



The Effect of Captopril on Brain Apoptosis After Burn Injury in Rats

Yanık Sonrası Rat Beyninde Meydana Gelen Apoptozis Üzerine Kaptoprilin Etkisi

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ABSTRACT

AIM: The purpose of this study was to determine the possible protective effects of captopril treatment against apoptosis in the brain induced by burn injury.

MATERIAL and METHODS: Under ether anaesthesia, Wistar albino rats (200-250 g) were exposed to a 900C (burn) or 250C (sham) water bath for 10 s. The ACE group was treated with i.p. 10 mg/kg captopril immediately after burn injury and this treatment was repeated twice daily. At the end of the 24 hours, brain samples were taken. Apoptotic brain cells marked by terminal deoxynucleotidyl transferase-mediated d-UTP-nick end labeling (TUNEL) were evaluated in the cerebellum and midbrain of rats.

RESULTS: Apoptotic cells in the cerebellum were significantly decreased after captopril treatment and found to be lower when compared to the burn group ($p<0.001$). In the midbrain of rats, the numbers of TUNEL-positive cells and apoptotic bodies were significantly increased in the burn group when compared to the control group ($p<0.001$). The burn-induced changes were reduced in the captopril-treated burn group ($p<0.01$).

CONCLUSION: Captopril has beneficial effects in burn injury and should be assessed as a therapeutic agent in the management of this condition.

KEYWORDS: Captopril, Apoptosis, Burn injury, TUNEL

ÖZ

AMAÇ: Yanığın indüklediği, beyinde meydana gelen apoptozise karşı kaptoprilin koruyucu etkisini araştırmak amaçlanmıştır.

YÖNTEM ve GEREÇLER: Anestezi altındaki Wistar albino sıçanlar (200-250 g) 900C (yanık) ya da 250C (sham-kontrol) su buharına 10 dakika süresince tutuldu. Sıçanlar, yanık oluşumunu takiben i.p. 10 mg/kg kaptopril ile günde iki kez olmak üzere tedavi edildi. Serebellum ve orta beyinden çıkarılan hücrelerde apoptozun saptanmasında terminal deoxynucleotidyl transferase- mediated d-UTP- (TUNEL) yöntemi kullanıldı.

BULGULAR: Yanık grubu ve sham-kontrol grubu serebellum bölgesinde oluşmuş olan apoptotik hücre sayısı açısından karşılaştırıldığında, TUNEL-positif apoptotik hücre sayısı yanık grubunda anlamlı olarak artmış bulundu ($p<0.001$). Serebellumdaki apoptotik hücre sayısının, kaptopril tedavisinden sonra, yanık grubu ile karşılaştırıldığında anlamlı olarak azaldığı görüldü ($p<0.001$). Ortabeyinde ise, apoptotik TUNEL-positif hücre sayısı yanık ve kontrol grubu karşılaştırıldığında, yanık grubunda anlamlı olarak arttığı gözlemlendi ($p<0.001$), yanığın indüklediği apoptotik değişiklikler ise kaptopril tedavisi ile anlamlı olarak azaldı ($p<0.01$).

SONUÇ: Kaptopril yanık hasarı ile beyinde meydana gelen apoptotik değişiklikleri geri döndürmüştür. Yanık hasarının tedavisinde faydalı bir ilaç olabilir.

ANAHTAR SÖZCÜKLER: Kaptopril, Apoptosis, Yanık hasarı, TUNEL

INTRODUCTION

Apoptosis, or programmed cell death, is an important gene-controlled cell condition. It is a distinct type of cell death characterised by a series of typical morphological events, such as shrinkage of the cell, fragmentation into membrane-bound apoptotic bodies and rapid phagocytosis into neighbouring

cells without induction of an inflammatory response (20). The biochemical hallmark of apoptosis is internucleosomal DNA fragmentation (46).

Angiotensin-converting enzyme (ACE) inhibitors have been routinely used to treat heart failure and hypertension. Traditionally, ACE inhibitors block the conversion of

angiotensin I to angiotensin II. Angiotensin II is a potent vasoconstrictor hormone; in addition to its known vital role in both cardiovascular and fluid homeostasis, several lines of evidence implicate angiotensin II in ischemic neuronal injury via the angiotensin II receptor subtype AT1 (AT1R) (42). Stimulation by angiotensin II of the AT2 receptors with activation of pathophysiological pathways apoptosis (47). It is known that apoptosis plays an important role in tissue remodelling in pathological conditions. Captopril has recently been found to inhibit Fas-induced apoptosis in human activated T cells (7) and lung epithelial cells (41). Moreover, the neuroprotective effects of ACE inhibitors have been verified in histological studies (45).

Burn injuries are a significant cause of death and disability around the world (11). A significant incidence of multiple organ (liver, thymus, spleen, skeletal muscle, lung, heart and brain) dysfunction and failure has been noted in both animals and humans surviving the initial insult of severe burn injury. This failure of different organ systems is thought to be, at least in part, due to increased apoptotic cell death (21). Following severe burn injury, there is also an increase in signalling pathways such as the p38 and Jnk MAPKs (mitogen-activated protein kinases), leading to an amplification of cytokine production and the initiation of apoptosis (8,10,49,50). With respect to the brain, previous studies have demonstrated that the brain is one of the remote organs subject to injurious effects following severe burn injury (4,12,24,31,33,50). In animal studies of burn injury, magnetic resonance imaging has identified marked changes in the brain up to three days post-burn injury, most notably brain swelling and lesions (24). Over the initial 24 hour post-burn period, rats were found to display decreased glucose utilisation in the brain; however, by week three, the glucose utilisation returned to baseline, indicating an acute dysregulation of glucose metabolism in the brain (2). In behavioural studies, such animals appear to have long-term cognitive deficits associated with a disruption in the blood-brain barrier (27,39,40), increased inflammation (31) and/or altered metabolism in the brain (2,50).

In the light of these findings, we aimed to investigate whether and to what extent captopril would provide protection against burn-induced apoptosis. This study was designed to determine the possible protective effect of captopril treatment against apoptosis in the brain induced by burn injury.

MATERIAL and METHODS

Animals and Laboratory

All experimental protocols were approved by the Marmara University Animal Care and Use Committee. Wistar albino rats of both sexes, weighing 200 to 250 g, were obtained from Marmara University School of Medicine Animal House. Rats were kept in a temperature-controlled room ($22 \pm 1^\circ\text{C}$) with 12-h: 12-h light and dark cycles; they were fed with standard rat chow and fasted for 12 h before the experiments.

Thermal Injury

Under brief ether anaesthesia, the dorsum of each rat was shaved and exposed to a 90°C water bath for 10 s. This procedure has been shown to result in a second-degree skin burn involving 30% of the total body surface area (36). To rule out the effects of anaesthesia, the same protocol was applied in the sham group, except that the dorsums 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 s.c.). Each group consisted of eight rats. ACE inhibitor captopril (10 mg/kg, 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. Control group rats did not receive captopril and eight rats in the burn group received captopril.

Rats were decapitated and brain samples were collected at 24 h after burn injury. The brain samples were sent to Baskent University Istanbul Hospital Pathology Laboratory in 10% formaldehyde tamponade solution. Samples from tissues of the cerebellum and midbrain were taken for routine tissue processing and paraffin blocks were prepared. Two micrometre-thick sections were affixed to slides.

TUNEL Staining

The level of DNA fragmentation was detected by *in situ* terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling (TUNEL) using an ApopTag *in situ* apoptosis detection kit (Millipore, USA & Canada) according to the manufacturer's instructions. Briefly, deparaffinised and rehydrated sections were pretreated with 20 $\mu\text{g}/\text{mL}$ proteinase K for 15 min at room temperature. After the slides were washed twice with PBS, the sections were incubated in equilibration buffer for 30 min. The sections were then incubated with the labelling solution containing terminal deoxynucleotidyl transferase in a humidified chamber for 1 h at 37°C . The reactions were terminated by rinsing the sections in a stop/wash buffer. The sections were incubated with anti-digoxigenin conjugate for 30 min at room temperature and then rinsed four times in PBS. The TUNEL-labelled slides were photographed using a fluorescence microscope.

Statistical Analysis

Statistical analysis of data was performed by one-way analysis of variance (ANOVA) with Tukey's test using a statistical software package (GraphPad Prism 3.0, San Diego, CA, USA). Values of $p < 0.01$ were regarded as significant.

RESULTS

Two brain areas were examined, the cerebellum and the midbrain, where TUNEL-positive cells indicating apoptosis were shown to increase at 24 h after a systemic inflammation model (35). A marked increase in the number of apoptotic cells was seen in the cerebellum and midbrain of burned rats 24 h after burn injury by *in situ* TUNEL staining. The apoptotic cells showed characteristic shrinking, chromatin clumping and nuclear fragmentation. Captopril treatment reduced the

numbers of apoptotic cells in both brain areas (Figure 1A-C, 2A-C).

In the cerebellum, the number of TUNEL-positive cells and apoptotic bodies in the burn group was found to be significantly higher than in the sham control group ($p < 0.001$). Apoptotic cells in the cerebellum were significantly decreased after captopril treatment and found to be lower when compared to the burn group ($p < 0.001$). However, in the captopril-treated burned rats, the number of TUNEL-positive cells was not significantly different from that in the control rats (Figure 3).

In the midbrain of rats, the numbers of TUNEL-positive cells and apoptotic bodies were significantly increased in the burn group when compared to the control group ($p < 0.001$). The burn-induced changes were reduced in the captopril-treated burn group ($p < 0.01$) (Figure 4).

DISCUSSION

Apoptosis is an orchestrated form of cell 'death by suicide'. It is essential in both the development and normal maintenance of tissue function. Apoptotic nerve cell death is implicated in the pathogenesis of several devastating neurodegenerative conditions. Neuronal apoptosis occurs

in several brain diseases, including Parkinson's disease (22), ischemia-reperfusion injury (48), encephalopathy and sepsis (19). Systemic inflammation induces apoptosis with variable vulnerability of different brain regions (35). Neuronal apoptosis has a potentially important role in burn injury.

The ACE inhibitor captopril blocks the conversion of angiotensin I to the potent vasoconstrictor angiotensin II and simultaneously inactivates the vasodilator peptide bradykinin. Besides its blood pressure-lowering properties, captopril has various immunomodulatory functions. The drug exhibits beneficial effects on rheumatoid arthritis (14,26) and prevents complications in insulin-dependent diabetes mellitus (23). Additionally, captopril successfully inhibits inflammation in schistosomiasis (44), experimental lupus diseases (13) and experimental autoimmune encephalomyelitis (EAE) (6). Some ACE inhibitors—including captopril—are capable of suppressing the production of monocytes/macrophage-derived proinflammatory cytokines such as tumour necrosis factor (TNF), interleukin (IL)-1, IL-6 and IL-12 (5,9,34). These immunomodulatory actions of captopril have been explained by several mechanisms, including anti-proliferation (1,3,29,37), anti-oxidant activity (15,16,32), inhibition of metalloproteases (28,38) and elevation of prostaglandin

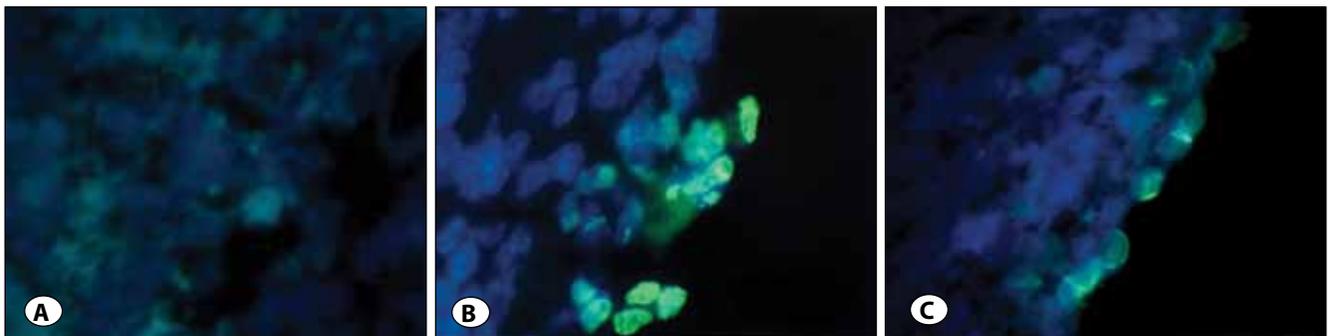


Figure 1: Detection of DNA fragmentation in the cerebellum was performed using the *in situ* terminal deoxynucleotidyl transferase dUTP nick-end labelling (TUNEL) method, as described in MATERIALS AND METHODS. TUNEL staining revealed increased numbers of fluorescent-stained nuclei consistent with apoptosis in the cerebellum of burned rats compared with sham control rats. Captopril treatment reduced the numbers of apoptotic cells. **A)** Control group, **B)** Burn group, **C)** Burn and captopril group.



Figure 2: Detection of DNA fragmentation in the midbrain was performed using the *in situ* terminal deoxynucleotidyl transferase dUTP nick-end labelling (TUNEL) method, as described in MATERIALS AND METHODS. TUNEL staining revealed increased numbers of fluorescent-stained nuclei consistent with apoptosis in the midbrain of burned rats compared with sham control rats. Captopril treatment reduced the numbers of apoptotic cells. **A)** Control group, **B)** Burn group, **C)** Burn and captopril group.

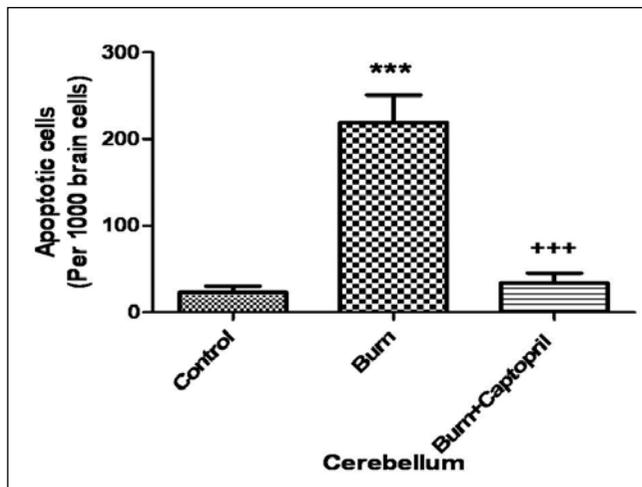


Figure 3: Apoptotic cell number as terminal deoxyuridine nick-end labelling (TUNEL)-positive cells or apoptotic bodies per 1,000 brain cells. The figure shows the number of TUNEL positive cells in the cerebellum of the burn, control and captopril-treated burn groups. Each group consists of four animals. *** $p < 0.001$; compared to control group. *** $p < 0.001$; compared to burn group.

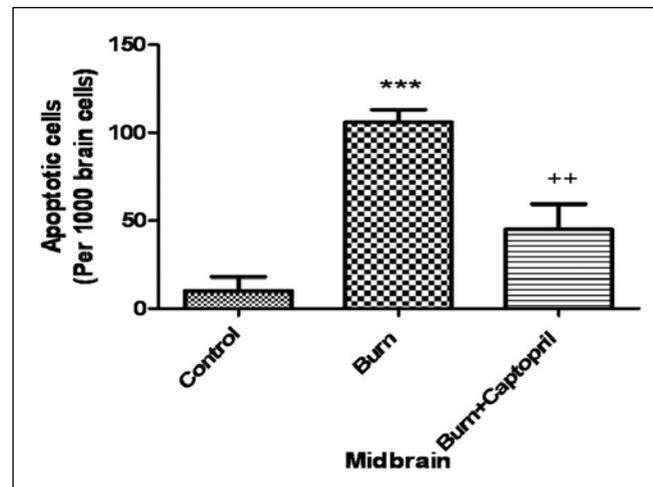


Figure 4: Apoptotic cell number as terminal deoxyuridine nick-end labelling (TUNEL)-positive cells or apoptotic bodies per 1,000 brain cells. The figure shows the number of TUNEL positive cells in the midbrain of the burn, control and captopril-treated burn groups. Each group consists of four animals. *** $p < 0.001$; compared to control group. ** $p < 0.01$; compared to burn group.

(17,18,25). Some of these properties may be related to the presence of thiol groups in its structure and are independent of its effect on the renin angiotensin system (30,43). As captopril has recently been found to inhibit Fas-induced apoptosis in human activated T cells (7) and lung epithelial cells (41), we hypothesise that prevention of apoptosis could be one of the mechanisms bringing about the efficacy of ACE inhibitors. Captopril has a long-acting effect, can be used once daily and is welcomed by clinicians and patients.

In conclusion, in this study, we showed that captopril inhibited two examined brain areas, the cerebellum and the midbrain, where TUNEL-positive cells indicating apoptosis were shown to increase at 24 h after a systemic inflammation model (35). Captopril may interfere with to provide protection from apoptotic cell death resulting from burn injuries. Our data indicate that captopril has beneficial effects in burn injury and should be assessed as a therapeutic agent in the management of this condition.

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