EXPERIMENTAL STUDY

Captopril protects against burn-induced cardiopulmonary injury in rats

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ABSTRACT

BACKGROUND: This study was designed to determine the possible protective effect of captopril treatment against oxidative damage in heart and lung tissues induced by burn injury.

METHODS: Under ether anesthesia, the shaved dorsum of Wistar albino rats was exposed to 90°C water bath for 10 seconds. Captopril was administered intraperitoneally (10 mg/kg) after the burn injury and repeated twice daily. In the sham group, the dorsum was dipped in a 25°C water bath for 10 seconds. At the end of the 24 hours, echocardiographic recordings were performed, then animals were decapitated and heart and lung tissue samples were taken for the determination of tumor necrosis factor-α (TNF-α) as a pro-inflammatory cytokine, malondialdehyde and glutathione levels and myeloperoxidase, caspase-3, and Na$^+$,K$^+$-ATPase activity in addition to the histological analysis.

RESULTS: Burn injury caused significant alterations in left ventricular function. In heart and lung tissues, TNF-α and malondialdehyde levels and myeloperoxidase and caspase-3 activities were found to be increased, while glutathione levels and Na$^+$, K$^+$-ATPase activity were decreased due to burn injury. Captopril treatment significantly elevated the reduced glutathione level and Na$^+$, K$^+$-ATPase activity, and decreased cytokine and malondialdehyde levels and myeloperoxidase and caspase-3 activities.

CONCLUSION: Captopril prevents burn-induced damage in heart and lung tissues and protects against oxidative organ damage.

Key words: Captopril; cytokine; lipid peroxidation; myeloperoxidase; thermal trauma.

INTRODUCTION

Thermal trauma produces profound systemic changes such as oligemic shock, anemia, renal failure, and metabolic disturbances. It causes direct tissue damage as well as inflammatory reactions.[1] Thermal trauma causes a progressive decrease in cardiac output and cardiac stroke volume, release of vasocon-strictor mediators such as vasopressin, altered baroreceptor and chemoreceptor reflexes, and reabsorption of fluid.[2] A number of experimental and clinical studies have shown that, despite aggressive fluid resuscitation and maintenance of pulmonary capillary wedge pressure, stroke work and ejection fraction (EF) often decrease after thermal trauma.[3,4] Several studies have indicated that decreased cardiac contractility is one of the important mechanisms for decreased cardiac output and cardiac dysfunction after severe thermal trauma.[5,6]

Angiotensin converting enzyme (ACE) catalyzes the conversion of angiotensin I (Ang I) to angiotensin II (Ang II). ACE also cleaves the terminal dipeptide of vasodilating hormone bradykinin to inactivate this hormone. Hyperactivity of the renin–angiotensin system (RAS), an endocrine system with critical roles in cardiovascular function, has been implicated in the etiology of high blood pressure, obesity and metabolic syndrome.[7] Therefore, inhibition of ACE is generally used as one of the

methods in the treatment of hypertension and chronic inflammation.[8,9] Pharmacologically, it is known that ACE inhibitors containing a sulfhydryl or a thiol radical, such as the orally active compound captopril, have an antioxidant activity.[10,11]

In the light of these findings, we hypothesized that captopril would provide protection against burn-induced cardiopulmonary damage. We aimed to investigate whether and to what extent captopril reduces this damage, by determining the presence of oxidative tissue injury using biochemical and histological parameters.

MATERIALS AND METHODS

Animals

Wistar albino rats of both sexes, weighing 200-250 g, were obtained from Marmara University School of Medicine Animal House. The rats were kept at a constant temperature (22±1°C) with 12-hour (h) light and dark cycles, fed with standard rat chow, and fasted for 12 h before the experiments, but were allowed free access to water. All experimental protocols were approved by the Marmara University Animal Care and Use Committee.

Thermal Injury and Experimental Design

This is a randomized controlled trial. All groups were elected in exactly the same way. Twenty-four animals were divided into three groups of eight rats each: One group served as controls. Two groups underwent burn injury and were treated with saline or captopril. Under brief ether anesthesia, the dorsum of the rats was shaved and exposed to 90° water bath for 10 seconds (s), which resulted in a second-degree burn involving 30% of the total body surface area.[12] To rule out the effects of the anesthesia, the same protocol was applied in the control group, except that the dorsa were dipped in a 25°C water bath for 10 s. After sham or burn injury, rats were resuscitated with physiological saline solution (10 ml/kg subcutaneous). ACE inhibitor captopril (10 mg/kg, intraperitoneal (i.p.); Sigma-Aldrich; St. Louis, MO, USA) or saline was given intraperitoneally to rats immediately after the burn injury, and the injections were repeated twice a day. Two groups (controls and the first burn group) received saline and the second burn group received i.p. captopril (10 mg/kg in saline) administered immediately after the burn, and the injections were repeated twice a day. Intraperitoneal administration of captopril could potentially have a systemic effect.[13] In both the saline- and captopril-treated burn groups, rats were decapitated at 24 h following burn injury. At the end of the experimental period, transthoracic echocardiography was performed to assess the cardiac function of the rats. Then, the animals were decapitated in order to evaluate the presence of oxidant injury in the heart, and lung tissue samples were taken and stored at -80°C for the determination of pro-inflammatory cytokine [tumor necrosis factor-α (TNF-α)], malondialdehyde (MDA) and glutathione (GSH)

levels and myeloperoxidase (MPO) and Na$^+$, K$^+$-ATPase and caspase-3 activities. For histological analysis, samples of the tissues were fixed in 10% (vol/vol) buffered p-formaldehyde and prepared for routine paraffin embedding. Tissue sections (6 μm) were stained with hematoxylin and eosin (H&E) and examined under a light microscope (Olympus-BH-2). An experienced histologist who was unaware of the treatment conditions performed the histological assessments.

Echocardiography

Echocardiographic imaging and calculations were done according to the guidelines published by the American Society of Echocardiography[14] using a 12 MHz linear transducer and 5-8 MHz sector transducer (Vivid 3, General Electric Medical Systems Ultrasound, Tirat Carmel, Israel). Under ketamine (50 mg/kg, i.p.) anesthesia, measurements were made from M-mode and two-dimensional images obtained in the parasternal long- and short axes at the level of the papillary muscles after observation of at least six cardiac cycles. Interventricular septal thickness (IVS), left ventricular diameter (LVD) and left ventricular posterior wall thickness (LVPW) were measured during systole (s) and diastole (d). EF, fractional shortening (FS) and left ventricular mass (LVM) and relative wall thickness (RWT) were calculated from the M-mode images using the following formulas: % EF = [(LVDd)^3 - (LVDs)^3/(LVDd)^3 x 100], % FS = [LVDd-LVDs/LVDd x 100], LVM= [1.04 x ((LVDd+LVPWd+IVSd)^3 - (LVDd)^3) x 0.8 + 0.14], and RWT = [2 x (LVPWd/LVDd)].

Determination of Tissue TNF-α Levels:

The levels of TNF-α in the tissues were measured by enzyme-linked immunosorbent assay (ELISA). Heart and lung tissues (100 mg) were homogenized in an ice-cold bath. After centrifugation, the supernatant was collected and total protein content was determined. Tissue TNF-α levels were measured with rat TNF-α ELISA kits (Invitrogen; Taastrup, Denmark) according to the manufacturer's instructions. Absorbance of standards and samples was determined on a plate reader at 405 nm. Results are expressed as pg/100 mg tissue.

Malondialdehyde and Glutathione Assays

Tissue samples were homogenized with ice-cold 150 mM KCl for the determination of MDA and GSH levels. The MDA levels were assayed for products of lipid peroxidation by monitoring thiobarbituric acid reactive substance formation as described previously.[15] Lipid peroxidation was expressed in terms of MDA equivalents using an extinction coefficient of 1.56×10^5 M^{-1} cm^{-1}, and results are expressed as nmol MDA/g tissue. GSH measurements were performed using a modification of the Ellman procedure.[16] Briefly, after centrifugation at 3000 rev/minute (min) for 10 min, 0.5 ml of supernatant was added to 2 ml of 0.3 mol/L Na$_2$HPO$_4$.2H$_2$O solution. A 0.2 ml solution of dithiobisnitrobenzoate (0.4 mg/ml 1% sodium citrate) was added, and the absorbance at 412 nm was measured immediately after mixing. GSH levels were calculated

using an extinction coefficient of 1.36×10^4 M^{-1} cm^{-1}. Results are expressed in μmol GSH/g tissue.

Myeloperoxidase Activity

Myeloperoxidase (MPO) is an enzyme found predominantly in the azurophilic granules of polymorphonuclear leukocytes (PMN). Tissue MPO activity is frequently utilized to estimate tissue PMN accumulation in inflamed tissues, and correlates significantly with the number of PMN determined histochemically in tissues. MPO activity was measured in tissues in a procedure similar to that documented by Hillegass et al.[17] Tissue samples were homogenized in 50 mM potassium phosphate buffer (PB, pH 6.0), and centrifuged at 41,400 g (10 min); pellets were suspended in 50 mM PB containing 0.5% hexadecyltrimethylammonium bromide (HETAB). After three freeze and thaw cycles, with sonication between cycles, the samples were centrifuged at 41,400 g for 10 min. Aliquots (0.3 ml) were added to 2.3 ml of reaction mixture containing 50 mM PB, o-dianisidine, and 20 mM H_2O_2 solution. One unit of enzyme activity was defined as the amount of MPO present that caused a change in absorbance measured at 460 nm for 3 min. MPO activity was expressed as U/g tissue.

Na$^+$, K$^+$-ATPase Activity

Since the activity of Na$^+$, K$^+$-ATPase, a membrane-bound enzyme required for cellular transport, is very sensitive to free radical reactions and lipid peroxidation, reductions in this activity can indicate membrane damage indirectly. Measurement of Na$^+$, K$^+$-ATPase activity is based on the measurement of inorganic phosphate released by ATP hydrolysis during incubation of homogenates with an appropriate medium containing 3 mM ATP as a substrate. The total ATPase activity was determined in the presence of 100 mM NaCl, 5 mM KCl, 6 mM $MgCl_2$, 0.1 mM EDTA, and 30 mM Tris HCl (pH 7.4), while the Mg^{2+}-ATPase activity was determined in the presence of 1mM ouabain. The difference between the total and the Mg^{2+}-ATPase activities was taken as a measure of the Na$^+$,

K$^+$-ATPase activity.[18] The reaction was initiated with the addition of the homogenate (0.1 ml), and a 5-min preincubation period at 37°C was allowed. Following the addition of Na$_2$ATP and a 10-min re-incubation period, the reaction was terminated by the addition of ice-cold 6% perchloric acid. The mixture was then centrifuged at 3500 g, and Pi in the supernatant fraction was determined by the method of Fiske and Subarrow.[19] The specific activity of the enzyme was expressed as μmol Pi mg-1 protein h-1. The protein concentration of the supernatant was measured by the Lowry method.[20]

Caspase-3 Activity

Caspase-3 activity was measured using ApoTargetCaspase-3/CPP32 Colorimetric Protease Assay (Invitrogen; Taastrup, Denmark) kit according to the manufacturer's instructions. Briefly, brain and lung tissue samples were homogenized and treated for 10 min with iced lysis buffer supplied by the manufacturer. The cell lysates were subjected to three freeze and thaw cycles and 2x10 s sonication to fully disrupt the cells and disperse cell debris. The cell lysate was then centrifuged at 20,000 g for 5 min and the supernatant transferred to new Eppendorf tubes. Following protein determination, cytosol extracts were diluted in 50μL lysis buffer and 50 μL reaction buffer (10mM dithiothreitol). Samples were added to wells and the microplate was equilibrated at 37°C for 10 min. The reaction was initiated by adding 5 μl of DEVD-pNA (Asp-Glu-Val-Asp p-nitroanilide) substrate (200 μM final concentration), and the reaction was carried out at 37°C for 2 h in the dark. The colorimetric release of p-nitroaniline (pNA) from the Ac-DEVD-pNA substrate was recorded at 405 nm using a microplate reader. Experiments were performed in triplicate. Results are presented as mean±SD of six separate experiments and expressed as fold-increase over pretreatment level (untreated samples).

Histopathological Analysis

For light microscopic investigations, heart and lung specimens

Table 1. Transthoracic echocardiography measurements in the experimental groups

Parameter	Control group	Burn group	
	Saline-treated	Saline-treated	Captopril-treated
Interventricular septal thickness (mm)	2.36±0.18	3.01±0.11**	2.45±0.08+
Left ventricular posterior wall thickness (mm)	1.73±0.12	2.55±0.11*	1.77±0.11+
Relative wall thickness	0.66±0.03	0.98±0.10*	0.71±0.08+
Left ventricular diameter in systole (mm)	2.63±0.27	3.96±0.15**	2.98±0.21+
Left ventricular diameter in diastole (mm)	4.09±0.16	5.18±0.14**	4.32±0.24+
Ejection fraction (%)	79.03±3.67	63.60±2.73*	77.78±3.44+
Fractional shortening (%)	42.27±3.54	26.61±2.24**	39.71±3.21+
Heart/body weight ratio (mg/g)	2.21±0.12	2.73±0.08**	2.29±0.09+

Data are the mean±SEM of six animals. *p<0.05, **p<0.01 compared to saline-treated control group; +p<0.05 compared with the saline-treated burn group.

were fixed in 10% buffered formalin for 48 h, dehydrated in an ascending alcohol series, and embedded in paraffin wax. Approximately 5-μm-thick sections were stained with H&E for general morphology. Histological assessments were made with a photomicroscope (Olympus BX 51; Tokyo) by an experienced histologist who was unaware of the experimental groups.

Statistics

Statistical analysis was carried out using GraphPad Prism 3.0 (GraphPad Software; San Diego, CA, USA). All data were expressed as means±SEM. Groups of data were compared with an analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. Values of $p<0.05$ were regarded as significant.
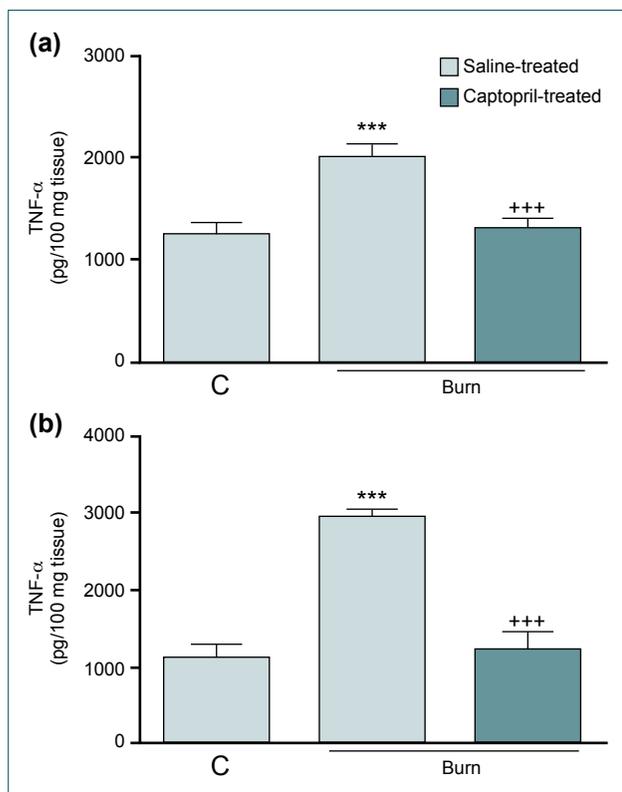
RESULTS

Table I summarizes the transthoracic echocardiography measurements of the experimental groups. In the saline-treated burn group, LVPW, LV end-diastolic and end-systolic dimensions and RWT, and heart/body weight ratio were increased significantly compared to the control group ($p<0.05$-0.01), while percent FS and EF were decreased significantly. On

the other hand, in the captopril-treated burn group, echocardiographic measurements were significantly different than those of the saline-treated burn group ($p<0.05$), but similar to those of the control group.
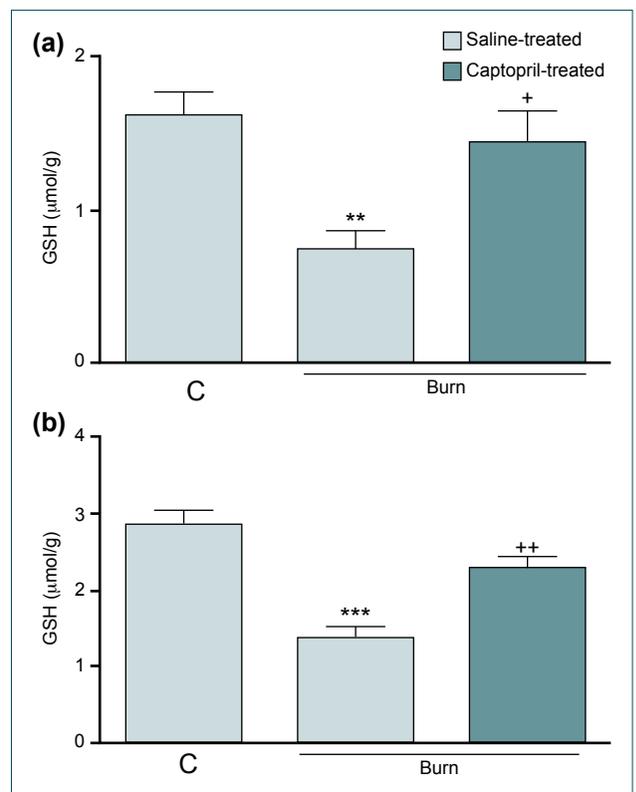
TNF-α levels in the heart and lung tissues of the saline-treated burn group were significantly increased, while TNF-α levels were found to be decreased in the captopril- treated burn group ($p<0.001$; Figs. Ia, b).

Burn injury caused significant decreases in the GSH levels of the heart and lung tissues of the saline-treated burn group ($p<0.01$-0.001), but this antioxidant was restored in the captopril-treated burn group ($p<0.05$-0.01; Figs. 2a, b). On the other hand, MDA levels in both tissues were significantly increased following burn injury ($p<0.01$-0.001), while treatment with captopril reversed burn-induced elevations in MDA back to the control levels ($p<0.05$-0.01; Figs. 3a, b). In the saline-treated burn group, MPO activities in both the heart and lung tissues were increased significantly ($p<0.001$), and treatment with captopril prevented these alterations ($p<0.01$-0.001; Figs. 4a, b).
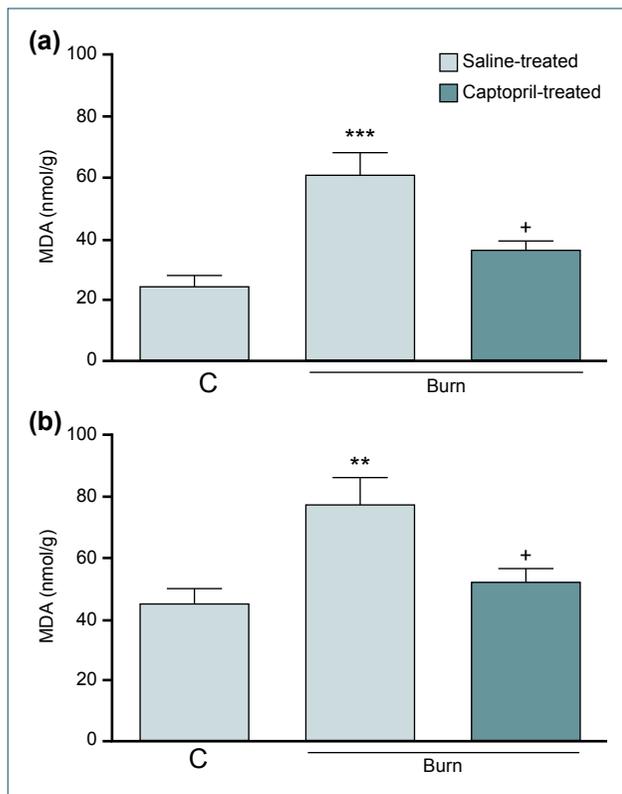
Na$^+$, K$^+$-ATPase activities measured in the cardiac and pulmo-

Figure 1. Tumor necrosis factor-α (TNF-α) levels in the **(a)** heart and **(b)** lung tissues of control (C) and saline- or captopril-treated burn groups. Each group consists of 8 animals. ***: $p<0.001$: compared to saline-treated control group; +++: $p<0.001$: compared to saline-treated burn group.

Figure 2. Glutathione (GSH) levels in the **(a)** heart and **(b)** lung tissues of control (C) and saline- or captopril-treated burn groups. Each group consists of 8 animals. **: $p<0.01$, ***: $p<0.001$: compared to saline-treated control group; + $p<0.05$, ++: $p<0.01$: compared to saline-treated burn group.

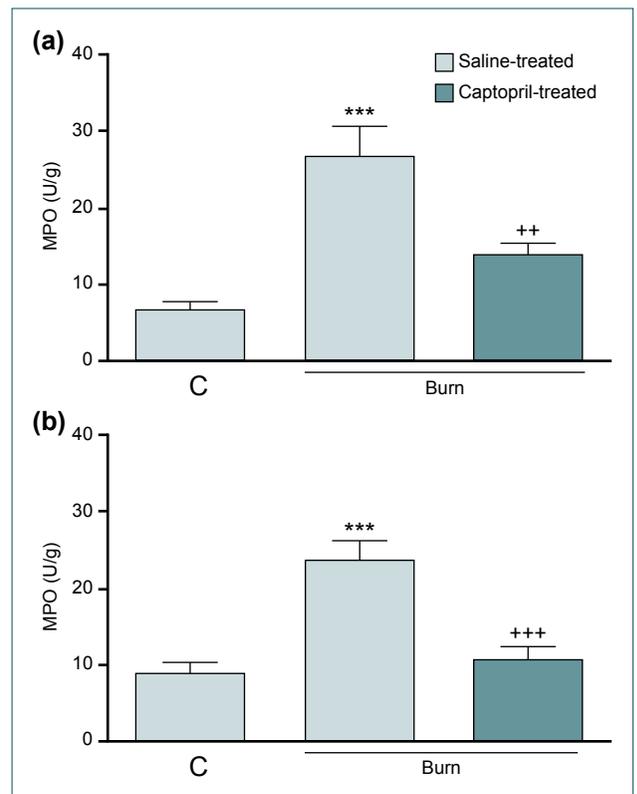**Figure 3.** Malondialdehyde (MDA) levels in the **(a)** heart and **(b)** lung tissues of control (C) and saline- or captopril-treated burn groups. Each group consists of 8 animals. **: $p<0.01$, ***: $p<0.001$: compared to saline-treated control group; +: $p<0.05$: compared to saline-treated burn group.

Figure 4. Myeloperoxidase (MPO) activity in the **(a)** heart and **(b)** lung tissues of control (C) and saline- or captopril-treated burn groups. Each group consists of 8 animals. ***: $p<0.001$: compared to saline-treated control group; ++: $p<0.01$, +++: $p<0.001$: compared to saline-treated burn group.

nary tissues were reduced in the saline-treated burn group ($p<0.001$, Figs. 5a, b). In the captopril-treated burned rats, the measured Na^+, K^+-ATPase activities in the studied tissues were increased ($p<0.05$-0.001).

Caspase-3 activities in both the heart and lung tissues were increased significantly ($p<0.001$), while treatment with captopril reduced these increases ($p<0.01$-0.001; Figs. 6a, b).

Light microscopic evaluation of the cardiac muscle cells in the control group revealed regular nuclei with central alignment (Fig. 7a). In the burn group, the capillaries present in the connective tissue of muscle fibers showed congestion in addition to vacuolization in the cytoplasm of most of the cardiomyocytes (Fig. 7b). On the other hand, in the captopril-treated burn group, vasocongestion was reduced and regular cellular morphology was settled (Fig. 7c).

In lung tissues, the control group demonstrated a well-designated morphology (Fig. 8a), whereas severe interstitial edema with bronchiolar hemorrhage and stretched alveolar walls were observed in the burn group (Fig. 8b), which was ameliorated by captopril treatment, revealing the reversal of degeneration in the interstitium (Fig. 8c).
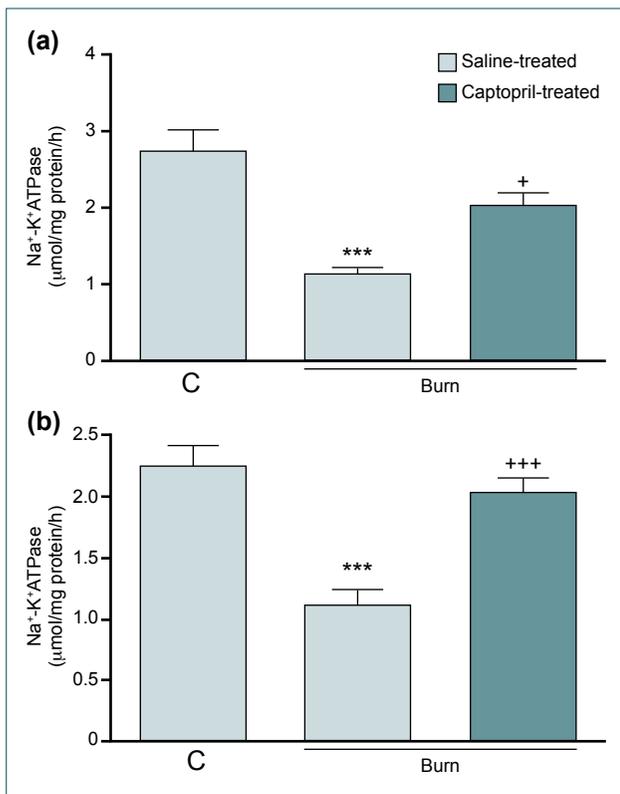
DISCUSSION

The aim of the present work was to explore the effect of captopril, a known ACE inhibitor, on the cardiopulmonary inflammatory process associated with burn in rats.
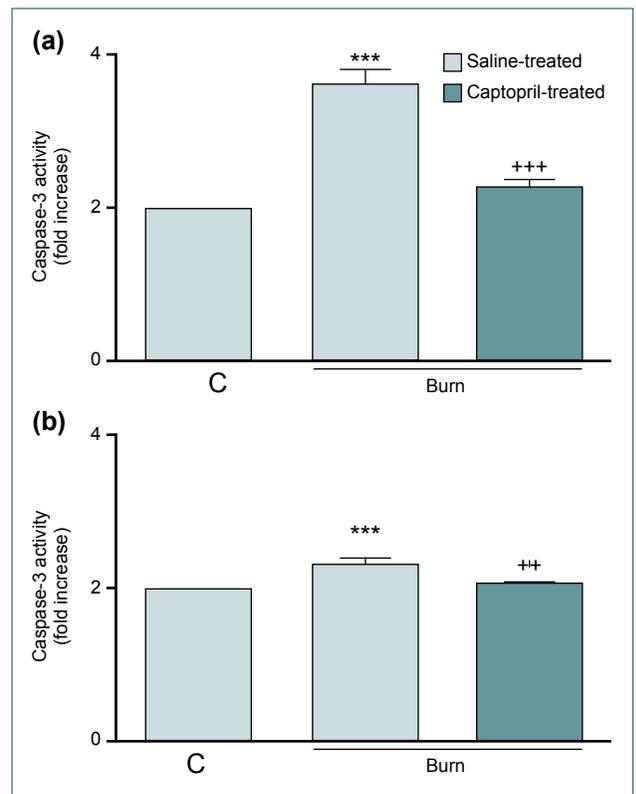
Thermal trauma is a stressful condition challenging whole body homeostatic mechanisms, accompanied by both local and distant effects leading to intense inflammation, tissue damage and infection. As local production of proinflammatory cytokines will activate non-specific host immunity, tissue injury or infection, cytokines are approached as being important components in the postburn pathophysiological process. [21-23] Accordingly, after thermal trauma, a great many cytokines are induced rapidly,[12,24-27] and ACE mediates inflammation to elicit T cell stimulation. On the other hand, the ACE inhibitor captopril inhibits conclusive immune functions and attenuates inflammation.[28,29] Our data above demonstrate that burn-induced cardiac and lung injury involve oxidant formation and inflammation, while captopril treatment greatly attenuated these responses. The ability of captopril to protect against thermal trauma was observed with significant decreases in tissue MDA and TNF-α levels and MPO and caspase-3 activities, which were found to be increased

Figure 5. Na⁺-K⁺ ATPase activity in the **(a)** heart and **(b)** lung tissues of control (C) and saline- or captopril-treated burn groups. Each group consists of 8 animals. ***: $p<0.001$: compared to saline-treated control group; +: $p<0.05$, +++: $p<0.001$: compared to saline-treated burn group.

Figure 6. Caspase-3 activity in the **(a)** heart and **(b)** lung tissues of control (C) and saline- or captopril-treated burn groups. Each group consists of 8 animals. ***: $p<0.001$: compared to saline-treated control group; ++: $p<0.01$, +++: $p<0.001$: compared to saline-treated burn group.

following thermal trauma. Furthermore, captopril treatment in the burned rats caused significant increases in both GSH levels and Na⁺, K⁺-ATPase activities, which were found to be depleted in the burn group.
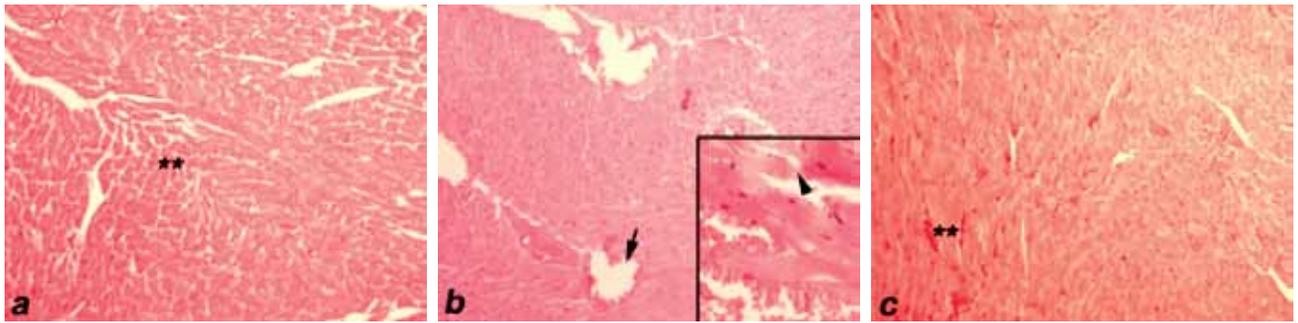
TNF-α is a cytokine excreted by macrophages and monocytes in response to different stimuli, and concentrations were found to be high in patients with heart disease, in association with noticeable activation of the RAS. Levine et al.[30-32] found increased concentrations of TNF-α in heart disease patients with high renin concentrations. In addition, they demonstrated that increased serum TNF-α, which plays an essential role in the inflammatory processes, was also decreased with captopril treatment. In our study, following burn injury, increased cytokine TNF-α was associated with impaired cardiac function since in the saline treated-burn group, LVPW, LV end-diastolic and end-systolic dimensions and RWT, and IVS thickness were significantly higher as compared to control rats ($p<0.05$-0.01), while FS and EF were found to be significantly decreased. Youn et al.[33] studied both ACE inhibition and angiotensin receptor antagonism in a rat model of acute infarction in which transforming growth factor (TGF) beta-1 mRNA expression was also increased. Their results demonstrated that both captopril and losartan suppressing the acute induction of TGF-beta1
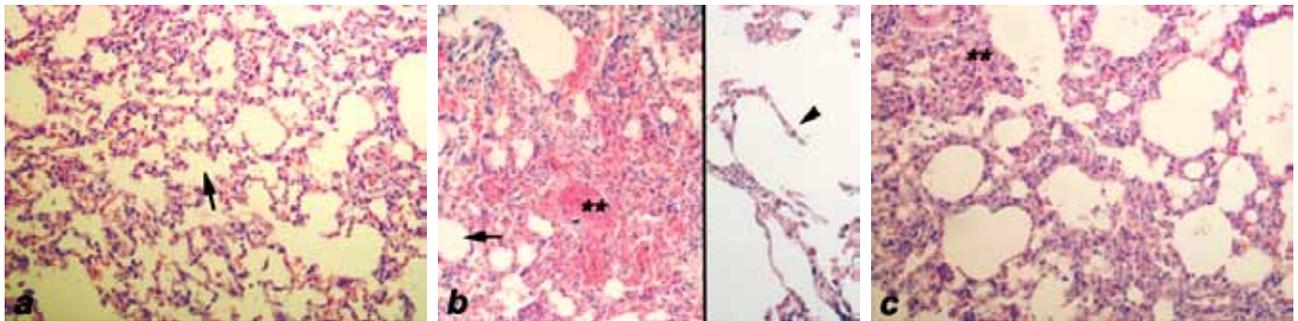
mRNA expressions attenuated LV remodeling. Captopril has been shown to express immune-regulating, antioxidant and anti-inflammatory properties.[34] In agreement with these studies, our results showed that depressing cytokine expression is associated with improved cardiac function.

Major burn injuries can lead to life-threatening end-organ dysfunction including cardiac dysfunction, which is a major contributor to mortality.[35,36] Despite prompt and adequate fluid replacement following burn injury, alterations in cardiovascular function such as reduced cardiac output, myocardial inflammation and an intrinsic defect in myocardial performance can cause multiple organ dysfunction syndrome. Numerous experimental studies have been performed to identify the molecular mechanisms involved in burn-related cardiac dysfunction with the end goal of creating novel therapeutic interventions and agents to reduce the incidence of life-threatening complications.[37,38] In our study, by improving cardiac function, captopril protected cardiac and lung tissues since oxidative injury observed through MDA and GSH levels and MPO, Na⁺, K⁺-ATPase and caspase activities were reversed in both tissues. One of the causative agents accountable for the development of burn shock and distant organ damage in animal models of burn injury are oxygen radicals.[27]

Figure 7. (a) Control group: regular alignment of muscle cells with centrally located nuclei (**). **(b)** Burn group: capillary vasocongestion among the connective tissue of the muscle cells (arrow), vacuolization in the cytoplasm of the cardiac muscle cells and degeneration of some gap-junctions (arrowhead). **(c)** Burn and captopril treatment: the capillary vasocongestion was reduced, and vacuolization in the cytoplasm of the cells was decreased (**), H&E staining, x200, insets x400.

Figure 8. (a) Control group demonstrated a regular alveolar structure (arrow) with no distention in its walls. **(b)** Burn group, severe congestion (**) and interstitial edema, which led to a decrease in alveolar space (arrow) in most areas of the tissue. In some regions, the alveoli united with each other resulting in large distended alveolar spaces (arrowhead-inset). **(c)** Burn and captopril group: reduced interstitial congestion and edema (**) and expanded alveolar areas and no congestion present.

The distant organs involved in the thermal damage during experimental skin burn are thought to be the lungs, heart, liver, kidney, and gastrointestinal mucosa. In all cases of thermal trauma, neutrophils diffuse these organs, being the potent source of reactive oxygen metabolites.[39] As is known, accumulation and activation of neutrophils induce tissue damage and release of different cytotoxic proteins into the extracellular fluid. MPO activity, an indirect marker of neutrophil infiltration,[40] has been shown to be significantly increased in various tissues of burned rats.[12,41] Similarly, in the present study, increased MPO activity in both heart and lung tissues suggests that neutrophil accumulation in these tissues assist in organ injury distant from the original wound. On the other hand, in the captopril- treated burn groups, MPO activities were reduced. The effects of captopril on MPO activity were studied previously in different experimental models of inflammation. Leor et al.[42] studied the effects of captopril experimentally, and proposed that captopril did not decrease neutrophil-induced myocardial injury following coronary occlusion and reperfusion. Nonetheless, Van Antwerpen et al.[43] in their *in vitro* study showed that captopril, by inhibiting the MPO/HOCl system, was able to significantly reduce the oxidative modification of low-density lipoprotein in a dose-dependent manner. Furthermore, Li et al.[44] demonstrated

that pathological injury of the lung in rats with intense acute pancreatitis was decreased with captopril treatment.

Malondialdehyde (MDA), an end product of lipid peroxidation, is major evidence of lipid peroxidation, and in this study, its levels were found to be significantly advanced due to burn in both heart and lung tissues. Results from animal and human studies suggested that there is a relationship between tissue MDA levels and the degree of burn complications, including shock and remote organ damage.[25,41,45,46] Our results showed that captopril treatment in the burned rats caused significant reduction in MDA.

Esterified GSH is a significant component of the cellular antioxidant system. The GSH molecule acts as an electron acceptor for hydrogen peroxide by forming an oxidized thiol and then undergoing degradation by glutathione reductase.[47] In a previous study, GSH levels have been reported to be decreased in the lung, liver and kidney of rats after burn.[12,25,26,28,45] In this study, lessening effects of captopril on MDA concurred with preservation of tissue GSH level, which is an important antioxidant. Moreover, Gurer et al.,[48] studying GSH, suggested that captopril has a promising beneficial role in augmenting the reducing capacity of the cells by increasing GSH.

Apoptosis is an orchestrated form of cell 'death by suicide'. It is principal in both the development and normal continuation of tissue function. Apoptotic nerve cell death is related to the pathogenesis of several devastating neurodegenerative diseases. Recently, apoptosis was found to be important in the pathophysiological changes occurring after major burns. After thermal injuries, nearly every organ or tissue seems affected and manifests this peculiar cellular mechanism characterizing a "systemic apoptotic response".[49] Currently, ROS may mediate apoptosis through the starting of poly (ADP-ribose) polymerase (PARP), advances in mitochondrial permeability with the release of cytochrome C, and activation of caspases.[50,51] ROS-triggered release of lysosomal ingredients can also lead to apoptosis. Hydrogen peroxide can freely diffuse across cell membranes, including the lysosomal membrane. Inside the lysosome, hydrogen peroxide can react with iron to form the potent hydroxide radical, at least causing damage to the lysosomal membrane and leakage of contents. The translocation of the protease cathepsin D from the lysosome to the cytosol yields to the induction of apoptosis.[52] Since captopril has recently been found to inhibit Fas-induced apoptosis in human-activated T cells[53] and lung epithelial cells,[54] we hypothesized that suppression of apoptosis could be one of the mechanisms bringing about the efficacy of ACE inhibitors. In the present study, we examined the apoptosis measuring caspase-3 activity in the heart and lung tissues, and found that thermal trauma increased heart and lung apoptosis. On the other hand, captopril treatment effectively lessened caspase-3 activity and protected tissues, as also confirmed histologically.

Na$^+$, K$^+$-ATPase is a considerable membrane protein that carries out the coupled extrusion and uptake of Na$^+$ and K$^+$ ions across the plasma membranes. As the activity of Na$^+$, K$^+$-ATPase, a membrane-bound enzyme required for cellular transport, is very sensitive to free radical reactions and lipid peroxidation, decrease in this activity can show membrane damage indirectly. Na$^+$, K$^+$-ATPase activities measured in the cardiac and pulmonary tissues were reduced in the saline-treated rats ($p<0.001$), indicating damaged transport function in these tissues (Fig. 5a, 5b). In the captopril-treated burned rats, the measured Na$^+$, K$^+$-ATPase activities in the studied tissues were increased compared to those of the control rats ($p<0.05-0.001$). Ottlecz et al.[55] demonstrated that stimulation of retinal Na$^+$, K$^+$-ATPase activity in diabetes is most likely one of the mechanisms through which captopril can improve retinal complications. Our study has shown that Na$^+$, K$^+$-ATPase activities were significantly reduced in both cardiac and lung tissues of burned rats, while captopril treatment significantly protected the enzyme activity.

In conclusion, the findings of the present study demonstrated that captopril treatment protected multiorgan damage in thermal trauma through inhibition of pro-inflammatory and oxidative pathways. Furthermore, captopril treatment provided regular cellular morphology in lung and heart tissues,

which caused a concomitant decrease in lipid peroxidation and increase in tissue antioxidant defense. Therefore, these results suggest an anti-inflammatory, cardiopulmonary protective effect of captopril, with a reduction in the circulating proinflammatory markers and an increase in those antiinflammatory cytokines; these effects result in a benefit on the cardiopulmonary inflammatory process associated with burn-induced thermal trauma. Thus, captopril treatment merits consideration as a potential therapeutic agent for restoring organ damage following thermal trauma.

Conflict of interest: None declared.

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DENEYSEL ÇALIŞMA - *ÖZET*

Sıçanlarda yanıkla uyarılan kardiyopulmoner hasara karşı kaptoprilin koruyucu etkisi

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AMAÇ: Bu çalışma, kalp ve akciğer dokularında yanıkla uyarılan oksidatif hasara karşı kaptopril tedavisinin olası koruyucu etkisini saptamak için tasarlandı.

GEREÇ VE YÖNTEM: Eter anestezisi altında sırt derileri traş edilen Wistar albino türü sıçanlar 10 saniye süreyle 90°C suya tutuldu. Yanık hasarından sonra ve 12 saat sonra olmak üzere 10 mg/kg intraperitoneal kaptopril uygulandı. Kontrol grubunda ise sıçanların sırtı 10 saniye süreyle 25°C suya tutuldu. Yanık hasarından 24 saat sonra ekokardiyografik kayıtları alınan sıçanlar dekapite edildi ve kalp ve akciğer doku örnekleri çıkartılarak tümör nekroz faktör-α (TNF-α), malondialdehit ve glutatyon düzeyleri, miyeloperoksidaz, kaspaz-3 ve Na+, K+-ATPaz aktiviteleri tayini ile histolojik analizler yapıldı.

BULGULAR: Yanık sol ventrikül fonksiyonlarında önemli değişikliklere neden oldu. Kalp ve akciğer dokularında yanık hasarına bağlı olarak glutatyon düzeyleri ve Na+, K+-ATPaz aktivitelerinin azaldığı ve TNF-α ve malondialdehit düzeyleri ile miyeloperoksidaz ve kaspaz-3 aktivitelerinin arttığı bulundu. Kaptopril tedavisinin, azalmış glutatyon düzeyi ve Na+, K+-ATPaz aktivitesini anlamlı düzeyde yükselttiği ve sitokin ve malondialdehit düzeyleri ve miyeloperoksidaz ve kaspaz-3 aktivitelerini ise anlamlı düzeyde azalttığı bulundu.

TARTIŞMA: Kaptopril kalp ve akciğer dokularında yanıkla uyarılan hasarı önler ve oksidatif organ hasarına karşı korur.

Anahtar sözcükler: Kaptopril; lipid peroksidasyonu; miyeloperoksidaz; sitokin; yanık.