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Caffeic Acid Phenethyl Ester Improves Oxidative Organ Damage in Rat Model of Thermal Trauma

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Summary

Severe burn injuries cause functional impairment in distant internal organs. Although this mechanism is not clear, it is possible that free radical toxicity plays an important role. Research in animals and clinical studies have shown that there is a close relationship between a lipid peroxidative reaction and secondary pathological changes following thermal injury. It has been demonstrated that antioxidant treatment prevents oxidative tissue damage associated with thermal trauma. This study was designed to determine the possible protective effect of caffeic acid phenethyl ester (CAPE) treatment against oxidative damage in the kidney and lung induced by thermal injury. Rats were decapitated either 1, 3 or 7 days after burn injury. CAPE was administered intraperitoneally immediately after thermal injury. Kidney and lung tissues were taken for the determination of malondialdehyde (MDA) level, myeloperoxidase (MPO), catalase (CAT), superoxide dismutase (SOD) and xanthine oxidase (XO) activities. Severe skin thermal injury caused a significant decrease in SOD and CAT activities, as well as significant increases in MDA level, XO and MPO activities in tissues during the postburn period. Treatment of rats with CAPE (10 μmol/kg) significantly elevated the decreased SOD and CAT activities, while it decreased MDA levels and MPO as well as XO activity.

Key words

Burn • Oxidative damage • Lung • Kidney • CAPE • Honeybee extracts

Introduction

Thermal injury may lead to hypovolemia, ischemia and reperfusion (LaLonde *et al*. 1992). In addition, endotoxemia may occur by the damage of intestinal mucosa induced by burns (Demling et al. 1986). These changes start as a chain reaction such as sequestration of polymorphonuclear leukocytes, activation of neutrophils and xanthine oxidase system, increase in the metabolism of arachidonic acid, release of free metal ions (e.g. iron) which leads to hydroxyl radical production from hydrogen peroxide *via* the Fenton reaction, release of inflammatory cytokines (interleukin 1, tumor necrosis factor-α, etc.), platelet aggregation and other hormonal and metabolical changes (Friedl *et al.* 1989, Damtew *et al*. 1993, Kataranovski *et al*. 1999, Yamashita *et al*. 2000, Basoglu *et al*. 2002). These reactions are the factors triggering oxidative reactions and

cause excess production and release of reactive oxygen substances (ROS). Local and systemic oxidant changes are believed to provide a stimulus for increased tissue inflammation, with resultant neutrophil and macrophage sequestration in distant organs (Baskaran *et al*. 2000, Dries *et al*. 2001). On the contrary, the antioxidant system of tissues is damaged by injury and cannot cope against ROS in the following period (Demling and LaLonde1990, Dubick *et al*. 2002). The combination of increased oxidant with decreased endogenous nonenzymatical and enzymatical antioxidant activity corresponds to a decrease in cellular energetics and cell membrane lipid peroxidation. Membrane lipid peroxidation can lead to changes in membrane fluidity and permeability, and also to increased rates of protein and nucleic acid degradation, and these finally lead to cell lysis. Therefore, detrimental effects of burns are not only limited locally to the skin, but they also affect distant organs.

CAPE is an active component of honeybee propolis extracts and has been used in tradional medicine for many years. Recent studies have shown that CAPE has antiinflammatory, antioxidant, immunomodulatory, antimitotic and anticarcinogenic properties (Ilhan *et al*. 1999, Hepsen *et al*. 1997, 1999, Orhan *et al*. 1999, Uz *et al.* 2002).

In this study, we examined the effects of CAPE treatment on lung and kidney tissues after thermal injury in an animal model. To determine the efficacy of CAPE, the levels of MDA and activities of superoxide dismutase, catalase, xanthine oxidase and myeloperoxidase, were measured on the first, 3th and 7th day of the postburn period.

Methods

Male Wistar albino rats of the same age, weighing between 250-300 g, were obtained from the Zonguldak Karaelmas University Medical Faculty Experimental Research Center and housed in separate cages under standard conditions, with a 12/12 h light-dark regimen. The rats were given standard rat chow and water *ad libitum*.

Anesthesia was achieved by sodium pentobarbital (50 mg/kg, i.p.), and the skin on the dorsal surface of the back was depilated using an animal depilatory agent. The dorsal area of the back was scrubbed with Betadine following removal of the hairs.

Tested Drug

CAPE (Sigma, St. Louis, MO, USA) was administered intraperitoneally to rats in doses of 10 μmol/kg.

Thermal injury

Animals were subjected to a 25-30 % total body surface area full-thickness burn by brass probe. Under general anesthesia, the brass probe was immersed in boiling water (100 ^{o}C) until thermal equilibrium was achieved. It was then placed without pressure for 20 s on the back and flanks of the rats. All animals were resuscitated with 5 ml of lactated Ringer's solution intraperitoneally.

Experimental protocol

The rats were randomly separated into three groups. The first group served as controls with a control burn (control, n=8), and the second group (n=15) as burn control with burn injury without any therapy. The last group (n=18) was then subdivided into three subgroups according to the assessment time, either first day, 3rd day or 7th day. CAPE was administered intraperitoneally immediately after the burn injury and continued with the same dose one a day.

After scarification, all organs were washed two times with cold saline solution, placed into glass bottles, labeled, and stored in a deep freeze (–30 °C) until processing (maximum 10 h). Tissues were homogenized in four volumes of ice-cold Tris-HCl buffer (50 mM, pH 7.4) using a glass Soniprep 150 homogenizer (MSS 150 CX 3.5, Sanyo, UK) after cutting the organs into small pieces with scissors (for 2 min at 5000 rpm). The homogenate was then centrifuged at 5000 x *g* for 60 min to remove the debris. Clear upper supernatant fluid was taken and CAT activity and protein concentration were determined at this stage. The supernatant solution was extracted with an equal volume of an ethanol/chloroform mixture (5/3, v/v). After centrifugation at 5000 x *g* for 30 min, the clear upper layer (the ethanol phase) was taken and used in the SOD and CAT activities and protein assays. All preparation procedures were performed at +4 °C.

Malondialdehyde (MDA) determination

The tissue MDA levels were determined by the method of Draper and Hadley (1990) based on the reaction of MDA with thiobarbituric acid (TBA) at 95 ^{o}C.

In the TBA test reaction, MDA and TBA react by forming a pink pigment with an absorption maximum at 532 nm. The reaction was carried out at pH 2-3 at 95 °C for 15 min. The sample was mixed with 2.5 volumes of 10 % (w/v) trichloroacetic acid to precipitate the protein. The precipitate was pelleted by centrifugation and aliquot of supernatant was reacted with an equal volume of 0.67 % TBA in a boiling water-bath for 15 min. After cooling, the absorbance was read at 532 nm. Arbitrary values obtained were compared with a series of standard solutions (1,1,3,3 tetramethoxypropane). The results were expressed as nanomoles per milligram tissue (nmol/mg tissue).

Myeloperoxidase (MPO) activity determination

MPO activity was determined using 4-amino-antipyrine/phenol solution as the substrate for MPO-mediated oxidation by H_2O_2 and changes in absorbance were recorded at 510 nm. One unit of MPO activity is defined as that which degrades 1 μmol H_2O_2/ min at 25 ^oC. Data are presented as U/g protein (Wei and Frenkel 1993).

Xanthine oxidase (XO) activity determination

XO activity was assayed spectrophotometrically at 293 nm and 37 °C with xanthine as substrate (Prajda and Weber 1975). The formation of uric acid from xanthine increases absorbency. One unit of activity was defined as 1 μmol of uric acid formed per minute at 37 °C, pH 7.5, and expressed in units per gram tissue protein.

Superoxide dismutase (SOD) activity determination

Total (Cu-Zn and Mn) SOD (EC 1.15.1.1) activity was determined according to the method of Sun *et al.* (1988). The principle of the method is based on the inhibition of NBT reduction by the xanthine-xanthine oxidase system as a superoxide generator. Activity was assessed in the ethanol phase of the lysate after 1.0 ml ethanol/chloroform mixture (5/3, v/v) was added to the same volume of the sample and centrifuged. One unit of SOD was defined as the enzyme amount causing 50 % inhibition in the NBT reduction rate. SOD activity was also expressed as units per milliliter.

Catalase (CAT) activity

CAT activity was determined according to Aebi's method (1974). The principle of the method is based on the determination of the rate constant (s^{-1}, k) or the H_2O_2 decomposition rate at 240 nm. The results were expressed as k (rate constant) per gram protein.

Protein assays

Protein assays in the samples were determined by the method of Lowry *et al.* (1951).

Statistical analysis

All statistical analyses were carried out using SPSS statistical software (SPSS for Windows version 11.0). All data were presented in means ± SD. Differences in measured parameters among three groups were analyzed by a Kruskal-Wallis test. Dual comparisons between days that present significant values were evaluated with the Mann-Whitney U-test. The differences were considered to be significant when the probability was less than 0.05.

Results

Malondialdehyde levels

The lung and kidney MDA levels in the group with burns were significantly higher on the first day than in the control group. It was observed that these high levels went up to the end of the 3rd day and became normalized on the 7th day. No significant difference of tissue MDA levels was observed between the CAPE group and the control group in the postburn period (Fig. 1).

Myeloperoxidase activity

While MPO activities of the kidney and lung were found to be significantly higher on the 1st day of the postburn period in the group with burns than in the control group, they started to decrease and returned to normal levels by the 3rd day. No significant change of tissue MPO activities was observed in the CAPE group compared to the control group during the postburn period (Fig. 2).

Xanthine oxidase activity

While XO activities in the kidney and lung were found to be significantly higher on the 1st day of the postburn period than in the control group, they started to decrease and returned to normal levels on the following days. No significant change of tissue XO activities was observed in the CAPE group compared to the control group during the postburn period (Fig. 3).

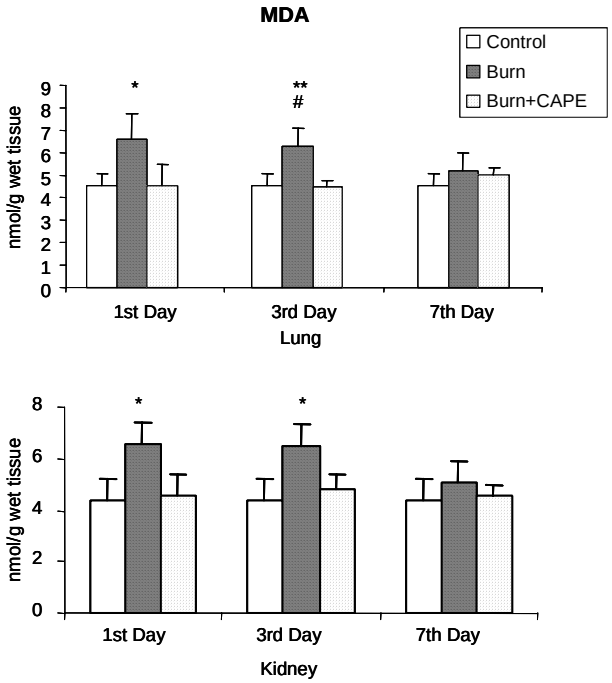

Fig. 1. Effects of burn and its treatment with CAPE on malondialdehyde levels of lung and kidney tissues. Data represent mean ± SD from three animal groups. *p<0.01 compared with control and burn+CAPE. **p<0.01 compared with control. #p<0.05 compared with burn+CAPE.

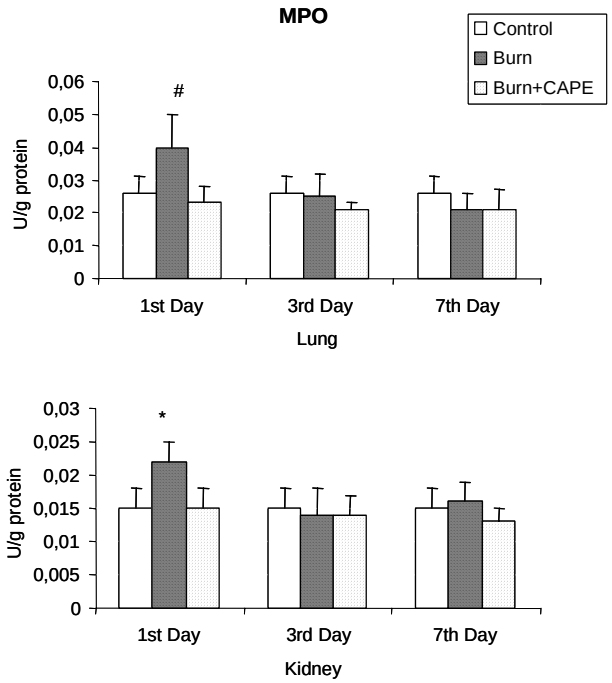

Fig. 2. Effects of burn and its treatment with CAPE on myeloperoxidase activities of kidney and lung tissues. Data represent mean ± SD from three animal groups. *p<0.01 compared with control and burn+CAPE. #p<0.05 compared with control and burn+CAPE.

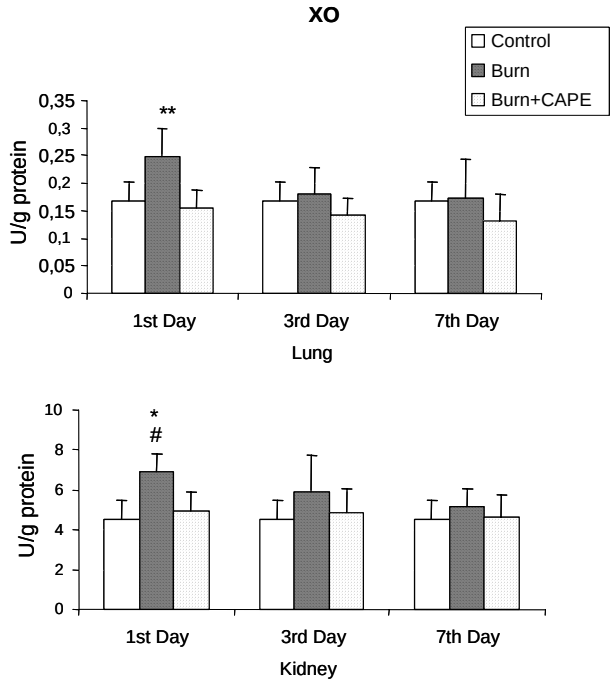

Fig. 3. Effects of burn and its treatment with CAPE on xanthine oxidase activities of kidney and lung tissues. Data represent mean ± SD from three animal groups. *p<0.01 compared with control. #p<0.05 compared with burn+CAPE. **p<0.01 compared with control and burn +CAPE.

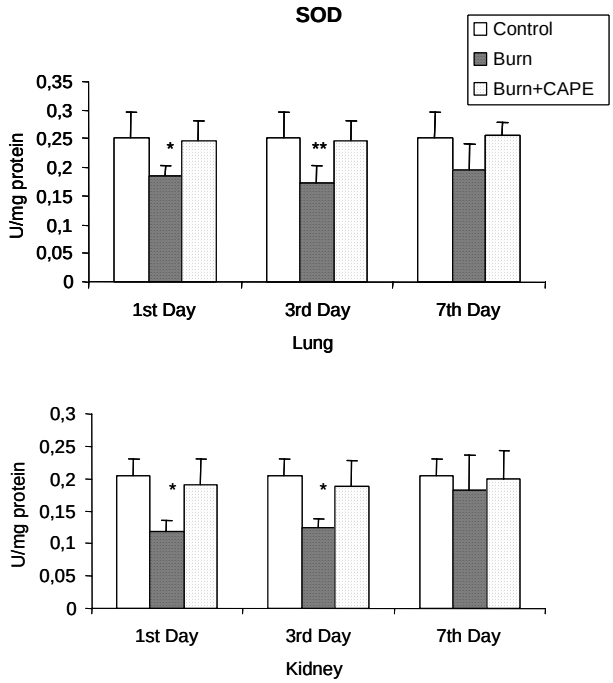

Fig. 4. Effects of burn and its treatment with CAPE on superoxide dismutase activities of lung and kidney tissues. Data represent mean ± standard deviation from three animal groups. *p<0.01 compared with control and burn+CAPE. **p<0.05 compared with control and burn+CAPE.

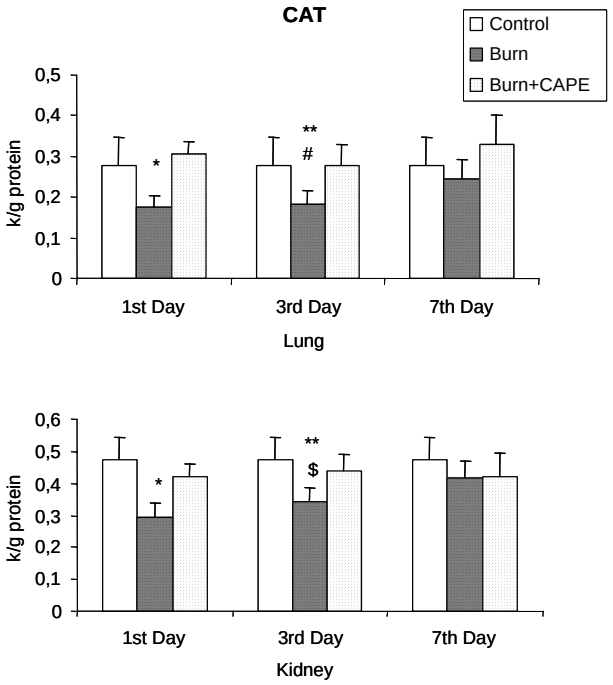

Fig. 5. Effects of burn and its treatment with CAPE on catalase activities of lung and kidney tissues. Data represent mean ± SD from three animal groups. $^{*}p<0.01$ compared with control and burn+CAPE. $^{**}p<0.05$ compared with control and burn+CAPE. $^{\$}p<0.01$ compared with control. $^{\#}p<0.05$ compared with control.

Superoxide dismutase activity

It was observed that the kidney and lung SOD activities in the burn group started to decrease on the first day of the postburn period. This decrease went on up to the 3rd day and SOD activities reached normal levels on the 7th day. No significant change of tissue SOD activities was observed in the CAPE group compared to the control group during the postburn period (Fig. 4).

Catalase activity

The kidney and lung CAT activities in the burn group started to decrease on the first day of the postburn period. This decrease continued up to the 3rd day and CAT activities returned to normal levels on the 7th day. No significant change of tissue CAT activities was observed in the CAPE group compared to the control group during the postburn period (Fig. 5).

Discussion

The generation of ROS is a crucial step in the pathogenesis of tissue damage. Thus, consequences of the attack of biomolecules by ROS, such as lipid peroxidation, could thereby result in an alteration of the structure of biological membranes. Research from animal and clinical studies have shown that there is a close relationship between a lipid peroxidative reaction and secondary pathological changes following thermal injury (Bertin-Maghit *et al*. 2000). A local burn insult produces oxidant-induced organ changes as evidenced by increased lipid peroxidation in remote organs (Youn *et al.* 1992). In the present study, the levels of MDA, an end-product of lipid peroxidation, are significantly increased in kidney and lung tissues. These results are in agreement with previous studies (Dubick *et al*. 2002, Sener *et al*. 2002). CAPE significantly decreased MDA levels in various tissues. This antilipoperoxidative effect of CAPE may be explained by its direct free radical scavenger property. Chen *et al*. (2001) demonstrated the selective scavenging activity of CAPE for H_2O_2 in human leukemic HL-60 cells. On the other hand, Frenkel *et al*. (1993) showed similar effects of CAPE in bovine lenses.

Generalized tissue inflammation is present in injured organs in the postburn period. It has been shown that neutrophil accumulation in the kidney, lung and liver may be involved in the pathogenesis of burn injury in these distant organs (Hansbrough *et al*. 1996, Dries *et al*. 2001). Neutrophils are likely the source of reactive oxygen metabolites as a result of the systemic inflammatory reaction to a local burn insult. In this study, the tissue-associated myeloperoxidase activity, which is an index of neutrophil infiltration, was increased in lung and kidney tissues at 24 h after burn injury. MPO plays an important role in the production of oxidants by neutrophils, which are a potential source of ROS and are considered to be the major effector cells in remote organ damage (Dib *et al.* 2002). According to our results, treating rats with CAPE attenuated the increase in the tissue levels of MPO and MDA caused by thermal injury. In addition, it has been suggested that CAPE exhibits antioxidant properties by blocking the production of ROS in human neutropils and suppresses the oxidative burst of human polymorphonuclear leukocytes (Frenkel *et al.* 1993). These effects may prevent damage to the cell membranes partly caused by oxygen-free radicals released from polymorphonuclear leukocytes.

Xanthine oxidase is the last enzyme in the pathway of degradation of purine derivatives from nucleic acids and the best documented biological source of oxygen radicals (Parks and Granger 1986). XO plays an important role in the pathogenesis of thermal injury by producing ROS that causes oxidative damage. Thermally injured rats showed increased XO activity that is the

source of oxygen radicals involved in edema formation (Till *et al*. 1989). Burton *et al.* (1995) conclude that XO may contribute to acute lung injury and a number of events associated with the development of acute lung leak following skin burns. Previous studies have reported that XO inhibiting therapy reduced postburn oxidative tissue damage (Demling and LaLonde 1990). We observed that XO activity was suppressed by CAPE in lung and kidney tissues in our study. This property of CAPE had been reported by Russo *et al.* (2002). Such an effect of CAPE may be an important factor in decreased oxidative damage in this animal model.

The antioxidation defense system is known to inhibit lipid peroxidation in mammalian tissues by destroying some of ROS that has an important role in initiation of the lipid peroxidation process The antioxidant defense system operates through enzymatic and nonenzymatic components. The system is affected by burns. It has been reported that nonenzymatic antioxidants, such as glutathione, α-tocopherol and selenium, are decreased in the serum and tissues after thermal injury (Cetinkale *et al.* 1997). Bekyarova and Yankova 1998). A similar condition has also been shown for antioxidant enzyme activities. Some authors have reported that SOD and CAT activities in the lungs gradually decrease after burns (LaLonde *et al*. 1996, Youn *et al.* 1998). Saitoh *et al*. (2001) reported that SOD activity increased after burn injury but the same authors reported a different result in another study. They demonstrated that SOD synthesis was inhibited in severe burn injuries despite a strong mRNA expression of SOD (Gotoh *et al*. 2003). The tissue enzyme activities were only decreased in the burn group when compared to the other two groups. This decrease may be related to the consumption of activated enzymes against oxidative stress. The CAPE treatment resulted in improved enzyme activities.

In conclusion, CAPE scavenges free oxygen radicals or decreases MPO activity in neutrophils or directly increase the antioxidant enzyme activity and prevent the inhibition of the activities of these enzymes. Considering our results, CAPE would be a beneficial agent in humans who suffer from thermal injury.

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