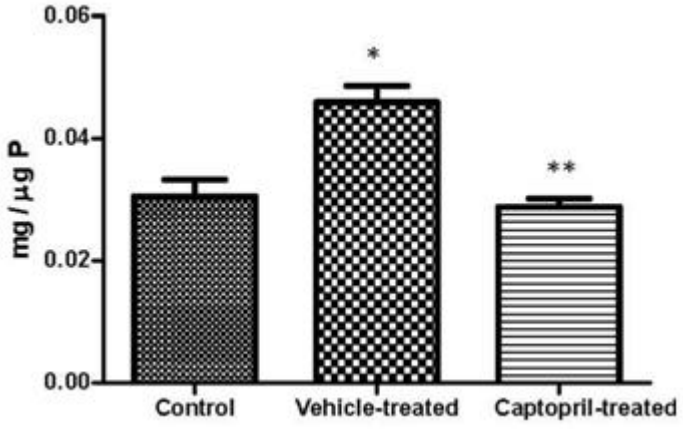

Figure 6. Kidney sialic acid levels of the control, vehicle-treated, and captopril treated burn groups.
*$p < 0.05$ significantly different from the control group, **$p < 0.05$ significantly different from the vehicle-treated burn group

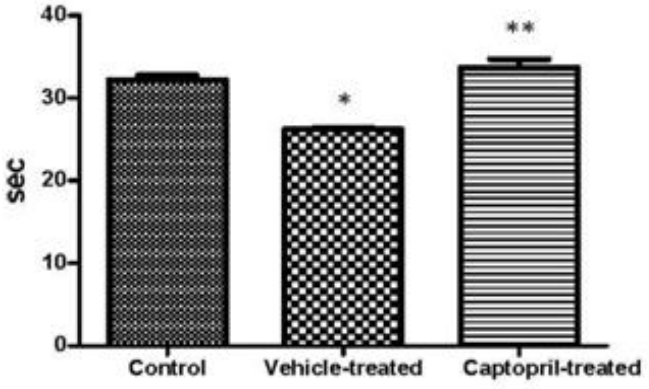

Figure 7. Kidney tissue factor activities of the control, vehicle-treated, and captopril treated burn groups.
*$p < 0.05$ significantly different from the control group, **$p < 0.05$ significantly different from the vehicle-treated burn group

Discussion

The results of the present study demonstrate that the burn-induced injury in the kidney tissue was ameliorated by captopril treatment, as evidenced by changes in GSH, SOD, Catalase, GST, sialic acid, and MDA levels. These findings suggest that captopril has a protective role in the burn-induced injury, which may be attributed to its antioxidant effects.

Thermal injury may cause damage to multiple organs distant from the original burn wound and may lead to multiorgan failure. Several studies demonstrated that burns initiate systemic inflammatory reactions by producing burn toxins such as ROS, and finally lead to peroxidation [6, 23, 24]. In many tissues such as skin, plasma, liver, and lung, burns are associated with lipid peroxidation, which is believed to be an important cause of oxidative damage to cellular membranes, and eventually cell death [25-27]. An intense affiliation has been demonstrated between the quantity of lipid peroxidation and the degree of burn complications such as remote organ damage and shock in skin and plasma [26]. A good indicator of oxidative injury and an end product of lipid peroxidation is the formation of MDA. Several studies demonstrated that burn and ischemia reperfusion injury is associated with elevated levels of MDA in different organs and tissues [28]. Significant increases in MDA may cause severe tissue injury. Cell membranes are rich sources of polyunsaturated fatty acids (PUFA), which are the most vulnerable targets of ROS attack. Thus lipid peroxidation is one of many changes associated with burns, with free radicals being the causative agents [27]. Our observation that MDA levels tended to increase in kidney tissue indicate that ROS contributes to organ injury distant from the original wound.

Glutathione, which is found in high concentrations in the kidney, provides major protection in oxidative injury by participating in the cellular system of defense against oxidative damage [29]. GSH is the most abundant thiol and functions as a co-substrate for glutathione peroxidase, the antioxidative enzyme that catalyzes the reduction of H_2O_2 and lipid hydroperoxides. Glutathione scavenges the superoxide anion and protects protein thiol groups from oxidation [30]. SOD, one of the important intracellular antioxidant enzymes present in aerobic cells has an antitoxic effect against superoxide anion. The presence of SOD in various fractions such as cytosol (cupro-zinc-SOD), mitochondria (mangano-SOD) and plasma (cupro-SOD) enables SOD to eliminate superoxide radicals immediately and protect the cell from oxidative damage [31]. Catalase, the heme containing antioxidant enzyme protects cells from accumulation of H_2O_2 by decomposing it to H_2O and O_2 [32]. GST is widely distributed in human tissues and plays a pivotal protective role against environmental damage. The increase of GST isoenzymes can reflect the degree of the hepatocellular and renal proximal tubular epithelium damage [33].

The results of the present study support the notion that depletion of renal GSH, SOD, and catalase is one of the major factors that permit lipid peroxidation and subsequent tissue damage. On the other hand, increased GST activity in the kidney reflects renal damage. In many inflammatory conditions including burns, the plasma sialic acid level rises as an acute responder [34]. Accordingly in our present study, kidney sialic acid levels increased significantly in the burn group.

Furthermore, the results also suggest that captopril has a preventive effect in thermal trauma by decreasing MDA and sialic acid levels, GST activities, and increasing the levels of GSH, and the activities of SOD and catalase significantly. Captopril ([S]-1-[3-mercapto-2-methyl-1-oxo-propyl]-l-proline) is the first marketed orally-active ACE inhibitor (ACEI) designed to treat hypertension by blocking the conversion of angiotensin I into angiotensin II [35]. The specific inhibitory properties of captopril have been attributed to its structure containing thiol groups that provide specific binding to ACE and to indirect suppression of the expression of the gene encoding ACE. In addition, this thiol compound can react with superoxide anion radicals, acting as a scavenger or with hydroxyl radicals, and improves the oxidative balance [36]. Ahmed et al. [37] treated portal vein-ligated rats with captopril and reported significant elevations in GSH content and SOD activity. Ha and Kim reported that captopril ameliorated the lipid peroxidation associated with diabetes [38]. Rodrigues de Araujo et al. [39] investigated the effects of captopril as a promoter in modulating the oxidant–antioxidant balance in rats with type 1 diabetes, and the influence of protein kinase C pathways in the production of ROS induced by bradykinin in type 1 diabetic rats. They suggested the antioxidant effect of captopril to be independent of bradykinin, and that captopril can modulate the production of ROS in circulating neutrophils.

The antioxidant effect of ACEI may be through direct effect on enzyme synthesis or activity or a secondary effect resulting from the consequences of ACE metabolic actions: inhibition of angiotensin II synthesis, inhibition of aldosterone formation and release, stimulation of renin production, increasing cellular sensitivity to catecholamine, and potentiation of bradykinins [40]. de Cavanagh et al. [41] showed that captopril treatment increased antioxidant enzymes and nonenzymatic antioxidants in several mouse tissues. Furthermore, in erythrocytes the augmentation of antioxidants by ACE inhibitors was associated with protection against oxidant damage.

Burn and septic injuries induce changes in coagulation status. Strong thromboplastic activity because of TF exposition or release (in or from burned tissues), can activate the extrinsic pathway of the coagulation system and trigger massive thrombin generation when present in sufficient concentration. This is the most plausible biological explanation supporting the development of intravascular coagulation in patients with burn injury. Severe burns, left untreated, might also lead to an immunological and inflammatory response because of the activation of the complement cascade, which can amplify fibrinolysis and blood clotting [42, 43]. In our present study, TF activity of the kidney increased significantly in the burn group and captopril treatment decreased TF activity significantly. Ravindranath et al. [42] examined changes in the plasma tissue factor pathway inhibitor and thrombin activatable fibrinolytic inhibitor levels in a rat model of burn and septic injuries. They suggested that burn, sepsis, and their combined injuries perturb the coagulation cascade and thrombotic process toward the procoagulant pathway by impairing fibrinolysis.

In the present study, burn-induced increases in lipid peroxidation, GST, sialic acid levels, and TF activity with concomitant decreases in GSH levels, SOD, and catalase activities were observed in kidney. Moreover, captopril, with its ability to neutralize a number of ROS and stimulate several antioxidative enzymes, demonstrated a protective effect in burn-induced renal injury. Captopril also exerted additional beneficial effects by normalizing the activity of TF, GST, and levels of sialic acid in kidney. Over the past 25 years, the indications for ACE inhibitors have spread into many fields. Based on the results of our present study, captopril, which scavenges reactive free radicals, normalizing the activity of TF and GST, seems to be a promising agent for restoring renal damage following thermal trauma.

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