ORIGINAL PAPERS

Adv Clin Exp Med 2015, **24**, 3, 409–417
DOI: 10.17219/acem/43761

© Copyright by Wroclaw Medical University
ISSN 1899–5276

Mubin Hosnuter[1, A, E, F], Cenk Melikoglu[2, B, C], Cem Aslan[1, B–D], Gulcan Saglam[3, B, C], Recep Sutcu[3, B, C]

The Protective Effects of Epigallocatechin Gallate Against Distant Organ Damage After Severe Skin Burns – Experimental Study Using a Rat Model of Thermal Trauma

[1] Department of Plastic, Reconstructive and Aesthetic Surgery, Katip Celebi University School of Medicine, Ataturk Training and Research Hospital, Izmir, Turkey
[2] Department of Plastic, Reconstructive and Aesthetic Surgery, Sifa University School of Medicine, Izmir, Turkey
[3] Department of Biochemistry, Katip Celebi University School of Medicine, Ataturk Training and Research Hospital, Izmir, Turkey

A – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation; **D** – writing the article; **E** – critical revision of the article; **F** – final approval of article; **G** – other

Abstract

Background. Epigallocatechin gallate (EGCG), a green tea polyphenol, has potent antioxidant properties.
Objectives. The purpose of the present study was to examine the possible preventative effects of EGCG against internal organ injury due to large-surface skin burns in a rat model.
Material and Methods. The study design involved three groups of rats: a sham group and two groups with 25–30% full-thickness burns: (a) the sham group without burns or treatment (n = 18); (b) the control burn group (burns + sterile saline, n = 18); and (c) the burn treatment group (burns + treatment with EGCG, n = 18). EGCG was administered intraperitoneally immediately after the thermal injury, and daily in 100 μmol/kg doses. Kidney and lung tissue samples were taken to determine the levels of malondialdehyde (MDA), superoxide dismutase (SOD), tumor necrosis factor-α (TNF-α), and glutathione peroxidase (GPX) after the first, third and seventh post-burn days.
Results. In the EGCG-treated burn group, SOD and GPX activity were significantly higher than in the burn control group. Additionally, MDA and TNF-α levels were significantly lower in the EGCG-treated burn group.
Conclusions. Based on this study, it might be anticipated that EGCG treatment may be beneficial in burn injury cases (**Adv Clin Exp Med 2015, 24, 3, 409–417**).

Key words: antioxidant, EGCG, free radicals, green tea, polyphenol.

The systemic inflammatory response to burn injuries is extremely complex, resulting in deleterious systemic effects on distant internal organs. The inflammation begins immediately after the burn injury and the systemic response progresses with time, usually peaking 5 to 7 days after the burn injury. The inflammatory mediators are responsible for many of the local changes and a majority of the distant changes [1–2].

Severe thermal injury leads to systemic inflammatory reactions by producing burn toxins, reactive oxygen species (ROS), and finally peroxidation occurs. The relationship between the levels of free radical scavengers and the amount of oxidative metabolism products determines the outcome of local and distant tissue damage and further distant organ failure in burn injuries [3].

There is a sensitive equilibrium between the production and destruction of ROS. When this equilibrium breaks down, ROS are produced excessively and tissues are exposed to oxidative damage [4].

Previous studies have demonstrated the protective effects of antioxidants in severe thermal

injury. Therapeutic application of both enzymatic and non-enzymatic antioxidants such as caffeic acid phenethyl ester, superoxide dismutase (SOD), alpha-tocopherol, ascorbic acid, ceruloplasmin, desferrioxamine, allopurinol and N-acetylcysteine have been shown to be effective [5–7].

The major bioactive compounds of green tea are (-)-epigallocatechin-3-gallate (EGCG), (-)-epigallocatechin (EGC), (-)-epicatechin-3-gallate (ECG) and (-)-epicatechin (EC)-like polyphenolic epicatechin. EGCG is the most effective and abundant catechin in green tea [8]. Several studies have demonstrated that EGCG has numerous biological effects, which include antioxidant [9–10], antimicrobial [11], anti-inflammatory [12–13], anti-allergic [14] and antineoplastic [15] activities.

In the present study, the protective effects of EGCG treatment on lung and kidney tissues after thermal trauma in a rat model were investigated. The levels of MDA, TNF-α and the activities of superoxide dismutase and glutathione peroxidase were measured on the 1st, 3rd and 7th day of the post-burn period to determine the efficacy of EGCG.

Material and Methods

The study protocol was approved by the Ege University Ethics Committee on Research Animal Use. Wistar albino rats of the same age, weighing between 250–300 g, were used in the study. They were obtained from the Experimental Research Center of the Uludag University School of Medicine and housed in separate cages under controlled conditions, with a 12/12 h light-dark regimen. The rats were fed with standard rat food pellets and water *ad libidum*. Anesthesia was achieved with a combination of ketamine (60 mg/kg) and xylazine (7.5 mg/kg). The dorsum of the rat was scrubbed with Betadine following removal of the dorsal hair with an animal depilatory agent.

The rats were randomly divided into 3 groups. The first group was designated the sham group, without burns (n = 20); the second group (n = 20) was the control burn group (burns + sterile saline), with burn injury without any therapy; the last group (n = 20) was the burn treatment group (burns + EGCG), with burns and EGCG therapy.

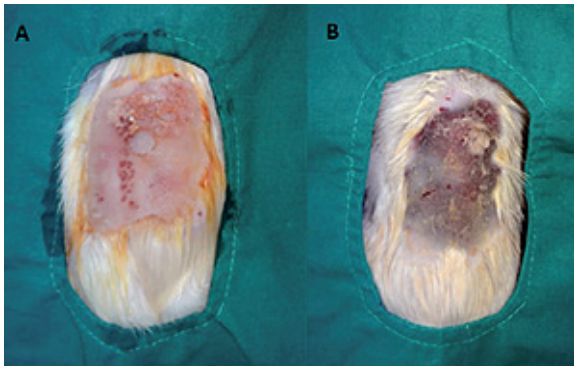

Fig. 1. View of the rat dorsum after thermal trauma. A – early period after thermal trauma; B – first day after thermal trauma

Thermal Injury

In the 2 burn groups, full-thickness burns were created on the backs of the rats with a metal probe. The burn area was 25–30% of the total body surface of each rat. The metal probe was placed in boiling water (100°C) until the same temperature was obtained. It was then placed on the dorsum of the rats without pressure for 20 s under general anesthesia (Fig. 1) [16, 17]. For animal fluid resuscitation, 5 mL sterile saline solution (0.9%) was used.

Tested Drug

EGCG (DSM Nutrients Inc., Turkey) was dissolved in sterile saline and administered intraperitoneally to the rats in the burn treatment group in doses of 100 μmol/kg. It was applied immediately after the burn injury and continued with the same dose daily (Fig. 2).

Experiment

All groups were subdivided into 3 subgroups according to the assessment time, either 1st day, 3rd day, or 7th day. Four rats of in the burn groups died

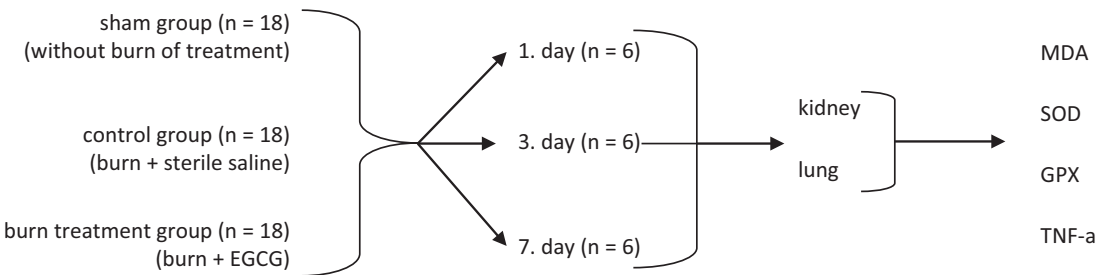

Fig. 2. Experimental design of the groups

on the first post-burn day and were excluded from the study. Two rats in the sham group were excluded from the study because they were exhibiting signs of excessive stress.

After the animals were sacrificed, all tissues were washed twice with cold saline solution, placed in a phosphate buffer, labeled and stored in a freezer (–30°C) until processing. The tissues were homogenized before biochemical evaluation. Lung and kidney tissues were weighed and homogenized (Ultra-turrax T25, Germany) on ice for 2 min at 10,000 rpm in a 1/9 ratio cold phosphate buffer (100 mmol/L, pH 7.0). Sonification was performed for 30 s on the homogenates obtained. Then the homogenates were centrifuged at 10,000 *g for 30 min at +4°C. After centrifugation, the clear upper supernatant fluid was taken to determine MDA and TNF-α levels, as well as enzyme (SOD, GPX) activity.

Determining Superoxide Dismutase Activity

Superoxide dismutase activity was measured based on the method described by Woolliams et al. [18]. The concentrations were determined by the use of a standard percentage inhibitory concentration chart that had been previously prepared. Enzyme activity was determined as U/mL. The results were obtained as U/mg-protein, by multiplying the values by the dilution coefficient and then dividing by the protein concentration.

Determining Glutathione Peroxidase Activity

Glutathione peroxidase activity was measured using the method described by Paglia and Valentine [19]. Glutathione peroxidase catalyzes the oxidation of glutathione. Oxidized glutathione is reduced by the glutathione reductase with the presence of NADPH. Meanwhile, NADPH is oxidized to NADP+. The enzyme activity was calculated by measuring the absorbance difference, which depends on the decrease of NADPH at 340 nm.

The amount of homogenate protein was determined by the Lowry [20] method and the results were obtained as U/mg-protein.

Determining MDA

MDA levels were measured using the thiobarbituric acid reactivity method described by Draper and Hadley [21]. MDA is the end product of fatty acid peroxidation. It reacts with 2-thiobarbituric acid (TBA) and forms a colored complex, which provides maximum absorbance at 532 nm.

MDA levels were found in nm/mL by using the absorption coefficient of the MDA-TBA complex at 532 nm (1.56 × 105 cm-1M-1). The results were obtained as nanomoles/(mg-protein) by dividing the previously obtained values by the homogenate protein.

Determining TNF-α

TNF-α levels were investigated using the enzyme-binding immunosorbent assay (ELISA) method in a semi-automatic device (Assay Rat TNF-α ELISA kit, lot #: 978614A, Invitrogen, USA).

Statistical Analysis

All statistical analyses were carried out using SPSS statistical software (SPSS for Windows v. 15.0). The variables between the 3 groups were compared using the Kruskal-Wallis *H*-test. Significant differences found in the Kruskal-Wallis *H*-test were checked by the Bonferroni-corrected Mann-Whitney *U*-test to determine which group or groups were responsible for the significant differences. A value of $p < 0.05$ in the Kruskal-Wallis *H*-test and a value of $p < 0.016$ in the Bonferroni-corrected Mann-Whitney *U*-test were considered statistically significant.

Results

The detailed statistical analysis can be seen in Tables 1–3. All the groups had 18 suitable rats for the study; and 6 in each group were sacrificed for analysis on the 1st, 3rd and 7th days (Fig. 2). The difference in the number of rats per group in the statistical analysis depends on the assessment day in each group. If statistical significance was found only on a single assessment day (the 1st, 3rd or 7th), the significant differences were found in only 6 rats in each group. If statistical significance was found on 2 assessment days (the 1st and 3rd or 3rd and 7th days), the significant differences were found in 12 rats in each group. Lastly if statistical significance was found on all 3 assessment days, the significant differences were found in the 18 rats in each group.

Malondialdehyde Levels

MDA levels were higher in the control burn group (burn + sterile saline, n = 18) than in the burn treatment group (burn + EGCG, n = 18) on the 1st, 3rd, and 7th days. These differences between the 2 burn groups were significant ($p < 0.016$). Kidney MDA levels in the control burn group

Table 1. Mean distribution of enzyme levels in the kidney tissues on the 1st, 3rd and 7th days

Enzyme	Measurement time	Sham group		Control burn		Burn treatment		p*
		avr. ± SD	median	avr. ± SD	median	avr. ± SD	median	
MDA (nm/mg-protein)	day 1	0.15 ± 0.12	0.10	0.42 ± 0.01	0.42	0.31 ± 0	0.31	0.004
	day 3	0.13 ± 0.07	0.10	0.28 ± 0.01	0.28	0.19 ± 0	0.19	0.004
	day 7	0.1 ± 0.01	0.10	0.12 ± 0.01	0.12	0.09 ± 0.01	0.09	0.005
SOD (U/mg-protein)	day 1	0.07 ± 0.02	0.07	0.04 ± 0	0.04	0.06 ± 0.01	0.06	0.004
	day 3	0.07 ± 0.01	0.07	0.04 ± 0.01	0.04	0.07 ± 0.01	0.07	0.004
	day 7	0.07 ± 0.01	0.07	0.06 ± 0.01	0.06	0.08 ± 0.01	0.08	0.012
GPX (U/mg-protein)	day 1	13.78 ± 3.37	15.05	6.14 ± 0.01	6.14	7.6 ± 0.03	7.60	0.006
	day 3	13.91 ± 3.02	15.05	7.06 ± 0.01	7.06	10.86 ± 0.01	10.86	0.005
	day 7	14.44 ± 1.61	15.05	10.8 ± 0.02	10.80	12.85 ± 0.01	12.85	0.006
TNF-α (pg/mg-protein)	day 1	20.96 ± 10.26	17.08	44.24 ± 0.04	44.24	31.89 ± 0.01	31.89	0.006
	day 3	19.31 ± 5.89	17.08	32.68 ± 0.01	32.67	23.85 ± 0.02	23.85	0.005
	day 7	18.09 ± 2.65	17.08	24.1 ± 0.02	24.09	20.37 ± 0.01	20.37	0.005

* Kruskal Wallis *H*.
The variables between the 3 groups were compared using the Kruskal-Wallis *H*-test. At least one of the variables was found to be statistically significant ($p < 0.05$).

Table 2. Mean distribution of enzyme levels in the lung tissues on the 1st, 3rd and 7th days

Enzyme	Measurement time	Sham group		Control burn		Burn treatment		p*
		avr. ± SD	median	avr. ± SD	median	avr. ± SD	median	
MDA (nm/mg-protein)	day 1	0.11 ± 0.1	0.07	0.34 ± 0.01	0.34	0.21 ± 0.01	0.21	0.004
	day 3	0.09 ± 0.04	0.07	0.17 ± 0.01	0.17	0.12 ± 0.01	0.12	0.005
	day 7	0.08 ± 0.01	0.07	0.11 ± 0.01	0.11	0.09 ± 0.01	0.09	0.012
SOD (U/mg-protein)	day 1	0.22 ± 0.02	0.22	0.18 ± 0.01	0.18	0.21 ± 0.01	0.21	0.004
	day 3	0.22 ± 0.01	0.22	0.19 ± 0.01	0.19	0.22 ± 0.01	0.22	0.005
	day 7	0.22 ± 0.01	0.22	0.21 ± 0.01	0.21	0.28 ± 0.01	0.28	0.005
GPX (U/mg-protein)	day 1	14.5 ± 3.68	15.89	6.14 ± 0.01	6.14	7.59 ± 0.02	7.60	0.006
	day 3	15.17 ± 1.9	15.89	10.86 ± 0.02	10.86	12.85 ± 0.01	12.85	0.006
	day 7	15.53 ± 0.96	15.89	13.36 ± 0.01	13.36	15.53 ± 0.01	15.53	0.005
TNF-α (pg/mg-protein)	day 1	27.99 ± 15.74	22.04	63.7 ± 0.01	63.69	51.54 ± 0.07	51.55	0.005
	day 3	23.47 ± 3.77	22.04	32.02 ± 0.01	32.02	27.89 ± 0.01	27.88	0.005
	day 7	22.34 ± 0.77	22.04	24.08 ± 0.01	24.08	22.2 ± 0.01	22.20	0.005

* Kruskal Wallis *H*.
The variables between the 3 groups were compared by using the Kruskal-Wallis *H*-test. At least one of the variables was found statistically significant ($p < 0.05$).

(burn + sterile saline, n = 6) were significantly higher than in the sham group (n = 6) on the 1st day ($p < 0.016$). Kidney MDA levels in the burn treatment group (burn + EGCG, n = 6) were significantly lower than in the sham group (n = 6) on the 7th day ($p < 0.016$). There were no other significant differences in MDA levels between the sham and burn groups.

Table 3. Bilateral comparisons of enzyme levels in the lung and kidney tissues on the 1st, 3rd and 7th days

	Enzyme	Measurement time	Sham – control burn (p-value)	Sham – burn treatment (p-value)	Control burn – burn treatment (p-value)
Kidney	MDA (nm/mg protein)	day 1	0.010[a]	0.035	0.004[i]
		day 3	0.018	0.035	0.004[i]
		day 7	0.030	0.005	0.004[i]
	SOD (U/mg protein)	day 1	0.030	0.138	0.004[ii]
		day 3	0.018	0.366	0.004[ii]
		day 7	0.106	0.234	0.016[ii]
	GPX (U/mg protein)	day 1	0.018	0.035	0.004[ee]
		day 3	0.010[b]	0.035	0.004[ee]
		day 7	0.010[b]	0.035	0.004[ee]
	TNF-α (pg/mg protein)	day 1	0.018	0.035	0.004[i]
		day 3	0.005[c]	0.035	0.004[i]
		day 7	0.010[c]	0.035	0.004[i]
Lung	MDA (nm/mg protein)	day 1	0.005[d]	0.035	0.004[i]
		day 3	0.005[g]	0.035	0.004[i]
		day 7	0.018	0.035	0.016[i]
	SOD (U/mg protein)	day 1	0.018	0.138	0.004[ii]
		day 3	0.018	0.628	0.004[ii]
		day 7	0.106	0.001[h]	0.004[ii]
	GPX (U/mg protein)	day 1	0.018	0.035	0.004[ee]
		day 3	0.010[e]	0.035	0.004[ee]
		day 7	0.010[e]	0.035	0.004[ee]
	TNF-α (pg/mg protein)	day 1	0.005[f]	0.035	0.004[i]
		day 3	0.005[f]	0.035	0.004[i]
		day 7	0.005[f]	0.035	0.004[i]

Bonferroni-corrected Mann Whitney *U* test.

Significant differences that measured in Kruskal Wallis *H* test were checked by the Bonferroni-corrected Mann Whitney U test to find which group or groups were responsible for the significant differences.

[a] Kidney MDA levels in the control burn group (burn + sterile saline) were significantly higher than in the sham group on the 1st day ($p < 0.016$). [d] Lung MDA levels in the control burn group were significantly higher than in the sham group on the 1st and 3rd days ($p < 0.016$). [g] Kidney MDA levels in the burn treatment group (burn + EGCG) were significantly higher than in the sham group on the 7th day ($p < 0.016$). [i] All MDA and TNF-α levels in the burn treatment group were significantly lower than in the control burn group on the 1st, 3rd and 7th days ($p < 0.016$). [ii] All enzyme levels in the burn treatment group were significantly higher than in the control burn group on the 1st, 3rd and 7th days ($p < 0.016$). [h] Lung SOD levels in the burn treatment group were significantly higher than in the sham group on the seventh day ($p < 0.016$). [b] Kidney GPX activity in the control burn group was significantly lower than in the sham group on the 3rd and 7th days ($p < 0.016$). [e] Lung GPX activity in the control burn group was significantly lower than in the sham group on the 3rd and 7th days ($p < 0.016$). [ee] All enzyme levels in the burn treatment group were significantly higher than in the control burn group on the 1st, 3rd and 7th days ($p < 0.016$). [c] Kidney TNF-α levels in the control burn group were significantly higher than in the sham group on the 3rd and 7th days ($p < 0.016$). [f] Lung TNF-α levels in the control burn group were significantly higher than in the sham group on the 1st, 3rd and 7th days ($p < 0.016$).

Superoxide Dismutase Activity

SOD activity was significantly higher in the burn treatment group (burn + EGCG, n = 18) than in the control burn group (burn + sterile saline, n = 18) on the 1st, 3rd, and 7th days ($p < 0.016$). Lung SOD levels in the burn treatment group (burn + EGCG, n = 6) were statistically higher than in the sham group (n = 6) on the 7th day ($p < 0.016$). There were no other significant differences in SOD levels between the sham and burn groups.

Glutathione Peroxidase Activity

No significant differences in GPX levels were observed between the burn treatment group (burn + EGCG, n = 18) and the sham group in the post-burn period. GPX activity was significantly higher in the burn treatment group (burn + EGCG, n = 18) than in the control burn group (burn + sterile saline, n = 18) on the 1st, 3rd and 7th days ($p < 0.016$). Kidney GPX activity in the control burn group (burn + sterile saline, n = 12) was statistically lower than in the sham group (n = 12) on the 3rd and 7th days ($p < 0.016$). Lung GPX activity in the control burn group (burn + sterile saline, n = 12) was statistically lower than in the sham group (n = 12) on the 3rd and 7th days ($p < 0.016$). There were no other significant differences in GPX levels between the sham and burn groups.

TNF-α Levels

No significant differences in TNF-α levels were observed between the burn treatment group (burn + EGCG, n = 18) and the sham group in the post-burn period. TNF-α levels in the kidney and lung tissues were significantly higher in the control burn group (burn + sterile saline, n = 18) compared to the burn treatment group (burn + EGCG, n = 18) on the 1st, 3rd and 7th days ($p < 0.016$). Furthermore, kidney TNF-α levels in the control burn group (burn + sterile saline, n = 12) were statistically higher than in the sham group (n = 12) on the 3rd and 7th days ($p < 0.016$). Lung TNF-α levels in the control burn group (burn + sterile saline, n = 18) were statistically higher than the sham group (n = 18) on the 1st, 3rd and 7th days ($p < 0.016$). There were no other significant differences in TNF-α levels between the sham and burn groups.

Discussion

A great many different plant extracts, e.g. grape seed, lemon, rosemary and jojoba, have been employed for healing wounds and increasing longevity. All of these plants have a common property, i.e., producing compounds with a phenolic structure. These phytochemicals ordinarily react with compounds such as oxygen free radicals and other macromolecules in order to neutralize free radicals and/or initiate biological effects [22].

Ample evidence indicates that green tea, which has anti-oxidant, anti-cancer, anti-aging and anti-inflammatory effects, could also prevent exaggerated collagen production and accumulation, and could induce changes in immune responses as well [23]. Most of these properties can be attributed to the plant's polyphenolic compounds – in particular, catechin compounds [24].

Previous studies have shown that there is a close relationship between lipid peroxidation and secondary pathological changes following thermal injury [25]. ROS (reactive oxygen species) are believed to be primarily responsible for this lipid peroxidation. A local burn insult produces oxidant-induced organ changes in remote organs [26]. Malondialdehyde (MDA) is a stable end product of lipid peroxidation, which shows the degree of toxicity [18]. Superoxide dismutase (SOD) catalyzes the transformation of superoxide radicals to hydrogen peroxide and oxygen, and is an important antioxidant enzyme that protects cells from damage due to superoxide radicals [27]. Glutathione peroxidase (GPX) is the common name of an enzyme family that contains many subspecies. GPX protects cells from damage mediated by free radicals by catalyzing the transformation of hydrogen peroxide to water [28].

The results of the current study presented above demonstrate that ROS-dependent tissue injury developed in response to remote thermal injury of the skin is greatly attenuated by treatment with EGCG. EGCG's ability to protect against thermal injury can be seen in the significant decreases in tissue MDA levels, and naturally higher levels in SOD and GPX activities. Moreover, increased TNF-α levels, which play a pivotal role in the inflammatory processes, were also reduced by EGCG treatment.

Evidence from animal and human studies suggested that there is a correlation between tissue MDA levels and the degree of burn complications, including shock and remote organ damage [29]. In this study, MDA levels were higher in the burn groups, especially on the 1st day after thermal trauma. MDA levels in the lung and kidney tissues were significantly higher in the control burn group (burn + sterile saline) than the burn treatment group (burn + EGCG) on all the measured days ($p < 0.016$). These results show that treating the burned rats with EGCG caused a significant reduction in MDA levels.

It was observed that kidney and lung SOD activity was lowest in the control burn group (burn + sterile saline) on the 1st and 3rd days. On the 7th day, the SOD activity of the control burn group and the sham group became equal, but enzyme activity in the burn treatment group (burn + EGCG) was significantly higher than the other groups. GPX activity was lowest in the control burn group, similar to SOD activity. This difference between the sham and control burn groups was significant only on the 3rd and 7th days. GPX activity did not normalize on the 7th day in the control burn group. No significant difference in GPX levels was observed between the burn treatment and the sham group in the post-burn period. SOD and GPX activity in the lung and kidney tissues was significantly lower in the control burn group than the burn treatment group on all the measured days ($p < 0.016$).

These findings may be due to ROS scavenging and the antioxidant effects of EGCG. In a rat lower extremity ischemia-reperfusion study, Büttemeyer et al. reported that superoxide radical formation was less than 50% in the EGCG-treated group than the control group. The same study emphasized that the free radical scavenging effect of EGCG is 100 times more than vitamin C [30]. Kakuta et al. researched the protective effects of EGCG in rats with renal ischemia reperfusion injury and reported that EGCG protects the kidneys by augmenting the HO-1 (heme oxygenase-1) gene and macrophage blocking [31]. Brückner et al. researched the therapeutic antioxidant effect of EGCG in a murine model of colitis. They reported that EGCG significantly reduces the loss of body weight, improves the clinical course and increases overall survival in comparison to untreated groups. They also reported that treatment with EGCG enhanced the expression of SOD and GPX, and reduced the production of pro-inflammatory cytokines. These effects may prevent cell membrane damage caused by reactive oxygen species by augmenting the cell antioxidant defense systems [32].

It has been shown that macrophage activity is an important mechanism of distant organ injury in severe thermal trauma. Activated macrophages are another source of ROS and inflammatory cytokines [2]. TNF-α, a polypeptide compound belonging to the cytokine family, is primarily synthesized by activated macrophages during inflammation. It has many systemic effects during inflammation, such as aggregation and activation of neutrophils, nitric oxide synthesis, fibroblast activation and the release of proteolytic enzymes from mesenchymal cells [33]. In this study, no significant differences in TNF-α levels were observed between the burn treatment group (burn + EGCG) and the sham group in the post-burn period. Furthermore, the TNF-α levels in the lung and kidney tissues were significantly higher in the control burn group (burn + sterile saline) than the burn treatment group on all the measured days ($p < 0.016$). These anti-inflammatory effects of EGCG are supported by previous studies. In 2 separate studies, Giakoustidis et al. demonstrated EGCG's protective effects against ischemia-reperfusion injury to the liver and intestine by downregulating c-Jun and NF-kappaB [34, 35]. Townsend et al. carried out an experimental study on cell cultures prepared from the rat's myocardium. They reported that EGCG protects the cells from reperfusion injury by inhibiting the activation of the STAT-1 signaling protein [36]. In the same way, EGCG also protects the tissues from excessive inflammatory reactions.

In addition, epicatechin gallate has been shown to have a beneficial effect on wound healing quality, leading to a more pleasant scar, which probably results from its ability to increase levels of vascular endothelial growth factor, accelerate vessel formation and enhance nitric oxide and cyclooxygenase expression [37].

EGCG, as mentioned above, protects cells from free radical-mediated damage and excessive inflammatory reaction through more than one mechanism. The findings of the present study demonstrate for the first time that EGCG treatment, prevented multiorgan damage in thermal trauma by inhibiting proinflammatory and oxidative pathways, which causes a concomitant decrease in lipid peroxidation and an increase in tissue antioxidant defense. Thus, EGCG treatment merits consideration as a potential therapeutic agent for organ damage following thermal injury.

References

[1] **Çakır B, Yegen BC:** Systemic Responses to Burn Injury. Turk J Med Sci 2004, 34, 215–226.

[2] **Nerlich M, Flynn J, Demling R:** Effect of thermal injury on endotoxin induced lung injury. Surgery 1983, 92, 289–296.

[3] **Cetinkale O, Konukoglu D, Senel O, Kemerli GD, Yazar S:** Evaluation of lipid peroxidation and total antioxidant status in plasma of rats following thermal injury. Burns 1997, 23, 114–116.

[4] **Aust SD, Thomas CE, Morehouse LA, Saito M, Bucher JR:** Active oxygen and toxicity. Adv Exp Med Biol 1986, 197, 513–526.

[5] **Senel O, Bulan R:** The effect of antioxidant therapy on cell-mediated immunity following burn injury in an animal model. Burns 1999, 25, 113–118.

[6] **Matsuda T, Tanaka H, Yuasa H, Forrest R, Matsuda H, Hanumadass M:** The effects of high dose Vitamin C therapy on postburn lipid peroxidation. J Burn Care Rehabil 1993, 14, 624–629.
[7] **Hoşnuter M, Gürel A, Babucçu O, Armutcu F, Kargi E, Isikdemir A:** The effect of CAPE on lipid peroxidation and nitric oxide levels in the plasma of rats following thermal injury. Burns 2004, 30, 121–125.
[8] **Jin JY, Park SH, Bae JH, Cho HC, Lim JG, Park WS:** Uncoupling by (-)-epigallocatechin gallate of ATP-sensitive potassium channels from phosphatidylinositol polyphosphates and ATP. Pharmacol Res 2007, 56, 237–247.
[9] **Ramesh E, Elanchezhian R, Sakthivel M, Jayakumar T, Senthil Kumar RS, Geraldine P:** Epigallocatechin gallate improves serum lipid profile and erythrocyte and cardiac tissue antioxidant parameters in Wistar rats fed an atherogenic diet. Fundam Clin Pharmacol 2008, 22, 275–284.
[10] **Katiyar SK, Elmets CA:** Green tea polyphenolic antioxidants and skin photoprotection (Review): Int J Oncol 2001, 18, 1307–1313.
[11] **Matsumoto Y, Kaihatsu K, Nishino K, Ogawa M, Kato N, Yamaguchi A:** Antibacterial and antifungal activities of new acylated derivatives of epigallocatechin gallate. Front Microbiol 2012, 3, 53–63.
[12] **Shin HY, Kim SH, Jeong HJ, Kim SY, Shin TY, Um JY:** Epigallocatechin-3-gallate inhibits secretion of TNFalpha, IL-6 and IL-8 through the attenuation of ERK and NF-kappaB in HMC-1 cells. Int Arch Allergy Immunol 2007, 142, 335–344.
[13] **Katiyar S, Mukhtar H:** Green tea polyphenol (2)-epigallocatechin-3-gallate treatment to mouse skin prevents UVB-induced infiltration of leukocytes, depletion of antigen-presenting cells, and oxidative stress. J Leukoc Biol 2001, 69, 719–726.
[14] **Yoshino K, Miyase T, Sano M:** Preventive effects of C-2 epimeric isomers of tea catechins on mouse type I allergy. J Nutr Sci Vitaminol (Tokyo) 2010, 56, 211–215.
[15] **Lim YC, Cha YY:** Epigallocatechin-3-gallate induces growth inhibition and apoptosis of human anaplastic thyroid carcinoma cells through suppression of EGFR/ERK pathway and cyclin B1/CDK1 complex. J Surg Oncol 2011, 104, 776–780.
[16] **Tavares Pereira Ddos S, Lima-Ribeiro MH, de Pontes-Filho NT, Carneiro-Leão AM, Correia MT:** Development of animal model for studying deep second-degree thermal burns. J Biomed Biotechnol 2012, Article ID 460841, 7 pages.
[17] **Neder Meyer T, Lázaro Da Silva A:** Ketamine reduces mortality of severely burnt rats, when compared to midazolam plus fentanyl. Burns 2004, 30, 425–430.
[18] **Woolliams JA, Wiener G, Anderson PH, McMurray CH:** Variation in the activities of glutathione peroxidase and superoxide dismutase and in the concentration of copper in the blood various breed crosses of sheep. Res Vet Sci 1983, 34, 69–77.
[19] **Paglia DE, Valentine WN:** Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 1967, 70, 158–169.
[20] **Lowry OH, Rosebrough NJ, Farr AL, Randall RJ:** Protein measurement with the Folin phenol reagent. J Biol Chem 1951, 193, 265–275.
[21] **Drapper HH, Hadley M:** Malondialdehiyde determination as index of lipid peroxidation. Methods Enzymol 1990, 186, 421–431.
[22] **Hsu S:** Green tea and the skin. J Am Acad Dermatol 2005, 52, 1049–1059.
[23] **Jankun J, Selman SH, Swiercz R, Skrzypczak-Jankun E:** Why drinking green tea could prevent cancer. Nature 1997, 387, 567.
[24] **Frei B, Higdon JV:** Antioxidant activity of tea polyphenols *in vivo*: evidence from animal studies. J Nutr 2003, 133, 3275–3284.
[25] **Bertın-Maghıt M, Goudable J, Dalmas E, Steghens JP, Bouchard C, Gueugnıaud PY:** Time course of oxidative stress after major burns. Intensive Care Med 2000, 26, 800–803.
[26] **Youn Yk, Lalonde C, Demlıng R:** Oxidants and the pathophysiology of burn and smoke inhalation injury. Free Radic Biol Med 1992, 12, 409–415.
[27] **McCord JM, Fridovich I:** "Superoxide dismutase: the first twenty years (1968–1988)". Free Radic Biol Med 1988, 5, 363–369.
[28] **Nakagawa Y:** Role of mitochondrial phospholipid hydroperoxide glutathione peroxidase (PHGPx) as an antiapoptotic factor. Biol Pharm Bull 2005, 27, 956–960.
[29] **Cevik O, Oba R, Macit C, Cetinel S, Kaya OT, Sener E:** Lycopene inhibits caspase-3 activity and reduces oxidative organ damage in a rat model of thermal injury. Burns 2012, 38, 861–871.
[30] **Büttemeyer R, Philipp AW, Schlenzka L, Mall JW, Beissenhirtz M, Lisdat F:** Epigallocatechin gallate can significantly decrease free oxygen radicals in the reperfusion injury *in vivo*. Transplant Proc 2003, 35, 3116–3120.
[31] **Kakuta Y, Okumi M, Isaka Y, Tsutahara K, Abe T, Yazawa K:** Epigallocatechin-3-gallate protects kidneys from ischemia reperfusion injury by HO-1 upregulation and inhibition of macrophage infiltration. Transpl Int 2011, 24, 514–522.
[32] **Brückner M, Westphal S, Domschke W, Kucharzik T, Lügering A:** Green tea polyphenol epigallocatechin-3-gallate shows therapeutic antioxidative effects in a murine model of colitis. J Crohns Colitis 2012, 6, 226–235.
[33] **Mitchell RN, Cotran RS:** Acute and Chronic Inflammation. In: Robbins Basic Pathology Eds.: Kumar V, Cotran RS, Robbins SL, Saunders Elsevier Inc., Philadelphia, 2003, 7th Edition, 33–61.
[34] **Giakoustidis AE, Giakoustidis DE, Koliakou K, Kaldrymidou E, Iliadis S, Antoniadis N:** Inhibition of intestinal ischemia/repurfusion induced apoptosis and necrosis *via* down-regulation of the NF-kB, c-Jun and caspace-3 expression epigallocatechin-3-gallate administration. Free Radic Res 2008, 42, 180–188.

[35] Giakoustidis DE, Giakoustidis AE, Iliadis S, Koliakou K, Antoniadis N, Kontos N: Attenuation of liver ischemia/reperfusion induced apoptosis by epigallocatechin-3-gallate *via* downregulation of NF-kappaB and c-Jun expression. J Surg Res 2010, 159, 720–728.

[36] Townsend PA, Scarabelli TM, Pasini E, Gitti G,Menegazzi M, Suzuki H: Epigallocatechin-3-gallate inhibits STAT-1 activation and protects cardiac myocytes from ischemia/reperfusion-induced apoptosis. FASEB J 2004, 18, 1621–1623.

[37] Kapoor M, Howard R, Hall I, Appleton I: Effects of epicatechin gallate on wound healing and scar formation in a full thickness incisional wound healing model in rats. Am J Pathol 2004, 165, 299–307.

Address for correspondence:

Cem Aslan
Katip Celebi University School of Medicine
Ataturk Training and Research Hospital
Department of Plastic and Reconstructive Surgery
Karabaglar 35150
Izmir
Turkey
Tel.: +90 532 712 67 69
E-mail: cemsln@yahoo.com

Conflict of interest: None declared

Received: 15.08.2013
Revised: 8.04.2014
Accepted: 8.05.2015