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Roles of the Nrf2/HO-1 pathway in the anti-oxidative stress response to ischemia-reperfusion brain injury in rats

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Abstract. – OBJECTIVE: The aim of this study was to investigate the roles of the Nrf2/HO-1 pathway in the responses to the oxidative stress created by ischemia-reperfusion brain injury in rats.

MATERIALS AND METHODS: 54 healthy, adult, male SD rats were included in the study. Eighteen (18) rats were placed in the sham group. The ischemia-reperfusion model was created in the other 36 rats, among whi received injections of Nrf2 agonist bef surgery. The suture method was used to te artery occlusions in the right brain of the and reperfusion was done after 90-minute is emia (MCAO); while no suture was inserted the sham group. At 3, 6, 12, 24 72 hou after the modeling, their ne gic nctions were evaluated. Also, at rent ti points, rats were decapitated, their brain tissues were used to dete e i percentages by TTC ning e brain water contents by th -wet we ethod. The SOD contents in ain tissue w easured by Xanthine o y. RT-PCR used to detect the mR expr n of HO-1 in the brain tissues, a western bl thod was used to detect th xpression leve O-1 and Nrf-2.

RESULTS: Th rats in the ham group had no c us ne gical defects; while those in the O g showed significant neurological d all time nts. The MCAO group h hig urolo evaluation scores than ham staining showed that infa n the group kept increasing over tim nd peake at 24h. Measurements of SOD fo the sham group had the highest SOD ree groups, and showed no significant fluctuation over time. The MCAO group much lower SOD activities than the sham g at all the time points. The higher the level of O-1mRNA and protein expression in the brain tissue of rats in each group, the higher the degree of brain injury, but the lower the level of Nrf2 protein expression and the lower degree of brain inju Nrf2 a t markedly improved all these indicators in th which underwent the MC ery.

CONCLUSIONS: The e ression of HO-1 after i emia-reperfusion brain injury may contribute to the incre d infarct volume. Activation rf2 could i ove the prognosis of isch-e eperfusi rain injury.

Key Wo
Ischemia-reperfusion brain injury, Nrf-2, HO-1.

Introduction

Ischemic or hemorrhagic cerebrovascular disease is a type of severe neurological disease. Each year about 1 million people die of cerebrovascular diseases in our country. About 75% of those who survive have varying degrees of disabilities or complete loss of self-care abilities, seriously affecting the quality of patients' lives[1]. Ischemia-reperfusion brain injury is a complex pathological process; and its underlying mechanism connects and interact. The current research results showed that many mechanisms were involved in ischemia-reperfusion brain injury. They were interrelated and affected one another, forming a "mesh" structure, and the "main pathway" of the pathogenesis remained unclear. Therefore, investigating the incidence of ischemia-reperfusion injury is still the main focus of research in this field[2,3].

The Keapl-Nrf2/ARE pathway was one of the important mechanisms for intracellular antioxidant function and cytotoxic defense. It had a wide range of cytoprotective functions, including anti-tumor, anti-oxidative stress, regulating GSH synthesis, anti-apoptotic, anti-inflammatory, anti-atherosclerotic, regulating heart cerebrovascu-

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lar reactivity and neuroprotection[4,5]. Transcription factor NFE2 related factor 2 (Nrf2) and its cytoplasmic adapter protein Keapl are the central regulators of cellular antioxidant responses. By interacting with the antioxidant response element ARE, Nrf2 appears to be an important effector molecule for the maintenance of healthy blood vessels and the prevention of cardiovascular diseases through NO-mediated signal transduction. Nrf2 is also an important effector molecule to lower the risk of stroke by antagonizing NO-induced apoptosis[6-8]. Upregulation of Nrf2 plays a protective role in neurons by inducing some antioxidant enzymes and detoxification enzymes to accelerate the enzymatic reactions, as well as by increasing the expression levels of GSH and SOD and other antioxidants[9]. Heme oxygenase-1 (HO-1) pathway is an important oxidative stress pathway. In tissues with ischemic damages, the over activation of the HO-1 pathway can have protective functions. As a rate-limiting enzyme in the metabolism of heme, HO catalyzes the oxidative degradation of heme into carbon monoxide (CO), iron and biliverdin. Subsequently, biliverdin is converted to bilirubin by bilirubin reductase. There are three sub[illegible]s of HO, namely HO-1, HO-2, and HO-3 (Hsp32) could be induced by stress in almo[illegible]y-ery cell type[10]. A key regulatory element of H[illegible] expression is Bach-1, which is a highly conser[illegible] leucine zipper protein with a h[illegible]ding sit[illegible] Using small interfering RNA[illegible]RI[illegible]o target Bach-1 significantly reduc[illegible]e mRN[illegible]nd protein level of Bach-1, and[illegible]ced th[illegible]ion of HO-1[11]. Based on al[illegible]ese[illegible]research[illegible]s, we have further in[illegible]gated th[illegible]ctions of the Nrf2/HO-1 pathw[illegible]ischemia-re[illegible]sion brain injury, in an e[illegible]vide theoret[illegible] support for clinical tre[illegible]ment.

Mate[illegible]ls and Methods

Expe[illegible] Anima[illegible]

Fifty-[illegible] SPF[illegible]e healthy male adult SD r[illegible]ith w[illegible]tween 250 and 300 g were pur[illegible]sed fro[illegible]edical Science Experiment Animal Institute of Chinese Academy of Medical [illegible] study obtained the Ethics Committee's approval of Linyi People's Hospital Affiliated to Shandong University.

Materials and Equipment

The following materials and equipment were use: Total SOD detection kit (Nanjing Jiancheng Biological Engineering Technology Co., Ltd.); 1 M Tris-Hcl (pH = 6.8), DNA Marker, Tris, Trizol reagent, Diethyl dicarbonate ester, PVDF membrane (0.45 μM), PMSF (phenylmethyls[illegible]l fluoride), Western blotting blocking b[illegible] body dilution buffer for Western bl[illegible]g, IP cel[illegible] lysis buffer, Hypersensitivity ECL[illegible]miluminescence kit, Rabbit anti-rat β-actin m[illegible]onal antibody (Beyotime Biotechnol[illegible]y Co. [illegible]hanghai, China); 37°C constant[illegible]perature in[illegible] Vortex mixers (Beijing [illegible] Scient[illegible]c Instru[illegible] Factory, Beijing, Chi[illegible] × dN[illegible] DMSO, EB, Reverse transcripta[illegible]e, [illegible]se agor[illegible] (Sigma-Aldrich, St. [illegible]s, M[illegible]A); 40[illegible]ter-Bis (39: 1), 0.1M[illegible] BCA pro[illegible]s[illegible]kit, SDS (sodium d[illegible]ulfate), β-[illegible]ptoethanol; AP (amm[illegible]um p[illegible]fate), Horseradish peroxidase-labeled goat anti[illegible]t IgG antibody, Mortar, Edet[illegible]odium, Isopr[illegible]lcohol, Chloroform, E[illegible]ol, Tween solution, Glycine, Glycerol, Meth[illegible] (Chongqing [illegible]ingguo Biological Company, [illegible]ngqing, Chi[illegible] FBS (Promega, Madison, WI, U[illegible] PageRule[illegible]estained Protein Ladder (Thermo [illegible]ific [illegible]ham, MA, USA); PCR thermocycler, [illegible]horesis apparatus, Electrophoresis [illegible]ank, Gel scanning and analysis system, Power [illegible] (Bio-Rad, Hercules, CA, USA); Taq DNA [illegible]ase, TEMED (Amresco, Solon, OH, USA); Glass homogenizer (Zhuozhou Changhong Glass Instrument Factory, Zhuozhou, China); Ultra-low temperature freezer (Sanyo, Tokyo, Japan); Low temperature centrifuge CR21 (Hitachi, Tokyo, Japan); Multifunctional microplate reader Model 680 (Bio-Rad, Hercules, CA, USA); High-speed refrigerated centrifuge (Eppendorf, Hamburg, Germany); Gel imaging system (Kodak, Tokyo, Japan); Boric acid (Southwest Chemical Reagent Company, Chongqing, China); Nrf2 agonist, Agarose (Sigma-Aldrich, St. Louis, MO, USA); Horizontal electrophoresis tank (Beijing Liuyi Scientific Instrument Factory, Beijing, China); Image analysis system software (Imaging, Eagan, MN, USA); Rabbit polyclonal anti-rat Nrf2 (Bioworld, USA); Bromphenol blue (Amresco, Solon, OH, USA).

Methods

Animal grouping and drug administration

Fifty-four healthy adult male SD rats, weighing between 250 to 300 g, were used in this study. All rats were numbered and grouped by the random number table method. Eighteen rats were placed in the sham group. The ischemia-reperfusion model was created in the other 36 rats, among which 18 rats received injections of Nrf2 agonist before the sur-

gery. At 3h, 6h, 12h, 24h, 48h and 72h after the modeling, their neurological functions were evaluated. A modified suture method was used to create MCAO model on the right side of the rats. The rats were fasted 12 hours before the operation but the water was allowed. Reperfusion was done 90 minutes after ischemia. Hereinafter the model group would be referred to as the MCAO group. The rats in the sham group received the same surgery operations except that no suture was inserted. The drug-administered group received an intraperitoneal injection of Nrf2 agonist at the dose of 20 mg/kg at 48h and 24h before the MCAO, hereinafter referred to as MCAO + Nrf2 agonist group. The rats in the sham group and the MCAO got 3 ml intraperitoneal injection of 3 ml saline at the same time points.

Creation of experiment animal model

Preoperative preparation

The animals were fasted 12 hours before the surgery, but the water was allowed. Fishing line with a diameter of about 0.26 mm was used to make sutures. The head ends were made spherical with fire, and the length of the sutures was about 40 mm. A black marker was used to mark the 18-24 mm part. The sutures were air-dried, disinfected with 75% alcohol and stored in physiological saline.

Model creation

Intraperitoneal injection of an appropriate amount of 3.5% chloral hydrate (10 mg/kg) was used to anesthetize the rats. The right common carotid artery, internal and external carotid arteries were exposed and separated. Ligation of the common carotid artery was made at the proximal end, about 0.7 cm to the bifurcation. A loose sparing suture line was placed between this surgical ligature and the bifurcation. A combination of the external carotid artery and clipping of the internal carotid artery. A small incision was cut between the bifurcation and the prepared suture line. A prepared suture was inserted until the mark of the part that was marked by the black marker. After the spare suture had been tightened together, the clip on the internal carotid artery was loosened, and the excess suture line was cut off. The incision was sutured and disinfected. The same operations except that no suture was inserted, were performed in the sham group. After the surgeries, the rats were placed into clean cages, kept warm, well fed and given enough water. Ninety minutes after the blocking ischemia, the suture was gently pulled out and reperfusion occurred.

Measurement of infarct volume percentages

After the rats had been anesthetized, they were decapitated and their brains were separated on the ice. The brains were frozen in a -20°C refrigerator for 20 minutes. Each brain was cut into 2 mm sections. The sections were then placed in 2% TTC with a foil cover and incubated in a 37°C incubator for 15-30 minutes, making sure the brain tissues uniformly contact the staining solution. A digital camera and the image analysis system were used for the image acquisition and analyses. The infarct volume percentage (%) = the accumulated infarct volume / volume of the bilateral hemisphere.

Determination of brain water contents

After the rats had been anesthetized and decapitated, the entire brain was taken out. 4 mm frontal pole was removed with coronal cut and reserved for brain water content determination. Each foil piece was weighed beforehand (W1). Each brain tissue was wrapped in a piece of foil and the total weight was demined (W2). Wet weight = W2-W1. The brain tissues wrapped in foil were then placed. After the packages had gone back to room temperature, their weights were taken and referred to as W3. Dry weight = W3-W1. Brain water content was calculated by the formula: (W3-W1) / (W2-W1) × 100%.

Determination of brain tissue SOD contents

Animals were sacrificed and decapitated to remove the right brains. An appropriate amount of brain tissues was set aside after the cerebellum and medulla oblongata were removed. The brain tissue was weighed and put in a pre-cooled (at -20°C) glass homogenizer. Precooled saline was added and the homogenization was carried on an ice tray until no suspended solids were visible. The homogenate was transferred to a centrifuge tube (10 m) with a pipette and centrifuged at 4°C at 3,500 rpm for 15 minutes. The supernatant was used to measure the SOD content. All operations followed the manufacturer's instructions and the readings were taken at the wavelength of 550 nm.

Evaluations of neurological functions

Neurological functions were evaluated at 3 h, 6 h, 12 h, 24 h, 48 h and 72 h after successful surgeries. Rats with 2, 3, 4 points were included in the MCAO group. The evaluation was based on the following six-point scale of improved Longa assessment: 0 point, no neurological defect; 1 point, failure to fully extend the contralateral (paralyzed) side forelimb; 2 points, failure to ex-

tend the contralateral (paralyzed) side forelimb; 3 points, slight circular motion to the contralateral (paralyzed) side (big circle); 4 points, obvious circular motion to the contralateral (paralyzed) side (small circle); 5 points, falling to the contralateral (paralyzed) side. The higher the score, the more severe the behavioral disorders[1].

Sample preparation and procedures of RT-PCR

The total RNAs were extracted and the concentrations and integrities were determined according to the instructions of the Trizol kit. PCR amplification contained 1 µl cDNA (obtained by reverse transcription of RNA), 1 µl upstream primer, 1 µl downstream primer and 12.5 µl master mix. Double distilled water was used to bring the volume to 25 µl. The PCR reaction conditions for Nrf2 and HO-1 were: 94°C denaturation for 5 min, 30 cycles of 95°C for 30 seconds, 57°C for 30 seconds, and 72°C for 30 seconds. The RT-PCR primers were synthesized by Shanghai Sango Biotech. The primer sequences were: HO-1 gene primer: The upstream sequence: 5'-AGCCCCACCAAGTTCAAACA-3': downstream sequence: 5'-TGCCAACAGGAAGCTGAGA[illegible]3' The Amplified fragment was 321 bp, β-act[illegible] primer: The upstream: 5'-GAGACCTT[illegible]A-CACCCCAGC-3' downstream sequence: 5'-C[illegible]CAGGATTCCATACCCAA-3'. The Ampli[illegible] fragment was 446 bp.

Sample preparation and procedures of Western [illegible]ng

The animals wer[illegible]ne[illegible]and st[illegible]e fixed. Heart perfusi[illegible]as done[illegible]saline (0.9% NaCl, containing[illegible]mg/ml NaC[illegible]l heparin. Then, the rats[illegible]e[illegible]pitated and[illegible]r brains were removed. After c[illegible]is and homogenization, prot[illegible]were extrac[illegible]rom brain tissues with th[illegible]A method, and th[illegible]e proteins were trans[illegible]ed to[illegible]membrane. The membrane wa[illegible]bate[illegible]th the diluted primary antibody (Nrf2[illegible]O-1 1:1[illegible]r β-actin 1:500) at 4°C [illegible]over[illegible]. The[illegible]mbrane was then washed with TPBS solution with shaking for 5 min, and the wash was repeated two more times. Diluted horseradish peroxidase-labeled secondary antibodies (Nrf2 1:3000, HO-1 1:3000, or β-actin 1:5000) were added and the membran[illegible] bated at room temperature for 2 hou[illegible]ne mem[illegible] brane was again washed 3 times [illegible]PBS solution with shaking. The membrane [illegible]veloped by following the instructions[illegible]the hyp[illegible]sitivity ECL chemiluminescen[illegible]t. The gel s[illegible] analysis system was use[illegible]obtain[illegible]e imag[illegible] to do the analysis.

Statistical Ana[illegible]s

RT-PCR [illegible]Western b[illegible]g [illegible]ults were processed [illegible]Image Lab [illegible]are.The optical dens[illegible]f ea[illegible]d was collected and then statistically analyzed [illegible]the SPSS 12.0 statistical [illegible]e (IBM, Ar[illegible]NY, USA). Data of n[illegible]logical function, infa[illegible]ct volume, and SOD a[illegible]ity were col[illegible]cted, and statistical analyses [illegible] conducted. [illegible]ults of RT-PCR and Western b[illegible]g underw[illegible]ANOVA analysis and SNK-q test[illegible]05 [illegible]ificance was set at $p<0.05$.

Results

Evaluation of neurological functions

All modeled rats had a score between 2-4. The rats in the sham group had no obvious symptoms of neurological defects. The rats in the MCAO group and the MCAO + Nrf2 agonist group exhibited evident neurological defects and higher evaluation scores than the sham group at each time point. The differences were significant (p <0.05) (Table I), indicating the successful establishment of the ischemia-reperfusion model. The scores increased with time and peaked at 24h. The MCAO + Nrf2 agonist group had improved neurological functions compared with the MCAO group at each time point. They had significantly lower scores than the MCAO rats (p <0.05) (Figure 1).

T[illegible]n of neurological functions.

Group	n	3h	6h	12h	24h	48h	72h
[illegible]m	18	0.12±0.45	0.37±0.21	0.53±0.32	1.57±0.43	1.16±0.45	0.33±0.51
[illegible]O	18	3.12±0.43	3.68±0.51	4.18±0.56	4.58±0.42	3.82±0.67	3.02±0.42
MCAO+Nrf2 agonist	18	2.76±0.64	3.23±0.42	2.43±0.65	3.08±0.66	2.51±1.25	2.02±0.46
F-value	-	41.265	55.487	32.763	23.872	23.434	14.387
p-value	-	0.000	0.000	0.000	0.000	0.000	0.000

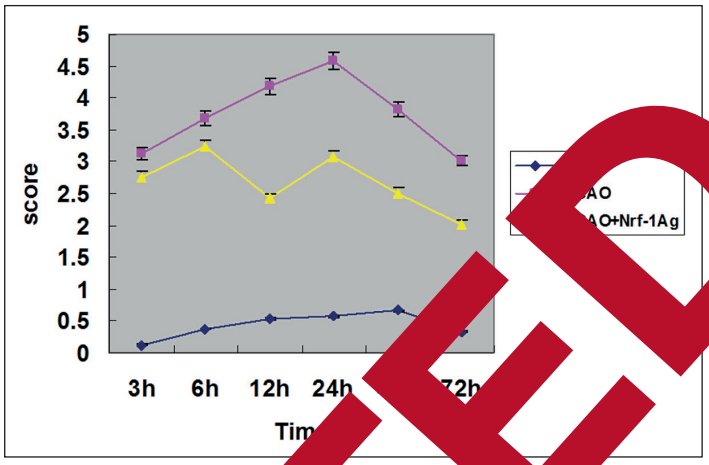


Figure 1. Neurological function scores over time after modeling. The MCAO and MCAO + Nrf-2 agonist group reached the peak scores 24 hours after the surgeries. Compared with the sham group, the differences were statistically significant (p<0.05).

Post-reperfusion Infarct volume percentages detected by TTC staining

Normal brain tissues would be red after TTC staining, while infarct would appear white. The sham group had no obvious brain infarct, while large areas of infarct were observed in the MCAO group and the MCAO + Nrf2 agonist group. The differences were significant (p <0.05) (Table II), indicating that the establishment of the experimental model was successful. The MCAO group showed evident increased brain infarct volume percentage at 3h; the percentage peaked at 24h and started to decrease slowly at 48h. Compared with the MCAO group, the infarct volume percentage of the MCAO + Nrf2 agonist group was lower at each time point and the differences were significantly different (p <0.05) (Table 2 and 3), suggesting that the Nrf2 agonists could significantly reduce infarct volume percentages, mitigate the damage caused by cerebral ischemia and have protective effects on cerebral ischemia.

Determination of brain tissue water contents

The sham rats showed no obvious increase in the brain water content. The rats in the MCAO group had higher brain water contents than the sham group at all the studied time points. The significant increase started at 3h and peaked at 24h. Slow declining started at 48h. The differences were statistically significant (p <0.05) (Table III). Compared with the sham group, the MCAO + Nrf2 agonist group showed no significant difference in brain water content at 3h. Their brain water contents began to rise significantly at 6h, reached the peak at 24h and began to decline at 48h. Compared with the MCAO group, the MCAO + Nrf2 agonist group had lower brain water contents, and the differences were significant (p <0.05) (Figure 4).

Determination of SOD in the brain tissues of each group

The rats in the sham group showed the highest brain tissue SOD and did not change much over time. The MCAO group and MCAO + Nrf2 agonist group both had lower brain tissue SOD activity than the sham group, and the differences were significant (p <0.05) (Table IV). The SOD activity in the MCAO rats began to decrease at 3h, and did not show recovery until 72h after the surgery. The rats in the MCAO + Nrf2 group showed comparable SOD activity compared with the sham group at 3h. Their SOD activity started to show decline at 6h. Also, the MCAO + Nrf2 group showed higher SOD activity than the MCAO group at each time point with significant differences (p <0.05) (Figure 5).

Tarct volume percentages of each group of rats (X ± SD).

Group	n	3h	6h	12h	24h	48h	72h
	18	3.14±0.00	2.37±0.01	4.59±0.01	4.68±0.00	2.38±0.00	2.46±0.00
	18	24.12±2.43	32.68±1.51	38.18±1.56	37.58±0.42	43.82±3.67	34.38±3.8
MCAO+Nrf2 agonist	18	19.76±3.64	25.23±1.42	31.43±1.65	35.08±7.66	37.51±5.25	24.33±2.18
F-value	-	341.265	2055.487	5132.763	3223.872	3923.434	3614.387
p-value	-	0.000	0.000	0.000	0.000	0.000	0.000

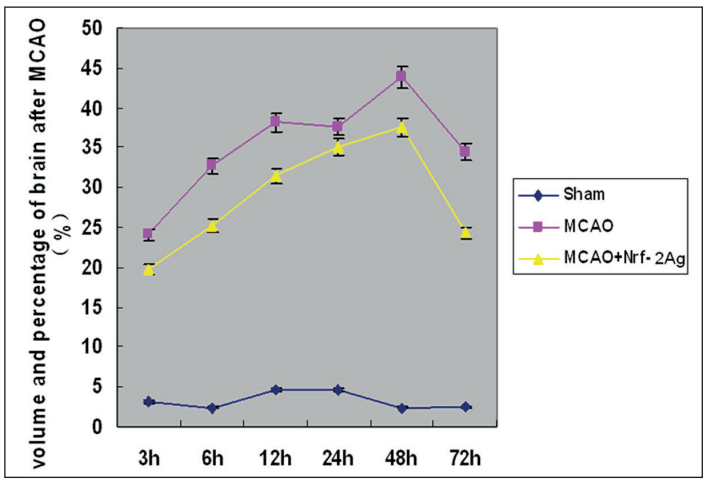


Figure 2. Infarct volume percentages over time after modeling. The MCAO and MCAO + Nrf2 agonist group had the highest percentages 24 hours after the surgery. Compared with the sham group, the differences were statistically significant ($p<0.05$).

Figure 3. Results of TTC staining. Normal brain tissue showed red after TTC staining, while infarct was white. The ham group had no significant brain infarct; while large areas of infarct were observed in the MCAO group and the MCAO + Nrf-2 agonist group. The differences were significant ($p<0.05$).

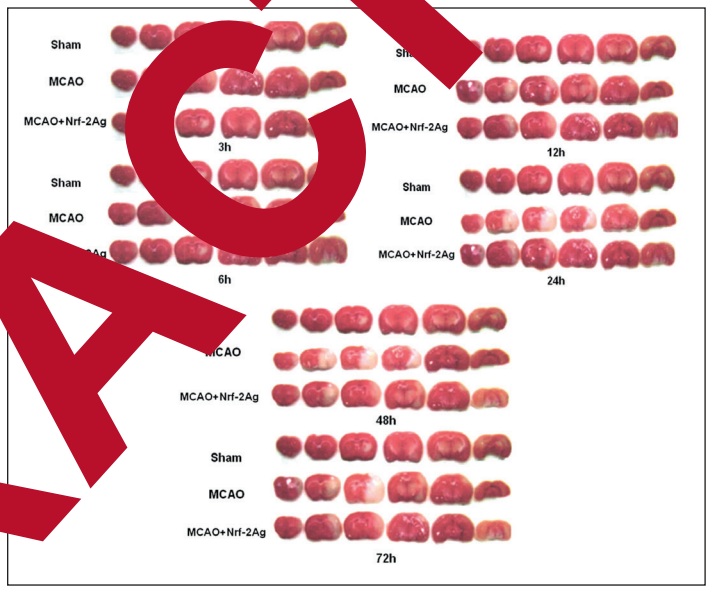


Table III. Brain [illegible] water [illegible]nts.

Group	n	3h	6h	12h	24h	48h	72h
Sham	1	[illegible]±0.00	68.37±0.01	69.59±0.01	70.68±0.00	69.38±0.00	68.46±0.00
MC	1	78.12±2.41	79.68±2.51	81.18±1.56	85.58±0.48	84.82±3.67	83.38±3.8
M Nrf2 nist	1	69.76±3.84	71.23±1.42	76.43±1.65	79.08±7.68	75.51±5.25	74.33±1.18
F-va		168.265	78.487	96.763	164.872	323.234	174.287
value		0.000	0.000	0.000	0.000	0.000	0.000

Table IV. [illegible]mination of SOD in brain tissues of each group.

roup	n	3h	6h	12h	24h	48h	72h
m	1	117.14±3.98	118.37±0.98	116.59±0.01	117.68±0.00	115.38±1.69	116.46±1.43.
O	1	85.12±2.41	77.68±2.51	72.18±1.56	68.58±0.48	64.82±3.67	68.38±3.28
M O+Nrf2 agonist	1	116.76±2.84	101.23±1.48	99.43±2.65	89.08±7.68	87.51±3.85	78.33±1.18
F-value	-	198.265	1378.487	480.763	339.872	701.234	342.887
p-value	-	0.000	0.000	0.000	0.000	0.000	0.000

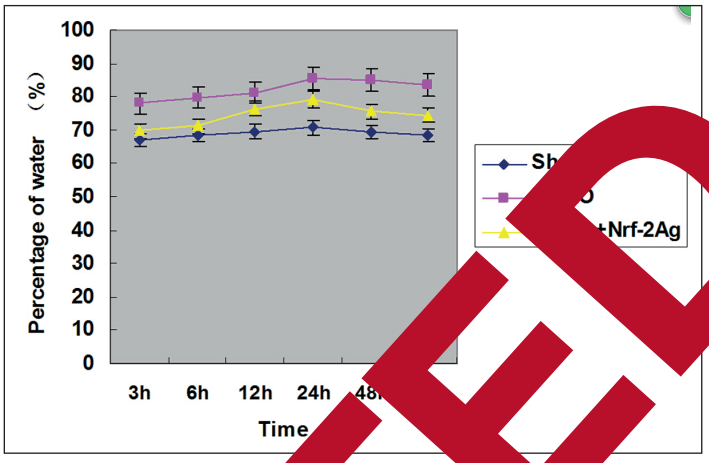


Figure 4. Changes of brain water contents in each group. Compared with the sham group, the brain water contents of the MCAO + Nrf-2 agonist group showed no significant change at 3h. It began to rise significantly at 6h, reached the peak at 24 h and started to decline at 48 h. Compared with the MCAO group, the MCAO + Nrf-2 agonist group had significantly decreased brain water contents; and the differences were significant ($p<0.05$).

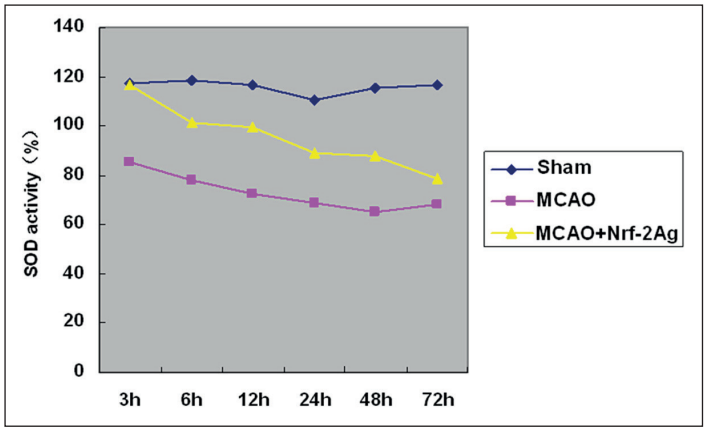
These results showed that the Nrf2 agonist could significantly increase SOD activity, mitigate the damages caused by cerebral ischemia and have protective effects on cerebral ischemia.

Expression of HO-1 mRNA and Nrf2 and HO-1 protein in the brain tissues of each group

The expression of HO-1mRNA did not much with time in the shame group. Com with the sham group, the MCAO showed incre HO-1 at 3h; the increase was significant at 12h the HO-1 expression peaked at Compare with the sham group, the MC agonists group had significantly high rf2 an O-1, and overtime, the expression ues t pared with the MCAO oup AO 2 group had significa ower H RNA expression at each time t and the d nces were statistically sig <0.05) (Fig 6 and 7). The change of xpressi el of HO-1 and Nrf2 protein in group on t ve time point was significant. The expre levels of HO-1 protein in b e of all time ts were significantly d sed after treated wi Nrf-2 agonist, while Nrf-2 level wa ignificantly improved, and the rence was stically significant ($p<0.05$). T xpression of Nrf-2 at each time point was the expression level of HO-1 was re using Nrf-2 agonist.

Discussion

Ischemia-reperfusion brain injury is a complicated pathological process[12]. The underlying mechanisms connect and interact with one another. The injuries include primary injury during ischemia and secondary injury from reperfusion; while ischemia and hypoxia are the factors to initiate these injuries. To date, research results showed that a lot of mechanisms were involved in ischemia-reperfusion brain injury, and they were interrelated and affected one another. A variety of



re 5. Changes of SOD in each group. Compared w sham group, the MCAO group had significant decreased SOD ($p<0.05$). While the MCAO + Nrf2 agonist improved the SOD level compared with the MCAO group. These results indicated that ischemia-reperfusion injury decreased the SOD activity.

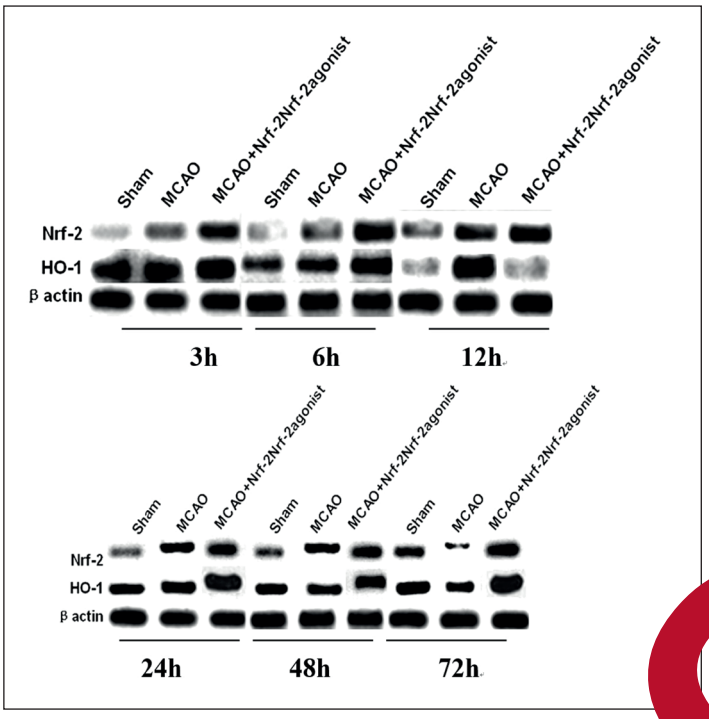


Figure 6. Protein expression of Nrf-2 and HO-1 detected by Western blotting. The Nrf2 agonists significantly decreased the HO-1 protein level, while the Nrf2 level was significantly increased, the differences were statistically significant ($p<0.05$). Nrf[illegible] may increase Nrf2 expression and decre[illegible] pression.

inflammatory cytokines and signaling path[illegible] seemed to be involved in the occurrence d[illegible] ment of ischemia-reperfusion injury[13].

This study found that the MCAO group[illegible] larger infarct than the sham group. Also, t[illegible] had decreased SOD activities [illegible]me poin[illegible] compared to the sham group[illegible]ng indicated that ischemia-reperf[illegible]n injur[illegible]creased the oxidative stress. But[illegible]agon[illegible] improved the SOD a[illegible]ity [illegible] ference ($p<0.05$) a[illegible]lso imp[illegible] the MCAO rats' neurologic[illegible]nctions. P[illegible]s studies suggested tha[illegible] the bioche[illegible]al reac-

tions i[illegible]Nrf2, Nrf2's interaction with protein kinase C (PKC) was more prominent[14]. PKC [illegible] to the serine/threonine protein kinase [illegible]and is widely present in the body cells. It is involved in cell skeleton, cell proliferation, differentiation, migration, and apoptosis. PKCα is the most prominent subtype in the PKC family. Studies have shown that PKCα could directly phosphorylate Nrf2, dissociate it with Kelch-like ECH-associated protein-1, promote its nuclear translocation so it could recognize and bind to ARE, thereby regulate the expression of its target genes. The Nrf2/heme oxygenase-1 (HO-1)

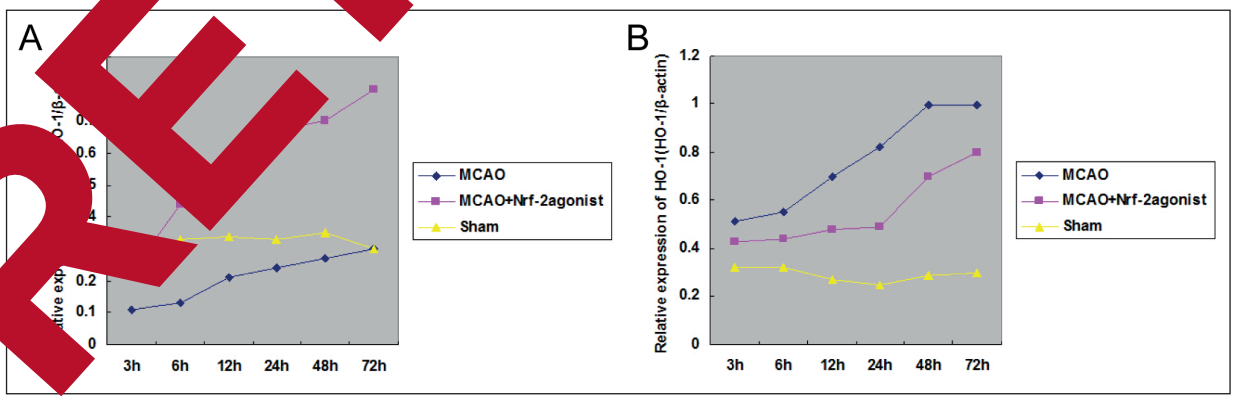


Figure 7. Changes of HO-1mRNA. The MCAO group had much higher HO-1 mRNA than the other groups. The sham group did not show much fluctuation of HO-1 mRNA. The MCAO + Nrf2 group significantly decreased the HO-1 mRNA.

pathway is an important intracellular anti-oxidative stress pathway. In ischemic tissue damages, over activation of this pathway could have some protective effects. The findings of this investigation also suggested that Nrf2 play a catalytic role in the hypoxic-ischemic brain injury in the ischemia-reperfusion brain injury model. Appropriate activation of Nrf2 can improve the prognosis of the disease and has some clinical significances[15].

Conclusions

We observed that the Nrf-2 agonists could protect brain function by increasing Nrf-2 level after ischemia-reperfusion injury.

Conflict of interest

Authors have no conflict of interest.

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