Özgün Deneysel Çalışma

Neuroprotective Effect of ACE Inhibitors Following Cerebral Ischemia in Rats: Histopathological Evaluation

Süleyman COŞKUN [1], Gökşin ŞENGÜL [1], Murteza ÇAKIR [1], Ahmet HACIMÜFTÜOĞLU [2], Fatih SARUHAN [2], Mustafa Kemal ÇOBAN [1], Muhammet ÇALIK [3]

[1] Ataturk University Medical Faculty, Departments of Neurosurgery, Erzurum
[2] Ataturk University Medical Faculty, Departments of Pharmacology, Erzurum
[3] Ataturk University Medical Faculty, Departments of Pathology, Erzurum

✔ **Objective:** Angiotensin converting enzyme (ACE) inhibitors are frequently used in the treatment of essential hypertension causing disorders especially in the cerebral circulation. In this study, neuroprotective effect, and mechanism of action of ACE inhibitors in focal cerebral ischemia created in rats via unilateral common carotid artery ligation were examined and their effects on behavior patterns were tested.

Methods: Thirty rats were used in this study. The rats were separated into 5 groups randomly. Following left common carotid artery ligation, Group 1 was given captopril, Group 2 ramipril, and Group 3 perindopril. Group 4 received normal saline for 7 days, and Group 5 was used as a control group. The rats were sacrificed after carrying out behavioral tests for 7 days. Their exenterated brains were histopathologically examined.

Results: Neuronal damage was lower in ACE inhibitor groupa in comparison to the normal saline group The lowest neuronal damage was observed in the perindopril group. The results of behavioral tests indicated that ACE inhibitors did not have any effect on behavior patterns in cerebral ischemia.

Conclusions: Our data indicate that perindopril, ramipril and captopril have beneficial effects in brain ischemia and should be tried as therapeutic agents in the management of this condition.

J Nervous Sys Surgery 2010; 3(2):66-71

ACE İnhibitörlerinin Serebral İskemi Sonrası Sıçan Beyin Hücrelerindeki Nöroprotektif Etkisi: Histopatolojik Değerlendirme

✔ **Giriş:** Anjiotensin dönüştürücü enzim (ACE) inhibitörleri özellikle serebral sirkülasyonda bozukluğa sebep olan esansiyel hipertansiyon tedavisinde sıkça kullanılmaktadır. Bu çalışmada, sıçanlarda tek taraflı karotis kommunis ligasyonu ile oluşturulan fokal serebral iskemide ACE inhibitörlerinin nöroprotektif etkisi ve etki mekanizması incelendi ve davranış üzerine olan etkileri test edildi.

Yöntem: Bu çalışmada 30 rat kullanıldı. Ratlar rastgele 5 gruba ayrıldı. Sol kommon karotid arter ligasyonunu takiben 7 gün boyunca 1. gruba kaptopril, 2. gruba ramipril, 3. gruba perindopril, 4. gruba serum fizyolojik verildi. Beşinci grup kontrol grubu olarak kullanıldı. Yedinci gün ratlara davranış testleri yapılarak, sakrifiye edildi. Çıkarılan beyinler histopatolojik olarak incelendi.

Bulgular: ACE inhibitörlerinin verildiği gruplarda nöronal hasar serum fizyolojik grubuna göre daha azdı. En az nöronal hasar ise perindopril verilen grupta gözlendi. Davranış testlerinin sonuçları serebral iskemide ACE inhibitörlerinin davranış üzerine etkisi bulunmadığını gösterdi.

Sonuçlar: Elde ettiğimiz veriler, perindopril, ramipril ve kaptoprilin beyin iskemisinde yararlı etkisi olduğunu ve bu durumun tedavisinde teröpatik olarak denenebileceğini göstermektedir.

Anahtar kelimeler: ACE inhibitörleri, nöron, nöroprotektif etkiler, serebral iskemi

J Nervous Sys Surgery 2010; 3(2):66-71

Alındığı tarih: 16.06.2011
Kabul tarihi: 27.06.2011
Yazışma adresi: Uzm. Dr. Süleyman Coşkun, M. Akif Ersoy Mah. İbrahim Hakkı Fen Lisesi Karşısı Makrokent Sitesi B-Blok Kat: 2 No: 11, Erzurum
e-posta: dr.scoskun@mynet.com

Stroke is the eventual outcome of occlusive cerebrovascular diseases. These diseases which can be defined as the dysfunction of the nervous system are caused by any pathology in the cerebral circulation. Occlusive cerebrovascular diseases. ranks as top third, and to some authors even second in frequency as a life-threatening disease group in the western world (mean 27 %) and most frequently it results in long term disability. Because of unique anatomic and physiological structure of cerebral blood flow, brain is protected from many disorders in circulation. However, if a disease emerges in this protective system, then it results in stroke [11]. It was previously indicated that ACE inhibitors prolonged survival in cerebral ischemia depending on common carotid artery occlusion in rats. Neuroprotective effects of ACE inhibitors have been verified in histological studies [20]. Although a lot of researches have been carried out on the protective effect of ACE inhibitors, their mechanisms of action are not completely known.

This study aimed at both in vivo demonstration of the neuroprotective effects of ACE inhibitors histopathologically with focal cerebral ischemia model created by unilateral common carotid artery ligation, and determination of their effects on animal behaviors as assessed with opto-varimex tests.

MATERIALS and METHODS

Experimental procedure and the animals used: This study was conducted at the Medical Experimental Research Center, Ataturk University. The Ethics Committee of Ataturk University approved the study protocol. All procedures were performed in accordance with the National Institute of Health Principles of Laboratory Animal Care. A total of 30 female Sprague-Dawley rats weighing 180-220 g were used. Animals were kept under a 12h light/12h dark cycle and allowed free access to food and water. The rats were anaesthetised with 30 mg/kg sodium thiopental (Abbott, Istanbul) intraperitoneally and allowed to breathe spontaneously throughout the procedure.

Persuant to skin cleaning in cervical region in the supine position, vertical incision was made. After passing skin, subcutaneous tissue and muscle, trachea and esophagus were retracted medially. Left carotid artery was dissected away from vagal nerve, exposed and ligated with a silk suture. The layers were closed and the process was terminated. The rats were divided into 5 groups randomly. Group 1, was given captopril (Deva, Istanbul, Turkey) 25 mg/kg/day i.p, Group 2, ramipril (Aventis Pharma, Italy) 20 mg/kg/day i.p, Group 3, perindopril (Servier, Istanbul, Turkey) 5 mg/kg/day i.p, Group 4, normal saline for 7 days. Normal saline was administered i.p to the rats in Group 5 for 7 days without carrying out carotid ligation. After 7 days, locomotor activity (opto-varimex behavioral tests) was evaluated. Following this, the brains of the rats anaesthetized with high-dose thiopental were exenterated. The brains were kept for 5 days in separate jars previously prepared in 10 % formaldehyde adjusted to an amount of 10 times for each sample with the aim of fixation.

Histopathological evaluation: The samples kept in separate jars were sent to histopathological examination. With three coronal incisions each brain specimens were divided in 3 pieces, labeled separately, and buried in paraffin-embedded blocks. At least three pieces were taken from each block in order to facilitate evaluation, and preparates were stained with hematoxylin–eosine. Neuronal damage occurring in cerebral cortex specimens after induction of cerebral ischemia was examined. Acidophilic neuronal cytoplasm, pycnosis and histomorphological appearance characterized (red neuron) with karyorrhexis were observed in the damaged neurons. While neuronal damage was evaluated, the number of damaged neurons were counted in 5 different microscopic fields, and the ratios of

damaged to undamaged neurons present in these areas were determined. The preparates were examined under Sony DXC-390P light microscope having a camera attachment and photographs were taken.

Evaluating Locomotor Activity: It was carried out by means of Locomotor activity meter (MAY ACT508 IR LOCOMOTOR ACTIVITY METER). The total of horizontal, vertical and ambulatory activities were recorded as total activity.

Statistical analysis: Windows compatible SPSS (13.0 version) program was used. The damage scorings as assessed during histopathological study, and digital data obtained from the behavioral tests were evaluated by One-way ANOVA test. The relationship between groups were accepted as significant if p was <0.05.

RESULTS

Histopathological findings: The degree of neuronal damage was less intense in the ACE inhibitor groups relative to the normal saline group . Minimal number of red neurons indicating neuron damage was found in the perindopril group (Figure 1). The number of red neurons was higher in the ramipril and captopril groups when compared with the perindopril, and significantly lower in comparison with the normal saline group (Figure 2, 3, 4). A statistically significant difference between ACE inhibitor groups, and normal saline group was found ($p<0.05$).

Results of Behavioral Tests

The results of opto-varimex behavioral tests indicated that ACE inhibitors captopril, ramipril and perindopril stereotypes did not have any effect on behavior in cerebral ischemia in terms of vertical, ambulatory and horizontal movements.

DISCUSSION

A common consensus could not be reached on the best therapeutic approaches which might alleviate and prevent disability caused by cerebral ischemia. In this study, focal cerebral ischemia created by unilateral carotis ligation in rats and the ability of ACE inhibitors; captopril, perindopril and ramiprilin to reverse neuronal damage were examined. In addition, this subject has been discussed in line with the literature by testing the effects of ACE inhibitors on behavior patterns in focal cerebral ischemia. The study was conducted considering the principle that

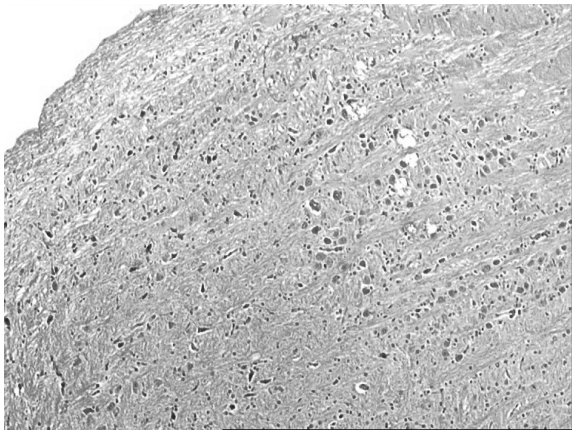

Figure 1. The light microscopic view of the brain cortex incision obtained from the group in which unilateral carotid ligation was carried out and given perindopril therapy. It is observed that red neurons are lower in comparison to the group given ramipril and captopril (HE 2.5*100).

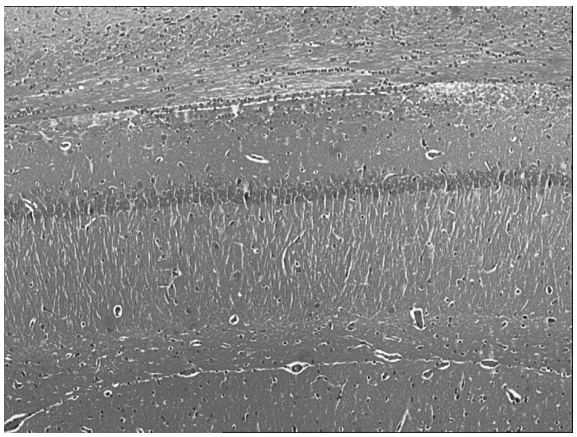

Figure 2. The light microscopic view of the brain cortex incision obtained from the group in which unilateral carotid ligation was carried out and given ramipril therapy. It is observed that red neurons are lower in comparison to the group given normal saline (HE 2.5* 100).

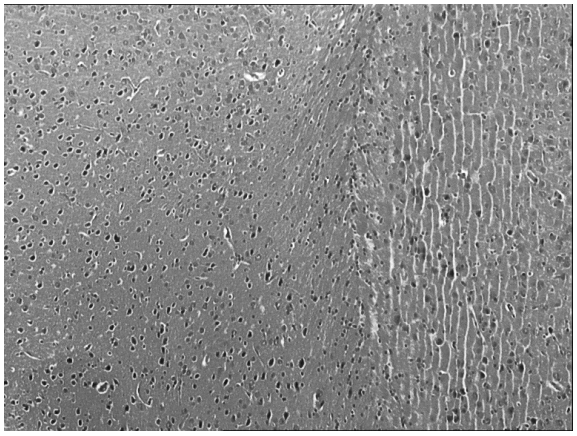

Figure 3. The light microscopic view of the brain cortex incision obtained from the group in which unilateral carotid ligation was carried out and given captopril therapy. It is observed that red neurons are lower in comparison to the group given normal saline (HE 4*100).

Figure 4. The light microscopic view of brain cortex incision taken from the groups given normal saline and in which unilateral carotid ligation was carried out. Red neurons are commonly seen (HE 2.5*100).

various drugs in different classes of ACE inhibitors may show different effects According to the data obtained from experimental results, ACE inhibitors demonstrate neuroprotective effect in focal cerebral ischemia only when compared with surgery.

The results of histopathological analyses of the brain tissue after experimental ischemia indicate that perindopril is superior to captopril with respect to their neuroprotective activities [18]. This situation might result from the fact that perindopril is a prodrug metabolized from liver. In the histopathological results obtained by us, perindopril is also superior to both captopril and ramipril as for neuroprotective activity. The neuroprotective effect of ramipril on cerebral white matter lesions manifests itself by reducing free radicals in the chronic ischemia model created by bilateral carotid ligation [7]. It was indicated that ramipril therapy for 14 days (20 mg/kg/day) healed white matter lesions and MDA level and GSSH/GSH ratios turned to normal in the rats with chronic ischemia. It was detected that white matter lesions increase in number in association with high level of MDA, and progressive formations of free radicals by damaged healthy neurons. In addition, a decrease was detected in glutathione levels [7]. Glutathione is both an essential tripeptide, and an endogenous antioxidant detected in all animal cells. It protects against superoxidal damage of hydroxy-radicals by reacting against free radicals. The gradual reduction in glutathione levels after marked increase in free radicals makes us think that this is a compulsory outcome of the combat against oxidative stressws. Increases in GSSH/GSH ratio might be the result of secondary MDA increments after bilateral common carotid artery occlusion. And it is also possible that other mechanisms might be concealing white matter lesions by showing neuroprotective effects. Ramipril might increase blood flow following vasodilatory effect in brain arterioles, and prevent the release of cytotoxic substances such as protease contributing to the healing of white matter lesions. Beneficial effects of ACE inhibitors on cell apoptosis can have a role in the pathogenesis of white matter lesions [20]. These data show similarities with histopathological neuroprotective effects of ramipril in our study. The results given above suggest us the formation of free radicals in white matter as a result of chronic hypoperfusion. A study asserted that ACE inhibitors such as enalapril and moexipril has a neuroprotective effect on white matter against toxic effects of free radicals in in vivo and in vitro focal ischemic injury models [18]. It is possible that chronic cerebral hypoperfusion in rats might have covered the effect of acute

ischemic damage and also masked the acute stage . Histopathological findings found out by us our in vivo focal cerebral ischemia models in rats also support these neuroprotective effects of ACE inhibitors.

ACE inhibitors are easily utilized in brain and frequently in basal ganglions, cerebral cortex, circumventricular organs, hypothalamic neurosensitive nucleus, dentate gyrus of hypocampus and cerebellum. In basal ganglions, ACE neurons and striatum are combined. In addition, ACE shows changes in different areas in postmortem brains in cases with neuropsychiatric problems. Surprisingly, one of the recent studies determined that ACE inhibitor perindopril inhibited striatal ACE, and increased dopamine levels 2-3 times which were released into rat striatum [8]. ACE inhibitors can pass blood-brain barriers very easily, and modulate levels of central neurotransmitters. Besides, central effects of ACE inhibitors can modulate dopaminergic neurotransmission. Current studies have indicated that ACE inhibitor perindopril increased the level of dopamine in patients with Parkinson disease. It was determined that perindopril reduced free radicals stemming from methyl-phenyl-tetrahydopiridine (MPTP) in the dopaminergic system and it was effective in the treatment of neurodegenerative diseases [8]. The relationship between ACE inhibition and neuronal protection is slightly known, and the research should be carried out to that end.

ACE inhibitors might show neuroprotective effects because of the drug's radical scavenger effect [14]. It was determined in various studies that captopril has also a radical scavenger effect with its sulphydryl group. This tissue protective effect of captopril is due to its sulphydryl group bound to thiol groups formed after ischemia [1,5,12,13,17]. In this study, since the neuroprotective effect of the drugs not containing sulphydryl group such as perindopril and ramipril in cerebral ischemia is stronger than captopril containing a sulphydryl group, we can deduce that chemical structure of ACE inhibitors is not an effective factor alone in the viability of neurons.

It was indicated that oxidative stress had a role in physiotherapy of stroke. It was also demonstrated that the free radicals destructed blood-brain barrier (BBB), caused brain edema, and directed inflammatory cells into ischemic area leading to changes in blood-flow. Hydroxyl radical and superoxide ions having oxygen free radicals are reactive and bound to nucleic acids, lipids, carbonhydrates and proteins in neurons manifesting destructive effects on them [3]. Captopril suppresses major oxidative stress processes (for example, lipid peroxidation and protein oxidation) and shows neuroprotective effect [10]. It was indicated that captopril inhibited lipid peroxidation triggerred in rabbits with FeCl3, and had protective effect in apoptosis model of oxidative stress in endothelium cells [2,21]. It was demonstrated that ramipril due to its lipophilic nature showed neuroprotective effects with a similar mechanism [16]. It was detected that the rats with suppressed NADPH oxidase activity were resistant to ischemic brain damage [3]. The oxidative stress emerging in cerebral ischemia triggers the production of local ACE and angiotensin II. Angiotensin II induces the formation of superoxide by increasing vascular NADPH oxidase. As a result, NO levels decrease, and oxidative stress increases [4,6]. It is possible that ACE inhibitors might reduce harmful effects of free radicals due to its antioxidant effect (by suppressing angiotensin II and NADPH oxidase activities). ACE inhibitors are kininaz-2 inhibitors and prevent destruction of bradykinin which is a vasodilatory peptide [14]. ACE inhibitors inhibit kininase activity providing bradykinin degradation and ensure the increase of prostanoids with vasodilatation as a result of phospholipase A2 activation dependant on bradykinin. In addition, endothelial B2 receptors are stimulated with the local increase in bradykinin levels, and nitric oxide release from endothelial cells which contribute to its vasodilatory effect [9,15]. It was determined that nitric oxide showed

neuroprotective effect by increasing blood build up in the ischemic area in the early stages of cerebral ischemia [19]. In this study, we think that ACE inhibitors can reduce neuron death by increasing NO release.

There is not any information in the literature about the effect of ACE inhibitors on behavioral patterns in cerebral ischemia. The data obtained by us during statistical evaluations of horizontal, vertical, ambulatory and stereotype activities were statistically significant, but their significance was not higher as we expected. We were hoping that total activity results obtained would be near to the normal group and there would be an increase in their neuroprotective effects. Consequently, we can express that ACE inhibitors have not a positive effect on behavior patterns in cerebral ischemia.

Conclusion: ACE inhibitors might have provided a neuroprotective effect against cerebral ischemia due to their antioxidant, and free radical scavenger effects, and also increments in NO release.

REFERENCES

1. **Bagchi D, Parsad R, Das DK.** Direct scavenging of free radicals by captopril, an Angiotensin converting enzyme inhibitor. Biochem Biophys Res Commun 1989; 158:52-7.
2. **Birincioglu M, Aksoy T, Olmez E, Acet A.** Protective effect of ACE inhibitors on ischemia-reperfusion-induced arrhythmias in rats: is this effect related to the free radical scavenging action of these drugs? Free Radic Res 1997; 27:389-96.
3. **Chan PH.** Reactive oxygen radicals in signaling and damage in the ischemic brain. J Cereb Blood Flow Metab 2001; 21:2-14.
4. **Egan TM, Minta JO, Scrimgeour KG, Cooper JD.** Captopril--a potential free radical scavenger: inhibition of PMN NADPH oxidase. Clin Invest Med 1988; 11:351-6.
5. **Goldschmidt JE, Tallarida RJ.** Pharmacological evidence that captopril possesses and endothelium-mediated component of vasodilation: Effect of sulfhydryl groups on endothelium derived relaxing factor. J Pharmacol Exp Ther 1991; 257:1136-45.
6. **Griffin SA, Brown WC, MacPherson F, McGrath JC, Wilson VG, Korsgaard N, et al.** Angiotensin II causes vascular hypertrophy in part by a non-pressor mechanism. Hypertension 1991; 17:626-35.
7. **Kim JS, Yun I, Choi YB, Lee KS, Kim YI.** Ramipril protects from free radical induced white matter damage in chronic hypoperfusion in the rat. J Clin Neurosci 2008; 15:174-8.
8. **Kurosaki R, Muramatsu Y, Kato H, Watanabe Y, Imai Y, Itoyama Y, et al.** Effect of angiotensin-converting enzyme inhibitor perindopril on interneurons in MPTP-treated mice. Eur Neuropsychopharmacol 2005; 15:57-67.
9. **Liu X, Lukasova M, Zubakova R, Lewicka S, Hilgenfeldt U.** Kallidin-like peptide mediates the cardioprotective effect of the ACE inhibitor captopril against ischaemic reperfusion injury of rat heart. Br J Pharmacol 2006; 148:825-32.
10. **Lopez-Real A, Rey P, Soto-Otero R, Mendez-Alvarez E, Labandeira-Garcia JL.** Angiotensin-converting enzyme inhibition reduces oxidative stress and protects dopaminergic neurons in a 6-hydroxydopamine rat model of Parkinsonism. J Neurosci Res 2005; 81:865-73.
11. **Mathiesen EB, Bonaa KH, Joakimsen O.** Echolucent plaques are associated with high risk of ischemic cerebrovascular events in carotid stenosis: the tromso study. Circulation 2001; 103:2171-5.
12. **Mira ML, Silva MM, Querioz MJ, Manso CF.** Angiotensin converting enzyme inhibitors as oxygen free radical scavengers. Free Radic Res Commun 1993; 19:173-81.
13. **Mittra S, Singh M.** Possible mechanism of captopril induced endothelium-dependent relaxation in isolated rabbit aorta. Mol Cell Biochem 1998; 183:63-7.
14. **Opie LH.** Angiotensin- Converting Enzyme Inhibitors: Scientific Basis for Clinical Use. New York, Willey-Liss, 1994.
15. **Ozer MK, Sahna E, Birincioglu M, Acet A.** Effects of captopril and losartan on myocardial ischemia-reperfusion induced arrhythmias and necrosis in rats. Pharmacol Res 2002; 45:257-63.
16. **Pines A, Fisman EZ.** ACE Inhibition with moexipril: a review of potential effects beyond blood pressure control. Am J Cardiovasc Drugs 2003; 3:351-60.
17. **Pi X-J, Chen X.** Captopril and ramiprilat protect against free radical injury in isolated working rat heart. J Mol Cell Cardiol 1989; 21:1261-71.
18. **Ravati A, Junker V, Kouklei M, Ahlemeyer B, Culmsee C, Krieglstein J.** Enalapril and oexipril protect from free radical-induced neuronal damage in vitro and reduce ischemic brain injury in mice and rats. Eur J Pharmacol 1999; 373:21-33.
19. **Stokinger HE.** Boron. In: Clayton GD, Clayton FE, eds. Patty's industrial hygiene and toxicology. Vol. 2B. Toxicology, 3rd ed. New York, NY, John Wiley & Sons, 1981: 2978-3005.
20. **Werner C, Hoffman WE, Kochs E, Rabito SF, Miletich DJ.** Captopril improves neurologic outcome from incomplete cerebral ischemia in rats. Stroke 1991; 22:910-4.
21. **Yu W, Akishita M, Xi H, Nagai K, Sudoh N, Hasegawa H, et al.** Angiotensin converting enzyme inhibitor attenuates oxidative stress-induced endothelial cell apoptosis via p38 MAP kinase inhibition. Clin Chim Acta 2006; 364:328-34.