Acta Neurol Belg (2014) 114:33–40
DOI 10.1007/s13760-013-0234-2

ORIGINAL ARTICLE

Effects of tadalafil on ischemia/reperfusion injury in rat brain

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Received: 13 March 2013 / Accepted: 17 July 2013 / Published online: 6 August 2013

Abstract Cerebral ischemia–reperfusion (I/R) injury is caused by lack of blood supply to the brain. The accumulation of toxic products such as reactive oxygen species (ROS) occurs on reperfusion, when the occlusion is removed. The resulting oxidative stress results in the initiation of pathways leading to necrotic and apoptotic cell death. Tadalafil (TAD) prevents the accumulation of ROS and increases antioxidant cellular protective mechanisms. The aim of this study was to investigate the effect of TAD treatment against short-term global brain I/R injury in rats. The study was carried out on 30 Wistar-albino rats, which were divided into three groups including a control group ($n = 10$), an I/R group ($n = 10$) and an I/R + TAD group ($n = 10$) (2 mg/kg/day for 4 days before ischemia). At the end of the experiment, tissue samples were collected for both biochemical and histopathological analyses. Malondialdehyde was significantly lower in the TAD-administered group (9.06 ± 0.15) than in the I/R group ($p < 0.05$). However, no significant difference was observed in nitric oxide levels in the TAD-administered group compared to the I/R group. The mean superoxide dismutase level was significantly higher in the I/R–TAD group than the I/R group. There was no statistically significant difference in glutathione peroxidase levels in I/R + TAD group compared to I/R group. Histopathologically, TAD-administered group provided significant morphological improvement compared to the I/R group. We concluded that TAD prevented I/R-induced neurotoxicity as shown by obtained biochemical and histopathological findings.

Keywords Tadalafil · Brain · Ischemia/reperfusion

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Introduction

Cerebrovascular disease is a leading cause of death and disability in many countries. Cerebral ischemia results from reduced cerebral blood flow due to a transient or permanent cerebral artery occlusion [1, 2]. Ischemic injury in brain leads to neuronal cell death [3, 4], and eventually causes neurological impairment. In addition, tissue damage following cerebral ischemia is caused by complex pathophysiological processes such as glutamate excitotoxicity, membrane depolarization, inflammation, and apoptosis [5]. When the brain is deprived of blood supply, the injury is not only caused by the temporary loss of oxygen and energy supply, but also by reactive oxygen species (ROS), which is generated through reactions with the reintroduced oxygen during reperfusion [1, 5]. The brain is very susceptible to damage by energy-depriving injuries, particularly oxygen radical-mediated injuries, because it has low energy levels, high aerobic metabolism, and low concentrations of radical-scavenging enzymes [6]. There is substantial experimental evidence that ROS is produced in the

brain during ischemia and reperfusion injury [7]. These reactive species are often divided into two groups: ROS and reactive nitrogen species (RNS).

Reactive oxygen species are potent oxidizing and reducing agents that directly damage cellular membranes through lipid peroxidation (LPO) [8]. Alternative approaches to demonstrate their involvement in cerebral ischemic damage have concentrated on measuring the rate of consumption of endogenous protective molecules or the formation of byproducts of LPO, such as malondialdehyde (MDA) [9]. The antioxidant enzyme capacity of the tissue affected by ischemia/reperfusion (I/R) is particularly important for primary endogenous defense against free radical (FR)-induced injury. Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) are endogenous antioxidants that play a role in the prevention of oxidative injury [10]. NO is beneficial as a messenger or modulator, but in conditions such as oxidative stress, it is potentially toxic. The toxic effects of NO may be attributed to $ONOO^-$, which is a reaction product of NO with superoxide (O_2^-). Nitric oxide synthase (NOS) activity and NO release are greatly increased in the acutely ischemic brain. There are several sources for NO overproduction caused by cerebral ischemia. The described elevation in intracellular Ca^{2+} after the ischemia is a likely source of the production of NO via nNOS activation. Therefore, an enhancement of antioxidant activity in brain tissues may be potentially beneficial for neuronal recovery from I/R injury [11].

Tadalafil (TAD) is a selective inhibitor of cyclic guanosine monophosphate (cGMP)-specific phosphodiesterase type 5 (PDE5) and represents a class of drugs that have lately been reported to possess neuroprotective features [12]. It crosses the blood–brain barrier and prevents the accumulation of ROS when their production is accelerated and increases antioxidant cellular protective mechanisms [12–14]. However, its neuroprotective effects have rarely been investigated in global cerebral ischemia in rats [15, 16]. Therefore, the aim of the present study was to investigate the effectiveness of TAD treatment in preventing brain I/R injury in rats.

Materials and methods

Animals and experimental procedures

The animals were procured, maintained, and used in accordance with the Animal Welfare Act and the *Guide for the Care and Use of Laboratory Animals* by Mustafa Kemal University, Animal Ethical Committee. Male Wistar rats weighing 200–250 g were housed in polycarbonate cages and given the standard laboratory chow and water at 24 °C with 42 ± 5 % relative humidity in a 12–12-h light–dark cycle. Body temperature was maintained around 37 ± 5 °C throughout the surgical procedure.

Experimental design

Rats were divided into three groups: (1) control ($n = 10$), (2) I/R ($n = 10$) (ischemia was induced by bilateral occlusion of the carotid arteries for 20 min and reperfusion was achieved by releasing the occlusion to restore the circulation for 20 min), (3) I/R + TAD ($n = 10$) (2 mg/kg/day for 4 days before ischemia). Ischemia was induced 12 h after last dose of TAD (Lifta; Abdi Ibrahim, Istanbul, Turkey), a long-acting phosphodiesterase type-5 (PDE-5).

Induction of cerebral ischemia

Rats were anesthetized with ketamine hydrochloride (75 mg/kg) and xylazine (8 mg/kg). Two common carotid arteries were exposed through lateral incisions and separated from the nervous vagus. Ischemia was induced by bilateral clamping of the common carotid arteries for 20 min. Reperfusion was achieved by declamping the arteries to restore the circulation for 20 min more. Blood pressure was monitorized using a femoral transducer.

Tissue samples

Brain tissues were rapidly excised, and parietal lobes of right hemispheres were used for microscopic examination. The left hemispheres were used for biochemical analyses. The tissues were weighed and homogenized in 4 volumes of ice-cold Tris–HCl buffer (50 mM, pH 7.4) containing 0.50 ml/l Triton X-100 with a homogenizer (IKA Ultra-Turrax T 25 Basic) for 2 min at 13,000 rpm at +4 °C. Tissue homogenates were centrifuged at 5,000 *g* for 60 min to remove debris, and the clear supernatant fluids were separated and kept at −40 °C until the enzyme activity measurements were performed (about a week later).

Biochemical determination

The brain tissue samples were stored at −70 °C until tissue analysis of MDA levels, NO, SOD, and GSH-Px activities. Brain tissues were homogenized for 2 min at 5,000 rpm in four volumes of ice-cold Tris–HCl buffer (50 mM, pH 7.4) using a glass Teflon homogenizer (Ultra-Turrax IKA T10 Basic, Germany) for MDA, NO, and protein measurement. The homogenates were then centrifuged at 5,000 *g* for 60 min to remove debris and obtain supernatant. Supernatant fluids were collected and used for measuring GSH-Px activities and protein concentration. The supernatant solutions were mixed with an equal volume of an ethanol/chloroform mixture (5/3, volume per volume v/v).

After centrifugation at 5,000 *g* for 30 min, the clear upper layer (the ethanol phase) was collected and used in the analysis of SOD activity and protein assays. All preparation procedures were carried out at +4 °C.

Determination of malondialdehyde level

The tissue MDA level was determined using a method from Esterbauer and Cheeseman based on its reaction with thiobarbituric acid (TBA) at 90–100 °C. In the TBA test reaction, MDA and TBA react to produce a pink pigment with an absorption maximum at 532 nm at pH 2–3 and at 90 °C for 15 min. The sample was mixed with 2 volumes of cold 10 % (w/v) trichloroacetic acid to precipitate the protein. The precipitate was centrifuged and an aliquot of the supernatant was reacted with an equal volume of 0.67 % (w/v) TBA in a boiling water bath for 10 min. After cooling, the absorbance was read at 532 nm. Results were expressed as nmol/g of wet tissue, by reference to a standard curve prepared from measurements made with a standard solution (1,1,3,3-tetramethoxypropane).

Determination of nitric oxide level in tissue samples

The method for plasma nitrite and nitrate levels was based on the Griess reaction. Samples were initially deproteinized with Somogy's reagent. Total nitrite (nitrite + nitrate) was measured by spectrophotometry at 545 nm after conversion of nitrate to nitrite by copperized cadmium granules. A standard curve was established with a set of serial dilutions (10^{-8} to 10^{-3} mol/l) of sodium nitrite. Linear regression was done using the peak area from nitrite standards. The resulting equation was used to calculate the unknown sample concentrations. The NO levels were expressed as μmol/g wet tissue.

Determination of superoxide dismutase activity

Total (Cu–Zn and Mn) SOD (EC 1.15.1.1) activity was determined based on the method of Sun et al. [13]. The principle of the method is based on the inhibition of nitro blue tetrazolium (NBT) reduction by the xanthine–xanthine oxidase system as a superoxide generator. Activity was assessed in the ethanol phase of the supernatant, after 1 ml of an ethanol–chloroform mixture (5:3, v/v) was added to the same volume of the sample and centrifuged. One unit of SOD was defined as the amount of enzyme causing 50 % inhibition in the NBT reduction rate. The SOD activity was expressed as U/g protein.

Determination of glutathione peroxidase activity

Glutathione peroxidase (EC 1.6.4.2) activity was measured. The enzyme reaction in the tube containing NADPH, reduced glutathione (GSH) and sodium azide, and glutathione reductase was initiated by the addition of H_2O_2. The change in absorbance at 340 nm was monitored by a spectrophotometer. Activity was expressed as U/g protein.

Histopathological examination

Brain samples were fixed in 10 % neutral buffered formalin for light microscopic examination. After dehydrating with the graded alcohol series, tissues were embedded in paraffin. Several 5 μm thick transverse sections were obtained from brain tissue blocks and stained with hematoxylin–eosin for histological evaluation. Three sections from each rat were evaluated and totally 30 sections per group were evaluated for histopathological scoring. Sections were examined and photographed with an Olympus DP20 camera-attached Olympus CX41 photomicroscope. In addition, a statistical analysis modified from Shi et al. [17] and Nanri et al. [18] was made according to two parameters: the percentage of neurons showing signs of ischemia such as shrunken eosinophilic cell cytoplasm and pyknotic nuclei, and signs of tissue degeneration such as hemorrhage and edema (0, no signs of ischemia and degeneration; 1, scattered ischemic neurons and mild degeneration; 2, nearly half of the neurons are ischemic and moderate degeneration; 3, extensive cell loss or most of the neurons show signs of ischemia and severe degeneration) (Table 1).

Statistical analysis

Data were analyzed by using a commercially available statistics software package (SPSS®15 for Windows). Distributions of the groups were tested using the one-sample Kolmogorov–Smirnov test. The measured parameters for all groups were normally distributed, and the parametric statistical method was used to analyze the data. A one-way ANOVA test was performed, and post hoc multiple comparisons were done by Scheffe's method for adjusting significance levels in a linear regression analysis. Results were presented as mean ± standard error of means (SEM). A p value lower than 0.05 was regarded as statistically significant. This method was used in similar studies that had a small number of subjects.

Results

Biochemical results (oxidants and antioxidants)

Mean levels of SOD, GSH-Px, MDA, and NO in the brain tissue are listed in Table 2. MDA levels were significantly increased in the I/R group in comparison with the control

Table 1 Histopathologic scoring

	Grade 0	Grade 1	Grade 2	Grade 3
Ischemia	No sign	Scattered ischemic neurons	Nearly 50 % of neurons	Extensive cell loss
Degeneration	No sign	Mild	Moderate	Severe

Table 2 Histopathologic scoring

	Grade 0	Grade 1	Grade 2	Grade 3
Ischemia	No sign	Scattered ischemic neurons	Nearly 50 % of neurons	Extensive cell loss
Degeneration	No sign	Mild	Moderate	Severe

group ($p < 0.001$). MDA was significantly lower in the TAD-administered group than in the I/R group ($p < 0.05$). NO levels in both the I/R and TAD groups increased significantly compared to the control group. However, no statistical difference regarding NO levels was observed in the TAD group compared to the I/R group. The mean SOD level significantly decreased in the I/R group, while the mean SOD level was significantly higher in the I/R–TAD group than the I/R group (Fig. 1). The mean GSH-Px level significantly decreased in the I/R group, while the mean GSH-Px level was higher in the I/R–TAD group than the I/R group.

Histopathological results

Microscopic examination revealed normal neuronal structure in the control group (Fig. 2). In the I/R group, significant cell body shrinkage, increased eosinophily, and condensation of cell nucleus was observed (Fig. 3). In addition, the pia mater was detached in some regions (Fig. 4) and hemorrhage was observed under the pia mater (Fig. 5). In the I/R + TAD group, cellularity was increased compared to the I/R group (Fig. 6). In addition, the general neuronal structure was preserved (Fig. 7). Statistically, I/R caused significant cell loss and degeneration compared to the control group and Group 3. However, TAD administration increased cellularity and improved general tissue histology compared to Group 2 (Fig. 8). Histopathologic scores for each group is shown in Table 3.

Discussion

Two major hypotheses have been proposed for the phenomenon of I/R-induced neuronal death. The first, a neurotransmitter hypothesis, is related to the role of excitotoxic amino acids in the acute period of ischemia [9, 11]. The second, a FR hypothesis, is directed at the events during reperfusion [10].The formation of ROS initiates a vicious cascade of tissue injury. In particular, ROS leads to peroxidation of lipids as clearly seen in our study with consecutive alteration of membrane structure. These events provide evidence to explain delayed neuronal death after periods of I/R [11]. In animal studies, it has been shown that endothelial adhesion of polymorphonuclear leukocytes (PMN), which generate ROS and RNS, significantly contribute to the pathogenesis of reperfusion injury after focal ischemia [7].

The central nervous system contains large amounts of lipids which are vulnerable to FR-induced LPO, a major pathophysiologic mechanism of secondary neuronal and glial damage [9, 19–21]. The balance between the consumption of endogenous protective molecules and the formation of products of LPO, such as MDA [20], determines tissue resistance to oxidative injury. SOD and GSH-Px are endogenous antioxidants, which function in the prevention of oxidative injury [21–23]. Therefore, an enhancement in antioxidant activity in brain tissues may be potentially beneficial for neuronal recovery from I/R injury.

Tadalafil inhibits the cGMP specific PDE5 which is responsible for degradation of cGMP which then causes smooth muscle relaxation. Tadalafil is predominantly metabolized by CYP3A4 to a catechol metabolite which is then excreted mainly in the feces. Its half life is 17.5 h and has rare side effects like headache, back pain, flushing, and vision changes. It is commonly used to treat a number of diseases such as erectile dysfunction, prostatic hyperplasia, chronic obstructive pulmonary disease, hypertension, and coronary heart disease [24]. The mechanism of PDE-5 inhibitors includes the active inhibition of the PDE-5 enzyme catalyzing the hydrolysis of cGMP, thereby causing an increase in cGMP [25]. PDE-5 inhibitors may also inhibit the activity and expression of NADPH oxidase by enhancing cGMP levels [26]. Perk et al. [27] analyzed the effects of sildenafil citrate administration on LPO and antioxidant redox enzymes in the blood of healthy men. They found that sildenafil significantly increased SOD and CAT activities and slightly decreased MDA levels at 6 and 24 h in animal models. If the activity and expression of

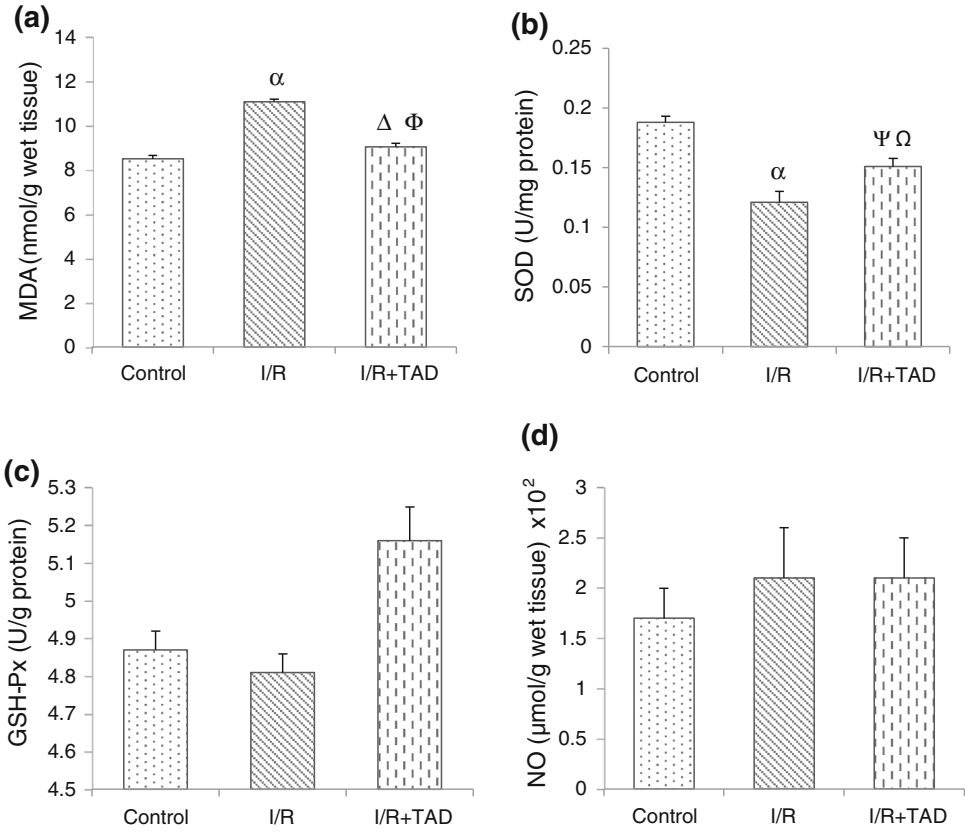

Fig. 1 Levels of MDA (**a**) and NO (**c**), and activities of SOD (**b**) and GSH-Px (**d**) enzymes from the control, I/R and I/R + TAD groups. All values represent mean ± SEM. *I/R* ischemia reperfusion-applied, *I/R + TAD* ischemia reperfusion + tadalafil-treated. $^{\alpha}p < 0.001$ compared to the control group; $^{\Delta}p < 0.05$ compared to the control group; $^{\Psi}p < 0.01$ compared with control group; $^{\Phi}p < 0.001$ compared to the I/R group; $^{\Omega}p < 0.001$ compared to the I/R group

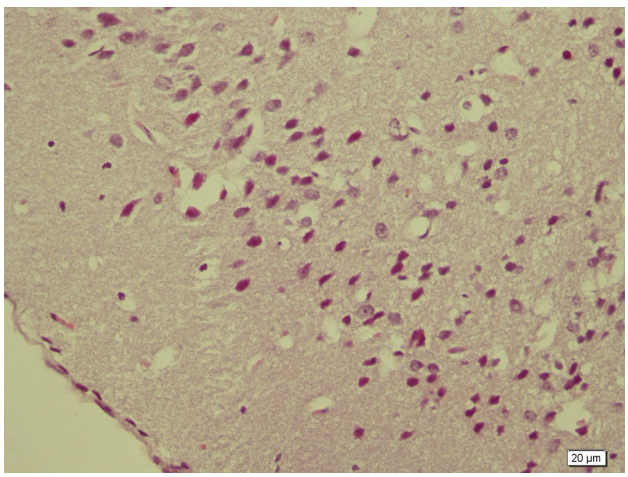

Fig. 2 Normal neuronal structure in control group

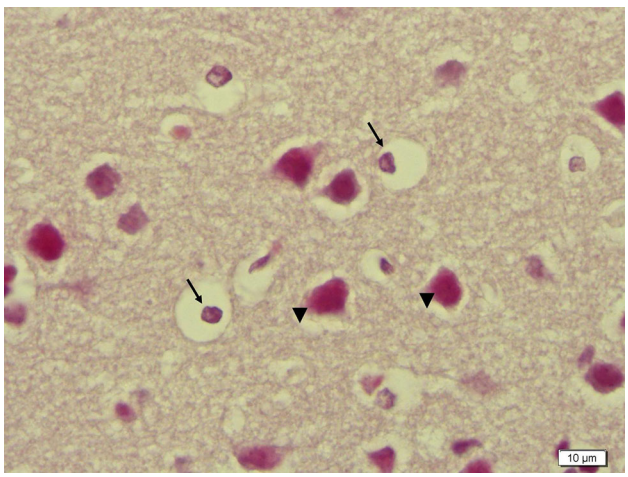

Fig. 3 Significant cell body shrinkage (*arrows*), increased eosinophily (*arrowheads*) and condensation of cell nucleus in I/R group

NADPH oxidase is inhibited, then the formation of ROS will decrease [27]. In several studies, it has been shown that cyclic adenosine monophosphate or cGMP analogs have protective properties against LPO in the plasma of rat neural [28] and renal [29] cells.

Tadalafil was previously reported to be beneficial in several I/R-induced brain and spinal cord pathologies [9, 12, 30]. Different PDE-5 inhibitors were also found to be effective in stroke models [31]. It was shown that TAD treatment decreased ischemia-induced neuronal cell death and apoptosis and enhanced cell proliferation in the hippocampus of rats [31, 32].

In our study, TAD administration decreased I/R-induced elevation in MDA levels and increased SOD level compared to the I/R group. Our biochemical results are in line with data from the existing literature. In addition, PDE-5 inhibitors have a protective effect on endothelial tissue by opening adenosine triphosphate-sensitive potassium (K-ATP)

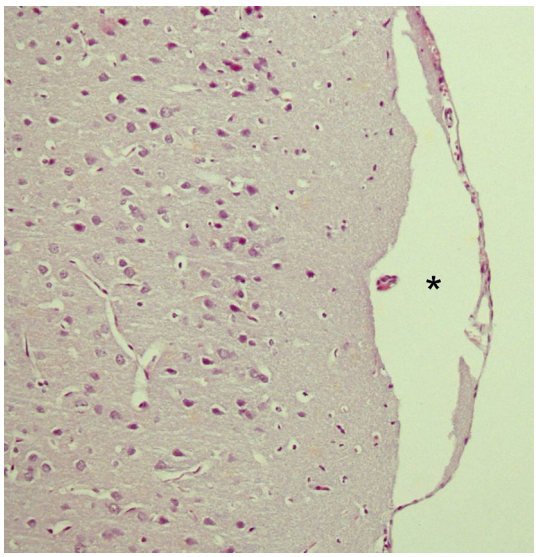

Fig. 4 Detached pia mater (*asterisk*) in I/R group

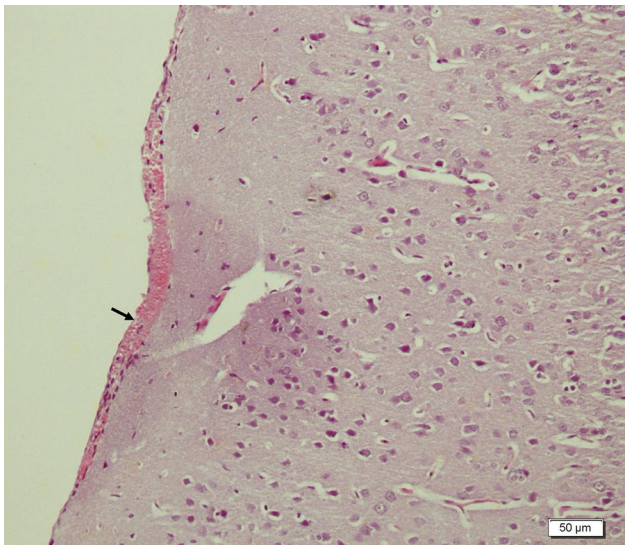

Fig. 5 Hemorrhage under pia (*arrow*) in I/R group

channels [33]. It was reported in another study that TAD increases the activity of antioxidant enzymes, such as SOD and GSH-Px, and decreases MDA levels after spinal cord injury in rats [34]. Sildenafil increased SOD, GSH-Px, and CAT levels and decreased MDA levels in testicular torsion/detorsion injury in rats [29]. Erol et al. [34] found an increase in MDA levels and a decrease in SOD and GSH-Px enzyme activities, a pattern of tissue damage, in an experimental SCI (spinal cord injury) study. Serarslan also reported similar findings in an experimental SCI model. Those levels were inverted by neuroprotective treatment. In another study, neuroprotective treatment significantly increased tissue GSH-Px and SOD levels between 22 and 26 h post-injury [35]. In a study by Ozdegirmenci et al.

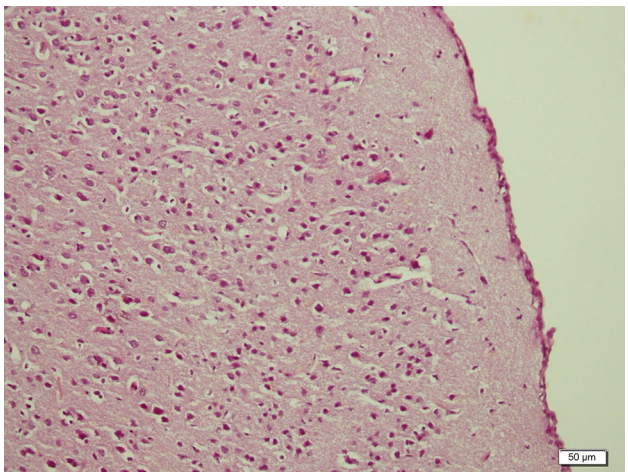

Fig. 6 Increased cellularity in TAD + IR group

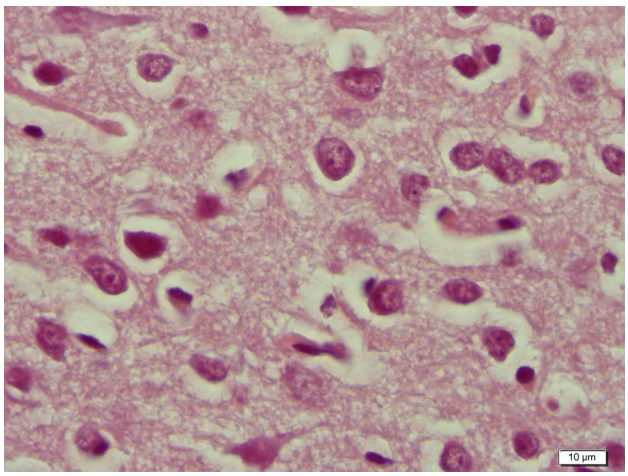

Fig. 7 Preserved neurons in TAD + IR group

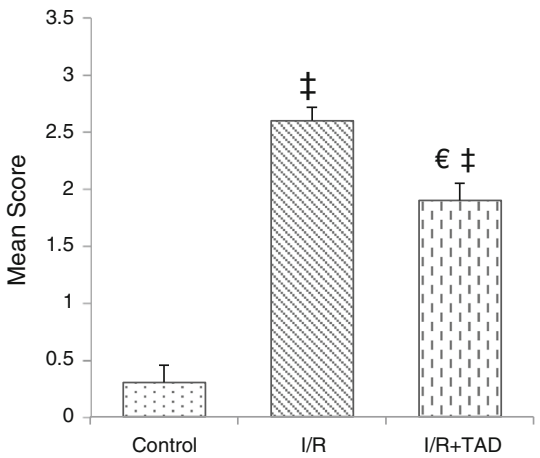

Fig. 8 Histopatologic scoring of the control, I/R and I/R + TAD groups. All values represent mean ± SEM. *I/R* ischemia reperfusion-applied, *I/R + TAD* ischemia reperfusion + tadalafil-treated. $^{\ddagger}p < 0.001$: compared to the control group; $^{\epsilon}p < 0.05$: compared to the I/R group

Table 3 Histopathologic scores of the groups

	Grade 0 (*n*)	Grade 1 (*n*)	Grade 2 (*n*)	Grade 3 (*n*)	Mean score ± SEM
Control (*n*: 10)	7	3	0	0	0.30 ± 0.48
I/R group (*n*: 10)	0	0	4	6	2.60 ± 0.51
I/R + TAD group (*n*: 10)	0	2	7	1	1.90 ± 0.56

$p < 0.001$: control vs I/R group, $p < 0.001$: control vs I/R + TAD group, $p < 0.05$: I/R vs I/R + TAD group. One-way ANOVA with post hoc Scheffe's test was used

[16], where the effects of sildenafil and TAD against I/R injury were compared in terms of biochemical parameters, TAD was found to be more potent than sildenafil by means of increasing GSH-Px activity significantly after I/R.

NO has an important role in modulating blood flow and tissue injury in normal and several pathological conditions. NO passes through cell membranes by several means without specific release or uptake mechanisms that induce changes in signal-related functions. The main signal transduction pathways of NO are the activation of the soluble guanylyl cyclases, the formation of cGMP, and the action of cGMP-dependent protein kinases in the nervous system. The intracellular level of cGMP is controlled by its rate of synthesis via guanylyl cyclases and/or by the rate of its degradation via PDE [36, 37].

Recently, it was shown that PDE-5 inhibitors augment the action of NO by preventing the hydrolysis of cGMP [38]. Nitric oxide is a potent inhibitor of NADPH oxidase expression, which in turn reduces O_2 formation in vascular smooth muscle cells from vascular tissue, an effect mediated by cGMP [39]. It follows that PDE-5 inhibitors, by increasing cGMP levels, may also inhibit the activity and expression of NADPH oxidase, and thus O_2 formation will be reduced; as a result, these would augment NO bioavailability.

Based on these mechanisms, it has been shown that PDE-5 inhibitors could increase NO levels in both humans [40] and rats with different tissue injuries. In our study, NO levels in the I/R and I/R + TAD groups increased significantly compared to the control group. But TAD didn't increase NO levels compared with the I/R group. In a study by Arıkan et al. [41], TAD administration increased NO levels in I/R injury compared to the control.

Histopathologically, one of the significant findings of ischemic encephalopathy is eosinophilic neurons (EN). EN was recognized as a neuron with cytoplasmic eosinophilia, exhibiting pyknosis, karyorrhexis, or karyolysis. It was reported by Sun et al. [42] that intense EN areas are prone to transform into infarct areas when compared to areas of low-density EN. In our study, the IR group showed a large number of EN compared to the I/R + TAD and control groups. In addition, we observed shrunken neurons with pyknotic nuclei, damaged vessels, and edema as reported in another study [42].

In conclusion, TAD prevented ischemia reperfusion-induced neurotoxicity as shown by our biochemical and histological findings, suggesting that ischemia reperfusion-induced neurotoxicity may be secondary to free oxygen radicals and that TAD's protective role may arise from its antioxidative and radical-scavenging effects. Thus, the concomitant use of TAD may help prevent ischemia–reperfusion-induced neurotoxicity.

Conflict of interest None.

References

1. Dirnagl U, Iadecola C, Moskowitz MA (1999) Pathobiology of ischaemic stroke: an integrated view. Trends Neurosci 22:391–397
2. Leker RR, Shohami E (2002) Cerebral ischemia and trauma-different etiologies yet similar mechanisms: neuroprotective opportunities. Brain Res Brain Res Rev 39:55–73
3. Benchoua A, Guegan C, Couriaud C, Hosseini H, Sampaio N, Morin D, Onteniente B (2001) Specific caspase pathways are activated in the two stages of cerebral infarction. J Neurosci 21:7127–7134
4. Lee JH, Lee YK, Ishikawa M, Koga K, Fukunaga M, Miyakoda G, Mori T, Hosokawa T, Hong KW (2003) Cilostazol reduces brain lesion induced by focal cerebral ischemia in rats—an MRI study. Brain Res 994:91–98
5. Connell BJ, Saleh MC, Khan BV, Saleh TM (2011) Apocynin may limit total cell death following cerebral ischemia and reperfusion by enhancing apoptosis. Food Chem Toxicol 49:3063–3069
6. Chen YH, Du GH, Zhang JT (2000) Salvianolic acid B protects brain against injuries caused by ischemia–reperfusion in rats. Acta Pharmacol Sin 21:463–466
7. Rodrigo J, Fernandez AP, Serrano J, Peinado MA, Martinez A (2005) The role of free radicals in cerebral hypoxia and ischemia. Free Radic Biol Med 39:26–50
8. Toyokuni S (1999) Reactive oxygen species-induced molecular damage and its application in pathology. Pathol Int 49:91–102
9. Schmidley JW (1990) Free radicals in central nervous system ischemia. Stroke 21:1086–1090
10. Akyol O, Herken H, Uz E, Fadillioglu E, Unal S, Sogut S, Ozyurt H, Savas HA (2002) The indices of endogenous oxidative and antioxidative processes in plasma from schizophrenic patients: the possible role of oxidant/antioxidant imbalance. Prog Neuropsychopharmacol Biol Psychiatry 26:995–1005

11. Chan PH (1996) Role of oxidants in ischemic brain damage. Stroke 27:1124–1129
12. Zhang L, Zhang Z, Zhang RL, Cui Y, LaPointe MC, Silver B, Chopp M (2006) Tadalafil, a long-acting type 5 phosphodiesterase isoenzyme inhibitor, improves neurological functional recovery in a rat model of embolic stroke. Brain Res 1118:192–198
13. Sun Y, Oberley LW, Li Y (1988) A simple method for clinical assay of superoxide dismutase. Clin Chem 34:497–500
14. Vignozzi L, Filippi S, Morelli A, Ambrosini S, Luconi M, Vannelli GB, Donati S, Crescioli C, Zhang XH, Mirone V, Forti G, Maggi M (2006) Effect of chronic tadalafil administration on penile hypoxia induced by cavernous neurotomy in the rat. J Sex Med 3:419–431
15. Ko IG, Shin MS, Kim BK, Kim SE, Sung YH, Kim TS, Shin MC, Cho HJ, Kim SC, Kim SH, Kim KH, Shin DH, Kim CJ (2009) Tadalafil improves short-term memory by suppressing ischemia-induced apoptosis of hippocampal neuronal cells in gerbils. Pharmacol Biochem Behav 91:629–635
16. Ozdegirmenci O, Kucukozkan T, Akdag E, Topal T, Haberal A, Kayir H, Oter S, Akyol M, Uzbay T (2011) Effects of sildenafil and tadalafil on ischemia/reperfusion injury in fetal rat brain. J Matern Fetal Neonatal Med 24:317–323
17. Shi LL, Chen BN, Gao M, Zhang HA, Li YJ, Wang L, Du GH (2011) The characteristics of therapeutic effect of pinocembrin in transient global brain ischemia/reperfusion rats. Life Sci 88: 521–528
18. Nanri K, Montécot C, Springhetti V, Seylaz J, Pinard E (1998) The selective inhibitor of neuronal nitric oxide synthase, 7-nitroindazole, reduces the delayed neuronal damage due to forebrain ischemia in rats. Stroke 29(6):1248–1253
19. Diaz-Ruiz A, Rios C, Duarte I, Correa D, Guizar-Sahagun G, Grijalva I, Madrazo I, Ibarra A (2000) Lipid peroxidation inhibition in spinal cord injury: cyclosporin-A vs methylprednisolone. NeuroReport 11:1765–1767
20. Kurt G, Ergun E, Cemil B, Borcek AO, Borcek P, Gulbahar O, Ceviker N (2009) Neuroprotective effects of infliximab in experimental spinal cord injury. Surg Neurol 71:332–336 (discussion 336)
21. Şahin Ş, Söğüt S, Özyurt H, Uz E, İlhan A, Akyol Ö (2002) Tissue xanthine oxidase activity and nitric oxide levels after spinal cord ischemia/reperfusion injury in rabbits: comparison of caffeic acid phenethyl ester (CAPE) and methylprednisolone. Neurosci Res Commun 31:111–121
22. Ozerol E, Bilgic S, Iraz M, Cigli A, Ilhan A, Akyol O (2009) The protective effect of erdosteine on short-term global brain ischemia/reperfusion injury in rats. Prog Neuropsychopharmacol Biol Psychiatry 33(1):20–24
23. Gorgulu A, Kiris T, Unal F, Turkoglu U, Kucuk M, Cobanoglu S (2000) Superoxide dismutase activity and the effects of NBQX and CPP on lipid peroxidation in experimental spinal cord injury. Res Exp Med (Berl) 199:285–293
24. van Driel MF (2006) Phosphodiesterase inhibitors: effectiveness and new applications. Ned Tijdschr Geneeskd 150:1613–1616 (abstract in English)
25. Corbin JD, Francis SH (2002) Pharmacology of phosphodiesterase-5 inhibitors. Int J Clin Pract 56:453–459
26. Jeremy JY, Ballard SA, Naylor AM, Miller MA, Angelini GD (1997) Effects of sildenafil, a type-5 cGMP phosphodiesterase inhibitor, and papaverine on cyclic GMP and cyclic AMP levels in the rabbit corpus cavernosum in vitro. Br J Urol 79:958–963
27. Perk H, Armagan A, Naziroglu M, Soyupek S, Hoscan MB, Sutcu R, Ozorak A, Delibas N (2008) Sildenafil citrate as a phosphodiesterase inhibitor has an antioxidant effect in the blood of men. J Clin Pharm Ther 33:635–640
28. Keller JN, Hanni KB, Mattson MP, Markesbery WR (1998) Cyclic nucleotides attenuate lipid peroxidation-mediated neuron toxicity. NeuroReport 9:3731–3734
29. Guzeloglu M, Yalcinkaya F, Atmaca S, Bagriyanik A, Oktar S, Yuksel O, Fansa I, Hazan E (2011) The beneficial effects of tadalafil on renal ischemia–reperfusion injury in rats. Urol Int 86:197–203
30. Serarslan Y, Yonden Z, Ozgiray E, Oktar S, Guven EO, Sogut S, Yilmaz N, Yurtseven T (2010) Protective effects of tadalafil on experimental spinal cord injury in rats. J Clin Neurosci 17: 349–352
31. Royl G, Balkaya M, Lehmann S, Lehnardt S, Stohlmann K, Lindauer U, Endres M, Dirnagl U, Meisel A (2009) Effects of the PDE5-inhibitor vardenafil in a mouse stroke model. Brain Res 1265:148–157
32. Baek SB, Bahn G, Moon SJ, Lee J, Kim KH, Ko IG, Kim SE, Sung YH, Kim BK, Kim TS, Kim CJ, Shin MS (2011) The phosphodiesterase type-5 inhibitor, tadalafil, improves depressive symptoms, ameliorates memory impairment, as well as suppresses apoptosis and enhances cell proliferation in the hippocampus of maternal-separated rat pups. Neurosci Lett 488:26–30
33. Gori T, Sicuro S, Dragoni S, Donati G, Forconi S, Parker JD (2005) Sildenafil prevents endothelial dysfunction induced by ischemia and reperfusion via opening of adenosine triphosphate-sensitive potassium channels: a human in vivo study. Circulation 111:742–746
34. Erol FS, Kaplan M, Tiftikci M, Yakar H, Ozercan I, Ilhan N, Topsakal C (2008) Comparison of the effects of octreotide and melatonin in preventing nerve injury in rats with experimental spinal cord injury. J Clin Neurosci 15:784–790
35. Taskiran D, Tanyalcin T, Sozmen EY, Peker GO, Gulmen V, Cagli S, Kanit L, Tekeli G, Barcin E, Zileli M, Kutay FZ (2000) The effects of melatonin on the antioxidant systems in experimental spinal injury. Int J Neurosci 104:63–73
36. de Vente J, Markerink-van Ittersum M, Vles JS (2006) The role of phosphodiesterase isoforms 2, 5, and 9 in the regulation of NO-dependent and NO-independent cGMP production in the rat cervical spinal cord. J Chem Neuroanat 31:275–303
37. Marsala J, Orendacova J, Lukacova N, Vanicky I (2007) Traumatic injury of the spinal cord and nitric oxide. Prog Brain Res 161:171–183
38. Rosen RC, Kostis JB (2003) Overview of phosphodiesterase 5 inhibition in erectile dysfunction. Am J Cardiol 92:9M–18M
39. Muzaffar S, Shukla N, Angelini G, Jeremy JY (2004) Nitroaspirins and morpholinosydnonimine but not aspirin inhibit the formation of superoxide and the expression of gp91phox induced by endotoxin and cytokines in pig pulmonary artery vascular smooth muscle cells and endothelial cells. Circulation 110: 1140–1147
40. Rosano GM, Aversa A, Vitale C, Fabbri A, Fini M, Spera G (2005) Chronic treatment with tadalafil improves endothelial function in men with increased cardiovascular risk. Eur Urol 47:214–220 (discussion 220–212)
41. Arikan DC, Bakan V, Kurutas EB, Sayar H, Coskun A (2010) Protective effect of tadalafil on ischemia/reperfusion injury of rat ovary. J Pediatr Surg 45:2203–2209
42. Sun L, Kuroiwa T, Ishibashi S, Katsumata N, Endo S, Mizusawa H (2006) Transition of areas of eosinophilic neurons and reactive astrocytes to delayed cortical infarcts after transient unilateral forebrain ischemia in Mongolian gerbils. Acta Neuropathol 111:21–28