Short Communication

Effects of Echinacoside on Histio-central Levels of Active Mass in Middle Cerebral Artery Occlusion Rats*

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Abstract

Objective To investigate the effects of echinacoside on the extracellular striatal levels of norepinephrine (NE), dopamine (DA), homovanillic acid (HVA), 3, 4-dihydroxyphenylethanoid acid (DOPAC), 5-hydroxyindoleacetic acid (HIAA), and 5-hydroxytryptamine(5-HT) in middle cerebral artery occlusion (MCAO rats.

Methods The middle cerebral artery was occluded in male Sprague-Dawley rats. Three days later microdialysis probes were placed into the right striatum of MCAO rat brains and the brains were perfused with Ringer's solution at a rate of 1.5 μL/min. Cerebral microdialysates were collected every 30 minutes from awake and freely moving rats before assaying for NE, DA, HVA, DOPAC, HIAA, and 5-HT levels by reverse phase HPLC with electrochemistry.

Results Three days after MCAO, the extracellular striatal levels of NE, DA, DOPAC, HIAA, HVA, and 5-HT of the MCAO rats increased significantly (at least $P<0.05$ *vs.* control). However, simultaneous treatment with echinacoside (30.0 or 15.0 mg/kg) attenuated these increases (at least $P<0.05$ *vs.* non-treated model rats).

Conclusion These results imply that echinacoside may protect striatal dopa minergic neurons from the injury induced by MCAO and may help prevent and treat cerebral ischemic diseases.

Key words: Echinacoside; Norepinephrine (NE) dopamine (DA) homovanillic acid (HVA) 3,4-dihydroxyphenylethanoid acid (DOPAC) 5-hydroxyindoleacetic acid (HIAA) 5-hydroxytryptamine (5-HT); Middle cerebral artery occlusion(MCAO); Brain microdialysis; rats

Biomed Environ Sci, 2012; 25(2):238-244 *doi:10.3967/0895-3988.2012.02.017* *ISSN: 0895-3988*

www.besjournal.com (full text) *CN:11-2816/Q*

INTRODUCTION

Previous studies have shown that neurotransmitters are released from dopaminergic neurons in the striatum of middle cerebral artery occlusion (MCAO) rats[1-2] and oxidative stress plays an important role in the ischemic injury process in the brain[3-5]. Reactive oxygen species (ROS) such as the superoxide anion radical (O2-), hydrogen peroxide (H_2O_2) and the hydroxyl radical (OH-) are produced rapidly and in large amounts during cerebral ischemia[6] These ROS cause serious oxidative damage to lipids, deoxyribonucleic acid (DNA), and proteins in the

*This research was supported by the National Natural Science Foundation of China (No.30560171, No.30860334).

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Received: January 5, 2011; Accepted: December 21, 2011

ischemic tissues including the substantia nigra, which releases dopamine from these neurons[7-8]. Based on this principle many ROS scavengers have been used to prevent cerebral oxidative lesion after MCAO.

Echinacoside (Figure 1) is a phenylethanoid glycoside which is isolated and purified from the stems of Cistanche salsa, a parasitic plant native to northwest China. C.salsa is used as a traditional Chinese herbal medicine with anti-oxidative and antifatigue effects[9]. Chinacoside may also prevent neurons from oxidative-stress-induced toxic injuries[10-15]. However, whether protective for cerebral ischemic injury and decreases the release of dopaminergic neurotransmitters and their metabolites in the are still unknown. The present study observed the effects of echinacoside on the extracellular levels of norepinephrine (NE), dopamine (DA), homovanillic acid (HVA), 3,4-dihydroxyphenyl ethanoid acid (DOPAC), 5-hydroxyindoleacetic acid (HIAA), and 5-hydroxytryptamine(5-HT) in MCAO rats.

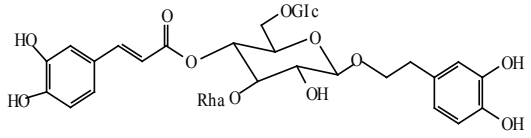

Figure 1. The chemical structure of echinacoside.

MATERIALS AND METHODS

Animals and Reagents

Male Sprague-Dawley rats, weighing 250-300 g, were housed individually in cages with food and water consumed ad libitum. The animals were kept at a temperature of 23±1 °C and a relative humidity of 40±5% with a 12 h light-dark cycle (lights on at 7:00 a.m.). All experiments were performed in accordance with the guidelines established by the European Community for the care and use of laboratory animals and were approved by the Animal Care Committee of Shihezi University.

Echinacoside from Cistanche salsa was kindly provided by Dr. TU Peng Fei (Peking University Modern Research Center for Traditional Chinese Medicine). The purity of the compound was more than 98% by high performance liquid chromatography (HPLC). Ligustrazine hydrochloride(CXQ) for parenteral injection was manufactured by Harbin Sanlian Pharmaceutical Co., Ltd (Harbin, China). NE, DA, DOPAC, HIAA, HVA, 5-HT, 1-heptanesulfonic acid sodium salt (HSA), and trisodium citrate were purchased from Sigma (St. Louis, MO, USA). Acetonitrile (ACN, HPLC grade) was purchased from Fisher (Pittsburgh, USA). Phosphoric acid (PA), citric acid and EDTA tetrasodium salt were obtained from Guoyao Group Co., Ltd. (Shanghai, China). Ringer's fluid was prepared in our laboratory. All the solutions were prepared by deionized water with at least 18.2 MΩ specific resistance.

Experimental Design

Animals were divided into five groups: control, model, echinacoside high and low doses and ligustrazine hydrochloride. The sham operation rats were used as the control and the ligustrazine hydrochloride-treated rats as positive drug group. Rats were given intraperitoneal injection (i.p.) of 0.9% saline (1 m/kg, for vehicle and model groups), 30.0 or 15.0 mg/kg echinacoside (as high or low dose groups, respectively) or 30 mg/kg ligustrazine hydrochloride (as the positive control group) respectively for 7 consecutive days. On the third day after drug administration, a guide cannula was implanted into the left striatum. At the end of the last drug administration, the left MCAO operation was performed on each rat (except for the sham operation group). On the 7th day, the microdialysis procedure was performed.

Middle Cerebral Artery Occlusion (MCAO)

Rats were anesthetized with chloral hydrate (350 mg/kg, i.p.). A polyethylene tube was inserted into the left femoral artery to continuously monitor blood pressure with a computer-assisted system (Medlab-U, Nanjing Medease Science and Technology Co., Nanjing, China). Serial measurements of arterial blood gases and pH (CIBA850, Corning, USA) and plasma glucose (Lifescan Co, New Brunswick, USA) were taken. Rectal temperature probes were used to keep the rectal temperature of the rats at 37±0.5 °C by using a heating pad during the entire operating procedure.

MCAO was induced using the intraluminal filament method[16]. First, a midline incision in the neck was given to expose the left common carotid artery (CCA). Then the external carotid artery (ECA) and the internal carotid artery (ICA) were dissected from the surrounding tissues. The distal parts of these arteries were then clamped temporarily with silver microvascular clips. A monofilament nylon suture with a 0.234 mm diameter was inserted through the proximal ECA lumen into the ICA and the circle of Willis, and finally occluded the MCA. The ECA stump was ligated with a silk suture to prevent bleeding. The rats in the control group received

similar sham surgeries but a nylon suture was inserted only into the proximal ECA lumen.

The Microdialysis Procedure

The rats were anesthetized with chloral hydrate (350 mg/kg, i.p.) and were fixed in a stereotaxic apparatus (SAS-4100, Bioanalytical Systems, Inc., West Lafayette, IN, USA). After the skull was exposed, a burr hole was drilled for the accommodation of the guide cannula (Microbiotech AB, Stockholm, Sweden). The cannula was implanted into the left striatum with the following coordinates: AP+0.2 mm, ML +3.0 mm, DV-3.5 mm from according to the brain atlas of Paxinos and Watson[18] (Paxinos and Watson, 1998), and was secured to the skull with screws and dental cement. Each rat was housed individually following the surgical operation.

Seven days later the dummy stylet in the guide cannula was pulled out and a microdialysis probe (MAB/6; O.D. 0.6 mm, membrane length 4 mm, cut-off 15 000 Da, Microbiotech AB) was inserted into the guide cannula while the rat was awake and freely-movin. The probe, which had been connected to a microinfusion pump (MD-1001 Baby Bee Syringe Drive and MD-1020 Bee Hive Controller, Bioanalytical Systems, Inc.), was perfused at a constant flow rate of 1.5 μL/min with Ringer's solution composed of 125 mmol/L NaCl, 3.3 mmol/L KCl, 2.4 mmol/L Mg_2SO_4, 1.25 mmol/L KH_2PO_4, 1.85 mmol/L $CaCl_2$ (pH=7.1). After an 80 min equilibrium period, dialysate samples were consecutively collected every 30 min into vials containing 5 μL saline and 0.1% ascorbic acid, which was added to prevent any oxidation of DA, DOPAC, NE, HIAA, HVA, and 5-HT. All samples were injected directly into the HPLC-ECD system and analyzed immediately or kept at -70 °C until analysis.

Chemical Assays

NE, DA, DOPAC, HIAA, HVA, and 5-HT were detected on a Shimadzu (Kyoto, Japan) LC-10ADvp HPLC system with a Shimadzu L-ECD-6A amperometric detector. The separations were performed using a Hypersil GOLD C18 column (ODS, 150×4.6 mm i.d., 5 μm, Los Angeles, USA). The column and detector were placed in the compartment of the Shimadzu CTO-10A column oven at a temperature of 28 °C. The analytes were detected at an oxidation potential of 0.75 V vs the in situ Ag/AgCl reference electrode. The isocratic mobile phase (pH=2.6 using the method of determining monoamine neurotransmitters as previously published[18]) consisted of 8.65 mmol/L HSA, 0.35% TEA, 0.4% PA, 6.25% ACN, and 0.26 mmol/L EDTA tetrasodium salt and was delivered at a flow rate of 0.7/min. A 25 μL sample volume was injected.

Proof of the Probe Position

After the completion of the microdialysis experiments, rats were sacrificed by rapid decapitation. Their brains were removed immediately and immersed in 4% paraformaldehyde overnight. The probe implantation sites were verified by careful visual inspection.

In Vitro Recovery Experiments

Prior to the *in vivo* microdialysis procedure, *in vitro* experiments were performed to examine the recovery of DA, DOPAC, NE, HIAA, HVA, and 5-HT through the dialysis membrane of the probe. The probe was immersed in the standard solution containing known concentration of DA, DOPAC, NE, HIAA, HVA, and 5-HT and then perfused at a flow rate of 1.5 μL/min with Ringer's solution at room temperature. The samples were collected at intervals of 20 min and measured with the same conditions as described before for the HPLC-ECD system. The recovery of DA, DOPAC, NE, HIAA, HVA, and 5-HT was 30.3%, 28.6%, 29.1%, 26.3%, 25.8%, and 25.6% respectively.

Statistical Analysis

The results were expressed as the mean±SEM of the concentrations. One way analysis of variance (ANOVA) followed by Dunnett' post-hoc test was used to compare the difference of the means between groupstatistical significance was set at $P<0.05$.

RESULTS

MCAO significantly elevated the extracellular levels of NE, DA, DOPAC, HIAA, HVA, and 5-HT in the rats of the model group three days after the operation, compared with that of the control group. Though the levels of these neurotransmiters and their metabolites in each group changed little throughout the entire perfusion period, the differences between groups were significant ($P<0.05$, $P<0.01$, or $P<0.001$ *vs.* model). Long term ischemia damaged the dopaminergic neurons in the striatum and caused the continuous release of these substances. He extracellular striatal DA and NE levels were 3 times more than the control. The levels of the neurotransmitters and metabolites of the control group (from high to low were NE, DA, DOPAC, HIAA, HVA, and 5-HT. The reason for

these levels may be related to the left ligated artery, the left brain ischemia, and injury when the probe was inserted where is the cannula was implanted. However, the high and low doses of echinacoside, as shown in figures 16, decreased the ischemic injury to the neurons and prevented the increased extracellular striatal levels of NE, DA, DOPAC, HIAA, HVA, and 5-HT ($P<0.05$, $P<0.01$, or $P<0.001$ *vs.* model). Ligustrazine hydrochloride decreased the content of these substances continuously ($P<0.05$, $P<0.01$, or $P<0.001$ *vs.* model) and manifested a time-dependent trend (Table 1-6).

Table 1. Effects of Echinacoside on the Extracellular Levels of NE in the Striatum of MCAO Rats ($ng \cdot \mu L^{-1}$, $\bar{x} \pm s$, $n=6$)

Dialysis Time (min)	Control	Model	ECH High Dose	ECH Low Dose	CXQ
0	194.23±55.23	286.46±53.52$^{\Delta}$	264.74±65.63	279.57±54.59	235.31±60.32
30	203.09±41.13	359.54±57.83$^{\Delta\Delta}$	325.09±65.71	338.83±48.85	313.36±69.53
60	209.43±50.43	498.82±66.79$^{\Delta\Delta}$	418.32±57.93*	477.37±18.79	385.64±37.92*
90	218.93±61.46	674.73±63.33$^{\Delta\Delta}$	506.47±61.73**	598.07±31.34**	452.47±28.94**
120	205.63±56.63	621.36±58.38$^{\Delta\Delta}$	475.95±55.74**	556.64±54.84**	428.72±48.73**
150	187.96±58.79	542.59±63.35$^{\Delta\Delta}$	429.69±48.96**	489.32±41.09*	398.54±59.49**
180	179.74±55.07	459.42±39.23$^{\Delta\Delta}$	384.45±20.42**	431.62±30.59	358.85±34.95**
210	175.32±57.83	369.66±57.04$^{\Delta\Delta}$	321.64±60.47	358.50±69.72	306.46±58.03*
240	171.30±41.42	327.53±47.22$^{\Delta\Delta}$	306.04±20.21	315.32±41.94	274.32±22.53*
270	169.73±38.93	305.32±46.62$^{\Delta}$	243.53±40.73*	279.42±20.83	236.53±32.64*
300	165.32±47.03	270.93±48.02$^{\Delta}$	225.83±48.03	258.64±33.22	238.75±29.03

Note. $^{\Delta}P<0.05$, $^{\Delta\Delta}P<0.01$ *vs.* control; $^{*}P<0.05$, $^{**}P<0.01$ *vs.* model.

Table 2. Effects of Echinacoside on the Extracellular Levels of DA in the Striatum of MCAO Rats ($ng \cdot \mu L^{-1}$, $\bar{x} \pm s$, $n=6$)

Dialysis Time (min)	Control	Model	ECH High Dose	ECH Low Dose	CXQ
0	421.65±58.83	491.74±59.65	476.36±63.73	487.74±45.74	452.65±52.64
30	465.75±51.73	637.75±64.73$^{\Delta}$	596.83±63.83	617.53±58.54	582.54±59.87
60	487.65±62.75	949.26±24.42$^{\Delta\Delta}$	886.83±37.73*	906.54±38.59	828.85±27.65*
90	498.85±63.75	1382.36±62.09$^{\Delta\Delta}$	1129.73±75.83**	1231.54±55.35**	1089.95±49.74**
120	467.75±52.75	1210.52±53.83$^{\Delta\Delta}$	989.54±83.84**	1069.54±55.74**	929.54±38.54**
150	462.83±62.93	997.83±24.73$^{\Delta\Delta}$	912.84±58.56*	964.84±44.83	849.74±34.73**
180	448.56±47.91	923.72±76.63$^{\Delta\Delta}$	877.73±28.85	901.64±39.69	808.63±43.84**
210	439.74±62.75	836.27±63.78$^{\Delta\Delta}$	803.64±44.73	819.65±35.53	748.84±58.04*
240	441.63±58.85	751.83±78.93$^{\Delta\Delta}$	704.63±37.74	726.53±23.73	679.64±28.04
270	434.74±59.92	684.63±43.73$^{\Delta\Delta}$	673.74±59.84	683.37±39.53	638.58±29.74
300	425.93±61.83	652.73±59.93$^{\Delta\Delta}$	627.74±68.74	638.37±57.24	612.64±56.85

Note. $^{\Delta}P<0.05$, $^{\Delta\Delta}P<0.01$ *vs.* control; $^{*}P<0.05$, $^{**}P<0.01$ *vs.* model.

Table 3. Effects of Echinacoside on the Extracellular Levels of DOPAC in the Striatum of MCAO Rats ($ng \cdot \mu L^{-1}$, $\bar{x} \pm s$, $n=6$)

Dialysis Time (min)	Control	Model	ECH High Dose	ECH Low Dose	CXQ
0	67.43±18.84	83.74±19.94$^{\Delta\Delta}$	71.72±22.74*	77.94±23.71	69.85±22.57*
30	72.76±23.83	92.55±21.63$^{\Delta\Delta}$	82.83±21.83	87.54±18.54	77.73±9.48*
60	79.55±22.75	108.76±27.04$^{\Delta\Delta}$	89.26±17.33**	98.62±18.84*	83.83±13.64**
90	85.74±9.83	135.38±32.54$^{\Delta\Delta}$	115.78±22.28**	128.27±15.75	107.97±29.64**
120	83.83±12.05	119.62±23.63$^{\Delta\Delta}$	105.34±23.34**	116.73±15.59	97.73±18.37**
150	81.52±10.73	106.84±24.83$^{\Delta\Delta}$	97.93±18.53*	108.38±14.83	93.84±14.33**
180	78.65±11.43	97.52±21.83$^{\Delta\Delta}$	91.38±11.38	94.37±19.83	86.57±13.62*
210	75.73±19.45	84.84±13.73$^{\Delta}$	88.74±14.37	86.94±10.84	79.38±8.04
240	71.73±23.83	78.63±28.73	74.58±17.83	81.48±13.57	71.68±19.69
270	63.63±26.52	68.74±11.84	65.83±9.83	69.49±9.60	65.38±19.72
300	56.86±21.54	65.72±9.03	61.38±18.89	63.06±17.60	59.38±12.03

Note. $^{\Delta}P<0.05$, $^{\Delta\Delta}P<0.01$ *vs.* control; $^{*}P<0.05$, $^{**}P<0.01$ *vs.* model.

Table 4. Effects of Echinacoside on the Extracellular Levels of HIAA in the Striatum of MCAO Rats ($ng \cdot \mu L^{-1}$, $\bar{x} \pm s$, n=6)

Dialysis Time (min)	Control	Model	ECH High Dose	ECH Low Dose	CXQ
0	164.54±12.43	182.64±9.85	174.76±23.73	179.64±19.73	171.62±22.64
30	171.41±10.83	243.74±24.53$^{\Delta\Delta}$	218.56±13.37*	229.63±18.74	207.62±19.87**
60	181.45±12.63	284.22±14.82$^{\Delta\Delta}$	246.83±17.23**	257.36±28.72*	251.67±17.25**
90	189.82±13.63	358.37±12.59$^{\Delta\Delta}$	317.74±15.73**	326.44±15.75**	305.27±9.94**
120	186.84±12.74	339.32±12.87$^{\Delta\Delta}$	306.84±13.94**	315.74±15.64**	283.62±18.624**
150	179.43±17.93	312.74±14.83$^{\Delta\Delta}$	286.54±18.86*	307.73±24.62	269.64±24.83**
180	172.36±14.91	297.57±13.73$^{\Delta\Delta}$	269.37±18.86*	289.83±29.09	254.72±13.74**
210	168.84±20.65	274.37±23.58$^{\Delta\Delta}$	252.38±24.71	264.38±25.62	235.82±17.74**
240	161.23±15.73	246.27±18.73$^{\Delta\Delta}$	225.84±17.54	236.28±13.61	216.47±18.04*
270	159.64±21.96	212.73±23.33$^{\Delta\Delta}$	196.37±29.84	207.84±21.52	197.53±26.73
300	152.45±20.03	192.36±19.03$^{\Delta\Delta}$	178.48±28.04	186.28±17.62	173.76±12.86

Note. $^{\Delta}P$<0.05, $^{\Delta\Delta}P$<0.01 *vs.* control; $^{*}P$<0.05, $^{**}P$<0.01 *vs.* model.

Table 5. Effects of Echinacoside on the Extracellular Levels of HVA in the Striatum of MCAO Rats ($ng \cdot \mu L^{-1}$, $\bar{x} \pm s$, n=6)

Dialysis Time (min)	Control	Model	ECH High Dose	ECH Low Dose	CXQ
0	163.55 ±31.13	191.54±24.75$^{\Delta}$	182.66±33.53	188.74±25.74	178.35±34.54
30	169.63±21.63	214.77±13.83$^{\Delta\Delta}$	196.82±13.73*	208.33±38.46	186.64±19.47*
60	174.37±56.71	266.36±54.48$^{\Delta\Delta}$	228.53±67.83**	235.74±68.56**	217.35±57.69**
90	183.75±53.62	354.32±63.79$^{\Delta\Delta}$	316.63±55.88**	339.24±65.31	307.93±59.34**
120	179.72±51.55	328.56±83.73$^{\Delta\Delta}$	301.62±47.74**	318.57±45.64	289.25±48.84**
150	176.73±22.63	294.43±24.76$^{\Delta\Delta}$	271.64±18.86*	284.74±24.67	254.63±24.63**
180	171.94±27.21	268.62±26.33$^{\Delta\Delta}$	236.73±18.95**	254.62±29.65	239.62±23.94**
210	168.28±12.65	251.72±23.73$^{\Delta\Delta}$	226.47±24.63*	247.85±25.63	224.82±18.44*
240	157.94±28.75	225.37±18.33$^{\Delta\Delta}$	207.73±17.94*	219.27±13.41	197.66±38.84*
270	146.38±23.62	195.56±23.63$^{\Delta\Delta}$	184.84±25.64	195.27±19.56	185.28±14.76
300	137.92±11.87	187.84±29.53$^{\Delta\Delta}$	174.48±28.76	184.34±17.64	175.24±26.45

Note. $^{\Delta}P$<0.05, $^{\Delta\Delta}P$<0.01 *vs.* control; $^{*}P$<0.05, $^{**}P$<0.01 *vs.* model.

Table 6. Effects of Echinacoside on the Extracellular Levels of 5-HT in the Striatum of MCAO Rats ($ng \cdot \mu L^{-1}$, $\bar{x} \pm s$, n=6)

Dialysis Time (min)	Control	Model	ECH High Dose	ECH Low Dose	CXQ
0	119.65±18.83	137.74±19.65	123.36±13.73	128.74±25.74	121.65±22.64
30	123.75±31.73	172.75±34.73$^{\Delta\Delta}$	151.83±23.83	158.53±48.54	147.54±29.87
60	136.65±12.75	223.26±24.42$^{\Delta\Delta}$	173.83±27.73**	189.54±38.59*	167.85±27.65**
90	161.85±23.75	286.36±22.09$^{\Delta\Delta}$	236.73±25.83**	257.54±35.35*	221.95±19.74**
120	153.75±22.75	263.52±13.83$^{\Delta\Delta}$	219.54±23.84**	243.54±25.74	203.54±28.54**
150	144.83±12.93	232.83±24.73$^{\Delta\Delta}$	194.84±28.56**	217.84±44.83	187.74±34.73**
180	135.56±17.91	217.72±16.63$^{\Delta\Delta}$	185.73±28.85**	195.64±19.69*	174.63±43.84**
210	122.74±22.75	192.27±33.78$^{\Delta\Delta}$	173.64±34.73	186.65±35.53	158.84±28.04**
240	116.63±18.85	182.83±28.93$^{\Delta\Delta}$	153.63±27.74**	169.53±23.73	142.64±28.04**
270	112.74±19.92	153.63±13.73$^{\Delta\Delta}$	143.74±19.84	149.37±29.53	128.58±19.74*
300	107.93±21.83	149.73±19.93$^{\Delta\Delta}$	128.74±38.74*	134.37±27.24	121.64±16.85**

Note. $^{\Delta}P$<0.05, $^{\Delta\Delta}P$<0.01 *vs.* control; $^{*}P$<0.05, $^{**}P$<0.01 *vs.* model.

DISCUSSION

Echinacoside is a biomonomer extracted from Cistanche salsa, a medicinal plant native to northwest China. Echinacoside is used as an antioxidant and to prolong life. The phenylethanoid glycosides are the major components of this herb[19]. Recently, echinacoside was found to have neuroprotective effects[12]. However, the cellular and molecular mechanisms that underlie these actions are not

fully understood, especially for its influence on neurotransmitters and their metabolites. We reported the neuroprotective effects of echinacoside on the striatal dopaminergic neurons injured by 6-OHDA[20], in which oxidative stress played an important damaging role in the neuronal pathogenesis. Because oxidative stress also influences the cerebral ischemia/reperfusion injury, the relationship between neurochemical changes and the neuroprotective effects of echinacoside on striatal dopaminergic neurons are of interest. The present study used cerebral microdialysis to collect and detect extracellular striatal NE, DA, DOPAC, HIAA, HVA, and 5-HT directly. The results showed that echinacoside prevented increases in the extracellular levels of NE, DA, DOPAC, HIAA, HVA, and 5-HT in the striatum of MCAO rats. The mechanisms behind these effects may be due to neuroprotective actions of echinacoside or its interaction with other factors so that it may protect the dopaminergic neurons from the ischemic injury.

Oxidative stress exists in the ischemic injury process in the brain[4-5]. Reactive oxygen species (ROS) or free radicals such as O_2-, H_2O_2, OH, and the peroxynitrite anion (ONOO−) are produced rapidly and in large amounts during cerebral ischemia[3,6]. These materials may damage lipidse the substantia nigra, and this damage release dopaminergic neurotransmitters[7-8]. Meanwhile, biogenic amines likely affect vasoregulation, cerebral blood flow(CBF), and cerebral metabolismafter brain ischemia[21]. Thus, we used the MCAO method to induce the rat cerebral ischemia model. Through in vivo microdialysis, the levels of NE, DA, DOPAC, HIAA, HVA, and 5-HT in the striatum of MCAO rat were elevated which could be diminished by echinacoside or ligustrazine hydrochloride treatments.

Echinacoside and ligustrazine hydrochloride have antioxidative stress effects, and are involved in the maintenance of mitochondria function and inhibition of caspase-3 activity[12,22]. These activities may constitute the basis of neuroprotective effects. Our study suggests that echinacoside may inhibit the oxidative damage of ROS to dopaminergic neurons and also may maintain the extracellular concentration of catecholamine neurotransmitters and their metabolites at normal or close to normal levels. The present study also demonstrated that consecutive administration of echinacoside for 7 days could prevent elevated extracellular striatal levels of NE, DA, DOPAC, HIAA, HVA, and 5-HT induced by MCAO. These results indicate that echinacoside may protect dopaminergic neurons in the striatum from ischemic injury. In conclusion, the results of this research showed that echinacoside may be a promising drug for the prevention and treatment of cerebral ischemic diseases.

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