Berberine and total base from *rhizoma coptis chinensis* attenuate brain injury in an aluminum-induced rat model of neurodegenerative disease

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ABSTRACT

الأهداف: التحقيق في العوامل الوقائية للقاعدة الكاملة من (CTB) وبيبرين (Ber) على الانتكاس العصبي المحرض بواسطة زيادة جرعة الألمنيوم في الجرذان.

الطريقة: أجريت هذه الدراسة بقسم الصيدلية بجامعة تشونقكينج الطبية – تشونقكينج – الصين، خلال الفترة ما بين فبراير 2005م وحتى مايو 2007م. تم تقسيم جرذان نوع ويستار إلى مجموعة التحكم، المجموعة المعالجة بعقار (Ber)، المجموعة التي تلقت عقار (CTB) بمقدار (55mg – 110mg/kg) والمجموعة التي تلقت عقار نيموديبين عدد=20. تم إجراء تلف لدماغ الجرذان بواسطة تلقيها مقدار 400mg من الألمنيوم مرة واحدة في اليوم وخمسة أيام في الأسبوع لمدة 12 أسبوع. تم إعطاء الجرذان عقار (Ber)، (CTB) و نيموديبين بعد 4 ساعات من تلقي الألمنيوم لمدة 12 أسبوع. تمت مراقبة التغيرات الشكلية للعصبونات لقرني آمون في الدماغ لدى الجرذان ومتغيرات وظائف الذاكرة والتعلم لديها. كما تم فحص أنشطة (AchE)، (ChAT)، (SOD) و (MAO–B) ومحتوى (MDA) وظهور (MAO–B) في دماغ الجرذان.

النتائج: يحسن عقار (CTB) و (Ber) ونيموديبين قصور قدرة التعلم والذاكرة وموت العصبونات بقرني آمون في الدماغ. كما يحد عقار (CTB) و (Ber) ونيموديبين من انخفاض انشطة (SOD) و(ChAT)) وزيادة محتوى (MDA) وانشطة (AchE) وظهور (MAO–B) والنشاط في الجرذان ذات الزيادة في عنصر الألمنيوم.

خاتمة: لدى عقاري (CTB) و (Ber) آثار وقائية على الانتكاس العصبي المحرض بواسطة زيادة الألمنيوم. لدى عقار (CTB) (110mg/kg) وقاية عصبية أكثر من عقار (Ber).

Objectives: To investigate the protective effects of the total base from *rhizoma coptis chinensis* (CTB) and berberine (Ber) on neurodegeneration induced by aluminum overload in rats.

Methods: The study took place in the Department of Pharmacology, Chongqing Medical University, Chongqing, China, between February 2005 and May 2007. Wistar rats were divided into control group, model group, Ber-treated group, CTB (55 mg/kg and 110 mg/kg)-treated group, and nimodipine-treated group (n=20). A rat brain damage model was established via intragastric administration of 400 mg/kg element aluminum once a day, 5 days a week for 12 weeks. The CTB, Ber, and nimodipine were intragastrically administered 4 hours after each aluminum administration for 12 weeks. The morphological changes of the neurons of the rat hippocampus and the changes of rat learning and memory functions were observed. The superoxide dismutase (SOD), choline acetyltransferase (ChAT), acetylcholinesterase (AchE), and monoamine oxidase-B (MAO-B) activities and malondialdehyde (MDA) content, as well as the MAO-B expression in the rat brain were examined.

Results: The CTB, Ber, and nimodipine significantly improved the learning and memory ability impairment and hippocampal neuronal death. The CTB, Ber, and nimodipine also significantly blunted the decrease of SOD and ChAT activities, and the increase of MDA content, AchE activities, and MAO-B expressions and activity in the aluminum-overload rats.

Conclusions: The CTB and Ber have protective effects on neurodegeneration induced by aluminum overload. The CTB (110 mg/kg) has more powerful neuroprotection than Ber.

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With the progress of society and rapid growth of an aging population, age-associated neurodegenerative diseases, such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), and amyotrophic lateral sclerosis (ALS), have become a worldwide problem that affects billions of people. It was reported that in some countries, AD was observed in more than 3% of individuals over the age of 65, and more than 20% of individuals over the age of 80. Previous studies showed that many factors, such as genetic factors, diet, aluminum, viruses and neuroinflammation and oxidative stress in the central nervous system (CNS) may play an important role in age-associated neurodegenerative diseases and in the normal aging.[1] However, the exact etiology and pathogenesis of these age-associated neurodegenerative diseases are still not clear. Thus, it is necessary to understand the mechanisms underlying the neurodegeneration and to develop effective protective agents. *Coptis chinensis Franch* is a well-known Chinese traditional herbal medicine widely used for hundreds of years. Berberin (Ber) is an isoquinoline alkaloid found in the root, rhizome, and stem bark of *Coptis chinensis*. Besides the Ber, coptis rhizoma contains other active alkaloids, such as balmatine, jatrorrizine, and coptisine. These alkaloids are known as total base from *Coptis chinensis (*CTB). In recent studies,[2] Coptis and Ber have been shown to have multiple pharmacological actions and bioactivities. In peripheral nervous system, has been used for treatment of dysentery, hypertension, anti-inflammation, and liver disease in China. Ber has antidiarrheal, antimicrobial, anti-inflammatory, antineoplastic, anti-arrhythmic, antiproliferative, and immunosuppressive properties. In the CNS, Ber is able to penetrate through the blood brain barrier and distribute to hippocampal neurons.[3] Studies showed that Ber improved scopolamine-induced amnesia by increasing the activity of peripheral and central cholinergic neuronal system activity, and that had an obvious antidepressive effect.[4] Also, it was reported that Ber attenuated repeated nicotine-induced behavioral sensitization in rats.[5] Moreover, Ber protected the hippocampal neurons from ischemic damage.[6,7] A previous study also showed that CTB had a protective effect on Abeta 25-35 induced leaning and memory dysfunction in rats.[8] However, the effects of CTB and Ber on chronic cerebral damage and neurodegeneration have not been investigated. Previous studies showed that protoberberine alkaloids also exhibit a wide variety of pharmacological and biological effects.[9] Therefore, we hypothesize that CTB and Ber have protection against neurodegeneration and a synergism exists among these alkaloids of CTB. The present study was designed to observe the protective effects of CTB and Ber against neurodegeneration, and whether the neuroprotection of CTB is more powerful than that of Ber in aluminum overloaded rats.

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Methods. ***Reagents.*** Ribonucleic acid (RNA)-Trizol reagent and reverse transcription polymerase chain reaction (RT-PCR) assay kit were purchased from Takara Co., Tokyo, Japan. Four percent aluminum gluconate solution was prepared by adding aluminum chloride hexahydrate (Dongfang Reagents Co., Chongqing, China) and natrium gluconate (Dongfang Reagents Co., Chongqing, China) to distilled water, and finally titrated to pH 6.0 with 0.1M sodium hydroxide (NaOH). Berberine (match number 940833, Chengdu 3rd Pharmaceutical Factory, Chengdu, China) with a purity of 98% was used. The CTB (match number 0012185) was prepared from *Coptidis rhizome* and was kindly provided by Associate Professor Jingchuan Shang, Department of Chemistry, Chongqing Medical University. The CTB consisted of Ber (87.6%), palmatine (2.7%), jatrorrhizine (3.6%), coptisine (5.2%), and other alkaloids (1%). The CTB, Ber, and nimodipine were dissolved with 0.5% carboxymethylcellulose (CMC).

Animals and experimental protocol. Male Wistar rats (n=120), 180-220 g, and 8 weeks old were purchased from the Laboratory Animal Care Center of Chongqing Medical University, Chongqing, China. They were housed in a regulated environment (25^0C ± 1^0C, 50% ± 2% humidity), with 12 hours light/dark cycles (light from 8:00-20:00). All experimental procedures were approved by the Chongqing Medical University Institutional Animal Ethics Committee and took place between February 2005 and May 2007. Rats were treated intragastrically with 4% aluminum gluconate solution (400 mg element aluminum/kg, pH 6.0-6.5) or saline. Four hours after the aluminum treatment, CTB (55 and 110 mg/kg, intragastrically), Ber (100 mg/kg, intragastrically), nimodipine (80 mg/kg, intragastrically) and vehicle (0.5% CMC) were administrated. The treatment was performed daily for 5 successive days per week and for 12 weeks. Each treatment group contained 20 rats. After the treatment, 10 rats from each group were used for behavioral studies and 10 rats were used for biochemical studies.

Behavioral tests. Passive avoidance task. At the end of 12 weeks of treatment, a step down type passive

avoidance task was conducted using DTT-2 apparatus (Institute of Materia Medica, Chinese Academy of Medical Sciences, Beijing, China). Briefly, 10 rats in each group were trained to learn avoiding electric stimuli (ES [36 volts]). Each rat was placed on the grid floor with its back against a platform. Intermittent ES were delivered to the grid floor. The rat can jump on the platform to avoid the ES. When they stepped down from the platform and placed all its paws on the grid floor, the rat was shocked with the intermittent ES and jumped on the platform again. The number of electric shock, for the rat to avoid the ES within 3 minutes was recorded as a standard to evaluate the ability. The higher the number of shock indicates the lower the leaning ability. Twenty-four hours after learning training, the retention test of memory was carried out. Each rat was placed on the safe platform on the grid floor without intermittent ES. The time for the rat to step down from the platform to the grid floor is called the step down latency. The step down latency was measured as memory retention. An upper cut-off time of 300 seconds (s) was set.

Morris water maze test. The next day after the step down type passive avoidance task, the Morris water maze test was conducted over 5 days with DMS-2 Morris water maze test apparatus (Institute of Materia Medica, Chinese Academy of Medical Sciences). In the first 4 days, acquisition test was performed, in which the rats (n=10 in each group) were sent into the water separately and forced to seek the platform (safety island) by swimming. If the rat found the platform within 180 s, it was allowed to stay on the platform for 15 s, otherwise, it was guided onto the platform. The training was carried out once a day. Twenty-four hours after the last test, the platform was removed and the rat was put into the water at the same place as before. The time for the rat to pass through the place, where the platform was previously placed, was called as the time for exploring platforms. The time for exploring platforms was recorded using a video tracking device integrated with Morris water maze test apparatus. The cut out time was 180 s.

Cerebral histological sections. After behavioral evaluation, rats (n=3) were anesthetized with sodium pentobarbital, 40 mg/kg intraperitoneally and transcardially perfused with 100 mL of 0.9% saline containing heparin (250 U) followed by 200 mL of fixing solution containing 3.5% formaldehyde and 0.1 M phosphate buffer (pH 7.2). The brain was removed and cut into coronal sections of 4 μm thickness. The sections were stained with hematoxylin and eosin. The morphology of neurons in the rat hippocampus was observed with light microscope.

Biochemistry. At the end of week twelfth of aluminum and drug administration, 10 rats from each group were sacrificed and the hippocampi were dissected. The hippocampus (100 mg) was homogenized with saline (tissue: saline=1:9). The malondialdehyde (MDA) content and the activities of superoxide dismutase (SOD), acetylcholinesterase (AchE), choline acetyltransferase (ChAT) and monoamine oxidase-B (MAO-B) were measured using the biochemistry assay kit (Jiancheng Bioengineering Ltd, Nanjing, China) according to the manufacturer's manual. The protein content of samples was measured using biuret reaction.

Reverse transcription polymerase chain reaction assay for MAO-B mRNA. To determine the expression of the MAO-B mRNA, a RT-PCR was conducted. Total RNA was extracted from cerebral tissue of rats using RNA-Trizol reagents according to the manufacturer's directions. The yield of the total RNA was determined by measuring the absorbance at 260 nm and 280 nm. First strand cDNA was synthesized according to the TaKaRa-2 steps method RT-PCR system manual (TaKaRa Co., Tokyo, Japan). In a total volume of 10 μL, 1 μL deoxynucleotide (dNTP) mix (approximately 100 μM each), 2 μL magnesium sulphate ($MgSO_4$), 0.5 μL recombinant avian myeloblastosis virus reverse transcriptase (RT), and 1 μL total RNA template, 0.25 μL RNAase inhibitor, 0.5 μL random primers, 1 μL for 10 x RT buffer were mixed. The RT reaction was carried out at 42^0C for 60 minutes followed by 74^0C for 5 minutes to stop the reaction. The PCR for MAO-B were conducted using MAO-B upstream primer 5'-TGCTAGATAAGATCTGCTGG-3'and downstream primer 5'-ATCCAATGTGTACGCAATTG-3' that produced a 535 bp fragment; nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) (a house-keep gene as control) was amplified with upstream primer 5'-TGAAGGTCGGTGTCAACGGATTTGGC-3' and downstream primer was 5'-CATGTAGGCCATGAGGTCCACCAC-3', which produced a 983 bp fragment. The PCR reaction was conducted in total volume of 40 μL, containing 28.25 μL RNAase freed H_2O, 10 μL 5 x PCR buffer, 0.25 μL TaKaRa Ex Taq™ HS (TaKaRa Co., Tokyo, Japan), 0.5 μL upstream/downstream primer, and 0.5 μL cDNA. The reaction was carried out for 27 cycles at 95^0C for one minute, at 60^0C for 55 seconds and at 72^0C at 90 seconds followed by a final extension step at 72^0C for 10 minutes. The reaction products were resolved by 1% low melt point agarose gel. The integrated density of bands was quantified using gel imaging and analysis system (Bio-Rad Laboratories, Inc., California, USA). The amounts of MAO-B mRNA were calculated as ratios of MAO-B mRNA amounts to the corresponding amounts of NADPH mRNA (MAO-B/NADPH).

Western blotting assay of MAO-B protein expression. The hippocampus was homogenated in a buffer solution

containing 10 mM Tris–HCl (pH 7.4), 0.15 M NaCl, 1% (weight/volume) nonidet P40 (NP-40), 0.1% (wt./vol) sodium dodecyl sulfate (SDS), 0.001 mg/ml leupeptin, 0.001 mg/ml pepstatin, 0.001 mg/ml aprotonin, and 10% (weight/volume) phenylmethyl sulfonylfluoride (PMSF), then centrifuged at 4°C 12,000 x g for 5 minutes. The supernatant was collected for Western blotting assay. The protein concentration was estimated by the method of Coomassie brilliant blue. A 50 μg protein was subjected to 12% (weight/volume) sodium dodecyl sulfate polyacrylamide gel electropheresis (SDS-PAGE) and transferred to a polyvinylidene difluoride (PVDF) membrane. The membrane was blocked for 2.5 hours in the PBS containing 5% fat-free (weight/volume) milk and 0.2% (vol/vol) tween 20. The blot was incubated for 2 hours at 37°C with either antibody of MAO-B and β-actin at the concentration of 1:400, followed by incubation for one hour at 37°C with secondary antibody (1:500). Immunoreactive bands of MAO-B and β-actin were visualized with horseradish peroxidase reagent. The optical density band of MAO-B and β-actin was detected using a gel imaging and analysis system (Bio-Rad Laboratories, Inc., California, USA). The amounts of MAO-B protein were calculated as ratios of MAO-B protein amounts to the corresponding amounts of β-actin protein (MAO-B/β-actin).

Statistical analysis. The data were presented as Mean±SD. The differences between groups were evaluated using the one-way analysis of variance, followed by Bonferroni's post-hoc analysis to compare between the groups with Statistical Analysis Software version 6.0. The 95% confidence intervals on the mean are computed based on the sample mean and sample standard deviation. Results were considered significant at a value of $p<0.05$.

Results. ***Effects of CTB and Ber on the behavioral changes induced by aluminum overload in rats.*** In passive avoidance task, the number of shocks during the training periods to learn to avoid the ES was significantly increased in the aluminum-overload rats when compared to the control group ($p=0.000$). Treatment with CTB (110mg/kg), Ber, and nimodipine significantly reduced the aluminum treatment-induced increase in the number of shocks ($p=0.000$). Step down latency in

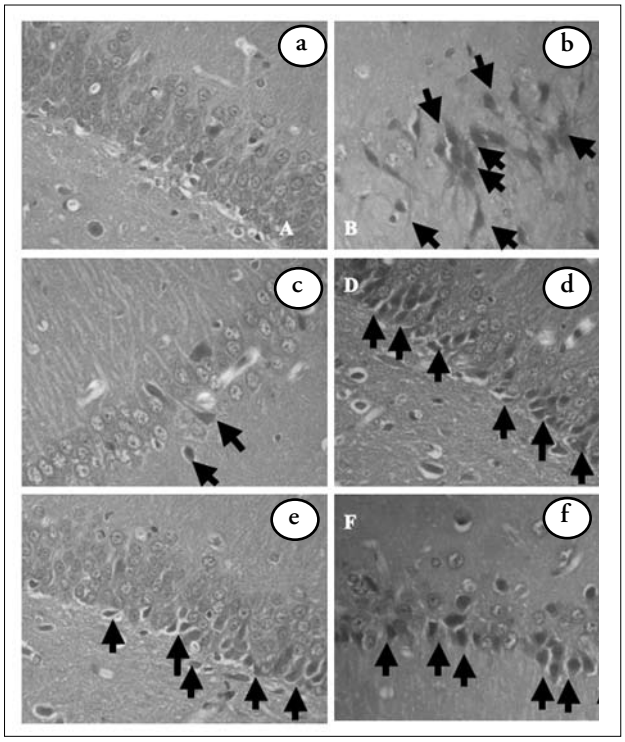

Figure 1 - Protection of hippocampal neurons from aluminum-induced damage by *rhizoma coptis chinensis (*CTB) and Ber in rats (Hematoxylin and Eosin×400). **a**) Vehicle-treated group; **b**) vehicle plus aluminum-treated group; **c**) CTB (110 mg/kg) plus aluminum-treated group; **d**) CTB (55 mg/kg) plus aluminum-treated group; **e**) Ber (100 mg/kg) plus aluminum-treated group. **f**) Nimodipine (80 mg/kg) plus aluminum-treated group. (Arrow indicates karyopyknosis of cells).

Table 1 - Effects of *rhizoma coptis chinensis* (CTB) and Berberin (Ber) on aluminum-induced impairment of learning and memory functions in rats (n=10).

Groups	Number of shocksduring training	Step down latencies (s)	Time for exploring platforms (s)
Control	1.25 ± 0.46	299.12 ± 30	19.75 ± 4.1
Model	5.65 ± 0.31*	26.18 ± 16.25*	36.13 ± 3.12*
CTB 55 mg/kg	4 ± 0.82†	215.57 ± 13.88‡	34.57 ± 2.75
CTB 110 mg/kg	1.83 ± 0.5‡	277.11 ± 17.31‡	9.44 ± 2.3‡
Ber 100 mg/kg	2.43 ± 0.49‡	232.83 ± 8.68‡	21.33 ± 6.62‡
Nimodipine 80 mg/kg	2.29 ± 0.48‡	223 ± 13.54‡	23.5 ± 23.5‡

Data are expressed as mean ± SD. *$p<0.01$ versus control group, †$p<0.05$ and ‡$p<0.01$, versus model group. Control group: treated with the 0.5% CMC followed by 0.9% normal saline. Model group: treated with aluminum gluconate followed by vehicle. CTB 110 mg/kg, CTB 55 mg/kg, Ber 100 mg/kg and nimodipine 80 mg/kg: treated with aluminum gluconate followed by CTB 110 mg/kg, CTB 55 mg/kg, Ber 100 mg/kg and nimodipine 80 mg/kg.

Table 2 - Effects of *rhizoma coptis chinensis* (CTB) and Berberin (Ber) on aluminum-induced changes of AchE and ChAT activities in rats (n=10).

Groups	AchE activity (nmol/mg protein)	ChAT activity (nmol/mg protein)
Control	0.31 ± 0.02	988.16 ± 90
Model	0.99 ± 0.13*	190.02 ± 34.88*
CTB 55 mg/kg	0.81 ± 0.09	225.4 ± 71.89
CTB 110 mg/kg	0.34 ± 0.16†	870.78 ± 96.36†
Ber 100 mg/kg	0.48 ± 0.09†	674.9 ± 119.26†
Nimodipine 80 mg/kg	0.45 ± 0.09†	614.98 ± 88.64†

Data are expressed as mean ± SD. *p<0.01 versus control group; †p<0.01 versus model group. Control group: treated with the 0.5% CMC followed by 0.9% normal saline. Model group: treated with aluminum gluconate followed by vehicle. CTB 110 mg/kg, CTB 55 mg/kg, Ber 100 mg/kg and Nimodipine 80 mg/kg: treated with aluminum gluconate followed by CTB 110 mg/kg, CTB 55 mg/kg, Ber 100 mg/kg, and Nimodipine 80 mg/kg.

Table 3 - Effects of *rhizoma coptis chinensis* (CTB) and Berberin (Ber) on aluminum-induced changes of malondialdehyde contents and SOD and MAO-B activities in rats (n=10).

Groups	SOD activity (Nu/mg prot)	Malondialdehyde contents (nmol/mg protein)	MAO-B activity (nmol/mg protein)
Control	39.63 ± 3.57	0.26 ± 0.13	0.43 ± 0.09
Model	14.04 ± 2.62*	1.93 ± 0.27*	1.87 ± 0.33*
CTB 55 mg/kg	25.54 ± 4.59	1.31 ± 0.19	1.29 ± 0.27
CTB 110 mg/kg	38.43 ± 1.91†	0.44 ± 0.2†	0.51 ± 0.1†
Ber 100 mg/kg	35.01 ± 3.52†	0.72 ± 0.15†	0.89 ± 0.1†
Nimodipine 80 mg/kg	28.52 ± 2.91†	0.77 ± 0.14†	0.76 ± 0.13†

Data are expressed as mean ± SD. *p<0.01 versus control group; †p<0.01versus model group. Control groups: treated with the 0.5% CMC followed by 0.9 % normal saline. Model group: treated with aluminum gluconate followed by vehicle. CTB 110 mg/kg, CTB 55 mg/kg, Ber 100 mg/kg and Nimodipine 80 mg/kg: treated with aluminum gluconate followed by CTB 110 mg/kg, CTB 55 mg/kg, Ber 100 mg/kg and Nimodipine 80 mg/kg.

Table 4 - Effects of *rhizoma coptis chinensis* (CTB) and Ber on aluminum-induced changes of monoamine oxidase-B (MAO-B) mRNA and protein expression in rats (n=3).

Groups	MAO-B mRNA expression	MAO-B protein expression
Control	0.539 ± 0.008	1.87 ± 0.24
Model	1.095 ± 0.008*	6.35 ± 0.11*
CTB 55 mg/kg	0.822 ± 0.014	5.71 ± 0.09
CTB 110 mg/kg	0.588 ± 0.006†	2.54 ± 0.17†
Ber 100 mg/kg	0.669 ± 0.032†	3.24 ± 0.28†
Nimodipine 80 mg/kg	0.653 ± 0.019†	3.35 ± 0.24†

Data are expressed as mean ± SD. *p<0.01 versus control group; †p<0.01 versus model group. Control: treated with the 0.5% CMC followed by 0.9 % normal saline. Model: treated with aluminum gluconate followed by vehicle. CTB 110 mg/kg, CTB 55 mg/kg, Ber 100 mg/kg and Nimodipine 80 mg/kg: treated with aluminum gluconate followed by CTB 110 mg/kg, CTB 55 mg/kg, Ber 100 mg/kg and Nimodipine 80 mg/kg.

aluminum-overload rats was significantly shorter than that in the control rats (p=0.000). The administration of CTB (110mg/kg), Ber, and nimodipine significantly decreased aluminum-overload-induced attenuation in step down latency (p=0.000) (**Table 1**). The water maze test showed that the latencies to find platform was significantly longer in aluminum-treated rats than that in the control group (p=0.0022). The CTB (110 mg/kg), Ber, and nimodipine administration significantly improved the spatial discrimination impairment induced by aluminum (p=0.000) (**Table 1**). The effects of CTB (110 mg/kg) on behavioral changes were more significant than that of Ber and showed a dose-dependent property (p=0.0024) (**Table 1**).

Effects of CTB and Ber on the pathomorphological changes of hippocampal neurons induced by aluminum

treatment. A karyopyknosis and loss of neurons were observed in the hippocampal CA1 subfield of aluminum-overloaded rats. The administration of CTB (110 mg/kg) as well as Ber and nimodipine significantly prevented the pathological changes of hippocampal neurons in a dose-dependent manner (**Figure 1**).

Effects of CTB and Ber on the changes in the AchE and ChAT activities caused by aluminum overload. Compared to the control group, AchE activity was significantly increased and ChAT activity was significantly decreased in the chronic aluminum-overload group (p=0.001). The administration of CTB (110mg/kg) (p=0.001), Ber (p=0.005), and nimodipine (p=0.004) significantly blunted the increase of AchE activity and the decrease of ChAT activity induced by aluminum treatment. The effects of CTB (110 mg/kg) on changes of AchE and ChAT activities were much stronger than that of Ber (p=0.00074) (**Table 2**).

Effects of total CTB and Ber on the changes of malondialdehyde contents and SOD activities caused by aluminum overload. Malondialdehyde contents were significantly increased and SOD activities were significantly decreased in aluminum-overload rats when compared to the control group, p=0.000. The administration of CTB (110 mg/kg), Ber, and nimodipine significantly attenuated the aluminum-induced increase in malondialdehyde content and decrease in SOD activity, p=0.000. When compared to Ber and nimodipine, CTB (110 mg/kg) had a stronger effect on the changes in the malondialdehyde content (p=0.002) and the SOD activity caused by chronic aluminum overload, p=0.009 (**Table 3**).

Effects of CTB and Ber on aluminum overload-induced changes in the function and expression of MAO-B. The MAO-B activity of the brain was significantly increased in aluminum-overloaded rats (p=0.000). Administration of CTB, Ber, and nimodine obviously blunted the effect of aluminum on MAO-B activity (p=0.000). The CTB (110 mg/kg) had a stronger effect in inhibition of MAO-B activity than Ber in aluminum-overloaded rats (p=0.00854) (**Table 3**).

The results of RT-PCR analysis showed that MAO-B mRNA (MAO-B/NADPH) remained at a relative low level in the normal control, whereas aluminum overload significantly increased the MAO-B mRNA in the cerebral tissue (p=0.000). The CTB, Ber, and nimodipine attenuated the aluminum-induced increase in MAO-B mRNA in the brain (p=0.000). The effects of CTB on MAO-B mRNA showed a dose-dependent manner. The CTB (110 mg/kg) had a much stronger inhibition on MAO-B mRNA than Ber and nimodipine (**Table 4**). Monoamine oxidase-B protein level in the hippocampus was significantly higher in aluminum overloading rats than that of control group (p=0.000). Treatment with nimodipine and Ber significantly inhibited the aluminum-induced increase in the MAO-B protein expression. Even though administration of CTB 55 mg/kg had no effect on the aluminum-induced elevation of MAO-B level, administration of CTB 110 mg/kg significantly reduced the aluminum-induced elevation of MAO-B protein level (p=0.000). The CTB (110 mg/kg), had much stronger effects on expression of MAO-B protein than Ber and nimodipine groups (**Table 4**).

Discussion. Behavior dysfunction and loss of neurons in the regions of the brain are common characteristics of chronic cerebral injury and neurodegeneration. In a previous study, repeat intracerebroventricular injection of aluminum cause acute cerebral damage and impairment of learning and memory function in mice.[10] In the present study, a neurodegeneration rat model was established by repeated intragastrically administration of aluminum and showed that aluminum overload induced the significant impairment of passive avoidant learning and memory function, and the spatially orient ability of rats. Simultaneously, aluminum administration also produced a significant neuronal death in hippocampus CA1 subfield and the increase of AchE activity and decrease of ChAT activity. These results indicate that administration of aluminum damages to the cholinergic neurons of rat hippocampus, and finally results in neurodegeneration.

Oxidative metabolism can supply energy to neurons. At the same time, this process may produce a lot of reactive compounds such as hydrogen peroxide and oxyradicals, which may result in peroxidation of biomembrane lipids, DNA damage, and neuronal death. Neuroinflammation and oxidative stress in the CNS may play an important role in neurodegenerative diseases and in the normal aging.[1] The results of the present study showed that aluminum overload induced significant decrease of SOD activity and significant increase of malondialdehyde content. These results further confirmed that oxidative stress contributed to aluminum induced neurodegeneration. In CNS, MAO-B is mainly responsible for metabolism of catecholamines (namely, dopamine and noradrenaline) and serotonin. While dopamine has been shown to be degraded, MAO-B can catalyze the dopamine to produce a large amount of hydrogen peroxide, which can further be turned to hydroxy radical via Fenton reaction. Therefore, the increase of MAO-B activity can not only obviously decrease the neurotransmitter in CNS but also produce obvious oxidative stress.[11] Studies showed that the increase of MAO-B activity was related to symptoms of AD.[12] Aluminum significantly increased the MAO-B

activity of rat brain homogenate in a dose-dependent manner.[13] The present studies also showed that not only the MAO-B activity but also the MAO-B mRNA and protein expression were significantly increased in the aluminum overload rat brain. This may be involved in the mechanisms of malondialdehyde content increase and neurotoxicity of aluminum.

In this study, results revealed, for the first time, that CTB and Ber protected rats from the repeat intragastrically exposure of aluminum induced neuronal damage that leads to deficits in learning and memory and eventual neuronal death. The protective mechanism of CTB and Ber was involved in antagonizing the aluminum-induced increase in brain oxidative stress and downregulating MAO-B mRNA and protein expression, as well as inhibition of MAO-B activity in a dose-dependent manner. These results also showed that the protective effects of CTB (110 mg/kg) were much stronger than those of Ber. The results suggest that a synergy exists among these alkaloids of CTB, but further research is required for confirmation.

In summary, these results confirm our hypothesis that CTB and Ber may play an important pharmacological role in some neurodegenerative diseases, and that CTB and Ber may be a candidate targets for neuroprotective therapy for chronic cerebral damage and neurodegenerative processes.

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