**Original Article**

# Inhibition of Angiotensin-Converting Enzyme Reduces Cerebral Infarction Size in Experimental-Induced Focal Cerebral Ischemia in the Rat

H. Panahpour, A.A. Nekooeian[1], G.A. Dehghani[2]

## Abstract

**Background:** The role of Renin Angiotensin System (RAS) in ischemic/reperfusion (I/R) injuries is not fully elucidated. Furthermore, it is not clear whether inhibition of RAS by Angiotensin-Converting Enzyme (ACE) inhibitors has beneficial effects in terms of protecting the brain from I/R injuries. In this study enalapril is used as an ACE inhibitor to evaluate the role of RAS in I/R injuries in the rat.

**Methods:** ACE inhibition was performed one hour before induction of ischemia using a single IP injection of 0.03 mg/kg or 0.1 mg/kg enalapril in the rats. Transient focal cerebral ischemia was induced by 60 min occlusion of the middle cerebral artery followed by reperfusion. Neurological deficit score (NDS) test was performed 24 hours after the start of reperfusion. Finally the animals were sacrificed under deep anesthesia, the brain removed and prepared for the evaluation of cortical and striatal infarction volumes using Triphenyltetrazolium chloride staining method.

**Results:** Pre-ischemic inhibition of ACE with non-hypotensive dose of enalapril (0.03mg/kg) significantly reduced cortical and striatal infarction volumes of ischemic rats by 41.6% and 52.7% respectively with concomitant improvements in NDS. However, no improvement was observed when ACE inhibition accompanied with arterial hypotension.

**Conclusion:** In the rat model of transient focal cerebral ischemia, ACE inhibition seems to reduce the severity of I/R injuries. Therefore, it is plausible to conclude that renin-angiotensin-system may participate in ischemic/reperfusion injuries.
**Iran J Med Sci 2007; 32(1): 12-17.**

**Keywords** ● Cerebral ischemia ● angiotensin-converting enzyme ● enalapril ● angiotensin II ● rat

Departments of physiology,
[1]Pharmacology, and [2]Medicinal&Natural Products Chemistry Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.

**Correspondence:**
Gholam Abbas Dehghani PhD,
Department of Physiology,
Shiraz University of Medical Sciences,
Shiraz, Iran.
**Tel/Fax:** +98 711 2302026
**Email:** dehghang@sums.ac.ir

## Introduction

Various therapeutic strategies have been developed to attenuate the stroke-induced neuronal injury and the subsequent neurologic deficits and disability.[1] Recent experimental and clinical studies have suggested that inhibition of the renin-angiotensin system (RAS) by Angiotensin Converting Enzyme (ACE) inhibitors or Angiotensin II type 1 (AT1) receptor antagonists may be effective in reducing the incidence of

injury after brain ischemia.[2] A large body of evidence suggests that angiotensin II (Ag II) is involved in the pathophysiology of the stroke in hypertensive rats.[3,4,5] Longterm treatment with ACE-inhibitors, or AT1 receptor antagonists, has been reported to prevent the occurrence of stroke in spontaneously hypertensive or salt loaded-Dahl salt-sensitive rats.[3,4,5] ACE-inhibitors are shown to ameliorate ischemic brain metabolism in spontaneously hypertensive rats by preventing the elevation accumulation of tissue lactate which is produced during ischemia or stabilizing the levels of ATP.[6] Moreover, ACE-inhibitors are reported to improve neurologic recovery from cerebral ischemia and reduce mortality rate in spontaneously hypertensive rats.[7,8,9]

Most of the investigations performed about the protective effects of ACE-inhibitors are done in genetically hypertensive rats.[3,4,5,10] Pathological remodeling of cerebral vessels is said to occur during chronic hypertension and this may interfere with the outcome of ACE inhibition and other neuroprotective agents.[5] To elucidate this possibility, therefore, we used transient focal cerebral ischemia as a model of stroke in normotensive rats and investigated whether pre-ischemic ACE inhibition, by systemic administration of enalapril, would be able to improve ischemia/reperfusion (I/R) injuries. Experimental-induced transient cerebral ischemia, after transient middle cerebral artery (MCA) occlusion, is used in the rat to examine the functional impairments that resemble those seen in human stroke.[11] Intraluminal (MCA) occlusion is widely used in experimental animals to induce I/R models of the stroke to evaluate interactions of endogenously induced substances which contribute to the improvement or exacerbation of ischemia in hypertensive,[3,4,5] or diabetic aggravated stroke in the rat.[12]

In this study we induced focal cerebral ischemia by (MCA) occlusion lasted for 60 min and followed by 24 hours reperfusion. We pretreated normotensive rats with a single intraperitoneal injection (IP) of enalapril at 1 hour before induction of cerebral ischemia and evaluated the effectiveness of complete ACE inhibition in improving I/R injuries. We also continuously monitored arterial blood pressure and blood gases to eliminate the possibility of their interactions with the outcome. After 24 hours reperfusion we quantified neurological status, cerebral, and striatal infarction volumes.

**Material and Methods**

Male normotensive Sprague-Dawley rats weighing 300-380 g obtained from the animal house of Shiraz University of Medical Sciences (Shiraz, Iran). Animals were kept under controlled conditions with respect to temperature, humidity and housed on a 12 hours light/12 hours dark cycle with having free access to food and water.

*Surgical procedures and instrumentation*

The animals were fasted overnight before the experiment but had access to water *ad libitum*. Anesthesia induced with chloral hydrate (400mg/kg IP). The trachea cannulated from mouth by a polyethylene catheter to prevent asphyxiation and hypoxia, and give oxygen when needed. Two temperature probes were inserted into the rectum and in the area of left temporalis muscle to record core and the cranial temperatures. Tow separate heating lamps were used to maintain rectal and cranial temperatures at 37±1°C during the experiment. In some rats, which selected randomly, posterior tail artery was cannulated for recording arterial blood pressure and blood sampling.

(MCA) occlusion was induced by intraluminal filament method.[13] In brief, the right common carotid artery was exposed through a midline neck incision and a Poly-L-Lysine-coated nylon thread (3-0) inserted into the internal carotid artery and advanced towards the origin of the middle cerebral artery (MCA) until occlusion occurred. After 60 min (MCA) occlusion, reperfusion continued for 24 hours by gently withdrawing the filament and suturing all insitions.

Arterial blood pressure was recorded continuously from 30 min before (MCA) occlusion until 30 min after termination of occlusion, during reperfusion period. Arterial blood pH, $PaO_2$, $PaCO_2$ saturation ($SaO_2$) was measured 10 min before ischemia, 30min after (MCA) occlusion and 10 min after termination of (MCA) occlusion. Plasma glucose levels were also determined with Blood Glucose Meter (Glucometer, Miles, USA).

After thirty minutes of termination of (MCA) occlusion, the tail catheter was removed and the vessel ligated. After suturing the incisions at the neck region the animal was allowed to recover from anesthesia and returned to a warm cage until the evaluation of its neurological outcome.

*Experimental design*

Rats were randomly assigned to four groups. Group I (n=10) was sham, in which the animal was anesthetized and surgery performed at the neck without inducing (MCA) occlusion or receiving any drug. Group II (n= 6) was ischemic, receiving the vehicle (an IP injection of 1ml/kg distilled water) at 1 hour before (MCA) occlusion followed by 60 occlusion and 24 hours reperfusion. Groups III (n=6) and IV (n= 5) received 0.03 mg/kg and 0.1 mg/kg enalapril (Sigma Chemicals, UK) respectively at

1 hour before (MCA) occlusion, followed by 24 hours reperfusion. From a total 45 rats assigned for the study, 18, rats died during reperfusion period and their results discarded from the study.

*Evaluation of neurological deficit score (NDS)*

Evaluation of NDS with minor modification was carried out 24 hours after termination of (MCA) occlusion.[13,14] The neurological grading system had a five-point scale as the following: Rats with no observable neurological deficits, for example, sham-operated rats, were graded 1. Rats with flexion of contralateral torso or forelimb upon lifting by the tail or failure to extend forepaw when suspended vertically, forelimb flexion and shoulder adduction were graded 2. Grade 3 was assigned to dysfunctional rats circling to contralateral side of the occlusion while having normal posture at rest. Grade 4 was rats with loss of righting reflex and decreased resistance to lateral push, and finally grade 5 consisted rats having no spontaneous motor activity.

*Quantification of infarction volume*

Twenty-four hours after (MCA) occlusion, rats were killed by a high dose of sodium thiopental and decapitated quickly. The brain was removed and cooled by immersing in 4 ℃ normal saline and keeping for 30 min in the refrigerator. Then the brain was sectioned coronally into six 2-mm thick slices using a brain Matrix.[11,12] Afterwards, slices were immersed in 2% Triphenyltetrazolium chloride (TTC) solution at 37℃ for 30 min in a water bath, and then transferred to 10% buffered formalin. Twenty four hours later, slices were photographed with a digital camera connected to a computer, cortical and striatal infarction

ion areas were measured, in $mm^2$, using an Image Analyzer Software.[13] The infarction ion volume, in $mm^3$, of each slice was calculated by multiplying the infarction area by its thickness. The total cortical and striatal infarction volumes of each brain was calculated as the sum of the infarction volumes of all six slices.[13] Since brain edema might significantly affect the accuracy of the estimation of infarction volumes, the calculated infarction volumes were then corrected for brain edema using the formula described by Swanson and colleagues.[15]

*Statistical analysis*

Data are presented as Mean±SEM. Infarction volumes, blood gas values, and other physiological variables are compared using one-way analysis of variance (ANOVA). Where a significant difference was found with ANOVA, the source of difference was located with Sidak and Tukey tests. A value of $p < 0.05$ was considered to be statistically significant.

**Results**

*Physiological parameters*

The values of $PaO_2$, $SaO_2$, $PaCO_2$, pH, blood glucose, as well as head and core temperatures, before and during (MCA) occlusion, as well as the first hour of reperfusion period were in normal physiological range and were not significantly different from each other (table 1).

*Mean arterial blood pressure (MAP)*

The measured MAP before, during (MCA) occlusion, and the beginning of reperfusion period, with the exception of group IV, were in the accepted physiological range and no statistical differences existed among them (table 1). In group IV, administration of 0.1mg/kg enalapril

**Table1:** Physiological parameters before (pre), during (MCA) occlusion and reperfusion in sham (G I), control ischemic (G II) and enalapril treated (0.03 mg/kg; G III) and (0.1 mg/kg; G IV) rats.

| Parameters | G I n=5 | Pre ischemia | | | (MCA) occlusion | | | Reperfusion | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | G II n =5 | G III n=4 | G IV n=5 | G II n =5 | G III n=4 | G IV n=5 | G II n =5 | G III n=4 | G IV n=5 |
| $PaO_2$ (mmHg) | 111 ±9 | 103 ±17 | 110 ±9 | 108 ±13 | 119 ±13 | 104 ±8 | 101 ±8 | 112 ±9 | 108 ±5 | 116 ±18 |
| $SaO_2$ (%) | 97.3 ±0.9 | 96 ±0.9 | 97 ±0.5 | 97 ±0.7 | 98 ±0.6 | 97.3 ±0.7 | 96 ±1 | 98 ±0.3 | 97.7 ±0.4 | 97 ±0.8 |
| $PaCO_2$ (mmHg) | 40.4 ±1.7 | 38 ±3 | 40.3 ±1 | 42 ±0.8 | 38 ±2 | 39.1 ±1.4 | 41 ±1 | 36 ±3 | 36.8 ±2.4 | 37 ±3 |
| pH | 7.31 ±0.01 | 7.33 ±0.02 | 7.34 ±0.02 | 7.32 ±0.01 | 7.31 ±0.02 | 7.33 ±0.02 | 7.29 ±0.02 | 7.31 ±0.02 | 7.32 ±0.01 | 7.27 ±0.04 |
| BG (mg/dl) | 135 ±17 | 136 ±10 | 145 ±33 | 146 ±10 | 148 ±11 | 139 ±19 | 150 ±9 | 147 ±15 | 149 ±41 | 139 ±10 |
| Body T (℃) | 37.2 ±0.12 | 37.4 ±0.1 | 37.17 ±0.12 | 37.3 ±0.06 | 37.2 ±0.09 | 37.3 ±0.15 | 37.3 ±0.07 | 37.2 ±0.1 | 37.5 ±0.1 | 37.2 ±0.1 |
| Head T (℃) | 37.4 ±0.1 | 37.1 ±0.1 | 37.1 ±0.12 | 37.1 ±0.04 | 37.2 ±0.1 | 37.2 ±0.12 | 37.2 ±0.1 | 37.2 ±1 | 37.3 ±0.15 | 37.3 ±0.1 |
| MAP (mmHg) | 94 ±7 | 96 ±4 | 90 ±3 | 59 ±5* | 96 ±2 | 88 ±7 | 67 ±4* | 91 ±5 | 82 ±12 | 60 ±7* |

Values are Mean ± SEM BG = blood glucose; T = temperature; MAP = mean arterial blood pressure, * = values are significantly different from those of group I at P<0.05.

induced, sever hypotension (MAP decreased from 96±7 to 59±5 mmHg) was observed shortly after injection which persisted during (MCA) occlusion and reperfusion period. In this group MAP was significantly lower than of its own pretreated condition and MAP of other groups (table 1).

*Neurological deficit score*

In group I (sham) NDS was 1. In group II (ischemia) NDS increased to 2.83±0.31 (fig 1). Inhibition of ACE with non-hypotensive dose of enalapril (group III) reduced NDS in average by 46%, and improved the neurological outcome (1.33±0.33). However, inhibition of ACE in conjunction with hypotension (group IV) could not improve the observational movements of the animal and did not statistically reduce NDS (fig 1).
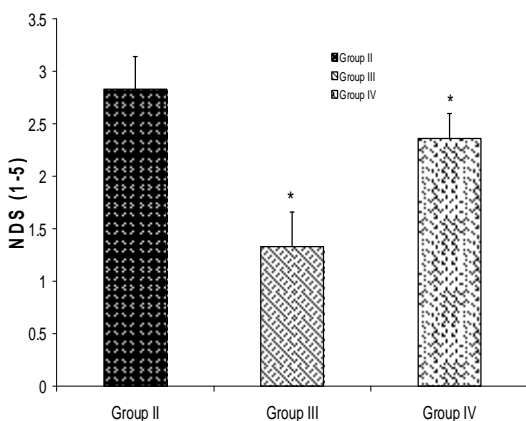


**Fig 1:** Values are mean±SEM for each group. Neurological deficit score (NDS; 1-5) of ischemic rats receiving of rats receiving saline (group II) or enalapril at dose 0.03 mg/kg (Group III) or 0.1 mg/kg (Group IV) at 1 hour before (MCA) occlusion. *denotes significant difference from group II (p<0.05).

*Effect of pre-ischemic ACE inhibition on the cortical and subcortical infarction*

Cortical and striatal infarction volumes of sham operated rats (group I) were zero. Sixty min (MCA) occlusion (group II) produced ischemic lesions both in cortical (206±18 mm$^3$) and striatal regions (72±10 mm$^3$) respectively (fig 2). Pre-ischemic ACE inhibition, with non-hypotensive dose of enalapril (group III) significantly lowered cortical and striatal infarction volumes by 41.6% and 52.7% respectively (fig 2). However, inhibition of ACE in hypotensive conditions (group IV) did not have such protective effects on the infarction volumes of these two regions (fig 2).

**Discussion**

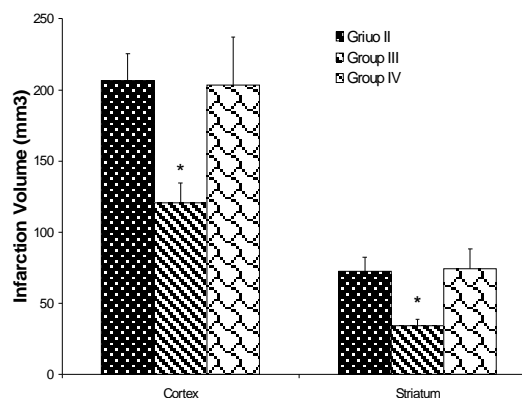Besides the critical role of renin–angiotensin system (RAS) and Ag II in cardiovascular and



**Fig 2:** Values are mean±SEM for each group. Cortical and striatal infarction volumes of ischemic rats receiving saline (group II) or enalapril at dose 0.03 mg/kg (Group III) or 0.1 mg/kg (Group IV) at 1 hour before (MCA) occlusion. * denotes significant difference from group II (p<0.05).

fluid hemostasis, some evidences exist about the role of Ag II in ischemic neuronal injury.[16,17] It is suggested that inhibition of the RAS might be effective not only in reducing the incidence of stroke but also attenuating neuronal injury after brain ischemia.[2]

Enalapril is an ACE-inhibitor which is widely used to treat hypertension.[18] It has renoprotective action in diabetic rats,[19] and shows positive effects in myocardial infarction.[20] However, information concerning the influence of ACE-inhibitors on the ischemic/reperfusion (I/R) injuries is scarce, although several changes in the clinical condition of some patients have been observed with severe alteration in cerebral blood flow.[21] In this study, therefore, we have tried to examine the usefulness of ACE inhibition, using enalapril, on the neurological deficits and the infarction sizes of the I/R injuries in the rat model of cerebral postischemic reperfusion.

The results of the present study indicated that sham operation (group I) did not lead to neurological dysfunctions or cerebral infarction. Although, in this study we did not directly measure cerebral blood flow during (MCA) occlusion, through indirect observations we strongly believed, and also had the support of other investigators,[5,10,22] that the ischemic injuries found in our experimental groups were due to severe curtailment and/or complete obstruction of blood flow to the regions of ischemic areas of the cortex and the striatum.

Reports have specified that alteration of physiological parameters such as blood gases, body temperature, and blood glucose, etc. may exacerbate ischemic brain injuries.[23] To lessen these interactions we have tried to monitor some of these physiological variables like PaO$_2$, PaCO$_2$, pH, head and core temperatures during the course of the experiment. As

shown in table 1, these parameters were all at normal physiological range, and were not statistically different from each other either, in intra or inter-group comparisons, indicating that they did not affect cerebral injuries, as concluded elsewhere.[13]

Arterial blood pressure is an important parameter that influencing regional cerebral blood flow during I/R injuries. Reports have pointed to the fact that mechanisms which regulate regional blood flow are impaired by disorders triggered during ischemia in the cerebral arterioles; hence, cerebral blood flow becomes directly dependent on the arterial blood pressure.[24] Therefore, we think under these conditions alteration of MAP may greatly influence the impact ACE inhibition on the neurological outcome and the cerebral infarction size.

We tested the above conclusion by using non-hypotensive (0.03mg/kg), and hypotensive (0.1 mg/kg) doses of enalapril. Our results implicitly indicated that when ACE inhibition was not accompanied with arterial hypotension, there was significant reductions in the cerebral infarction volumes and considerable improvement in the neurological outcome of I/R injuries (group III; fig 2). However, when severe hypotension was accompanied by ACE inhibition, (group IV; table 1), it did not allow ACE inhibition to illuminate its neuroprotective actions on the ischemic brain (fig 2). This finding is in accordance with the results of other investigators which indicated ACE inhibition with moexipril, when accompanied with hypotension, could not exert its protection against ischemic damage in permanent focal cerebral ischemic rats.[25] Our study too showed that higher doses of enalapril severely reduced mean arterial pressure (table 1), consequently, the neuroprotective actions of enalapril could not improve the neurological outcomes or diminish cerebral infarction volumes of the ischemic rats (fig 2). Hence, from the results of this study and the observations of other investigators, we conclude that RAS activity and the level of Ag II may have a direct correlation with the severity of I/R injuries.[26,17] In addition, it is important to emphasize that the protective action of ACE inhibition will be achieved only if arterial blood pressure does not decrease bellow its physiological levels.[6,24]

## Conclusion

The results of the present study are indicating that pre-ischemic ACE inhibition with non-hypotensive doses of enalapril reduces I/R injuries and improves neurological outcome in the rat model of transient focal ischemia. Therefore, we can conclude that angiotensin II may participate in the exacerbation of ischemic/reperfusion injuries.

## References

1   Alberts MJ. Update on the treatment and prevention of ischaemic stroke. *Curr Med Res Opin* 2003; 19: 438-41.

2   Culman J, Blume A, Gohlke P, Unger T. The renin-angiotensin system in the brain: possible therapeutic implications for AT(1)-receptor blockers. *J Hum Hypertens* 2002; 16: S64-70.

3   Zhou J, Pavel J, Macova M, et al. AT1 receptor blockade regulates the local angiotensin II system in cerebral microvessels from spontaneously hypertensive rats. *Stroke* 2006; 37: 1271-6.

4   Zhou J, Ando H, Macova M, Dou J, Saavedra JM. Angiotensin II AT1 receptor blockade abolishes brain microvascular inflammation and heat shock protein responses in hypertensive rats. *J Cereb Blood Flow Metab* 2005; 25: 878-86.

5   Ito T, Yamakawa H, Bregonzio C, et al. Protection against ischemia and improvement of cerebral blood flow in genetically hypertensive rats by chronic pretreatment with an Angiotensin II AT1 antagonist. *Stroke* 2002; 33: 2297-303.

6   Sadoshima S, Fujii K, Ooboshi H, et al. Angiotensin converting enzyme inhibitors attenuate ischemic brain metabolism in hypertensive rats. *Stroke* 1993; 24: 1561-6.

7   Fujii K, Weno BL, Baumbach GL, Heistad DD. Effect of antihypertensive treatment on focal cerebral infarction ion. *Hypertension* 1992; 19: 713-6.

8   Lee RM, Wang H, Smeda JS. Perindopril treatment in the prevention of stroke in experimental animals. *J Hypertens Suppl* 1996; 14: S29-33.

9   Werner C, Hoffman WE, Kochs E, et al. Captopril improves neurologic outcome from incomplete cerebral ischemia in rats. *Stroke* 1991; 22: 910-4.

10  Nishimura Y, Ito T, Saavedra JM. Angiotensin II AT1 blockade normalizes cere-

brovascular autoregulation and reduces cerebral ischemia in spontaneously hypertensive rats. *Stroke* 2000; 31: 2478-86.

11  Yamori Y, Horie R, Handa H, et al. Pathogenetic similarity of stroke–prone spontaneously hypertensive rats and humans. *Stroke* 1976; 7: 46-53.

12  Kusaka I, Kusaka G, Zhou N, et al. Role of AT1 receptors and NAD(P)H oxidase in diabetes aggravated ischemic brain injury. *Am J Physiol Heart Circ Physiol* 2004; 286: H2442–51.

13  Vakili A, Nekooeian AA, Dehghani GA. L-NAME and 7-Nitroindazole reduces brain injuries in transient focal cerebral ischemia in the rat. *Iran J Med Sci* 2004; 29: 109-15.

14  Vakili A, Nekooeian AA, Dehghani GA. Aminoguanidine reduces infarct volume and improves neurological dysfunction in transient model of focal cerebral ischemia in rat. *Daru* 2006; 14: 31-6.

15  Swanson RA, Morton MT, Tsao-Wu G, et al. A semiautomated method for measuring brain infarct volume. *J Cereb Blood Flow Metab* 1990; 10: 290-3.

16  Unger T, Badoer E, Ganten D, et al. Brain angiotensin: pathways and pharmacology. *Circulation* 1988; 77: I40-54.

17  Walther T,Olah L, Harms C, et al. Ischemic injury in experimental stroke depends on angiotensin II. *FASEB J* 2002; 16: 169-76.

18  Marzo A, Dal Bo L, Mazzucchelli P, et al. Pharmacokinetic and pharmacodynamic comparative study of zofenopril and enalapril in healthy volunteers. *Arzeinmittelforschung* 2002; 52: 233-42.

19  Kalender B, Ozturk M, Tuncdemir M, et al. Renoprotective effects of valsartan and enalapril in STZ-induced diabetes in rats. *Acta Histochem* 2002; 104: 123-30.

20   Walter T, Helber U, Bail D, et al. Influence of ACE inhibition on myocardial damage, the kallikrien-kinin system and hemostasis during cardiopulmonary bypass surgery. *Thorac Cardiovasc Surg* 2002; 50: 150-4.

21  Videbech P, Ravnkilde B, Pedersen TH, et al. The Danish PET/depression project: clinical symptoms and cerebral blood flow. A region-of-interest analysis. *Acta Psychiatr Scand* 2002; 106: 35-44.

22  Hamai M, Iwai M, Ide A, et al. Comparison of inhibitory action of candesartan and enalapril on brain ischemia through inhibition of oxidative stress. *Neuropharmacology* 2006; 51: 822-8.

23  Alonso de Lecinana M, Diez-Tejedor E, et al. Cerebral ischemia: from animal studies to clinical practice. Should the methods be reviewed? *Cerebrovasc Dis* 2001; 11: 20-30.

24  Shima T, Hossman KA, Date h. Pail arterial pressure in cats following midlle cerebral artery occlusion. 1. Relationship to blood flow, regulation of blood flow and electrophysiological function. *Stroke* 1983; 14: 713-9.

25  Ravati A, Junker V, Kouklei M, et al. Enalapril and moexipril 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.

26  Unger T. Inhibiting angiotensin receptors in the brain: possible therapeutic implications. *Curr Med Res Opin* 2003; 19: 449-51.