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Resuscitation from experimental traumatic brain injury by magnolol therapy

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ABSTRACT

Background: The purpose of the present study was to determine whether magnolol, a free radical scavenger, mitigates the deleterious effects of traumatic brain injury (TBI).
Material and methods: Traumatic brain injuries were induced in anesthetized male Sprague-Dawley rats using fluid percussion, and the rats were divided into groups treated with magnolol (2 mg/kg, intravenously) or vehicle. A group of rats that did not undergo TBI induction was also studied as controls. Biomarkers of TBI, including glycerol and 2,3-dihydroxybenzoic acid, were evaluated by microdialysis. Infraction volume, extent of neuronal apoptosis, and antiapoptosis factor transforming growth factor β1 (TGF-β1) were also measured. Functional outcomes were assessed by motor assays.
Results: Compared with the rats without TBI, the animals with TBI exhibited higher hippocampal glycerol and 2,3-dihydroxybenzoic acid. Relative to the vehicle-treated group, the magnolol-treated group showed decreased hippocampal levels of glycerol and hydroxyl radical levels. The magnolol-treated rats also exhibited decreased cerebral infarction volume and neuronal apoptosis and increased antiapoptosis-associated factor TGF-β1 expression. These effects were translated into improved motor function post TBI.
Conclusions: Our results suggest that intravenous magnolol injection mitigates the deleterious effects of TBI in rats based on its potent free radical scavenging capability, and the mechanism of anti–neuronal apoptosis is partly due to an increase in TGF-β1 expression in the ischemic cortex.

1. Introduction

In secondary damage after traumatic brain injury (TBI), reactive oxidative injury plays an important role in early neuronal damage [1]. It has been suggested that TBI triggers overproduction of reactive oxygen species, such as free radical formation, leading to cell damage through cell membrane lipid peroxidation and destruction, cleavage of DNA, the oxidation of proteins, and loss of their functions. Eventually, cells die through the apoptotic pathway [2,3]. This hypothesis is based on the brain's being highly sensitive to oxidative stress because of the following: (1) it possesses a higher oxygen

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metabolic rate than any other organ in the body, resulting in intense reduction of reactive oxygen metabolites [4]; (2) membrane lipids in the brain have high levels of polyunsaturated fatty acids as a source for lipid peroxidation reactions [5]; and (3) antioxidant activity in the brain is relatively scarce [1,5]. These hypotheses raise the possibility that high levels of oxidative stress will result in neuronal degeneration or death in so-called reactive oxygen species–dependent apoptosis or caspase-independent apoptosis [6]. Hence, exogenous administration of pharmacologic agents that promote the neutral antioxidant system has been regarded as a useful strategy to prevent secondary injury induced by TBI.

Magnolol, a blood-brain barrier–permeable hydroxylated biphenyl compound, was isolated and purified from the cortex of *Magnolia officinalis* [7–9]. Tsai *et al.* demonstrated that magnolol has clear peripheral and central nervous system pharmacokinetic effects; its half-life (2 mg/kg) in one bolus is 54.15 ± 5.14 min. The mean hippocampus magnolol concentration in rats 10 min after magnolol administration (5 mg/kg) was approximately 13.41 ± 1.10 μg/g [9]. Magnolol is a Chinese medicinal herb that is commonly used in China for the relief of stroke, myocardial infarction, headache, anxiety, diarrhea, and fever [10]. In addition to being an effective scavenger of free radicals, such as hydroxyl radicals [11], magnolol has been demonstrated to attenuate heatstroke-induced increased free radical production and lipid peroxidation in the brain [12], in rat liver mitochondria [13], and in rat heart mitochondria [14]. These results imply that magnolol might play a role in protecting against secondary cerebral damage, as well as in scavenging free radicals and subsequently reducing the lipid peroxidation associated with TBI.

To date, the therapeutic applications of magnolol have been used in many fields but not in neurotrauma. No direct mechanism has been proposed for the effects of magnolol on traumatic brain injury. In the current study, we chose magnolol specifically because of its potent antioxidant effects and because it is blood-brain barrier permeable, it has clear pharmacokinetic activity in the central nervous system, and it is a commonly used Chinese medicinal herb; most importantly, the therapeutic applications of magnolol have been used in many fields but not in neurotrauma. In this study, we hypothesized that magnolol would have therapeutic effects on TBI based on its being a powerful free radical scavenger. To test this hypothesis, experiments were conducted to assess the therapeutic effects of magnolol on brain cellular damage and ischemia, using an intracerebral microdialysis technique. In addition, this study compared motor deficits, cerebral infarction, and neuronal apoptosis and antiapoptosis-associated factor transforming growth factor β1 (TGF-β1) expression during TBI in rats with or without magnolol therapy.

2. Materials and methods

2.1. Animals

Adult male Sprague-Dawley rats weighing 300–400 g were used in these experiments. The animals were kept under a 12/12-h light/dark cycle and were allowed free access to food and water. All of the experimental procedures were approved by the Animal Research Committee of the Chi-Mei Medical Center, in compliance with the U.S. National Institutes of Health's regulations for the use of animals in research, to minimize discomfort to the animals during surgery and during the recovery period. At the end of the experiments, the control rats and any rats that survived TBI were sacrificed with an overdose of urethane.

2.2. Traumatic brain injury

The animals were anesthetized with intraperitoneal administration of sodium pentobarbital (50 mg/kg), ketamine (intramuscular, 44 mg/kg), rompun (intramuscular, 6.77 mg/kg), and atropine (intramuscular, 0.026 mg/kg). A craniectomy 2 mm in radius, 4 mm from the bregma and 3 mm from the sagittal sutures and in the right parietal cortex, was performed *via* stereotaxic framing. After craniectomy and implantation of an injury cannula, a fluid percussion injury (FPI) device was connected to the animal *via* Luer-loc fitting. Moderate FPI (2.2 atm) and 25 ms of percussion were produced by rapidly injecting a small volume of saline into the closed cranial cavity with a fluid percussion device (VCU Biomedical Engineering, Richmond, VA). This procedure created a moderate severity of brain trauma, as originally described by McIntosh *et al.* [15]. A transient hypertensive response, apnea, and seizure were observed immediately following fluid percussion injury, and these reactions were used as the criteria for separating the animals into sham-operated groups.

2.3. Drug administration

Magnolol was purchased from Yoneyama Pharmaceutical Industries, Ltd (Osaka, Japan) (lot no. MZD1030). It was freshly prepared prior to use and was dissolved in 0.5% carboxymethylcellulose (CMC). To determine and choose the effective dosage, magnolol was injected *via* the femoral vein at a dosage of 0.2 mg/kg or 2 mg/kg immediately after TBI.

2.4. Experimental groups

The animals were randomly assigned to the sham group, the FPI brain CMC (vehicle) group, or the FPI brain magnolol group. All of the tests were performed with the investigators blinded to the study groups, which were revealed only at the end of analyses. In the FPI vehicle- and magnolol-treated groups, the animals were treated, respectively, with 3 cc CMC or 2 mg/kg magnolol *via* the right femoral vein immediately after injury.

2.5. Measurement of extracellular glycerol and 2,3-dihydroxybenzoic acid in the hippocampus

The total recorded time was 4 h from stable to 2 h post injury in the acute stage. A microdialysis probe (4 mm in length, CMA/2; Carnegie Medicine, Stockholm, Sweden) was stereotaxically and obliquely (anterior 4.3 mm) implanted into the right hippocampus, according to the atlas and coordinates of Paxinos and Watson [16]: P, 8 mm; R, 3 mm; and H, 5 mm. According to the methods described previously [17,18],

microdialysis was perfused at 2.0 μL/min, and the dialysates were sampled in microvials. The dialysates were collected every 20 min in a CMA/140 fraction collector (Carnegie Medicine). Aliquots of the dialysates (5 μL) were injected onto a CMA 600 microdialysis analyzer (Carnegie Medicine) for the measurement of lactate, glycerol, pyruvate, glutamate, and hydroxyl radical.

For the measurement of extracellular hydroxyl radicals in the hippocampus, the implanted probe was perfused with artificial cerebrospinal fluid containing 10 mM salicylic acid, using a high-pressure pump (CMA/Microdialysis; RosLagsvägen, Stockholm, Sweden) at a flow rate of 1.2 μL/min [19]. It is known that salicylate can react with hydroxyl radicals to generate stable dihydroxybenzoic acid (DHBA) derivatives, in particular 2,3-DHBA, which can be used as an *in vivo* index of hydroxyl radical levels [20]. The volume of 2,3-DHBA in dialysates was measured by high-performance liquid chromatography using a two-channel electrochemical detector (LC-4C; Bioanalytical Systems, West Lafayette, IN). Only experiments in which the hippocampal localization of the microdialysis probes was confirmed histologically were included in the results.

2.6. Cerebral infarction assay

Infarct volume was assessed using 2,3,5-triphenyltetrazolium chloride (TTC) (Sigma, St, Louis, MO) staining, as demonstrated in a previous study [17]. Three days after injury, all of the animals were sacrificed. The brain slices in 2-mm sections were incubated in 2% TTC, dissolved in phosphate-buffered saline for 30 min at 37°C, and then transferred to 5% formaldehyde solution for fixation. The volume of infarction, as revealed by negative TTC staining (pale color), was measured in each slice and was totaled using computerized planimetry (PC-based Image Tools software; Media Cybernetics, Inc). The infarction volume was calculated as 2 mm (thickness of the slice) × (sum of the infarction area in all brain slices [mm^2]).

2.7. Neuronal apoptotic assay

Three days after injury, neuronal apoptotic cells were identified by double staining with terminal deoxynucleotidyl transferase–mediated dUTP-biotin nick end labeling (TUNEL) and the neuronal nuclear marker Neu-N [21]. These procedures followed those described previously [3]. The number of TUNEL/Neu-N-positive cells was calculated in five coronal sections from each rat and was totaled using computerized planimetry (PC-based Image Tools software). The following antibodies were used in this study: monoclonal mouse anti-Neu-N antibody (MAB377; Chemicon Millipore Corporation, Billerica, MA), detected with Alexa-Fluor 568 anti-mouse (IgG) antibody (A11031; Life Technologies Co, Grand Island, NY).

2.8. Neuronal apoptosis–associated TGF-β1 expression

TGF-β1 expression in neuronal cells was detected using immunofluorescence. Adjacent 50-μm sections, corresponding to coronal coordinates 2.0–7.0 mm posterior to the bregma, were obtained consecutively. These procedures followed those described previously [3]. Polyclonal rabbit anti-TGF-β1 antibody (ab92486; Abcam, Boston, MA) was used, which was then detected with Alexa-Fluor 488 anti-rabbit (IgG) antibody (A11034; Life Technologies Co). The numbers of labeled cells were calculated in five coronal sections from each rat and were expressed as the mean numbers of cells per section. For negative coronal sections, all of the procedures were performed in the same manner but without the primary antibodies.

2.9. Functional outcomes

An inclined plane was used to measure limb strength. The animals were placed facing right and then facing left, perpendicular to the slope of a 20 × 20-cm rubber-ribbed surface of an inclined plane, starting at an angle of 55 degrees [22]. The angle was increased or decreased in 5-degree increments to determine the maximal angle at which an animal could hold to the plane. The data for each day consisted of the mean of left and right side maximal angles.

2.10. Statistical analysis

The results are expressed as the means ± standard errors of the means for n experiments. Two-way analysis of variance for repeated measurements (in the same animals) was used for factorial experiments, whereas the Dunnett test was used for *post hoc* multiple comparisons among means. A value of $P < 0.05$ was considered to indicate a statistically significant difference.

3. Results

3.1. Magnolol (2 mg/kg, intravenously) significantly reduced FPI-induced cerebral infarction volume

From the preliminary experiments to determine the effective dosage of magnolol in FPI rats, compared with that in the sham and FPI controls, and due to treatment with 0.2 mg/kg intravenously (iv), the FPI-induced infarction volume (183.8 ± 17.7 mm^2) was significantly decreased by magnolol treatment with 2 mg/kg iv (110.5 ± 7.2 mm^2) but not treatment with 0.2 mg/kg iv (169.7 ± 5.9 mm^2) ($P < 0.05$; $n = 8$) (Fig. 1). Thus, the therapeutic dosage of 2 mg/kg magnolol was based on this preliminary experiment.

3.2. Magnolol significantly attenuated FPI-induced hydroxyl radical formation

Treatment with magnolol (2 mg/kg, iv) immediately after FPI significantly attenuated the TBI-induced overproduction of hydroxyl radicals in the hippocampus ($P < 0.05$; $n = 8$) (Fig. 2).

3.3. Magnolol decreased cell damage markers during FPI

Treatment with magnolol (2 mg/kg, iv) immediately after FPI significantly attenuated the TBI-induced overproduction of cellular injury markers (glycerol) in the hippocampus ($P < 0.05$; $n = 8$) (Fig. 3).

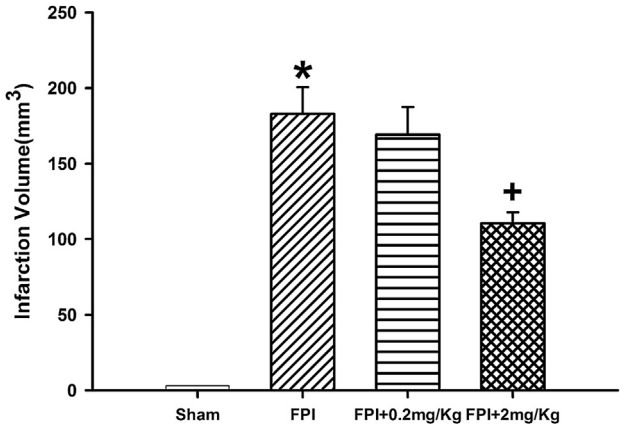

Fig. 1 – Effects of magnolol (2 mg/kg, iv) treatment on FPI-induced cerebral infarction volume. *P < 0.05 compared with the sham group; +P < 0.05 compared with magnolol (2 mg/kg, iv) treatment in the FPI group.

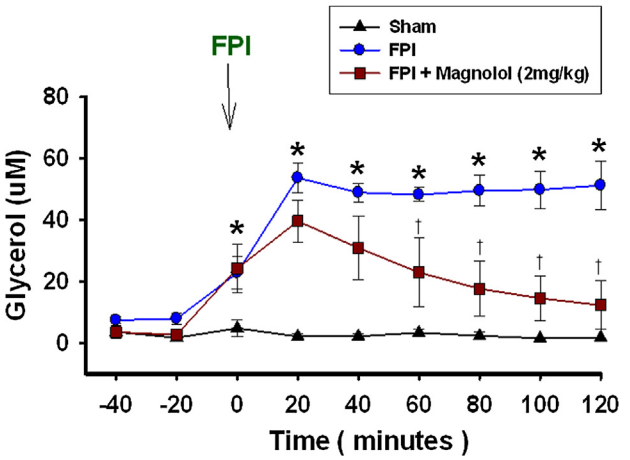

Fig. 3 – Effects of magnolol (2 mg/kg, iv) on the FPI-induced extracellular levels of glycerol in the hippocampus. *P < 0.05 compared with the sham group; +P < 0.05 compared with magnolol (2 mg/kg, iv) treatment in the FPI group.

3.4. *Magnolol significantly decreased FPI-induced neuronal cell apoptosis (TUNEL plus Neu-N stain assay)*

The positive TUNEL plus Neu-N in neuronal cells from the ischemic cortex were significantly increased ($P < 0.05$; $n = 8$), compared with those in the sham controls, at 72 h after TBI. However, the number of TUNEL plus Neu-N–positive cells among neuronal cells in the cortex resulting from FPI induction was significantly decreased ($P < 0.05$; $n = 8$) by magnolol therapy (Fig. 4).

3.5. *Magnolol significantly increased TGF-β1 expression in neuronal cells in the cortex (TGF-β1 plus Neu-N stain assay) after FPI*

The positive TGF-β1 expression in neuronal cells in the ischemia cortex was significantly decreased ($P < 0.05$; $n = 8$) compared with that in the sham controls at 72 h after FPI. However, the number of TGF-β1–positive cells among the neuronal cells in the cortex resulting from FPI induction was significantly increased ($P < 0.05$; $n = 8$) by magnolol therapy (Fig. 5).

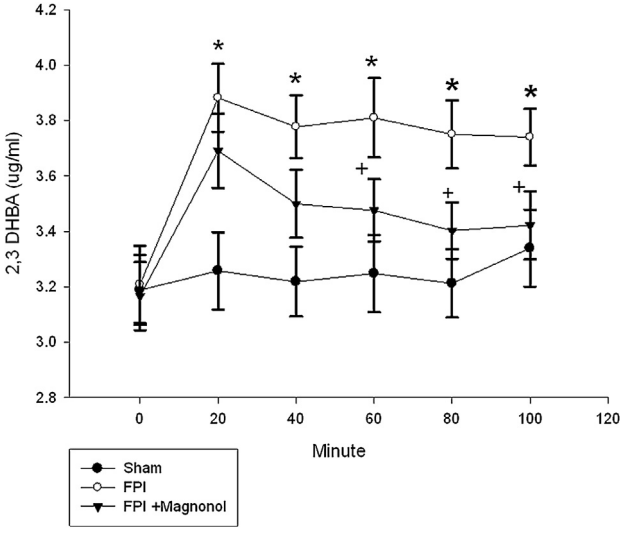

Fig. 2 – Effects of magnolol (2 mg/kg, iv) treatment on the FPI-induced hydroxyl radical overproduction. *P < 0.05 compared with the sham group; +P < 0.05 compared with magnolol (2 mg/kg, iv) treatment in the FPI group.

3.6. *Magnolol significantly attenuates FPI-induced motor deficits*

Three days after TBI, behavioral tests ($P < 0.05$; $n = 8$) revealed that vehicle-treated TBI rats had significantly lower performance on motor function tests than the sham-operated controls. FPI-induced motor dysfunction was significantly reduced by magnolol (2 mg/kg, iv) therapy (Fig. 6).

4. Discussion

4.1. *Novelty of the present study*

In the current study, magnolol was administered at a single dose of 2 mg/kg to counteract the cell damage, neuronal apoptosis in the brain, and neurologic deficits induced by TBI in rats by exerting its potent free radical scavenging abilities. To our knowledge, this is the first study to present neuroprotective effects using magnolol injection in traumatic CNS injury. We further demonstrated that magnolol's mechanism of anti–neuronal apoptosis occurs partly due to an increase in TGF-β1 expression in the ischemic cortex.

4.2. *Magnolol's effects on FPI-induced hydroxyl radical formation*

In the present study, a microdialysis probe was implanted into the hippocampus of the ipsilateral brain, which is most susceptible to cerebral ischemia and injury after FPI [23].

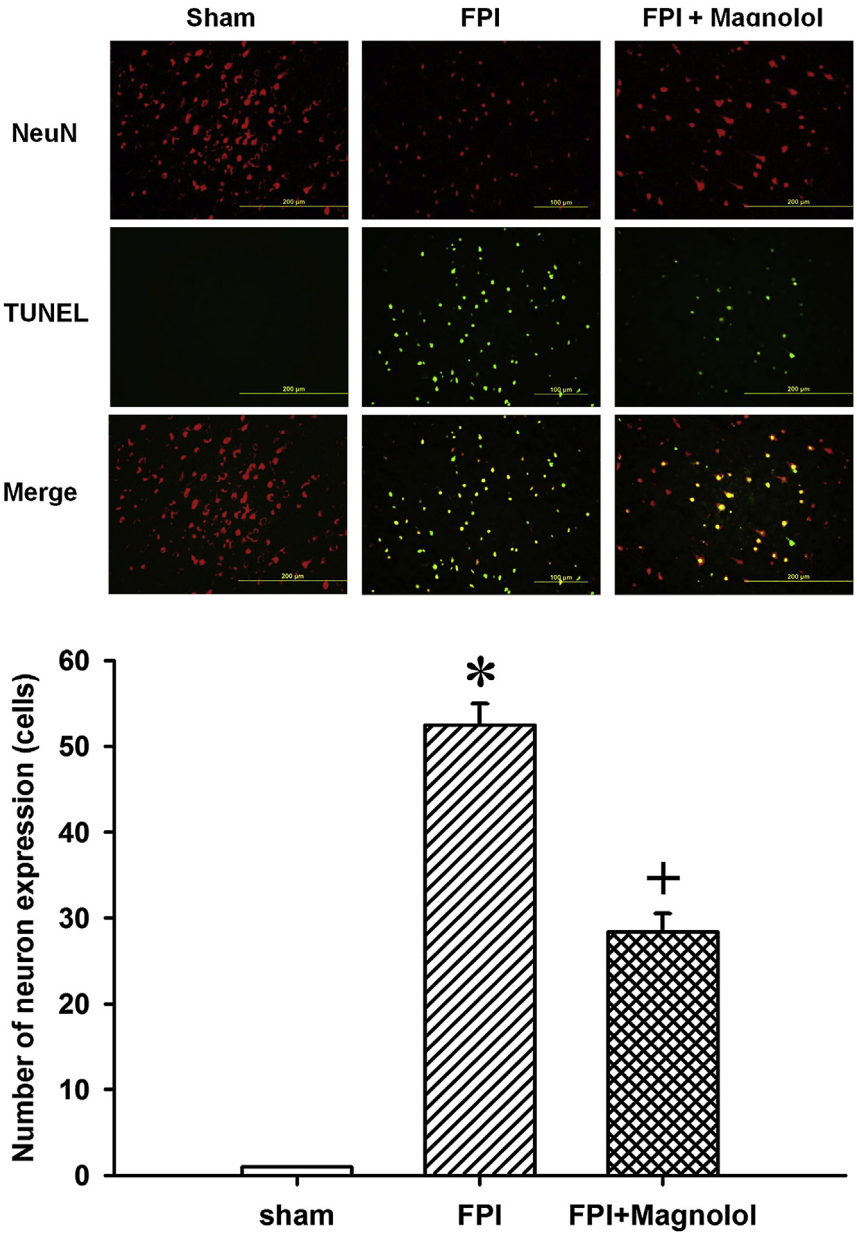

Fig. 4 – Effects of magnolol (2 mg/kg, iv) treatment on the FPI-induced neuronal apoptosis at 3 d following FPI. Top panels depict representative positive Neu-N and TUNEL staining for one sham rat, one FPI rat, and one FPI + magnolol-treated rat. $^{*}P < 0.05$ compared with the sham group; $^{+}P < 0.05$ compared with magnolol (2 mg/kg, iv) treatment in the FPI group.

The microdialysates obtained from the hippocampal region were assayed for the measurement of hydroxyl free radical formation and glycerol during FPI. 2,3-DHBA is regarded as specific for hydroxyl radical production, and it can be estimated with high-pressure liquid chromatography [20]. Although free radical formation and damage to neurons are secondary events, hydroxyl radical concentrations could be observed in the cortex of injured rats at 5 min post injury [24]. In our studies, we first measured the 2,3-DHBA level at 20 min. Thus, injection of magnolol immediately after injury could mitigate the effects of free radicals. We also found that, in the acute stage of FPI, 2,3-DHBA was detected at the maximum of 20 min after TBI (Fig. 2), and it significantly decreased at 60 min after magnolol administration. The hydroxyl radical level was correlated with the cell damage marker glycerol [25], a cellular marker of how severely cell membranes are damaged by ongoing pathology over 100 min of measurement (Fig. 3), as reported by Chang *et al.* [12]. The present data indicate that early treatment with magnolol could attenuate TBI by reducing the excessive accumulation of hydroxyl free radicals in the brain. These findings are consistent with those of several other animal models. For example, treatment with magnolol has proved neuroprotective in rat endothelial cells [26] and liver ischemia-reperfusion injury [27]. These results

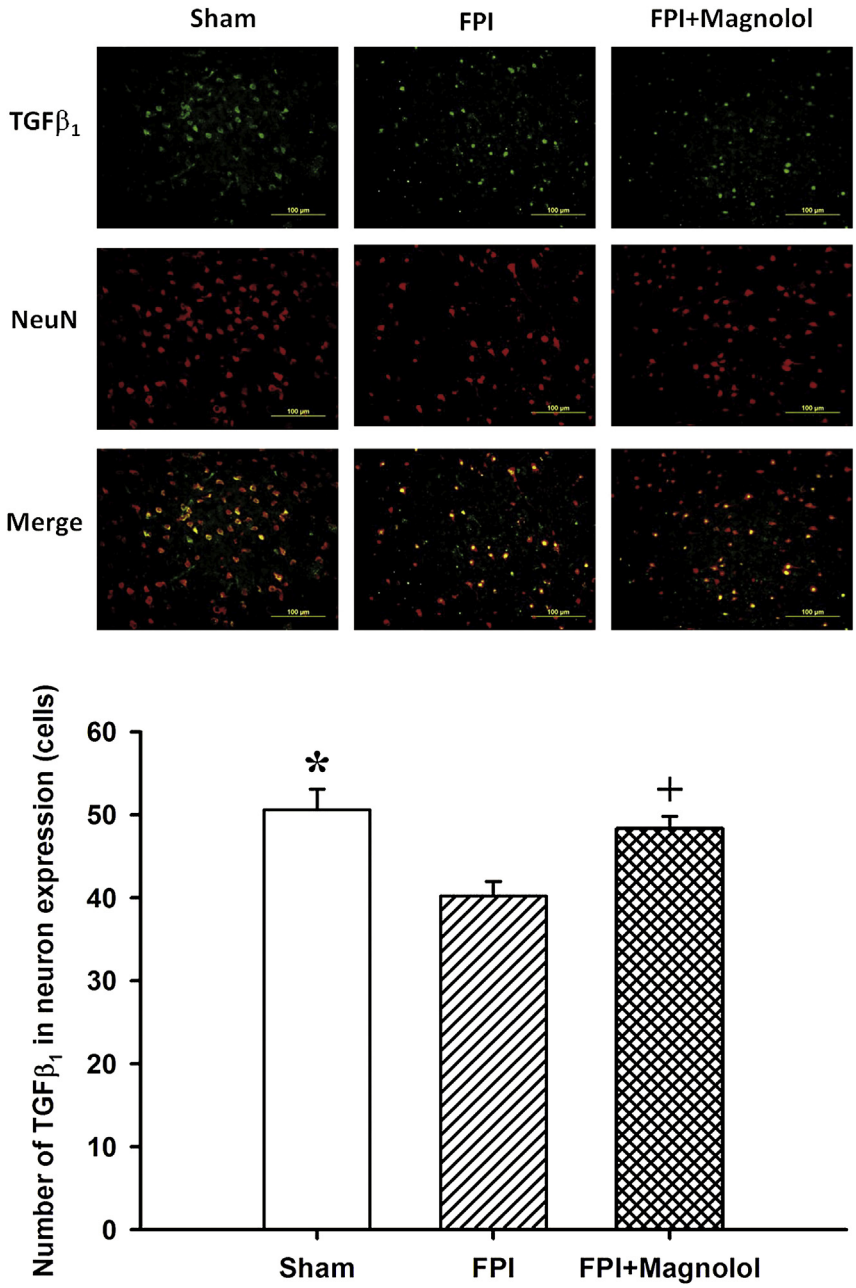

Fig. 5 – Effects of magnolol treatment on TGF-β1 expression in neuronal cells in the cortex (TGF-β1 plus Neu-N stain assay) at 3 d after FPI. Top panels depict representative positive Neu-N and TGF-β1 staining for one sham rat, one FPI rat, and one FPI + magnolol-treated rat. $^{*}P < 0.05$ compared with the sham group; $^{+}P < 0.05$ compared with magnolol (2 mg/kg, iv) treatment in the FPI group.

also supported that magnolol has potentially therapeutic effects in TBI *via* its powerful free radial scavenger ability.

4.3. *Magnolol's effects on neuronal cell protection*

After TBI, the secondary injury mechanisms are very complex and include early free radical–induced reactive oxidative injury and, finally, apoptotic cell death [1,28]. The present results demonstrated that magnolol therapy at a single dose of 2 mg/kg caused attenuation of TBI-induced early free radical formation (as evidenced by increased 2,3-DHBA levels), cell damage markers (evidenced by increased glycerol concentration), and, later, neuronal apoptosis (evidenced by increased numbers of TUNEL-positive cells) and cerebral infarction volume (evidenced by TTC staining) when evaluated 3 d after FPI. Magnolol therapy also demonstrated improvement in motor deficits. Altogether, in addition to the powerful free radical scavenger ability of magnolol, the neuroprotective effects of magnolol could also result from its antiapoptotic effects. Our results supported the idea that any agent that can

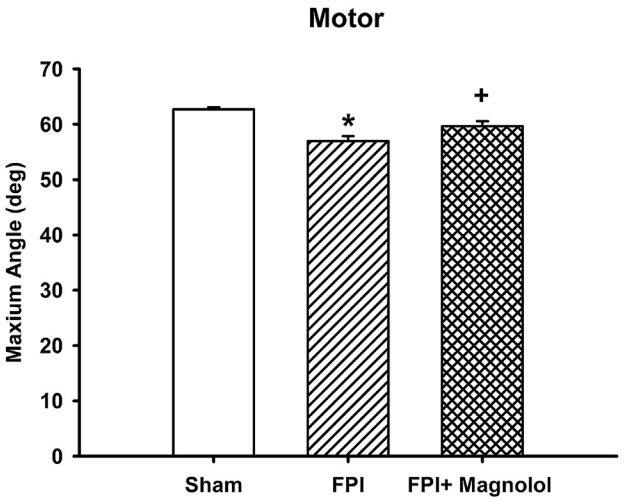

Fig. 6 – Effects of magnolol (2 mg/kg, iv) treatment on TBI-induced motor deficits evaluated by maximum angle in inclined plane grasp at 3 d after FPI. $^{*}P < 0.05$ compared with the sham group; $^{+}P < 0.05$ compared with magnolol (2 mg/kg, iv) treatment in the FPI group.

concurrently attenuate oxidative stress and exerts anti-apoptotic effects, such as magnolol, constitutes a promising strategy for the rescue of neurons following TBI [6].

4.4. *Magnolol's effects on FPI-induced TGF-β1 expression in neuronal cells*

According to a review of the related literature, TGF-β1 is involved in the signaling pathway of cell apoptosis. As a protective factor, TGF-β1 levels are elevated acutely after injury [29–31]. Furthermore, injection of the anti-inflammatory cytokine TGF-β1 after injury in rodents reduced inducible nitric oxide synthase (iNOS) production [32], reduced lesion size, and improved function [33]. In the current study, magnolol increased TGF-β1 expression in neuronal cells. It can also decrease neuronal apoptosis and, finally, improve motor function. We believe that increased level of TGF-β1 in the injured cortex, resulting in a decrease in neuronal apoptosis, is one mechanism by which functional recovery might occur.

Magnolol, in addition to being a free radial scavenger, has demonstrated several protective mechanisms in other animal models. For example, it decreased lung tumor necrosis factor α levels in rats submitted to septic shock [34], suppressed lung iNOS expression in rats with mesentery ischemia-reperfusion [35], attenuated heat stroke–increased levels of glutamate in a rat model [12], and activated the apoptotic biomarker ERK1/2 and Bcl-xl proteins [36]. In our previous studies, we found reactive astrogliosis and microgliosis, upregulated expression of iNOS and neuronal NOS, and caspase-3 and neurogenesis activation following fluid percussion injury–induced TBI [3,37,38]. Whether magnolol treatment has the same effects as noted above on TBI rats needs to be evaluated.

5. Conclusion

Based on our results, we conclude that magnolol, administered as a single dose of 2 mg/kg, counteracted the oxidative brain injury, apoptosis, and neurologic deficits induced by TBI in rats, by exerting its potent free radical scavenging ability. We also suggest that the antiapoptosis mechanism of magnolol might occur in part due to an increase in TGF-β1 expression in the ischemic cortex.

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REFERENCES

[1] Takuma K, Baba A, Matsuda T. Astrocyte apoptosis: implications for neuroprotection. Prog Neurobiol 2004;72:111.
[2] Raghupathi R, Graham DL, McIntosh TK. Apoptosis after traumatic brain injury. J Neurotrauma 2000;17:927.
[3] Kuo JR, Lo CJ, Lin KC, Lin MT, Chio CC. Agmatine-promoted angiogenesis, neurogenesis, and inhibition of gliosis reduced traumatic brain injury in rats. J Trauma 2011;71:87.
[4] Ames BN, Shigenega MT, Hagen M. Oxidants, antioxidants and the degenerative disease of aging. Proc Natl Acad Sci U S A 1993;90:7915.
[5] Floyd RA. Antioxidants, oxidative stress, and degenerative neurological disorders. Proc Soc Exp Biol Med 1999;222:236.
[6] Lau A, Arundine M, Sun HS, Jones M, Tymianski M. Inhibition of casepase-mediated apoptosis by peroxynitrite in traumatic brain injury. J Neurosci 2006;26:11540.
[7] Fujita M, Itokawa H, Sashida Y. Honokiol, a new phenolic compound isolated from the bark of Magnolia obobata THUNB. Chem Pharm Bull 1972;20:212.
[8] Lin SP, Tsai SY, Lee Chao PD, Chen YC, Hou YC. Pharmacokinetics, bioavailability, and tissue distribution of magnolol following single and repeated dosing of magnolol to rats. Planta Med 2011;77:1800.
[9] Tsai TH, Chou CJ, Chen CF. Pharmacokinetic and brain distribution of Magnolol in the rat after intravenous bolus injection. J Pharm Pharmacol 1996;48:57.
[10] Hong CY, Huang SS, Tsai SK. Magnolol reduces infarct size and suppresses ventricular arrhythmia in rats subjected to coronary ligation. Clin Exp Pharmacol Physiol 1996;23:660.
[11] Taira J, Ikemoto T, Mimura K, Hagi A, Murakami A, Makino K. Effective inhibition of hydroxyl radicals by hydroxylated biphenyl compounds. Free Radic Res Commun 1993;19:S71.
[12] Chang CP, Hsu YC, Lin MT. Magnonol protects against cerebral ischemia injury of rat heatstroke. Clin Exp Pharmacol Physiol 2003;30:387.
[13] Chiu JH, Wang JC, Lui WY, Wu CW, Hong CY. Effect of magnonol on in vitro mitochondria lipid peroxidation and isolated cold-preserved warm-reperfused rat livers. J Surg Res 1999;82:11.
[14] Lo YC, Teng CM, Chen CF, Chen CC, Hong CY. Magnolol and honokiol isolated from Magnolia officinalis protect rat heart mitochondria against lipid peroxidation. Biochem Pharmacol 1994;47:549.
[15] McIntosh TK, Vink R, Noble L, et al. Traumatic brain injury in the rat: characterization of a lateral fluid-percussion model. Neuroscience 1989;28:233.

[16] Paxinos G, Watson C. The rat brain in stereotaxic coordinates. New York: Academic Press; 1982.
[17] Kuo JR, Lo CJ, Chio CC, Chang CP, Lin MT. Resuscitation from experimental traumatic brain injury by agmatine therapy. Resuscitation 2007;75:506.
[18] Hsu SF, Niu KC, Lin CL, Lin MT. Brain cooling causes attenuation of cerebral oxidative stress, systemic inflammation, activated coagulation, and tissue ischemia/injury during heatstroke. Shock 2006;26:210.
[19] Huang WT, Lin MT, Chang CP. A NMDA receptor-dependent hydroxyl radical pathway in the rabbit hypothalamus may mediate lipopolysaccharide fever. Neuropharmacology 2006;50:504.
[20] Halliwell B, Kaur H, Ingelman-Sundberg M. Hydroxylation of salicylate as an assay for hydroxyl radicals: a cautionary note. Free Radic Biol Med 1991;10:439.
[21] Mullen RJ, Buck CR, Smith AM. NeuN, a neuronal specific nuclear protein in vertebrates. Development 1992;116:201.
[22] Hallam TM, Floyd CL, Folkerts MM. Comparison of behavioral deficits and acute neuronal degeneration in rat lateral fluid percussion and weight-drop brain injury models. J Neurotrauma 2004;21:521.
[23] Carbonell WS, Grady MS. Regional and temporal characterization of neuronal, glial, and axonal response after traumatic brain injury in the mouse. Acta Neuropathol 1999;98:396.
[24] Smith SL, Andrus PK, Zhang JR, Hall ED. Direct measurement of hydroxyl radicals, lipid peroxidation, and blood-brain barrier disruption following unilateral cortical impact head injury in the rat. J Neurotrauma 1994;11:393.
[25] Hillered L, Valtysson J, Enblad P, Persson L. Interstitial glycerol as a marker for membrane phospholipids degradation in the acutely injured human brain. J Neurol Neurosurg Psychiatry 1998;64:468.
[26] Ou HC, Chou FP, Sheu HH, Hsu SL, Lee WJ. Protective effects of magnolol against oxidized LDL-induced apoptosis in endothelial cells. Arch Toxicol 2007;81:421.
[27] Jawan B, Goto S, Pan TL, et al. The protective mechanism of magnolol, a Chinese herb drug, against warm ischemia–reperfusion injury of rat liver. J Surg Res 2003;110:378.
[28] Nortje J, Menon DK. Traumatic brain injury: physiology mechanism and outcome. Curr Opin Neurol 2004;17:711.
[29] Csuka E, Morganti-Kossmann MC, Lenzlinger PM, Joller H, Trentz O, Kossmann T. IL-10 levels in cerebrospinal fluid and serum of patients with severe traumatic brain injury: relationship to IL-6, TNF-alpha, TGF-beta1 and blood-brain barrier function. J Neuroimmunol 1999;101:211.
[30] Morganti-Kossmann MC, Rancan M, Stahel PF, Kossmann T. Inflammatory response in acute traumatic brain injury: a double-edged sword. Curr Opin Crit Care 2002;8:101.
[31] Yu CH, Yhee JY, Kim JH, et al. Pro- and anti-inflammatory cytokine expression and histopathological characteristics in canine brain with traumatic brain injury. J Vet Sci 2011;12:299.
[32] Hamada M, Naomoto Y, Fujiwara T, Kamikawa Y, Tanaka N. Suppressed apoptotic induction in esophageal squamous cell carcinomas expressing extensive p53 protein. Jpn J Clin Oncol 1996;26:398.
[33] Tyor WR, Avgeropoulos N, Ohlandt G, Hogan EL. Treatment of spinal cord impact injury in the rat with transforming growth factor-beta. J Neurol Sci 2002;200:33.
[34] Shih HC, Wei YH, Lee CH. Magnolol alters cytokine response after hemorrhagic shock and increases survival in subsequent intra-abdominal sepsis in rats. Shock 2003;20:264.
[35] Shih HC, Huang MS, Lee CH. Magnolol attenuates the lung injury in hypertonic saline treatment from mesenteric ischemia reperfusion through diminishing iNOS. J Surg Res 2012;175:305.
[36] Jin YC, Kim KJ, Kim YM, et al. Anti-apoptotic effect of Magnolol in myocardial ischemia and reperfusion injury requires extracellular signal-regulated kinase1/2 pathways in rat *in vivo*. Exp Biol Med 2008;233:1280.
[37] Kuo JR, Lo CJ, Chang CP, Lin HJ, Lin MT, Chio CC. Brain cooling - stimulated angiogenesis and neurogenesis attenuated traumatic brain injury in rats. J Trauma 2011;69:1467.
[38] Kuo JR, Lo CJ, Chang CP, Lin MT, Chio CC. Attenuation of brain nitrostative and oxidative damage by brain cooling during experimental traumatic brain injury. J Biomed Biotechnol 2011;2011:145214.