Does inhibition of angiotensin function cause neuroprotection in diffuse traumatic brain injury?

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A R T I C L E I N F O	**A B S T R A C T**
	Objective(s): Neuroprotection is created following the inhibition of angiotensin II type 1 receptor (AT1R). Therefore, the purpose of this research was examining AT1R blockage by candesartan in diffuse traumatic brain injury (TBI). ***Materials and Methods:*** Male rats were assigned into sham, TBI, vehicle, and candesartan groups. Candesartan (0.3 mg/kg) or vehicle was administered IP, 30 min post-TBI. Brain water and Evans blue contents were determined, 24 and 5 hr after TBI, respectively. Intracranial pressure (ICP) and neurologic outcome were evaluated at -1, 1, 4 and 24 hr after TBI. Oxidant index [malondialdehyde (MDA)] was determined 24 hr after TBI. ***Results:*** Brain water and Evans blue contents, and MDA and ICP levels increased in TBI and vehicle groups in comparison with the sham group. Candesartan attenuated the TBI-induced brain water and Evans blue contents, and ICP and MDA enhancement. The neurologic score enhanced following candesartan administration, 24 hr after TBI. ***Conclusion:*** The blockage of AT1R may be neuroprotective by decreasing ICP associated with the reduction of lipid peroxidation, brain edema, and blood-brain barrier (BBB) permeability, which led to the improvement of neurologic outcome.

Introduction

Traumatic brain injury (TBI), as a global health problem, occurs in both industrialized and developing countries and has been considered as a major cause of mortality and morbidity (1). Half of the deaths after TBI are caused by unsuccessfully controlling the brain edema and increased intracranial pressure (ICP) (2, 3), which ultimately lead to brain ischemia. Most TBIs result from blunt impacts and the remaining are induced by penetrating injury (4). Diffuse and focal mechanical damages inflicted on the brain at the time of the impact are primary damages of TBI, which result in the immediate and irreversible neuronal death (5). Primary damage is exacerbated by activating many different signaling pathways in minutes to days following injury. The activation of these pathways results in secondary injury by blood–brain barrier (BBB) damage, edema formation, increased inflammatory response and ICP, oxidant activity, and cell death (6). Therefore, a drug with multimodal action would be helpful against the multiple harmful secondary pathways activated by brain injury.

The modulation of the renin–angiotensin system (RAS) leads to anti-inflammatory, antioxidant, anti-apoptosis, anti-edema, angiogenesis, neuroprotective, hypotensive, and vasodilator effects in neurovascular disorders (7). Neuroprotection in the presence of reduced angiotensin (Ang) II formation has been proposed in stroke (8). The blockage of angiotensin II type 1 receptor (AT1R) within 4 hr after controlled cortical impact (CCI) effectively reduced secondary brain injury and neurologic disturbance (9).

RAS is expressed in the brain, in addition to the systemic one (10, 11). Angiotensin II results from angiotensinogen in the brain (12). The up-regulation of gene expression of angiotensinogen and AT2R occurs following experimental TBI (13). AT1R plays a key role in the creation of secondary brain injury post-TBI (13). The overstimulation of AT1R results in vasoconstriction, pro-oxidant, cell death, and inflammation leading to neuronal injury (7). In contrast, AT2R promotes vasodilation, anti-inflammation, neurogenesis, angiogenesis, antioxidant, and differentiation effects (7).

AT1R antagonists, angiotensin II receptor blockers (ARBs) or sartans, have been proposed for neuroprotection in animal models of stroke (14) via improvement in neurological outcome and brain circulation (15, 16), and a reduction in inflammatory and oxidative response and

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also apoptosis (17, 18). Candesartan, an antagonist of AT1R, was partially protective in animals subjected to controlled cortical impact (CCI) when given before or after injury (13, 19). ARBs have multiple mechanisms of action within the brain by binding two receptors, AT1R and peroxisome proliferator-activated receptor γ (PPARγ) (9).

The useful properties of ARBs and their known efficacy in treating stroke have led to investigation of their potential for treating TBI. Since the effect of ARBs in diffuse TBI has not been determined, therefore, the aim of this research was to investigate the effect of candesartan on brain edema, oxidant response, and neurologic recovery after diffuse TBI.

Materials and Methods

Experimental groups
The protocol was approved by an ethical committee (no. A/94/27) in Kerman University of Medical Sciences, in agreement to internationally approved guidelines for animal use and care, as indicated in the European community guidelines (EU Directive of 2010; 010/63/EU) or US guidelines (NIH publication #85-23, revised in 1985). The male adult Wistar rats weighing 250–300 g were bought for this interventional-experimental study. The animals were maintained in a light (on 7:00 a.m. to 7:00 p.m.) and temperature (21 ± 1 °C) controlled environment with food and water available.

Rats were randomly divided into sham (control), TBI, vehicle of candesartan (Veh), and candesartan (Can) groups (12 per group). Six rats in each group were assigned for determining BBB permeability and the other six rats for evaluating brain edema, ICP, and neurological outcome. All evaluations were done by an expert blinded to the study groups.

Candesartan (LKT, USA) (0.3 mg/kg) (20) was dissolved in 0.1% saline and 0.1 N Na2CO3 at pH=7.4 and injected intraperitoneally, 30 min after TBI. Saline and Na2CO3 were injected in the vehicle group instead of candesartan.

TBI protocol
All animals were intubated before surgery. Diffuse TBI was induced by the Marmarou method in animals anesthetized with ketamine (60 mg/kg) and xylazine (10 mg/kg), except the sham group. The TBI protocol has been explained in detail in our previous studies (21, 22). Briefly, a moderate TBI was performed by dropping a 250–300 g weight, from a 2 m height onto a metal disk attached to the animal's skull. Then, the rats with respiratory problems were connected to a respiratory pump (TSA animal respiratory compact, Germany). In the sham group, all stages of the TBI protocol were performed except the weight-drop. The mortality rate was recorded during the experiment (20–25%).

Brain edema assessment
The brain edema was measured by calculating brain water content as previously detailed (22). Briefly, the brain of anesthetized animals was removed 24 hr after TBI and the injured half was weighed (wet weight). The brain sample was then dried in an oven (Memmert, Germany) at 60 °C for 72 hr and was reweighed (dry weight). The percentage of brain water was then calculated using the following formula: (100 × [(wet weight–dry weight) / wet weight]).

Evaluation of BBB permeability
The brain extravascular leakage of the injected Evans blue (EB) dye was determined for evaluating BBB permeability as previously described in detail (23). Briefly, 20 mg/kg Evans blue dye 2% was injected into a jugular vein of anesthetized rats, 4 hr after surgery. Five hours after surgery, intravascular Evans blue was removed by perfusion. Then, the brain was weighed, homogenized, and inserted in a solution containing sodium sulfate and acetone on a shaker for 24 hr. In the next step, Evans blue absorbance of the supernatant followed by centrifuge was determined at 620 nm. The brain extravascular leakage of the dye was calculated as micrograms per gram brain tissue.

Intracranial pressure level
The recording of ICP level was performed as previously stated in detail (24). Briefly, a 20-gauge needle connected to a pressure transducer in a recording system (AD Instruments, Australia) was placed in the cisterna magna of the animal post-anesthesia. The ICP levels of all groups were recorded at -1, 1, 4, and 24 hr post-TBI.

Motor function evaluation
The motor performance was reported according to a motor score of veterinary coma scale (VCS) similar to another study (24). Scoring range was from 1 to 8 as 1: Flaccid to stimuli; 2: Extensor posturing (spontaneous or to stimuli); 3: Spontaneous pedaling; 4: Withdraws or pedals to pinch; 5: Lethargic, withdraws to pinch, and lifts head with attention to visual stimuli; no sternal recumbence; 6: Lethargic, unable to stand, but maintains sternal recumbence; 7: Mildly drowsy with spontaneous, purposeful movements; 8: Normal movement. The assessment of motor function was performed at -1, 1, 4, and 24 hr post-TBI.

Brain level of malondialdehyde (MDA)
The level of MDA was obtained using the thiobarbituric acid method (25). Briefly, a cerebral hemisphere was precipitated in 10% trichloroacetic acid (TCA), and the pink color resulting from the thiobarbituric acid reaction was assessed at 535 nm. The level of MDA was expressed as nanomoles per milligram (nmol/mg) using the standard curve of tetramethoxypropane.

Statistical analysis
Data of the study were described as mean ± SEM. Shapiro–Wilk's W test was performed for checking the normality of the data. The comparison of groups was performed each time using one-way analysis of variance (ANOVA) and Tukey's *post hoc* test due to interaction between the groups and the times, for evaluating ICP and motor function, the same as analyzing the permeability of BBB and the brain edema. $P < 0.05$ was considered statistically significant.

Results

Brain water content
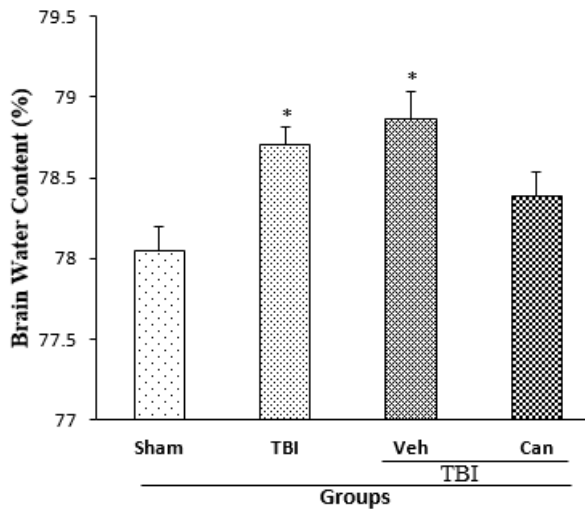The change in brain water content by candesartan is

Figure 1. The effect of candesartan administration on brain water content in male rats, 24 hr after traumatic brain injury (TBI) (n=6 in each group). Data are described as mean±SEM. *$P<0.05$ vs sham group. Veh: vehicle; Can: candesartan

Figure 2. The effect of candesartan administration on Evans blue content in male rats, 5 hr after traumatic brain injury (TBI) (n=6 in each group). Data are described as mean±SEM. #$P<0.05$ vs TBI group; †$P<0.05$ vs Veh group; **$P<0.01$ vs sham group; ***$P<0.001$ vs sham group. Veh: vehicle; Can: candesartan

represented in Figure 1. TBI resulted in increased brain water content in TBI and vehicle groups in comparison to the sham group, 24 hr post-TBI ($P<0.05$). The amount of brain water was not statistically different between the candesartan group and other groups.

Brain Evans blue content

The effect of candesartan administration on brain EB content is reported in Figure 2. TBI increased the brain EB content in TBI and vehicle groups compared to the sham group 5 hr post-TBI ($P<0.001$, $P<0.01$, respectively). The administration of candesartan decreased TBI-induced BBB disruption ($P<0.05$). The brain EB content was significantly different between candesartan and sham groups ($P<0.001$).
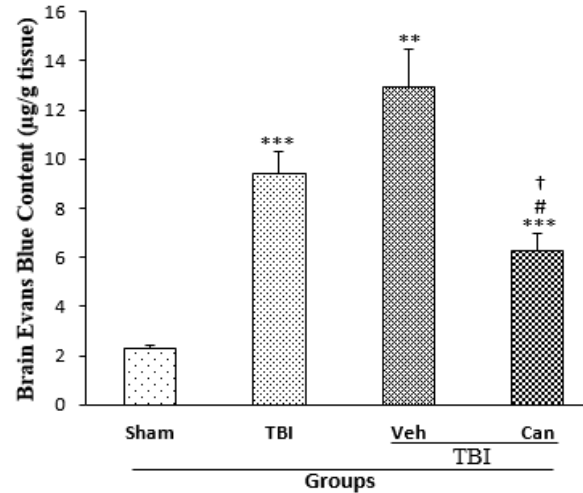
ICP level

The ICP levels of different groups at -1, 1, 4, and 24 hr post-TBI are shown in Figure 3. Before TBI (at -1 hr post-TBI), there was no statistical difference in ICP levels between the groups. TBI resulted in an increase of ICP levels in TBI and vehicle groups compared to that of the sham group post-TBI ($P<0.001$). The ICP level of the TBI group was significantly different from that of the vehicle group at 1 and 4 hr post-TBI ($P<0.001$, $P<0.001$, respectively). The candesartan diminished TBI-induced ICP increase ($P<0.001$). The ICP level was different between candesartan and sham groups ($P<0.001$).

Motor function of veterinary coma scale

The motor scores in different groups at -1, 1, 4, and 24 hr post-TBI are indicated in Figure 4. The motor score
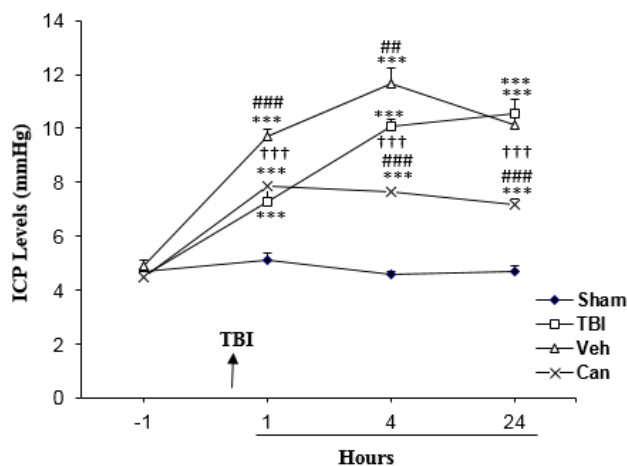

Figure 3. The intracranial pressure (ICP) level of study groups at different times after traumatic brain injury (TBI) (n=6 in each group). Data of ICP levels are presented as mean±SEM. ## $P<0.01$ vs TBI group at 4 hr after TBI; ***$P<0.001$ vs sham group at 1, 4, and 24 hr after TBI; ###$P<0.001$ vs TBI group at 1, 4, and 24 hr after TBI; ††† $P<0.001$ vs Veh group at 1, 4, and 24 hr after TBI. Veh: vehicle; Can: candesartan
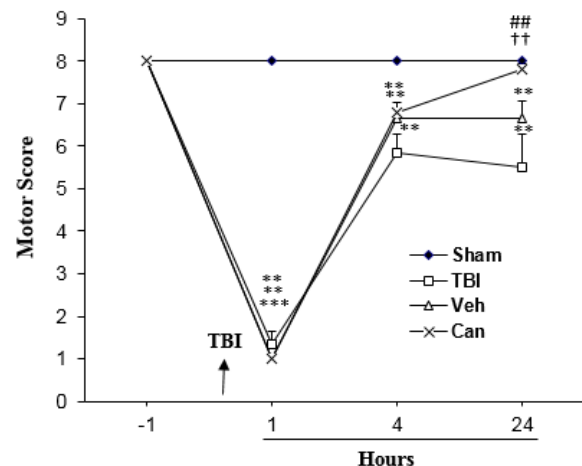

Figure 4. The effect of candesartan on the motor score of veterinary coma scale (VCS) before traumatic brain injury (TBI) and at different times after TBI (n=6 in each group). Data of study groups are described as mean±SEM. **$P<0.01$ vs sham group at 1, 4, and 24 hr after TBI; ##$P<0.01$ vs TBI group at 24 hr after TBI; ††$P<0.01$ vs candesartan group at 24 hr after TBI; ***$P<0.001$ vs sham group at 1 hr after TBI. Veh: vehicle; Can: candesartan
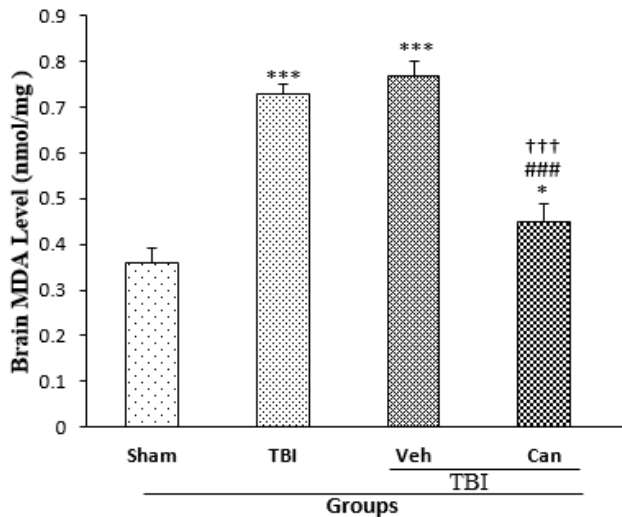
Figure 5. The effect of candesartan administration on brain MDA level in male rats, 5 hr after traumatic brain injury (TBI) (n=6 in each group). Data of brain MDA levels are described as mean±SEM. ***P < 0.001 vs sham group; ###*P*<0.001 vs TBI; †††*P*<0.001 vs Veh group; *P*<0.05 vs sham group. Veh: vehicle; Can: candesartan, MDA: malondialdehyde

was not statistically different among the groups, before surgery. Brain injury decreased motor score in TBI and vehicle groups compared to that of the sham group post-TBI (*P*<0.01). Candesartan could not recover motor function at 1 and 4 hr post-TBI, but it happened at 24 hr post- TBI in comparison with that of TBI and vehicle groups. The motor score was not different between candesartan and sham groups at 24 hr post- TBI.

Level of brain MDA

The effect of candesartan administration on the MDA level is represented in Figure 5. Injury increased the brain MDA level in TBI and vehicle groups in comparison to the sham group, 24 hr post-TBI (*P*<0.001). The MDA level declined following candesartan administration post-TBI (*P*<0.001). The brain MDA level was different between candesartan and sham groups (*P*<0.05).

Discusion

Inflammation is a major part of the pathophysiology of TBI (26, 27). The anti-inflammatory action of ARBs has been illustrated in various disorders (28). It has been suggested that angiotensin II signaling through the AT1Rs may play a major role in the progression of TBI (9). In the current study, for the first time, the neuroprotective effect of candesartan administration, as an ARB, was investigated in experimental diffuse TBI. In this survey, the administration of candesartan post-TBI attenuated brain edema, lipid peroxidation, BBB permeability, and ICP enhancement, and improved neurologic disturbance. Interestingly, candesartan administration did not alter mean arterial blood pressure in treated rats (data not indicated).

The neurologic outcome is induced in TBI due to neuro-inflammatory responses including, the development of brain edema, the disruption of BBB (29), an acute increase in pro-inflammatory cytokines (30), and ICP (31). Brain edema is a life-threatening event

in brain disturbances that significantly worsens the brain injury (32) via increased ICP level (33). Therefore, prevention of the brain edema expansion may decrease brain injury and mortality in TBI.

In the present research, TBI- increased BBB permeability and lipid peroxidation, and ICP level significantly declined by candesartan. Candesartan did not result in significantly decreased brain water content in comparison to the vehicle group, but water content was not different between sham and candesartan groups. The reduction in cerebral infarction volume and brain edema by candesartan in transient MCA (middle carotid artery) occlusion is in agreement with the current study (20). However, candesartan did not decrease brain edema in mice with CCI (13). The controversial findings could be attributed to the difference in the method of injury induction, dose and method of treatment, and the animal studied.

Studies showed that an increase in oxidant (34) and inflammatory activities (34, 35) results in the BBB disruption. Also, evidence suggests that activation of the RAS, especially Ang II causes a prolonged increase in the permeability of BBB mediated by AT1-Rs (36) probably via the production of superoxide and peroxynitrite (37). Candesartan suppressed lipid peroxidation and increased endogenous antioxidant defense capacity in a model of TBI (20). Also, inhibition of inflammatory mediators (i.e IL-1β, IL-6, and TNF-α) by candesartan was indicated in CCI mice (13). Brain edema aggravates the primary brain injury by negatively affecting the perfusion of penumbra due to the compression of cerebral vasculature via increased ICP (38, 39). Therefore, it is proposed that candesartan could reduce brain edema and the next ICP by antioxidant and anti-inflammatory effects on the BBB integrity in diffuse TBI. However, this suggestion needs more investigation for confirmation.

In line with the brain edema and ICP level results at 24 hr after TBI, neurologic impairment evaluated by the motor score of VCS was improved by candesartan administration in the present research. It is known that biochemical and molecular changes in TBI are accompanied by the enhancement of brain edema and brain infarction, deterioration of neurological function, and higher mortality (40). There is a number of reports supporting a neuroprotective role for candesartan in CNS disturbances. Candesartan decreased lipid peroxidation and cerebral infarction, and improved neurological outcome in ischemic brain injury (40). Administration of candesartan after experimental TBI reduced cerebral inflammation and improved neurologic recovery (13). A hypotensive dose of candesartan could improve functional outcome and reduce edema in animals with large strokes (7). However, a hypotensive dose (1 mg/kg) of candesartan was not advantageous in a model of TBI, possibly because of the blood pressure reduction (13). Brain edema is a prime cause of neurologic function impairment post-TBI (41). Also, the improvement of neurologic outcome can occur following ICP decrease in brain injury (42). According to the results of current research, it is supposed that candesartan could improve neurologic outcome, which is mediated by brain edema reduction.

Candesartan crosses BBB and causes a long-lasting blockade of cerebral AT1 receptors (43, 44). The neuroprotective mechanisms of candesartan in diffuse TBI might be various. Such effects might be attributed to the protection of BBB integrity (45), anti-apoptotic mechanisms (46), a reduction in the production of ROSs (47), attenuation of brain inflammation and microglia activation, reduction in central sympathetic tone (45), and activation of PPAR-γ (48). Another possible mechanism of candesartan is the effect of AngII through the AT2R receptor. It has been reported that inhibition of AT1R receptor triggers AngII function through AT2R with anti-inflammatory activity (49).

Conclusion

The present findings indicate that inhibition of AT1 after diffuse TBI results in neurologic improvement probably mediated by decreasing brain edema, BBB disruption, oxidant activity, and ICP level. These results indicate that AT1 has a major role in the development of secondary brain damage after diffuse TBI. Investigation of exact neuroprotective mechanisms of candesartan in diffuse TBI is the subject of possible future research.

Conflict of Interest

The authors have no conflicts of interest to declare.

Acknowledgment

References

1. Teasdale G, Jennett B. Assessment of coma and impaired consciousness: a practical scale. Lancet 1974; 304:81-84.
2. Feickert H-J, Drommer S, Heyer R. Severe head injury in children: impact of risk factors on outcome. J Trauma Acute Care Surg 1999; 47:33-38.
3. Stahel PF, Kariya K, Shohami E, Barnum SR, Eugster H-P, Trentz O, *et al.* Intracerebral complement C5a receptor (CD88) expression is regulated by TNF and lymphotoxin-α following closed head injury in mice. J Neuroimmunol 2000; 109:164-172.
4. Faul M, Xu L, Wald M, Coronado V. Traumatic brain injury in the United States: emergency department visits, hospitalizations, and deaths. Atlanta: Centers for Disease Control and Prevention. National Center for Injury Prevention and Control 2010.
5. Yakovlev AG, Faden AI. Mechanisms of neural cell death: implications for development of neuroprotective treatment strategies. NeuroRx 2004; 1:5-16.
6. O'Connor WT, Smyth A, Gilchrist MD. Animal models of traumatic brain injury: a critical evaluation. Pharmacol Ther 2011; 130:106-113.
7. Fouda AY, Artham S, El-Remessy AB, Fagan SC. Renin–angiotensin system as a potential therapeutic target in stroke and retinopathy: experimental and clinical evidence. Clin Sci 2016; 130:221-238.
8. Maeda A, Okazaki T, Inoue M, Kitazono T, Yamasaki M, Lemonnier FA, *et al.* Immunosuppressive effect of angiotensin receptor blocker on stimulation of mice CTLs by angiotensin II. Int Immunopharmacol 2009; 9:1183-1188.
9. Villapol S, Balarezo MG, Affram K, Saavedra JM, Symes AJ. Neurorestoration after traumatic brain injury through angiotensin II receptor blockage. Brain 2015; 138: 3299-3315.
10. Saavedra JM. Brain and pituitary angiotensin. Endocr Rev 1992; 13:329-380.
11. Wright JW, Harding JW. Brain renin-angiotensin—a new look at an old system. Prog Neurobiol 2011; 95:49-67.
12. Paul M, Mehr AP, Kreutz R. Physiology of local renin-angiotensin systems. Physiol Rev 2006; 86:747-803.
13. Timaru-Kast R, Wyschkon S, Luh C, Schaible E-V, Lehmann F, Merk P, *et al.* Delayed inhibition of angiotensin II receptor type 1 reduces secondary brain damage and improves functional recovery after experimental brain trauma. Critical care medicine 2012; 40:935-944.
14. Thöne-Reineke C, Steckelings UM, Unger T. Angiotensin receptor blockers and cerebral protection in stroke. J Hypertens 2006; 24:S115-S121.
15. Bennai F, Morsing P, Paliege A, Ketteler M, Mayer B, Tapp R, *et al.* Normalizing the expression of nitric oxide synthase by low-dose AT1 receptor antagonism parallels improved vascular morphology in hypertensive rats. J Am Soc Nephrol 1999; 10:S104-115.
16. Ishrat T, Pillai B, Soliman S, Fouda AY, Kozak A, Johnson MH, *et al.* Low-dose candesartan enhances molecular mediators of neuroplasticity and subsequent functional recovery after ischemic stroke in rats. Mol Neurobiol 2015; 51:1542-1553.
17. Kozak W, Kozak A, Johnson MH, Elewa HF, Fagan SC. Vascular protection with candesartan after experimental acute stroke in hypertensive rats: a dose-response study. J Pharmacol Exp Ther 2008; 326:773-782.
18. Jung K-H, Chu K, Lee S-T, Kim S-J, Song E-C, Kim E-H, *et al.* Blockade of AT1 receptor reduces apoptosis, inflammation, and oxidative stress in normotensive rats with intracerebral hemorrhage. J Pharmacol Exp Ther 2007; 322:1051-1058.
19. Villapol S, Yaszemski AK, Logan TT, Sánchez-Lemus E, Saavedra JM, Symes AJ. Candesartan, an angiotensin II AT1-receptor blocker and PPAR-γ agonist, reduces lesion volume and improves motor and memory function after traumatic brain injury in mice. Neuropsychopharmacology 2012; 37:2817-2829.
20. Panahpour H, Bohlooli S, Motavallibashi S. Antioxidant activity-mediated neuroprotective effects of an antagonist of At1 receptors, candesartan, against cerebral ischemia and edema in rats. Neurophysiol 2013; 45:441-447.
21. Soltani Z, Khasksari M, Shahrokhi N, Nakhaei N, Shaibani V. Effect of combined administration of estrogen and progesterone on brain edema and neurological outcome after traumatic brain injury in female rats. IJEM 2009; 10:629-638.
22. Khaksari M, Soltani Z, Shahrokhi N, Moshtaghi G, Asadikaram G. The role of estrogen and progesterone, administered alone and in combination, in modulating cytokine concentration following traumatic brain injury. Can J Physiol Pharmacol 2010; 89:31-40.
23. Soltani Z, Khaksari M, Shahrokhi N, Mohammadi G, Mofid B, Vaziri A, *et al.* Effect of estrogen and/or progesterone administration on traumatic brain injury-caused brain edema: the changes of aquaporin-4 and interleukin-6. J Physiol Biochem 2016; 72:33-44.
24. Soltani Z, Khaksari M, Jafari E, Iranpour M, Shahrokhi N. Is genistein neuroprotective in traumatic brain injury? Physiol Behav 2015; 152:26-31.
25. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979; 95:351-358.
26. Finnie J. Neuroinflammation: beneficial and detrimental effects after traumatic brain injury. Inflammopharmacology 2013; 21:309-320.
27. Johnson VE, Meaney DF, Cullen DK, Smith DH. Animal models of traumatic brain injury. Handb Clin Neurol 2015; 127:115-128.
28. Benigni A, Cassis P, Remuzzi G. Angiotensin II revisited: new roles in inflammation, immunology and aging. EMBO Mol Med 2010; 2:247-257.
29. Ballabh P, Braun A, Nedergaard M. The blood–brain barrier: an overview: structure, regulation, and clinical implications. Neurobiol Dis 2004; 16:1-13.

30. Sarkaki AR, Khaksari Haddad M, Soltani Z, Shahrokhi N, Mahmoodi M. Time-and dose-dependent neuroprotective effects of sex steroid hormones on inflammatory cytokines after a traumatic brain injury. J Neurotrauma 2013; 30:47-54.

31. Shahrokhi N, Khaksari M, Soltani Z, Mahmoodi M, Nakhaee N. Effect of sex steroid hormones on brain edema, intracranial pressure, and neurologic outcomes after traumatic brain injury. Can J Physiol Pharmacol 2010; 88:414-421.

32. Schuier F, Hossmann K. Experimental brain infarcts in cats. II. Ischemic brain edema. Stroke 1980; 11:593-601.

33. Dirnagl U, Iadecola C, Moskowitz MA. Pathobiology of ischaemic stroke: an integrated view. Trends Neurosci 1999; 22:391-397.

34. Gasche Y, Copin J-C, Sugawara T, Fujimura M, Chan PH. Matrix metalloproteinase inhibition prevents oxidative stress-associated blood–brain barrier disruption after transient focal cerebral ischemia. J Cereb Blood Flow Metab 2001; 21:1393-1400.

35. Soustiel JF, Larisch S. Mitochondrial damage: a target for new therapeutic horizons. Neurotherapeutics 2010; 7:13-21.

36. Pelisch N, Hosomi N, Ueno M, Nakano D, Hitomi H, Mogi M, *et al.* Blockade of AT1 receptors protects the blood–brain barrier and improves cognition in Dahl salt-sensitive hypertensive rats. Am J Hypertens 2011; 24:362-368.

37. Gohlke P, Kox T, Jürgensen T, von Kügelgen S, Rascher W, Unger T, *et al.* Peripherally applied candesartan inhibits central responses to angiotensin II in conscious rats. Naunyn Schmiedebergs Arch Pharmacol 2002; 365:477-483.

38. Marmarou A. A review of progress in understanding the pathophysiology and treatment of brain edema. Neurosurg Focus 2007; 22:1-10.

39. Miao X, Wei S, XU Q-p. Aquaporin-4 and traumatic brain edema. Chin J Traumatol 2010; 13:103-110.

40. Kusaka I, Kusaka G, Zhou C, Ishikawa M, Nanda A, Granger DN, *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-H2451.

41. Patel HC, Menon DK, Tebbs S, Hawker R, Hutchinson PJ, Kirkpatrick PJ. Specialist neurocritical care and outcome from head injury. Intensive Care Med 2002; 28:547-553.

42. Khaksari M, Mahmmodi R, Shahrokhi N, Shabani M, Joukar S, Aqapour M. The effects of shilajit on brain edema, intracranial pressure and neurologic outcomes following the traumatic brain injury in rat. Iran J Basic Med Sci 2013; 16:858-864.

43. Culman J, Blume A, Gohlke P, Unger T. The renin-angiotensin system in the brain: possible therapeutic implications for AT1-receptor blockers. J Hum Hypertens 2002; 16:S64-S70.

44. Groth W, Blume A, Gohlke P, Unger T, Culman J. Chronic pretreatment with candesartan improves recovery from focal cerebral ischaemia in rats. J Hypertens 2003; 21:2175-2182.

45. Nishimura Y, Ito T, Saavedra JM. Angiotensin II AT1 blockade normalizes cerebrovascular autoregulation and reduces cerebral ischemia in spontaneously hypertensive rats. Stroke 2000; 31:2478-2486.

46. Blume A, Herdegen T, Unger T. Angiotensin peptides and inducible transcription factors. J Mol Med 1999; 77:339-357.

47. Sugawara T, Kinouchi H, Oda M, Shoji H, Omae T, Mizoi K. Candesartan reduces superoxide production after global cerebral ischemia. Neuroreport 2005; 16:325-328.

48. Baranov D, Armstead WM. Selective blockade of AT1 receptor attenuates impairment of hypotensive autoregulation and improves cerebral blood flow after brain injury in the newborn pig. Anesthesiology 2003; 99:1118-1124.

49. Liu H, Kitazato KT, Uno M, Yagi K, Kanematsu Y, Tamura T, *et al.* Protective mechanisms of the angiotensin II type 1 receptor blocker candesartan against cerebral ischemia: *in-vivo* and *in-vitro* studies. J Hypertens 2008; 26:1435-1445.