

# The effects of two-stage carotid occlusion on spatial memory and pro-inflammatory markers in the hippocampus of rats

Mehrnoush Moghaddasi<sup>1</sup> · Majid Taati<sup>2</sup> · Payman Asadian<sup>3</sup> · Ali Reza Khalatbary<sup>4</sup> · Raheleh Asaei<sup>1</sup> · Naser Pajouhi<sup>1</sup>

Received: 27 December 2015 / Accepted: 13 July 2016  
© The Physiological Society of Japan and Springer Japan 2016

**Abstract** The purpose of this study was to evaluate the effects of cerebral hypoperfusion on cognitive ability, TNF $\alpha$ , IL1 $\beta$  and PGE2 levels in both hippocampi in a modified two-vessel occlusion model. Both common carotid arteries of adult male Wistar rats were permanently occluded with an interval of 1 week between occlusions. Learning and memory were significantly decreased after 1 month. This reduction was not significant after 2 months, which may be attributed to blood flow compensation. The TNF $\alpha$  level was significantly increased after 3 h and 1 day. IL1 $\beta$  was significantly increased after 1 day. After a week there was no significant difference in pro-inflammatory

levels. Furthermore, there was no difference between right and left hippocampi. It is possible that TNF $\alpha$  and IL1 $\beta$  elevation initiates pathologic processes that contribute to memory impairment.

**Keywords** Cerebral hypoperfusion · Inflammation · Interleukins · Spatial memory · Hippocampus

## Introduction

Reduction of cerebral blood flow has been shown to contribute to the physiopathological mechanisms of cognitive impairment in age-related disease [1–4], but the role of cerebrovascular pathology in the development of neural dysfunction has not yet been clearly defined [5]. Recently, inflammation, particularly pro-inflammatory cytokines and eicosanoids such as PGE2, has attracted attention for its role in neurodegenerative disorders [5–10].

The pro-inflammatory cytokines have important roles in regulation of synaptic function, and particularly TNF $\alpha$ , IL1 $\beta$  and IL-18 elevation have all been shown to suppress LTP (long-term potentiation) [11]. In studies on postoperative memory deficit, IL1 $\beta$  and TNF $\alpha$  have been involved in a failure to consolidate new spatial memory following an inflammatory surgical injury [12, 13]. IL1 $\beta$  plays a key role in CNS inflammatory response to CNS insults and TNF $\alpha$  has been consistently involved in memory impairment [14, 15]. A number of mechanisms for pro-inflammatory cytokines have been elucidated that may contribute to a neuronal injury [5], including: oxidative stress, abnormal neurotransmission, induction of nitric oxide, and apoptosis. For instance, much evidence suggests that TNF $\alpha$  plays an important role in the disruption of vascular circulation both in vivo and ex vivo. The enhanced expression of TNF $\alpha$

✉ Mehrnoush Moghaddasi  
m\_moghaddasi@hotmail.com

Majid Taati  
taatimajid@yahoo.com

Payman Asadian  
asadianp@gmail.com

Ali Reza Khalatbary  
khalat90@yahoo.com

Raheleh Asaei  
asaee\_ra@yahoo.com

Naser Pajouhi  
naserpajoh@yahoo.com

<sup>1</sup> Department of Physiology, Razi Herbal Medicines Research Center, Lorestan University of Medical Sciences, Khorramabad, Iran

<sup>2</sup> Department of Pathobiology, Faculty of Veterinary Medicine, Lorestan University, Khorramabad, Iran

<sup>3</sup> Ontario Veterinary College, University of Guelph, Guelph, Canada

<sup>4</sup> Department of Anatomy, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran

induces reactive oxygen species (ROS) production, leading to endothelial dysfunction in various pathophysiological conditions [16].

Cyclooxygenases (COXs) are the essential enzymes in the formation of prostaglandins from arachidonic acid [12]. COX2 is a well-known mediator of neuroinflammation [17, 18]. It is considered to be an important enzyme implicated in brain injury following cerebral ischemia [19]. COX2 inhibitors demonstrate neuroprotective effects in neurodegenerative models, suggesting PGE<sub>2</sub>, one of the COX products, can contribute to the neurodegeneration processes [20–22]. PGE<sub>2</sub> is involved in inflammation and the apoptosis of neural cells [18, 23, 24]. There is evidence that pathologically elevated PGE<sub>2</sub> can induce memory dysfunction in fear conditioning [25].

Cell-culture experiments have also demonstrated that pro-inflammatory cytokines, TNF $\alpha$  and IL1 $\beta$ , enhance COX2 expression and PGE<sub>2</sub> in microglia, astrocytes and neurons. On the other hand, the enhanced expression of PGE<sub>2</sub> and COX2 elevates the production of prostanoids, cytokines and also mediators of oxidative stress, which in turn leads to increased cell loss in different culture models [8, 26]. Furthermore, it has been indicated that prostaglandins and cytokines are directly involved in memory impairment in stressful conditions [27]. Nevertheless, it has been reported that the inhibition of particular inflammatory pathways can enhance amyloid deposition in a mouse model of Alzheimer's disease (AD) [10, 28, 29]. It appears that there are conflicting data about the role of inflammation in age-related diseases. For instance, in CSF of individuals with AD, both increased and decreased levels of PGE<sub>2</sub> have been observed [26, 30, 31]. Therefore, it seems further study is required to clarify the role of inflammation in cognitive dysfunction.

Chronic cerebral hypoperfusion can be induced in the rat by permanent occlusion of the two common carotid arteries (2-vessel occlusion, 2VO), simultaneously. Reperfusion injury does not happen and the induced cerebral hypoperfusion is global [32]. It seems that the chronic phase of the model resembles more closely the cerebral hypoperfusion observed in age-related disease [32]. A modified method permitting the two-stage establishment of cerebral hypoperfusion [32, 33] has been suggested, with a 1-week interval between the occlusion of both common carotid arteries. Kaliszewski et al. [34] showed that gradual vessel occlusion induces less severe tissue injury due to a more efficient development of collateral circulation. In confirming Cechetti et al.'s studies [33], our previous experience showed that survival of animals using the modified method was much greater than with the 2VO method. There are few studies that have applied this technique on rats in order to reduce blood flow to the brain. As far as we know, this study

investigates for the first time the alterations in inflammation markers in both hippocampi following cerebral hypoperfusion produced by the modified 2VO method. Therefore, the current study was designed to investigate spatial learning and memory impairment following chronic cerebral hypoperfusion induced by the modified 2VO method. Furthermore, alterations in the inflammatory markers, as potential candidates leading to cognitive dysfunction, and their temporal pattern in both hippocampi were investigated after inducing cerebral hypoperfusion.

## Materials and methods

### Animals

Male Wistar rats (weight 250–300 g) were accommodated four per cage and kept on a 12–12 h light–dark cycle in an air-conditioned room and maintained at (23  $\pm$  1  $^{\circ}$ C) for at least 10 days before any experimental procedure. Food and water were provided ad libitum. Experimental procedures were conducted in accordance with the Guidelines for Reporting Animal Research [35].

### Surgery

The animals were divided into two groups. In the first group ( $n = 36$ ), cerebral hypoperfusion was induced by modified 2VO, the second group served as sham-operated controls (sham,  $n = 36$ ). The animals were anesthetized with 400 mg/kg chloral hydrate i.p. Briefly, after a ventral midline neck incision, carotid arteries were exposed, gently freed from their sheath, then double ligated just below the bifurcation with 4–0 silk sutures. At first, the right common carotid was occluded, and then the left one was occluded 1 week later. The animals in the sham group underwent all the surgical procedures except ligation of the carotid arteries. Lidocaine (1 %) was applied as local anesthetic. During the operation, their body temperature was maintained at 37  $\pm$  0.5  $^{\circ}$ C by means of a heating lamp. Then the animals in both groups were divided into five groups, the first group after 3 h, the second and third groups after 1 day and 1 week, respectively, were deeply anesthetized with chloral hydrate, the brains were removed and the samples were quickly taken from the areas of the hippocampus. Then the samples were quickly frozen (until further processing) to assess inflammatory markers (IL1 $\beta$ , TNF $\alpha$  and PGE<sub>2</sub>) by ELISA technique later. In the fourth group (after 1 month) and fifth group (after 2 months), cognitive function was evaluated using the Morris water maze test (MWM) [36].

### Testing of spatial learning and memory in the Morris water maze

The rats were trained in a standard MWM task [36]. The maze consisted of a black circular pool, 200 cm in diameter, filled with water (temperature around 23 °C, depth 40 cm) located in a room with visual cues on the walls. A black platform, 10 cm in diameter, was submerged in the water (2 cm below the water surface) and the pool was conceptually divided into four quadrants and had four points designed as beginning starting positions (N, S, W or E) [36]. The rats performed four trials per day for 4 consecutive days. In the trials, each individual rat was released gently into the water at a randomly chosen quadrant. The rat swam and learned how to reach the hidden platform within 60 s. Upon arrival, the rat was permitted to stay on the platform for 15 s and was then taken back into the cage. The rats were placed on the platform, if they could not escape to the platform within 60 s by themselves, their escape latency was accepted as 60 s. During the inter-trial intervals, animals were kept in a dry home cage for 60 s. The time to reach the platform (latency), the length of traveled path, and the swim speed were recorded semi-automatically with a video tracking system. Twenty-four hours after the last day of training, subjects were examined on a probe trial, during which the platform was removed and the time spent in the target quadrant was measured for a 60-s trial.

### Determination of pro-inflammatory markers

For the assessment of cerebral cytokine levels, hippocampus samples were homogenized in Tris HCl buffer (0.02 M) containing DTT (0.1 mM), EDTA (0.1 mM), sucrose (0.25 M), pH 7.5 and 10 µl of 10 % Triton X-100 at 0 °C. Protein concentration was measured by the method of Bradford [37] in which bovine serum albumin (BSA) was used as the standard. ENZO life sciences ELISA test kits (USA) were utilized for measurement of rat IL1β, TNFα and PGE2 concentrations. The assays were conducted in accordance with the manufacturer's protocol. All ELISA measurements were conducted in duplicate, and the means of the two readings for each sample were utilized in the statistical analysis.

### IL1β determination

The IL1β (rat) kit (ENZO life sciences, USA) used an antibody to rat IL1β fixed on a microtiter plate to attach the rat IL1β in the sample or standards. The extra sample or standard was rinsed, after a short incubation, and a biotinylated antibody to rat IL1β was included. This antibody attached to the rat IL1β captured on the plate. After a

short incubation the extra antibody was rinsed and streptavidin conjugated to horseradish peroxidase (HRP) was added, which attached to the biotinylated rat IL1β antibody. Extra conjugate was rinsed and substrate was added. The enzyme reaction was stopped after a short incubation and the color developed was read at 450 nm. The determined optical density was directly proportional to rat IL1β concentration in samples or standards [38, 39]. IL1β concentration was expressed in pg/mg of protein.

### TNFα determination

In this protocol, samples and standards were added to wells coated with a monoclonal antibody specific for rat TNFα. The plate was then incubated, then rinsed, leaving only attached rat TNFα on the plate. A solution of biotinylated polyclonal antibody to TNFα was added to bind the TNFα attached on the plate. The plate was then incubated. The excess antibody was removed by washing the plate. A solution of HRP conjugate was added to the wells, attaching to the rat TNFα polyclonal. The plate was then incubated, and after that the plate was washed to remove extra HRP conjugate. TMB substrate solution was added. The blue color generated was stopped by adding stop solution. The color generated was read at 450 nm. The measured signal was directly proportional to the TNFα level in the sample. TNFα concentration was expressed in pg/mg of protein.

### PGE2 determination

The PGE2 high sensitivity kit (ENZO life sciences, USA) used a monoclonal antibody to PGE2 to attach the PGE2 in the sample or an alkaline phosphatase molecule which has PGE2 covalently bound to it, in a competitive manner. After a simultaneous incubation at 4 °C the extra reagents were rinsed away and substrate was added. After incubation at 37 °C, the enzyme reaction was stopped and the resulting color was measured on a microplate reader at 405 nm. The intensity of the color was inversely proportional to the PGE2 level in either samples or standards. For calculating PGE2 concentration, the measured optical density was used [38, 39]. PGE2 concentration was expressed in pg/mg of protein.

### Statistical analysis

Statistical analysis of data was performed by applying one-way analysis of variance (ANOVA) followed by a Tukey test for biochemical parameters and behavioral tests at different time intervals, unpaired *t*-test for behavioral and biochemical comparison between two groups and paired *t*-test for comparing between both

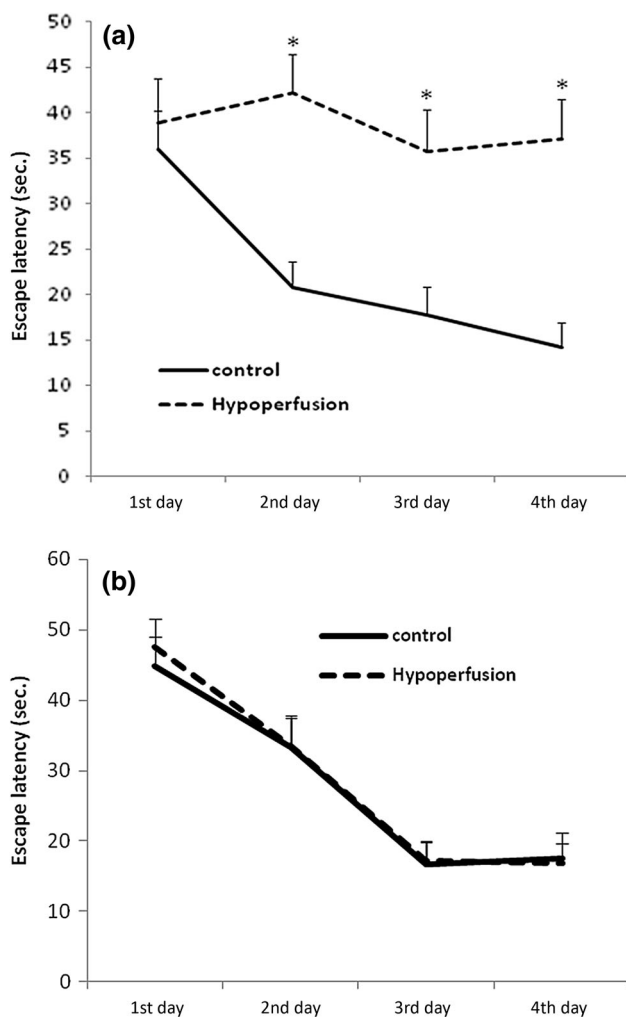
hippocampi. A  $P$  value of  $<0.05$  was considered statistically significant. To present the data, mean  $\pm$  standard error of the mean (SEM) was applied.

## Results

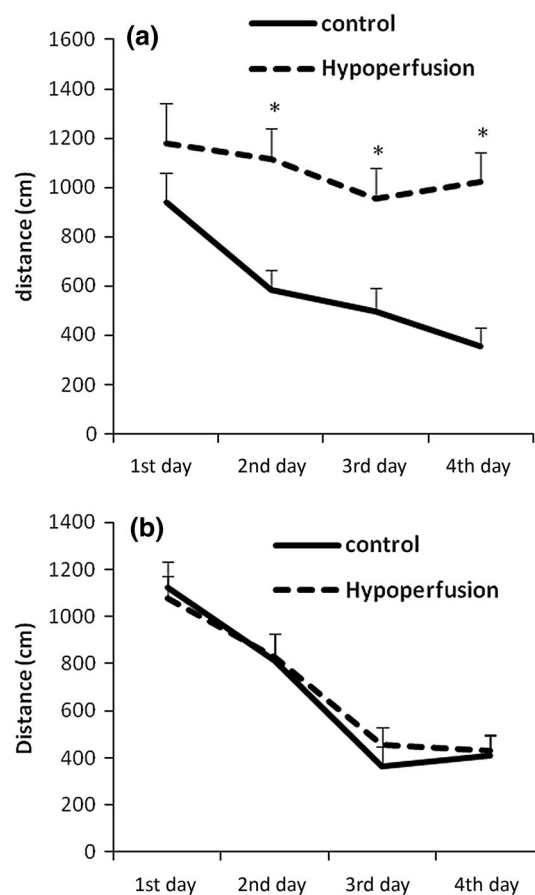
### Behavioral results

There were no differences between sham and non-operated groups (data are not shown); therefore, the cognitive performance of the hypoperfusion groups was compared to that of the sham operated groups, referred to as control groups. Figure 1a shows the effects of cerebral hypoperfusion on learning memory performance of rats measured 1 month after occluding both common carotids. In the first session of the escape latency trial, wherein the latency to find the platform was assessed, the statistical analysis did

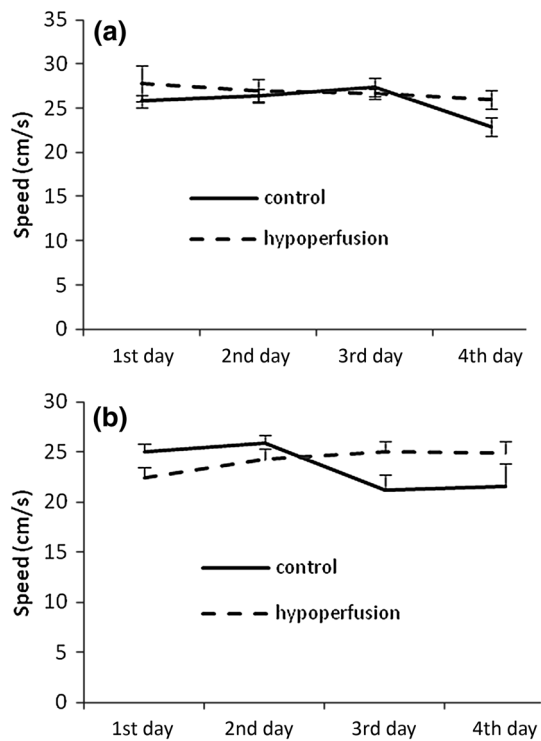
not reveal any significant difference between the two groups ( $p > 0.05$ ). As shown in Fig. 1a, escape latency decreased significantly during training ( $p < 0.01$ ) in the control groups and there was a significant difference between two groups after the 2nd day ( $p < 0.01$ ). Analysis of the swim path distance (traveled distance) (Fig. 2a) revealed a statistical difference between the groups ( $p < 0.05$ ), again after the 2nd day, indicating a cognitive impairment within the hypoperfusion group: rats swam longer distances in the tank before they found the platform compared to the controls ( $p < 0.05$ ). This discrepancy continued throughout the whole training period. To determine whether the difference between two groups was due to differences in swimming ability, swim speed was measured for each group, there was no significant difference between the two groups (Fig. 3a). In the probe trials, as shown in Fig. 4, there was a significant difference in time spent in the target quadrant between the two groups ( $p < 0.05$ ). As shown in Figs. 1, 2, 3, and 4b, 2 months after surgery, there were no significant differences between the two groups ( $p > 0.05$ ).



**Fig. 1** The effect of cerebral hypoperfusion on escape latency. **a** 1 month after surgery. **b** 2 months after surgery. \* $p < 0.05$  in comparison to the control



**Fig. 2** The effect of cerebral hypoperfusion on traveled distance in MWM test. **a** 1 month after surgery. **b** 2 months after surgery. \* $p < 0.05$  in comparison to the control



**Fig. 3** The effect of cerebral hypoperfusion on swimming speed in MWM test. **a** 1 month after surgery. **b** 2 months after surgery

**Inflammatory markers**

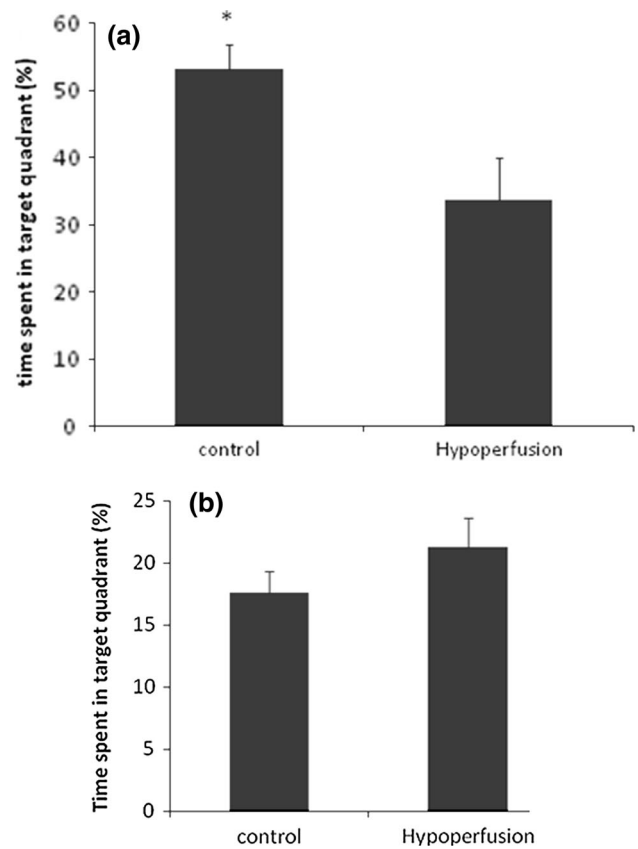
Since the two common carotids were occluded separately with a 1-week interval, we measured the inflammatory marker concentrations in both hippocampi. The results showed that there was no significant difference between the left and right hippocampi ( $p > 0.05$ ) (Table 1). In the present study, for convenience we considered only the right hippocampus in the statistical analysis.

**TNF $\alpha$**

As shown in Fig. 5, the TNF $\alpha$  levels in the hypoperfusion group reached their peak 3 h after surgery, and were significantly elevated as compared with the control groups ( $p < 0.001$ ), then markedly decreased by 1 day, but still were significantly elevated in comparison to the control group ( $p < 0.01$ , Fig. 5). There was no significant difference between both hypoperfusion and control groups after a week ( $p > 0.05$ ).

**IL1 $\beta$**

The IL1 $\beta$  level in the hippocampus of the hypoperfusion group tended to increase significantly 1 day after surgery as compared with that in the control group ( $p < 0.05$ ), then the significant difference was abolished 1 week after surgery



**Fig. 4** The effect of modified two-vessel occlusion on time spent in target quadrant (%). **a** 1 month after surgery. **b** 2 months after surgery. \* $p < 0.05$  in comparison to the hypoperfusion group

(Fig. 6). There were no significant differences in IL1 $\beta$  concentrations between the different time intervals ( $p > 0.05$ ).

**PGE2**

The hippocampus PGE2 level in the hypoperfusion group was not significantly different as compared with that in the control group (Fig. 7). Although the PGE2 concentration in both hippocampi increased after a week as compared to the control group, this difference was not significant.

**Survival rate**

In this study, we lost only one animal from the total animals studied (74 animals), and the survival rate was 98.64 %.

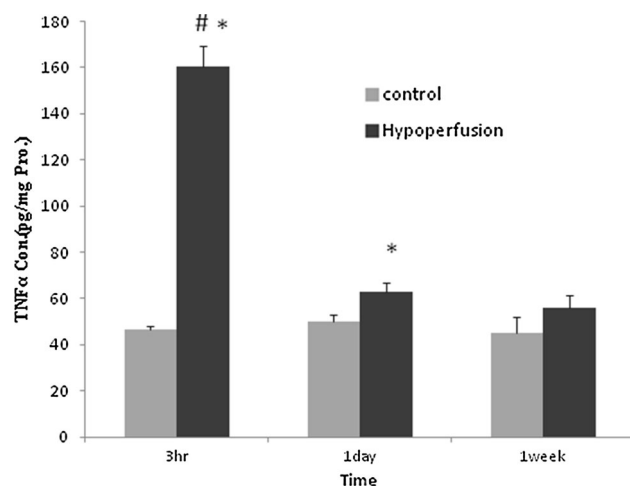
**Discussion**

Chronic cerebral hypoperfusion is an early feature of age-related diseases including AD, mild cognitive impairment (MCI), and vascular dementia (VD) [40–42]. The obtained

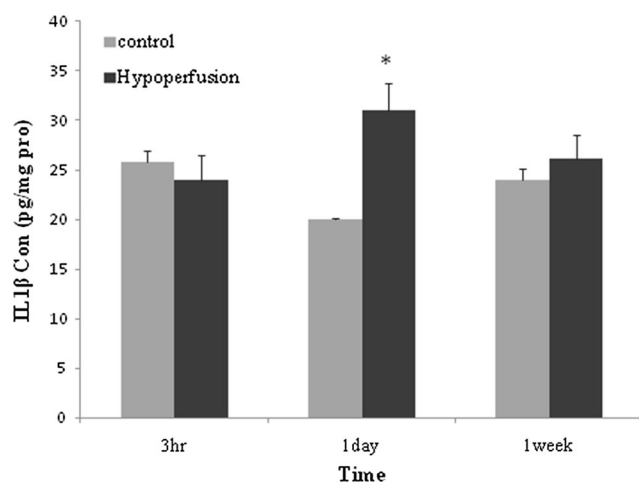
**Table 1** The effect of cerebral hypoperfusion on pro-inflammatory markers in both rat hippocampi

Time after 2VO	TNF (pg/mg protein)		IL1 $\beta$ (pg/mg protein)		PGE2 (pg/mg protein)	
	R	L	R	L	R	L
3 h	160.5 $\pm$ 8.76	161.05 $\pm$ 11.482	23.982 $\pm$ 2.438	29.550 $\pm$ 2.567	64.401 $\pm$ 2.575	68.768 $\pm$ 4.691
1 day	62.685 $\pm$ 4.384	57.473 $\pm$ 7.890	30.715 $\pm$ 3.039	23.891 $\pm$ 2.085	64.815 $\pm$ 1.882	70.145 $\pm$ 4.592
1 week	55.827 $\pm$ 5.765	57.713 $\pm$ 10.87	26.122 $\pm$ 2.340	19.635 $\pm$ 2.267	69.655 $\pm$ 2.929	68.138 $\pm$ 4.399

R, right hippocampus; L, left hippocampus

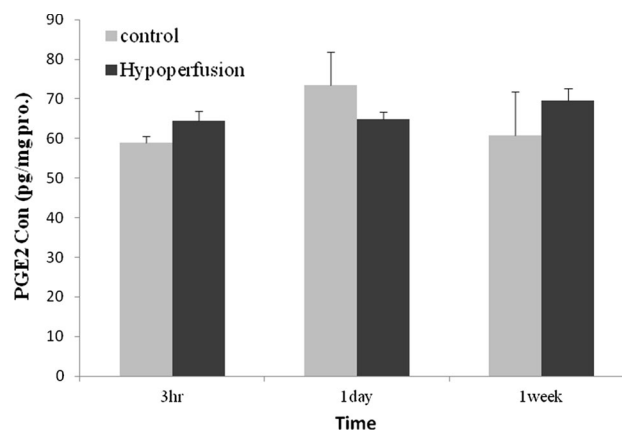


**Fig. 5** The effect of cerebral hypoperfusion on TNF $\alpha$  concentration in the hippocampus. \* $p < 0.05$  in comparison to the control. #  $p < 0.05$  in comparison to other time intervals (1 day and 1 week)



**Fig. 6** The effect of cerebral hypoperfusion on IL1 $\beta$  concentration in right hippocampus. \* $p < 0.05$  in comparison to control

results showed that 1 month postoperatively (the modified 2VO), learning and memory in MWM were significantly impaired. The data showed TNF $\alpha$  and IL1 $\beta$  levels were elevated in the hypoperfusion group. However, the levels



**Fig. 7** The effect of cerebral hypoperfusion on PGE2 concentration in the hippocampus

returned to basal levels within 1 week and there were no significant changes in the PGE2 levels of the hypoperfusion group in comparison with the control groups.

Several animal models have been developed to investigate the effects of chronic cerebral hypoperfusion [32, 43–45]. In association with cerebral hypoperfusion, there are neuronal loss, progressive cognitive dysfunction, astrogliosis, and cholinergic dysfunction in the hippocampus [45–47]. Consistent with the published reports, data presented in Figs. 1, 2, 3, and 4 show spatial learning and memory impairments 1 month after the modified 2VO, as evaluated in the MWM [36]. Synaptic and neuronal loss in the hippocampus reasonably impairs animal performance in the MWM [12].

Liu et al. [42] have shown that 2VO rats in a MWM task performed significantly worse than the control group 4 weeks after 2VO. In addition, cognitive ability of the rats significantly deteriorated as time passed. However, our behavioral results were partly in contrast with their results. After 2 months, learning and memory improved to the control level, potentially due to the establishment of collateral blood supply, as found in chronic vascular occlusion studies, particularly in the modified 2VO [48]. Cerebral blood flow was significantly lower than the control values 4 weeks after 2VO occlusion. Between 8 weeks and

3 months, no significant reduction was reported [32, 49]. In the modified 2VO, a stronger compensatory system is expected because there is more time to compensate for the reduced blood flow. Furthermore, in this study, we used 3-month-old animals, which are considered young animals. The cardiovascular and inflammatory potential of young animals are particularly stronger than old animals. Occluding the first artery possibly produces a type of cerebral preconditioning that reduces the damage of occluding the second artery. Taken together, it appears that the young age of animals and more subtle approach caused the complete recovery of learning and memory to the control level [32, 43].

A strong connection between neuroinflammation, mainly mediated by pro-inflammatory cytokines, and both AD and VD has been demonstrated [5, 50]. Our results showed that TNF $\alpha$  concentration was significantly increased 3 h postoperatively and this elevation persisted for 1 day. It has been reported that 20 min after 2VO operation (acute phase), the TNF $\alpha$  and IL1 $\beta$  levels in the cortex and hippocampus of rat were increased significantly in comparison with sham groups. But over chronic time, 3 weeks after permanent vessel occlusion, rat brain cytokine concentrations were completely recovered [51]. Zhu et al. [7] have shown that TNF $\alpha$  mRNA levels in mice reached the first peak at 3 h in the hippocampus after a transient 2VO operation. The peak levels of IL1 mRNA were observed 12 h after transient global ischemia and declined with time. This is similar to our data on TNF $\alpha$  and IL1 $\beta$  at the protein level in rats. The pro-inflammatory cytokine mRNA expressions measured in the study returned to control levels 72 h after ischemia [7].

TNF $\alpha$  has been shown to be the main inducer of IL1 $\beta$  [52, 53] and there is a temporal link between the two pro-inflammatory cytokines. Our results confirmed this temporal relationship. Brain damage induces inflammation with high levels of brain IL1, simultaneously with learning and memory impairment [27]. This adverse effect was observed in both in vivo and ex vivo studies [27]. Furthermore, the pro-inflammatory cytokines can regulate COX2 in the brain. During pathologic conditions, the high production of prostaglandins aggravates neuroinflammation [54].

A brief induction of COX2 after various transient brain injuries has been shown in numerous studies [19, 24, 54, 55]. Following transient global ischemia, the elevation of COX2 expression was observed for up to 48 h, which then returned to normal levels [55]. Alteration in PG concentration is intimately related to the severity of brain damage and its outcome [56]. In addition, it has been shown that the initial elevation of COX2 is adaptive, while the sustained augmentation seen after injury is pathological [57]. In this study, this alteration was not significant even though PGE2 concentration was increased (Fig. 7). It appears that brain

damage caused by this method was not severe enough to elevate the PGE2 concentration significantly and consistently for 1 week. Because IL1 $\beta$  and TNF $\alpha$  can suppress LTP in the hippocampus, the elevation of these cytokines alone may be enough to impair neuronal function. The results also showed the elevated inflammatory cytokine concentration returned to baseline level within a week after the occlusion. It has been reported that a single inflammatory insult has long-term effects on memory and cognition. Likewise, in animal models, a single inflammatory event impairs later memory and leads to long-term alterations in gene expression [15]. Adult neurogenesis and epigenetic modifications are two mechanisms which mediate these long-lasting effects. These processes are modulated by immune signaling [15, 58].

Additionally, pro-inflammatory cytokines mediate neurotoxic processes including enhancement of apoptosis, and impairments of synaptic function such as the inhibition of long-term potentiation [59]. Interruption in the neuron-glia signaling may also contribute to synaptic dysfunction in the injured CNS [60, 61]. In separate experiments, we observed the enhancement of apoptosis after 1 week in both hippocampi (unpublished data). Kitamura et al. [62] implanted an ameroid constrictor device on the common carotid arteries (CCAs). They showed that by gradually narrowing the CCAs (2-vessel gradual occlusion; 2VGO), MAG-immunopositive regions (indicating the degree of axon-glia integrity) declined in both 2VGO and 2VO groups when compared with sham, and the most obvious difference was at 28 days. The density of microglial and astrocyte cells and also white matter damage was significantly more in both 2VGO and 2VO groups compared with the sham. Spatial working memory was significantly impaired in both models. Comparing the modified 2VO method with the methods mentioned above, and regarding the timing of carotid occlusion, it is not surprising to observe memory deficits in the MWM test at 1 month after the second carotid occlusion.

In summary, this study showed that both (right and left) hippocampi reacted similarly to the separated occlusion of the two common carotids, regarding the pro-inflammatory cytokines. Moreover, the present study showed that the modified 2VO method resulted in a reduction in learning and memory. However, this effect was not sustained in the 2nd month. The obtained data confirmed a temporal relationship among these mediators of inflammatory damage; TNF $\alpha$  appears to be the inducer of IL1 $\beta$ . It is possible that TNF $\alpha$  and IL1 $\beta$  elevation initiate pathologic processes that contribute to memory impairment. However, further studies, particularly with longer times, are required to clarify the role of inflammatory factors in memory impairment. Using old animals for future studies is recommended.

**Acknowledgments** This study was supported by Lorestan University of Medical Sciences as a Grant (No. 10/89) to Mehrmouh

Moghaddasi. The authors are grateful to the directors in Razi Herbal Medicines Research Center for their assistance and collaboration.

### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

## References

- Wang J, Zhang HY, Tang XC (2010) Huperzine A improves chronic inflammation and cognitive decline in rats with cerebral hypoperfusion. *J Neurosci Res* 88(4):807–815
- de la Torre JC (2002) Vascular basis of Alzheimer's pathogenesis. *Ann N Y Acad Sci* 977:196–215
- Farkas E, Luiten PG (2001) Cerebral microvascular pathology in aging and Alzheimer's disease. *Prog Neurobiol* 64(6):575–611
- Matsuda H (2001) Cerebral blood flow and metabolic abnormalities in Alzheimer's disease. *Ann Nucl Med* 15(2):85–92
- Smith JA, Das A, Ray SK, Banik NL (2012) Role of pro-inflammatory cytokines released from microglia in neurodegenerative diseases. *Brain Res Bull* 87(1):10–20
- Berti R, Williams AJ, Moffett JR, Hale SL, Velarde LC, Elliott PJ, Yao C, Dave JR, Tortella FC (2002) Quantitative real-time RT-PCR analysis of inflammatory gene expression associated with ischemia-reperfusion brain injury. *J Cereb Blood Flow Metab* 22(9):1068–1079
- Zhu Y, Saito K, Murakami Y, Asano M, Iwakura Y, Seishima M (2006) Early increase in mRNA levels of pro-inflammatory cytokines and their interactions in the mouse hippocampus after transient global ischemia. *Neurosci Lett* 393(2–3):122–126
- Weggen S, Rogers M, Eriksen J (2007) NSAIDs: small molecules for prevention of Alzheimer's disease or precursors for future drug development? *Trends Pharmacol Sci* 28(10):536–543
- Zaremba J, Losy J (2004) Cytokines in clinical and experimental ischemic stroke. *Neurol Neurochir Pol* 38(1):S57–S62
- Wyss-Coray T (2006) Inflammation in Alzheimer disease: driving force, bystander or beneficial response? *Nat Med* 12(9):1005–1015
- Pickering M, O'Connor JJ (2007) Pro-inflammatory cytokines and their effects in the dentate gyrus. *Prog Brain Res* 163:339–354
- Cunningham C, Skelly DT (2012) Non-steroidal anti-inflammatory drugs and cognitive function: are prostaglandins at the heart of cognitive impairment in dementia and delirium? *J Neuroimmune Pharmacol* 7(1):60–73
- Cibelli M, Fidalgo AR, Terrando N, Ma D, Monaco C, Feldmann M, Takata M, Lever IJ, Nanchahal J, Faselow MS, Maze M (2010) Role of interleukin-1 $\beta$  in postoperative cognitive dysfunction. *Ann Neurol* 68:360–368
- Wilson CJ, Finch CE, Cohen HJ (2002) Cytokines and cognition—the case for a head-to-toe inflammatory paradigm. *J Am Geriatr Soc* 50(12):2041–2056
- Donzis EJ, Tronson NC (2014) Modulation of learning and memory by cytokines: signaling mechanisms and long-term consequences. *Neurobiol Learn Mem* 115:68–77
- Zhang H, Park Y, Wu J, Xp Chen, Lee S, Yang J, Dellsperger KC, Zhang C (2009) Role of TNF- $\alpha$  in vascular dysfunction. *Clin Sci (Lond)* 116(3):219–230
- Feng L, Sun W, Xia Y, Tang WW, Chanmugam P, Soyoola E, Wilson CB, Hwang D (1993) Cloning two isoforms of rat cyclooxygenase: differential regulation of their expression. *Arch Biochem Biophys* 307:361–368
- Phillis JW, Horrocks LA, Farooqui AA (2006) Cyclooxygenases, lipoxygenases, and epoxygenases in CNS: their role and involvement in neurological disorders. *Brain Res Rev* 52(2):201–243
- Nogawa S, Zhang F, Ross ME, Iadecola C (1997) Cyclo-oxygenase-2 gene expression in neurons contributes to ischemic brain damage. *J Neurosci* 17(8):2746–2755
- Gobbo OL, O'Mara SM (2004) Post-treatment, but not pre-treatment, with the selective cyclooxygenase-2 inhibitor celecoxib markedly enhances functional recovery from kainic acid-induced neurodegeneration. *Neuroscience* 125(2):317–327
- Drachman DB, Frank K, Dykes-Hoberg M, Teismann P, Almer G, Przedborski S, Rothstein JD (2002) Cyclooxygenase 2 inhibition protects motor neurons and prolongs survival in a transgenic mouse model of ALS. *Ann Neurol* 52(6):771–778
- Palumbo S, Toscano CD, Parente L, Weigert R, Bosetti F (2012) The cyclooxygenase-2 pathway via the PGE(2) EP2 receptor contributes to oligodendrocytes apoptosis in cuprizone-induced demyelination. *J Neurochem* 121(3):418–427
- Takadera T, Yumoto H, Tozuka Y, Ohyashiki T (2002) Prostaglandin E2 induces caspase dependent apoptosis in rat cortical cells. *Neurosci Lett* 317:61–64
- Manabe Y, Anrather J, Kawano T, Niwa K, Zhou P, Ross ME, Iadecola C (2004) Prostanoids, not reactive oxygen species, mediate COX-2-dependent neurotoxicity. *Ann Neurol* 55(5):668–675
- Hein AM, Stutzman DL, Bland ST, Barrientos RM, Watkins LR, Rudy JW, Maier SF (2007) Prostaglandins are necessary and sufficient to induce contextual fear learning impairments after interleukin-1 $\beta$  injections into the dorsal hippocampus. *Neuroscience* 150:754–763
- Hoozemans JJ, Veerhuis R, Rozemuller AJ, Eikelenboom P (2003) Non-steroidal anti-inflammatory drugs and cyclooxygenase in Alzheimer's disease. *Curr Drug Targets* 4(6):461–468
- Yirmiya R, Goshen I (2011) Immune modulation of learning, memory, neural plasticity and neurogenesis. *Brain Behav Immun* 25:181–213
- Golde TE (2002) Inflammation takes on Alzheimer disease. *Nat Med* 8(9):936–938
- Wyss-Coray T, Mucke L (2002) Inflammation in neurodegenerative disease—a double edged sword. *Neuron* 35(3):419–432
- Gasparini L, Ongini E, Wenk G (2004) Non-steroidal anti-inflammatory drugs (NSAIDs) in Alzheimer's disease: old and new mechanisms of action. *J Neurochem* 91(3):521–536
- Combrinck M, Williams J, De Berardinis MA, Warden D, Puopolo M, Smith AD, Minghetti L (2006) Levels of CSF prostaglandin E2, cognitive decline, and survival in Alzheimer's disease. *J Neurol Neurosurg Psychiatry* 77(1):85–88
- Farkas F, Luiten PGM, Bari F (2007) Permanent, bilateral common carotid artery occlusion in the rat: a model for chronic cerebral hypoperfusion-related neurodegenerative diseases. *Brain Res Rev* 54(1):162–180
- Cechetti F, Worm PV, Pereira LO, Siqueira IR, A Netto C (2010) The modified 2VO ischemia protocol causes cognitive impairment similar to that induced by the standard method, but with a better survival rate. *Braz J Med Biol Res* 43(12):1178–1183
- Kaliszewski C, Fernandez LA, Wicke JD (1988) Differences in mortality rate between abrupt and progressive carotid ligation in the gerbil: role of endogenous angiotensin II. *J Cereb Blood Flow Metab* 8(2):149–154
- Kilkenny C, Brown WJ, Cuthill IC, Emerson M, Altman DG (2010) Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. *PLoS Biol* 8(6):e1000412
- Morris R (1984) Developments of a water-maze procedure for studying spatial learning in the rat. *J Neurosci Methods* 11:47–60



37. Bradford MM (1976) Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72:248–254
38. Chard T (1990) An introduction to radioimmunoassay and related techniques, 4th edn. Elsevier, Amsterdam
39. Tijssen P (1985) Practice and theory of enzyme immunoassays. Elsevier, Amsterdam
40. Zhiyou C, Yong Y, Shanquan S, Jun S, Liangguo H, Ling Y, Jieying L (2009) Upregulation of BACE1 and  $\beta$ -amyloid protein mediated by chronic cerebral hypoperfusion contributes to cognitive impairment and pathogenesis of Alzheimer's disease. *Neurochem Res* 34:1226–1235
41. De Jong GI, De Vos RA, Steur EN, Luiten PG (1997) Cerebrovascular hypoperfusion: a risk factor for Alzheimer's disease? Animal model and postmortem human studies. *Ann NY Acad Sci* 826:56–74
42. Liu HX, Zhang JJ, Zheng P, Zhang Y (2005) Altered expression of MAP-2, GAP-43, and synaptophysin in the hippocampus of rats with chronic cerebral hypoperfusion correlates with cognitive impairment. *Brain Res Mol Brain Res* 139:169–177
43. Barros CA, Ekuni R, Moro MA, Pereira FM, Dos Santos Pereira MA, Milani H (2009) The cognitive and histopathological effects of chronic 4-vessel occlusion in rats depend on the set of vessels occluded and the age of the animals. *Behav Brain Res* 197(2):378–387
44. Ohta H, Nishikawa H, Kimura H, Anayama H, Miyamoto M (1997) Chronic cerebral hypoperfusion by permanent internal carotid ligation produces learning impairment without brain damage in rats. *Neuroscience* 79(4):1039–1050
45. Schmidt-Kastner R, Aguirre-Chen C, Saul I, Yick L, Hamasaki D, Busto R, Ginsberg MD (2005) Astrocytes react to oligemia in the forebrain induced by chronic bilateral common carotid artery occlusion in rats. *Brain Res* 1:28–39
46. Vicente E, Degerone D, Bohn L, Scornavaca F, Pimentel A, Leite MC, Swarowsky A, Rodrigues L, Nardin P, de Almeida LM, Gottfried C, Souza DO, Netto CA, Gonçalves CA (2009) Astroglial and cognitive effects of chronic cerebral hypoperfusion in the rat. *Brain Res* 1251:204–212
47. Bennett SA, Tenniswood M, Chen JH, Davidson CM, Keyes MT, Fortin T, Pappas BA (1998) Chronic cerebral hypoperfusion elicits neuronal apoptosis and behavioral impairment. *Neuroreport* 9(1):161–166
48. Eklöf B, Siesjö BK (1972) The effect of bilateral carotid artery ligation upon the blood flow and the energy state of the rat brain. *Acta Physiol Scand* 86(2):155–165
49. Otori T, Katsumata T, Muramatsu H, Kashiwagi F, Katayama Y, Terashi A (2003) Long-term measurements of cerebral blood flow and metabolism in a rat chronic hypoperfusion model. *Clin Exp Pharmacol Physiol* 30(4):266–272
50. Zhang HY, Zheng CY, Yan H, Wang ZF, Tang LL, Gao X, Tang XC (2008) Potential therapeutic targets of huperzine A for Alzheimer's disease and vascular dementia. *Chem Biol Interact* 175(1–3):396–402
51. Pläschke K, Grant M, Weigand MA, Zuchner J, Martin E, Bardenheuer HJ (2001) Neuromodulatory effect of propentofylline on rat brain under acute and long-term hypoperfusion. *Br J Pharmacol* 133(1):107–116
52. Muñoz-Fernández MA, Fresno M (1998) The role of tumor necrosis factor, interleukin 6, interferon- $\gamma$  and inducible nitric oxide synthase in the development and pathology of the nervous system. *Prog Neurobiol* 56(3):307–340
53. Hill JK, Gunion-Rinker L, Kulhanek D, Lessov N, Kim S, Clark WM, Dixon MP, Nishi R, Stenzel-Poore MP, Eckenstein FP (1999) Temporal modulation of cytokine expression following focal cerebral ischemia in mice. *Brain Res* 820(1–2):45–54
54. Gopez JJ, Yue H, Vasudevan R, Malik AS, Fogelsanger LN, Lewis S, Panikashvili D, Shohami E, Jansen SA, Narayan RK, Strauss KI (2005) Cyclooxygenase-2-specific inhibitor improves functional outcomes, provides neuroprotection, and reduces inflammation in a rat model of traumatic brain injury. *Neurosurgery* 56(3):590–604
55. Nakayama M, Uchimura K, Zhu RL, Nagayama T, Rose ME, Stetler RA, Isakson PC, Chen J, Graham SH (1998) Cyclooxygenase-2 inhibition prevents delayed death of CA1 hippocampal neurons following global ischemia. *Proc Natl Acad Sci* 95(18):10954–10959
56. Yang SY, Gao ZX (1999) Determination and clinical significance of plasma levels of prostaglandins in patients with acute brain injury. *Surg Neurol* 52(3):238–245
57. Strauss KI, Barbe MF, Marshall RM, Raghupathi R, Mehta S, Narayan RK (2000) Prolonged cyclooxygenase-2 induction in neurons and glia following traumatic brain injury in the rat. *J Neurotrauma* 17(8):695–711
58. Yamaguchi M, Seki T, Imayoshi I, Tamamaki N, Hayashi Y, Tatebayashi Y, Hitoshi S (2016) Neural stem cells and neurogliogenesis in the central nervous system: understanding the structural and functional plasticity of the developing, mature, and diseased brain. *J Physiol Sci* 66(3):197–206
59. Rosenberg PB (2005) Clinical aspects of inflammation in Alzheimer's disease. *Int Rev Psychiatry* 17(6):503–514
60. Chung WS, Welsh CA, Barres BA, Stevens B (2015) Do glia drive synaptic and cognitive impairment in disease? *Nat Neurosci* 18(11):1539–1545
61. Lana D, Melani A, Pugliese AM, Cipriani S, Nosi D, Pedata F, Giovannini MG (2014) The neuron-astrocyte-microglia triad in a rat model of chronic cerebral hypoperfusion: protective effect of dipyridamole. *Front Aging Neurosci* 6:322
62. Kitamura A, Fujita Y, Oishi N, Kalaria RN, Washida K, Maki T, Okamoto Y, Hase Y, Yamada M, Takahashi J, Ito H, Tomimoto H, Fukuyama H, Takahashi R, Ihara M (2012) Selective white matter abnormalities in a novel rat model of vascular dementia. *Neurobiol Aging* 33:1012–1035