

## POST-ISCHEMIC TREATMENT WITH CANDESARTAN PROTECTS FROM CEREBRAL ISCHEMIC/ REPERFUSION INJURY IN NORMOTENSIVE RAT

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### ABSTRACT

Large body of evidences has indicated that the renin – angiotensin system (RAS) and its effector peptide Angiotensin II may be involved in the pathophysiology of stroke. Previous studies showed that preischemic RAS inhibition reduced brain injury but effects of post-ischemic RAS inhibition on ischemic/reperfusion injuries have not completely been elucidated. Therefore, the present study has investigated the effects of post-ischemic AT1 receptor blocked with candesartan treatment on cerebral infarction and motor function following transient focal cerebral ischemia in normotensive rats.

Male normotensive rats were studied in three groups as sham, ischemic control and ischemic group which received candesartan (0.3mg/kg) at the beginning of reperfusion period. Transient focal cerebral ischemia was induced by 90 min occlusion of the left middle cerebral artery, followed by 24 hr reperfusion. Neurological deficit score (NDS ) evaluated at the end of the reperfusion period. Total, cortical and striatal infarct volumes were determined using TTC staining technique.

Animals in sham group had normal motor function and no ischemic lesions were observed in their cortical or striatal regions. MCAO in control ischemic group produced considerable infarctions in cortex and striatum in conjunction with severe impaired motor functions. AT1 receptor blocked with candesartan significantly reduced infarct volumes in the cortex and the striatum with improvement in NDS compared to control ischemic group.

In conclusion, the results of the present study indicated that AT1 receptor blocked with candesartan can decrease ischemic brain injury and improve neurological outcome.

**Keyword:** RAS, Candesartan, AT1 receptor, Focal cerebral ischemia, Rat.

### INTRODUCTION

Ischemic stroke remains the third leading cause of invalidism and death in industrialized countries. Ischemic brain injury is resulted from a complex sequence of pathophysiological events that develop over time and space <sup>1</sup>. Renin-angiotensin system (RAS) has traditionally been linked to the regulation of blood pressure and salt and water homeostasis. Beside its multiple physiological functions, RAS has been implicated in pathogenesis and outcome of ischemic injuries in vital organs such as heart <sup>2</sup> and kidney <sup>3</sup>. Furthermore, it is suggested that RAS may contribute in stroke related pathogenic mechanisms and involve in the ischemic brain damage <sup>4</sup>. It was showed that inhibition of the renin-angiotensin system might be effective not only in reducing the incidence of the stroke but also attenuating neuronal injury after stroke <sup>5</sup>. It was reported that pre-treatment with AT1 antagonists, improves neurological activity and reduces cerebral infarction volume in normotensive <sup>6</sup> and hypertensive rat <sup>7</sup>. As far as the literature is concerned, only a few studies have addressed the effects of post-ischemic RAS inhibition on ischemic brain injury. Therefore, this study designed to investigate the influence of AT1 receptor blocked by candesartan post-ischemic treatment on brain injury and neurological outcome following transient focal cerebral ischemia in normotensive rat.

### MATERIAL AND METHODS

Male normotensive Sprague Dawley rats (280-320 g) were obtained from central animal house facility of Ardabil University of Medical Sciences (Ardabil, Iran). All protocols of the study were approved by the institutional animal ethics committee of Ardabil University of Medical Sciences which follows the NIH guidelines for care and use of animals. Anesthesia made by injection of chloral hydrate (400mg/kg, IP). Body temperature was maintained at 37±1°C with a heating feedback control system.

#### Laser – Doppler Flowmetry

Regional cerebral blood flow (rCBF) was continuously monitored in the cerebral cortex of the left hemisphere in the supply territory

of middle cerebral artery (MCA) using laser Doppler flowmeter pencil probe(MNP100, AD instrument, Australia) <sup>8</sup> from 10 min before MCAO until 10 min after MCAO. Baseline CBF values measured before MCAO, were defined as 100%. MCAO was documented by a decrease in laser Doppler signals to lower than 20% of baseline.

#### Experimental protocol

Effects of post-ischemic candesartan treatment on brain injury and neurological score were investigated in randomly divided 3 groups of animals as follows:

Sham group (n=8), rats underwent the surgery at the neck region without being exposed to MCAO and received the vehicle, Sodium carbonate (SC) solution (0.1 normal) at the end of surgery.

Control ischemic group (n=8), rats experienced brain ischemia by 90 min MCAO followed by 24 hr reperfusion and received the vehicle (SC) at the beginning of reperfusion time.

Ischemic group post-treated with Candesartan (n=8), rats experienced ischemia and reperfusion as same as second group and received candesartan (0.3 mg/kg, IV, LKT laboratories, Inc, USA) at the beginning of reperfusion time.

#### Induction of transient focal cerebral ischemia

Ninety minutes MCAO and 24 hr reperfusion of the left cerebral hemisphere carried out by intra-luminal filament method described by Belayev et al <sup>9</sup>. Neurological deficits were assessed 24 hr after MCAO using 6 point scale: 1= Normal motor function, 2= Flexion of contralateral forelimb when suspended vertically by tail, 3= Circling when pulled with the tail to each side, 4= Spontaneous circling to each side, 5= Leaning to contralateral side and loss of righting reflex, 6= No spontaneous motor activity <sup>10</sup>.

After NDS assessment animals were slaughtered under deep anesthesia and brains removed. Frontal sections of 2-mm thick slices were prepared using a brain matrix and stained with

triphenyltetrazolium chloride (TTC). After staining, the slice images were digitized using a Cannon camera and cerebral infarction volume areas were determined using computer based NIH image analyzer software<sup>10,11</sup>.

#### Statistical analyses

Values are expressed as mean±SEM. Independent T test and one-way ANOVA with post-hoc of Duncan's test were used for comparisons. Statistical significance was accepted at  $P<0.05$ .

### RESULTS

#### Cerebral blood flow recording

rCBF was reduced to less than 20% baseline in Control and candesartan treated ischemic groups. There was no significant

difference in rCBF among groups during MCAO and beginning of the reperfusion period.

#### Evaluation of neurological deficit score

The NDS of control ischemic rats receiving vehicle was significantly higher than that of sham-operated rats. Post-ischemic treatment with candesartan significantly lowered NDS and improved motor function (Fig 1).

#### Assessment of cerebral infarct volumes

The sham-operated rats had no infarctions. Total infarct volume in ischemic rats receiving candesartan at 0.3mg/kg was significantly lower than control ischemic rats. Compared to control ischemic rats, candesartan-treated rats had significantly lower cortical and striatal infarct volumes (Fig 2, 3).

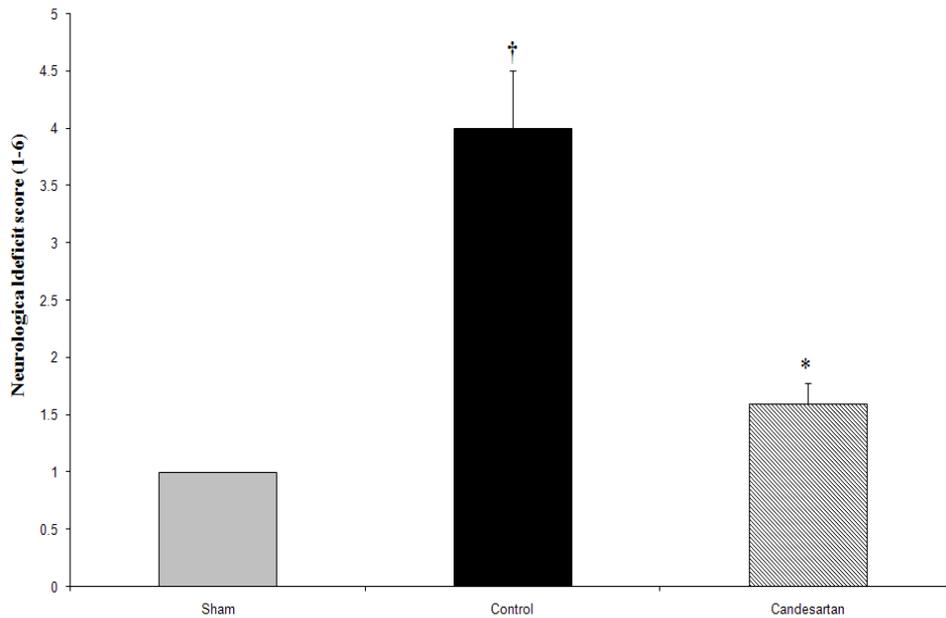


Fig. 1: Neurological deficit score in sham, control and candesartan treated rats. (\* $p<0.05$  vs. control, † $p<0.01$  vs. sham).

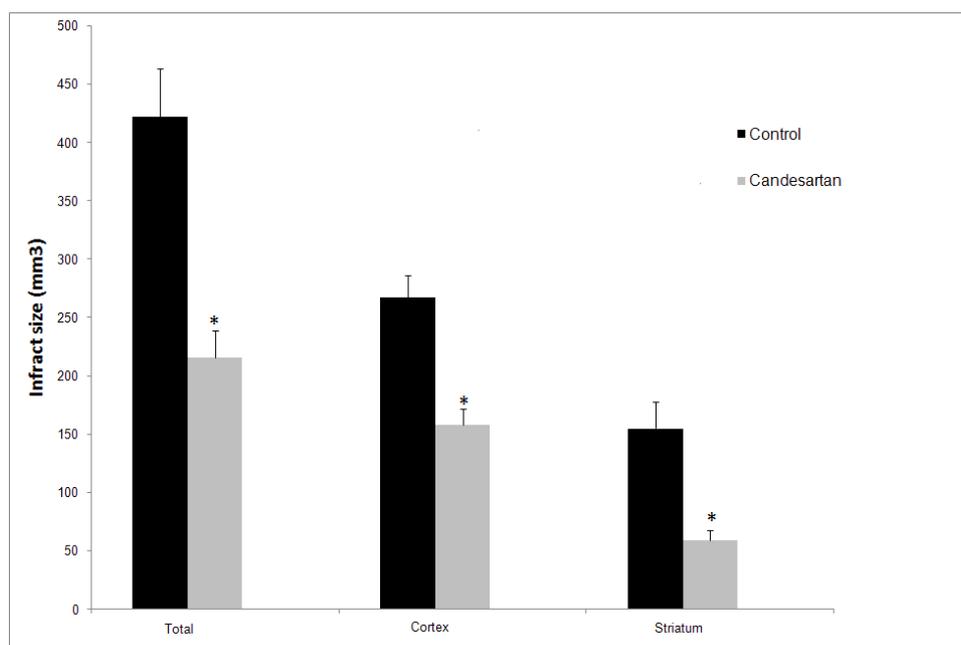


Fig. 2: Total, Cortical and striatal infarct volumes in control and candesartan treated rats. (\* $p<0.05$  vs. control).



**Fig. 3: Representative brain slices stained with TTC in sham, control and candesartan treated rats. Ischemic regions are colored white and non ischemic regions are red.**

## DISCUSSION

Most actions of Angiotensin II are mediated through AT1 receptor and AT1 receptor antagonists are widely used in treatment of cardiovascular diseases<sup>12</sup>. Since, these receptors contribute to stroke-related pathophysiological mechanisms such as hypertension, atherothrombosis and cardiac hypertrophy<sup>4</sup>, it is possible that Angiotensin II through stimulation of AT1 receptor aggravates ischemic/reperfusion injuries. Previous studies showed that pre-ischemic blockade of AT1 receptor produces protective effects on ischemic brain injuries<sup>6, 7</sup>. The results of this study revealed that the post-ischemic blockade of AT1 receptor reduced cortical and striatal infarct volumes and improved neurological motor deficits. These findings are in agreement with reports of other investigators demonstrating that AT1 receptor blockade reduced infarct size in transient cerebral ischemia in normotensive rats<sup>13</sup> or in hypertensive rats<sup>14,15</sup>. In contrast, Fu et al<sup>16</sup> reported that inhibition of AT1 receptor by treatment of the animal with candesartan did not reduce ischemic brain injuries in the rat. This discrepancy is said to be due to hypotension because these researchers saw a significant reduction in MAP after ischemia<sup>16</sup>. Profound drop in MAP hypoperfuses the ischemic area of the brain and prevent the reduction of cerebral infarct size<sup>17</sup>.

Various mechanisms might be responsible for the beneficial effects of AT1 receptor blockade in brain ischemia. Such effects might be partly attributed to their stabilization actions on the impaired cerebrovascular autoregulation in penumbra<sup>18-20</sup>. In addition, anti-apoptotic mechanisms may help the manifestation of protective effects of AT1 receptors blockade<sup>21</sup>. It was suggested that AT1 receptor activation initiates proapoptotic stimuli in neuronal cells. Thus, anti-apoptotic effects of AT1 receptor blockade may have beneficial effects on ischemic brain injury<sup>22</sup>. Oxidative stress is known to be responsible for molecular and cellular tissue damage mechanisms in wide range of diseases<sup>23</sup> including stroke. The beneficial effects of AT1 receptor blockade might also be attributed to the reduction of ROS production. The activation of AT1 receptor results in the production of superoxide, whereas its blockade was associated with the reduction of superoxide<sup>24</sup> and peroxynitrite<sup>25</sup>.

## CONCLUSION

In conclusion, the results of this study demonstrate that post-ischemic inhibition of the RAS by an AT1 receptor antagonist, candesartan reduces cerebral infarction volume, and improve neurological motor activity in normotensive rats exposed to transient MCA occlusion.

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