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The homeostasis of iron and suppression of HO-1 involved in the protective effects of nimodipine on neurodegeneration induced by aluminum overloading in mice

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Abstract

Aluminum intoxication can cause damage to the cognitive function and neurodegenerative diseases. In the present study, we investigated the role of iron homeostasis and heme oxygenase-1 (HO-1) expression in the protective effects of nimodipine on the neurodegeneration induced by aluminum overloading in mice. 2 μl of 0.25% aluminum chloride solution was intracerebroventricularly injected once a day for five days to induce the neurodegeneration of mice. Nimodipine was administered by intragastric gavage (80 mg/kg per day) for 30 days. We observed that nimodipine could improve the performance of behavior test related to the learning and memory function and ameliorate pathological changes of hippocampi caused by aluminum. Results of western blot, immunohistochemistry study, biochemical test and inductively coupled plasma-atomic emission spectrometry showed that nimodipine could suppress the increased expression of HO-1 protein, and decrease the elevation of both HO activity and iron level in hippocampi, induced by aluminum overloading. These results indicate that nimodipine can suppress the neurodegenerative development induced by aluminum overloading and the mechanism of its action is at least partly related to keeping the homeostasis of iron through blunting the expression of HO-1 in hippocampus.

Keywords: Aluminum; Iron; Heme oxygenase; Neurodegeneration; Nimodipine; Hippocampus

1. Introduction

Neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, etc., will increase with the increasingly ageing population in the next decades. It is believed that many factors, including genetics, cerebral vessel diseases, excessive reactive oxygen species, and abnormalities of aluminum, iron and other metal ions contribute to the onset of these diseases. Among them, aluminum for Alzheimer's disease and iron for Parkinson's disease have been well recognized (Borie et al., 2002; Feigin and Zgaljardic, 2002; Pratico et al., 2002).

Heme oxygenase (HO) functions as the rate-limiting enzyme in heme degradation (Abraham et al., 1998; Maines, 1988). There are three isoforms of HO: HO-1, HO-2 and HO-3 (Maines and Kappas, 1974; Yoshida et al., 1974; Maines et al., 1986; Trakshel et al., 1986; McCoubrey et al., 1997). HO-1 is an inducible form that can be induced by many factors (Wu, 2005) and expressed in different regions of brain, especially the hippocampus (Scapagnini et al., 2002). HO-1 is thought to be heavily involved in the catabolism of heme which is degraded into CO, biliverdin and iron. HO-2 that is a non-inducible isoenzyme of HO is thought to be particularly involved in signaling pathways. The physiological role of HO-3 is uncertain.

Nimodipine as a calcium channel blocker belongs to dihydropyridine compounds, which has a high selectivity to the cerebral vessel and shows an admiringly protective effect on the brain injury from cerebral ischemia and its complications (Ma and Zhang, 2006). It was reported that nimodipine could

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ameliorate the age-related working memory deficits in aged animals (Devo et al., 1989; Veng et al., 2003) and improve memory ability as well as attention ability of the patients with mild cognitive impairment (Wang et al., 2006). However, the mechanism is still unclear. It was reported that high concentration of glucose that affected the survival of pancreatic islet cells, elevated the expression of HO-1 in pancreatic beta cells through increasing calcium influx, which could be reversed by nimodipine (Jonas et al., 2003). Heme degraded by HO-1 is the main source of endogeneous iron. Our research has demonstrated that aluminum overloading caused damage to the brain of mice with significant elevation of iron (He et al., 2006). Documents also showed that the abnormal increase of iron involved the development of neurodegeneration (Ke and Qian, 2007). So far, the effects of nimodipine on neurodegeneration induced by aluminum overloading, hippocampal HO-1 expression and homeostasis of iron have not been elucidated.

Thus, our working hypothesis is that nimodipine may prevent the neurodegeneration induced by aluminum overloading through suppressing the expression of HO-1 and reducing the production of iron. Our analyses included behavior experiment and histological examination to evaluate the effect of nimodipine on neurodegeneration induced by aluminum overloading. Then, to clarify whether nimodipine affected HO, we examined the activity and expression of HO. Finally, the free iron was detected by inductively coupled plasma-atomic emission spectrometry to determine the effect of nimodipine on homeostasis of iron.

2. Materials and methods

2.1. Reagents

Glucose 6-phosphate, NADPH, Hemin and Glucose 6-Phosphate Dehydrogenase were purchased from Sigma Co. Rabbit anti rat HO-1 polyclonal antibody and Rabbit anti rat HO-2 polyclonal antibody were purchased from Oncogene Co. Nimodipine were purchased from Bayer Co.

2.2. Animals

The 55 days old male mice of the NIH strain (supplied by Laboratory Center, Chong Qing Medical University, Certificate No: scxk20020001) were fed in the long-day light cycle condition (16 h light/8 h dark) at the surrounding temperature of 20±4 °C. All experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by Chong Qing medical university.

2.3. Apparatus

The step down test apparatus came from Institute of Materia Medica, Chinese Academy of Medical Sciences. The apparatus was a rectangular box (10×15×40-cm) with copper grid floor electrified with 36 V alternating current when test was carried out and a rubber columnar platform (diameter: 3.0 cm, height: 3.5 cm) in one corner. Inductively coupled plasma-atomic emission spectrometry meter was purchased from PE Co, U.S.A.

2.4. Surgery

The surgery was performed according to the procedures of Toyoda's report (Toyoda et al., 1996). Briefly, mice were anesthetized with 4% (wt./vol) chloral hydrate solution (10 ml/kg, ip) and a stainless steel cylindrical cannula (outer diameter 0.6 mm; inner diameter 0.4 mm) with stopper was implanted into the left lateral ventricle of mice (1.3 mm lateral to the midline, 0.3 mm posterior to the bregma, 2.0 mm in depth). During the experiment, body temperature was monitored and maintained at 37±0.5 °C. The operated mice were allowed to rest for 7 days to recover from the surgery and then received intracerebroventricular injection.

2.5. Step down test

Mouse was placed on the platform. When it stepped down the floor and received a 36 V alternating current footshock, the mouse quickly jumped onto the platform to avoid the electric stimulation, which was counted as one error. The error number and electric shock time the mouse underwent during the experimental period of 10 min was recorded as learning score. 24 h later, the mouse was placed on the platform again without electricity on grid. Time the mouse took to put its two forefeet on the grid and time the mouse stayed on the platform in 5 min were recorded as step down latency and remaining time, respectively. The longer the step down latency and remaining time were, the better the memory ability of mouse was.

2.6. Histology

After being anesthetized with 4% (wt./vol) chloral hydrate solution (10 ml/kg, ip), mice were perfused transcardially with 100 ml of 0.9% (wt./vol) saline containing heparin (250 U) followed by 100 ml solution containing 3.5% (wt./vol) formaldehyde and 0.9% (wt./vol) saline in phosphate buffer (0.1 mol/L, pH 7.2). The brains were removed and kept in the same fixing solution for 5 days. Coronal sections of 4 μm in thickness from brain tissue were done with HE staining.

2.7. Immunohistochemistry study

According to the procedures of Maines's report (Maines et al., 1996), the immunohistochemistry of HO-1 was performed, except for antibody. Briefly, mice were anesthetized with 4% (wt./vol) chloral hydrate solution (10 ml/kg, ip) and perfused through the heart with 0.9% (wt./vol) saline, followed by 0.1 mol/L phosphate buffer containing 4% (wt./vol) paraformaldehyde and 1.5% (wt./vol) sucrose. Brains were fixed for 16 h (at 4 °C) prior to sequential equilibration in 10%, 20%, and 30% graded sucrose solutions (wt./vol, in 0.1 mol/L phosphate buffer pH 7.2). Brains were subsequently frozen, and 40 μm thick coronal sections were obtained using a sliding microtome and then cryopreserved in 0.1 mol/L phosphate buffer

Table 1
Effects of nimodipine on step down test

Group	Error number	Electric shock time (s)	Step down latency (s)	Remaining time (s)
Aluminum	7.1±0.82[b]	51.0±7.85[b]	33.5±12.18[b]	110.5±16.04[b]
Aluminum+Nimodipine	4.6±0.76[ac]	26.4±6.14[c]	97.7±29.68[ac]	150.5±26.80[a]
Control	2.2±0.44	12.7±2.12	189.2±24.59	252.2±18.54

Values represent the mean±S.E.M. of 10 animals in each group.
[a]$P<0.05$ and [b]$P<0.01$ vs Control group; [c]$P<0.05$ vs Aluminum group.

containing 30% (wt./vol) sucrose and 30% (wt./vol) ethylene glycol at −20 °C until use. HO-1 immunohistochemical staining of brain sections was carried out with peroxidase anti-peroxidase as previously described (Ewing and Maines, 1992). Immunohistochemical staining was obtained using 1/200 dilution of primary antibody in 0.1 mol/L phosphate buffer (pH7.2) containing 0.1% (vol/vol) Triton X-100 and 10% (vol/vol) normal goat serum for 24 h at 4 °C.

2.8. Measurement of HO activity

Hippocampi were dissected on the ice and stored in liquid nitrogen. The samples were homogenized with ultra-sonic homogenizer for 30 s in 3 volume of 0.01 mol/L Tris–HCl containing 0.01 mol/L sucrose and 0.0001 mol/L EDTANa_2 (4 °C, pH 7.4), then centrifuged at 4 °C 18,800 ×g for 15 min. 0.2 ml supernatant was added to the reaction mixture (2 mmol/L Glucose-6-Phosphate, 0.2 unit Glucose-6-Phosphate Dehydrogenase, 20 μmol/L Hemin, 2 mg rat liver cytoplasm, and 0.8 mmol/L NADPH) and the total volume was 1.0 ml. The mixture was aerobically incubated for 30 min at 37 °C in the dark and reaction was stopped by adding ice-cold chloroform 1.0 ml. The production of bilirubin was measured with a double-beam spectrophotometer at ΔOD 530 nm (extinction coefficient: 40 mM^{-1} cm^{-1} for bilirubin).

2.9. Determination of HO protein expression by western blotting

The hippocampus was lysed in a buffer solution containing 10 mM Tris–HCL (pH 7.4), 0.15 M NaCl, 1% (wt./vol) NP-40, 0.1% (wt./vol) SDS, 0.001 mg/ml Leupeptin, 0.001 mg/ml Pepstatin, 0.001 mg/ml Aprotonin, and 10% (wt./vol) PMSF, then centrifuged at 4 °C 20,000 ×g for 15 min. Protein concentration was estimated by Bradford method. 40 μg protein was subjected to 12% (wt./vol) SDS-PAGE and transferred to a nitrocellulose membrane. The membrane was blocked for 1 h in the PBS containing 5% fat-free (wt./vol) milk and 0.2% (vol/vol) Tween 20. The blot was incubated for 2 h at 37 °C with either antibody of HO-1, HO-2 or β-actin at the concentration of 1:400, followed by incubation for 1 h at 37 °C with secondary antibody. Immunoreactive bands of HO-1, HO-2 and β-actin were visualized with chemiluminescence reagent. The chemiluminescent signal of the band was detected using a luminogen image analyzer.

2.10. Inductively coupled plasma-atomic emission spectrometry (ICP-AES) analysis of iron and aluminum levels in hippocampus

After hippocampus was digested in tube with 1.0 ml concentrated nitric acid at room temperature for 4 h it was placed in a pre-heated block at 65 °C for 4 h. Then, the tube was stirred and diluted with 5 ml of deionized H_2O and centrifuged at 1500 ×g for 5 min. At last, the supernatant was transferred to a tube and the levels of iron and aluminum were determined at the emission wavelength 238.20 nm and 309.27 nm, respectively.

2.11. Experiment protocol

Mice were divided into three groups, *i.e.* sham control group, aluminum overloading group and aluminum overloading plus nimodipine group with 35 mice in each. 2 μl 0.25% (wt./vol) aluminum chloride solution was intracerebroventricularly (icv) injected once a day for 5 consecutive days, and then either 0.4% (wt./vol) nimodipine solution 0.02 ml/g (80 mg/kg) or 0.9% (wt./vol) saline 0.02 ml/g was given by intragastric gavage (ig) twice a day for 35 days to set up aluminum overloading group and aluminum overloading plus nimodipine group, respectively. Sham control group was established using similar procedure to that of aluminum overloading group except that 2 μl 0.25% (wt./vol) aluminum chloride solution was replaced by 2 μl artificial cerebrospinal fluid (aCSF). All protocols were approved by the ethics committee of Chong Qing medical university.

2.12. Data analysis

Data were represented as mean±S.E.M. The *t*-test and one way analysis of variance (ANOVA) was employed for

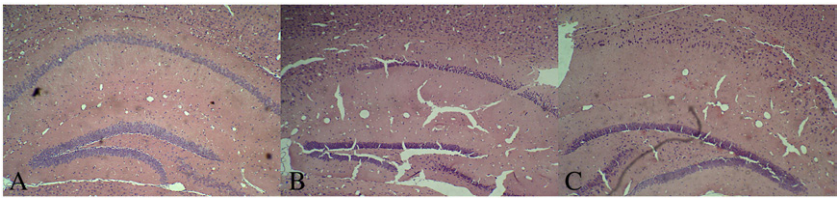

Fig. 1. Effects of nimodipine on pathomorphology in hippocampus (×100). A) Control group, B) Aluminum+Nimodipine group, C) Aluminum group. Aluminum caused chromatin concentrated, karyopycnosis and change of cellular morphology in hippocampus. Nimodipine alleviated the tissue injury significantly.

Table 2
Effects of nimodipine on the immunohistochemistry of hippocampal HO-1

Group	Mean optical density
Aluminum	0.290±0.007[a]
Aluminum+Nimodipine	0.208±0.008[ab]
Control	0.130±0.011

Values represent the mean±S.E.M. of 5 animals in each group.
[a]$P<0.01$ vs Control group; [b]$P<0.01$ vs Aluminum group.

Table 3
Effects of nimodipine on hippocampal HO activity

Group	HO activity (pmol bilirubin mg^{-1} protein h^{-1})
Aluminum	3422±137.50[a]
Aluminum+Nimodipine	2919±202.68[ab]
Control	2160±238.84

Values represent the mean±S.E.M. of 5 animals in each group.
[a]$P<0.01$ vs Control group; [b]$P<0.05$ vs Aluminum group.

comparison between two groups. $P<0.05$ was considered statistically significant.

3. Results

3.1. Effects of nimodipine on the learning and memory function

The step down test showed that the learning and memory ability of aluminum overloading mice was impaired significantly, compared with control group ($P<0.01$). Nimodipine (80 mg/kg ig) obviously decreased the damage to learning and memory function of mice caused by over loading aluminum ($P<0.05$, Table 1).

3.2. Effects of nimodipine on the pathomorphology in hippocampus

It was shown that the layer of CA1 pyramidal cells in aluminum overloading mice became thinner with pyknotic nuclei, compared with sham control, and administration of nimodipine dramatically relieved the phathomorphological changes induced by aluminum (Fig. 1).

3.3. Effects of nimodipine on the immunohistochemistry of hippocampal HO-1

Immunohistochemistry test showed that the positively staining of hippocampal HO-1 was much stronger in aluminum overloading mice than that of sham control mice ($P<0.01$), and nimodipine significantly blunted the positively staining of HO-1 ($P<0.01$). Results indicated that administration of nimodipine at least partly turned over excessive expression of HO-1 caused by aluminum overloading (Table 2, Fig. 2).

3.4. Effects of nimodipine on hippocampal HO activity

Table 3 showed that the HO activity of hippocampus was higher in aluminum overloading mice than that of sham control group ($P<0.01$), and nimodipine significantly suppressed the increasing HO activity caused by aluminum overloading ($P<0.05$).

3.5. Effects of nimodipine on hippocampal HO protein expression

HO-1 protein expression of hippocampus was higher in aluminum overloading mice than that of sham control group ($P<0.01$) and nimodipine clearly inhibited the increasing HO-1 protein expression induced by aluminum overloading ($P<0.01$). On the contrary, there was no difference of HO-2 protein expressions among all three groups ($P>0.05$, Table 4, Fig. 3).

3.6. Effects of nimodipine on aluminum and iron levels in hippocampus

The results showed that the levels of aluminum and iron were significantly increased in aluminum overloading mice, compared with sham control group ($P<0.01$). Administration of nimodipine had no effect on aluminum level ($P>0.05$), but distinctly decreased the elevation of iron level of hippocampus from aluminum overloading mice ($P<0.05$, Table 5).

4. Discussion

Aluminum is the third most abundant element in the earth crust and unavoidable for us to contact it. It is widely accepted that aluminum is a neurotoxin and could not only cause cognitive deficiency and dementia after it enters the body and accumulates in the brain, but also induce the aggregation of the tau protein and amyloid beta protein, as well as the impairment of synapse formation among the neurons (Banks et al., 2006; Kawahara, 2005). However, the neuro-toxicologic mechanism of aluminum is still quite unclear and the prevention of the neurotoxicity from aluminum overloading needs to be illustrated.

As well known that hippocampus is very important for high cognitive function. It was reported that there existed the highest

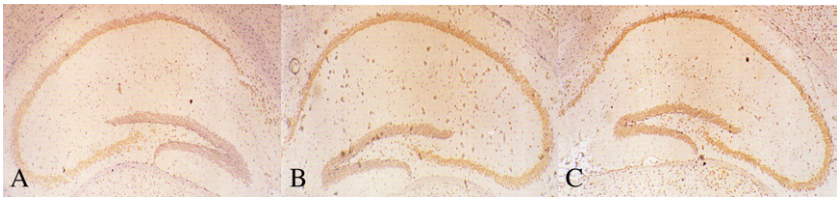

Fig. 2. Effects of nimodipine on the immunohistochemistry of hippocampal HO-1 (×40). A) Control group, B) Aluminum+Nimodipine group, C) Aluminum group. Aluminum induced the expression of HO-1 significantly and nimodipine down-regulated the expression of HO-1 induced by aluminum.

Table 4
Effects of nimodipine on hippocampal HO protein expression (ratio of HO integral optical density/β-actin integral optical density)

Group	HO-1	HO-2
Aluminum	0.23±0.009[b]	0.31±0.011
Aluminum+Nimodipine	0.14±0.007[ac]	0.32±0.016
Control	0.11±0.010	0.31±0.013

Values represent the mean±S.E.M. of 5 animals in each group.
[a]$P<0.05$ and [b]$P<0.01$ vs Control group; [c]$P<0.01$ vs Aluminum group.

Table 5
Effects of nimodipine on aluminum and iron levels in hippocampus

Group	Aluminum (μg/g tissue)	Iron (μg/g tissue)
Aluminum	2.08±0.10[b]	54.01±2.31[b]
Aluminum+Nimodipine	2.05±0.13[b]	36.09±2.85[ac]
Control	0.35±0.04	22.54±4.48

Values represent the mean±S.E.M. of 5 animals in each group.
[a]$P<0.05$ and [b]$P<0.01$ vs Control group; [c]$P<0.05$ vs Aluminum group.

concentration of aluminum among all parts of brain (Fattoretti et al., 2004). Therefore, the hippocampus is a crucial target to investigate the neurodegeneration associated with learning and memory (Savage et al., 2004). This was why we used the pathological changes of the hippocampus in combination of the results from behavior and biochemical tests as standards to evaluate the neurodegenerative development caused by aluminum overloading and investigate the improvement effects of drugs on these changes.

In present study, our results showed that there existed obviously concentrated chromatin, and karyopycnosis of hippocampal neurons and damage to the cognitive function in the aluminum overloading mice, compared with sham control group (Table 1,Fig. 1). In addition, we found that the iron level and HO-1 expression of the hippocampus increased significantly in aluminum overloading mice by ICP-AES, western blot and immunohistochemistry test (Tables 2, 4, 5).

HO-1 has been proved to be protective in oxidative stress response induced by acute ischemia and hypoxia (Takeda et al., 1994; Chen-Roetling and Regan, 2006; Li et al., 2007) and nimodipine can protect neuron from the injury induced by cerebral ischemia. However there has been no report about the effect and mechanism of nimodipine and HO-1 on neurodegeneration induced by aluminum overloading as yet.

HO-1 catalyzes heme to produce carbon monoxide, biliverdin, and ferrous iron. Biliverdin is believed to be the most powerfully endogenous antioxidant in the brain, which can scavenge peroxidatic radicals efficiently and protect cells from the damage caused by a 10,000-fold excess of hydrogen peroxide (Baranano et al., 2002). Ferrous iron can stimulate the synthesis of ferritin (Eisenstein et al., 1991) through binding its regulatory protein and activating iron response elements (Hanson et al., 1999; Pantopoulos et al., 1997). It has been estimated that each apo-ferritin molecule (450 kD) can sequester about 4500 iron atoms (Harrison and Arosio, 1996). In this way, ferritin possesses additional antioxidant capabilities (Balla et al., 1992, 1995).

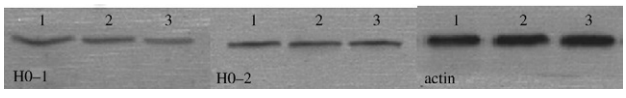

Fig. 3. Effects of nimodipine on hippocampal HO protein expression by western blotting. Lane 1 Aluminum group, Lane 2 Aluminum+Nimodipine group, Lane 3 Control group. Aluminum significantly enhanced the expression of HO-1 and nimodipine could partly suppress the up-regulation of HO-1 induced by aluminum. All pretreatments had no effect on HO-2.

However the function of HO-1 becomes paradoxical when there is abundance of aluminum. It was reported that aluminum blocked the synthesis of ferritin by stabilizing the iron-regulatory protein 2 (IRP-2) (Yamanaka et al., 1999). As a result, aluminum could influence the iron-regulatory pathway and up-regulate the level of free iron (Ward et al., 2001). Our research demonstrated that aluminum could increase the level of free iron and up-regulate the expression of HO-1. Although biliverin can eliminate reactive oxygen species effectively, the iron is not scavenged and it accumulates because of the up-regulation of HO-1 and the inhibition of aluminum on the synthesis of iron-binding protein. The elevation of iron will accelerate the generation of reactive oxygen species through Fenton reaction and the later causes damage to lipids, proteins, and nucleotides and leads to neuronal death (Radi et al., 1991; Salgo et al., 1995). As a result the balance between oxidative stress and antioxidant was disrupted.

Our data showed that administration of nimodipine could significantly relieve the decreasing learning and memory ability of mice and brain injury caused by aluminum overloading. The possible mechanism might be partly associated with suppressing the over expression of HO-1 induced by aluminum overloading and reducing the production of iron without influence on the content of aluminum which increased free iron level through blocking the synthesis of iron-binding proteins besides up-regulating HO-1 expression. Maybe it was why nimodipine could not make the iron recover to the normal level completely.

In conclusion, nimodipine is beneficial for keeping the homeostasis of iron by suppressing the expression of HO-1during the courses of neurodegenerative development induced by aluminum overloading in mice. It may be a promising candidate for the treatment of neurodegenerative diseases caused by the abnormalities of metal elements.

Acknowledgement

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References

Abraham, N.G., Lin, J.H., Schwartzman, M.L., Levere, R.D., Shibahara, S., 1998. The physiological significance of heme oxygenase. Int. J. Biochem. 20, 543–558.

Balla, G., Jacob, H.S., Balla, J., Rosenberg, M., Nath, K., Apple, F., Eaton, J.W., Vercellotti, G.M., 1992. Ferritin: a cytoprotective antioxidant stratagem of endothelium. J. Biol. Chem. 267, 18148–18153.

Balla, J., Nath, K.A., Balla, G., Juckett, M.B., Jacob, H.S., Vercellotti, G.M., 1995. Endothelial cell heme oxygenase and ferritin induction in rat lung by hemoglobin in vivo. Am. J. Physiol. 268, L321–L327.

Banks, W.A., Niehoff, M.L., Draqo, D., Zatta, P., 2006. Aluminum complexing enhances amyloid beta protein penetration of blood–brain barrier. Brain Res. 1116, 215–221.

Baranano, D.E., Rao, M., Ferris, C.D., Snyder, S.H., 2002. Biliverdin reductase: a major physiologic cytoprotectant. Proc. Natl. Acad. Sci. U. S. A. 99, 16093–16098.

Borie, C., Gasparini, F., Verpillat, P., Bonnet, A.M., Agid, Y., Hetet, G., Brice, A., Durr, A., 2002. Association study between iron-related genes polymorphisms and Parkinson's disease. J. Neurol. 249, 801–814.

Chen-Roetling, J., Regan, R.F., 2006. Effect of heme oxygenase-1 on the vulnerability of astrocytes and neurons to hemoglobin. Biochem. Biophys. Res. Commun. 10, 233–237.

Devo, R.A., Straube, K.T., Disterhoft, J.F., 1989. Nimodipine facilitates associative learning in aging rabbits. Science 243, 809–811.

Eisenstein, R.S., Garcia, M.D., Pettingell, W., Munro, H.N., 1991. Regulation of ferritin and heme oxygenase synthesis in rat fibroblasts by different forms of iron. Proc. Natl. Acad. Sci. U. S. A. 88, 688–692.

Ewing, J.F., Maines, M.D., 1992. In situ hybridization and immunohistochemical location of heme oxygenase-2 mRNA and protein in normal rat brain: differential distribution of isozyme 1 and 2. Mol. Cell. Neurosci. 3, 559–570.

Fattoretti, P., Bertoni, F.C., Balietti, M., Giorgetti, B., Solazzi, M., Zatta, P., 2004. Chronic aluminum administration to old rats results in increased levels of brain metal ions and enlarged hippocampal mossy fibers. Ann. N. Y. Acad. Sci. 1019, 44–47.

Feigin, A., Zgaljardic, D., 2002. Recent advances in Huntington's disease: implications for experimental therapeutics. Curr. Opin. Neurol. 15, 483–489.

Hanson, E.S., Foot, L.M., Leibold, E.A., 1999. Hypoxia posttranslationally activates iron-regulatory protein 2. J. Biol. Chem. 274, 5047–5052.

Harrison, P.M., Arosio, P., 1996. The ferritins: molecular properties, iron storage function and cellular regulation. Biochim. Biophys. Acta 1275, 161–203.

He, B.C., Teng, Y.Z., Yang, J.Q., Jiang, Q.S., Zhou, Q.X., 2006. Correlation between aluminum overload-induced brain damage and abnormal iron metabolism in brain of mice. Chin. J. Pharmacol. Toxicol. 20, 66–70.

Jonas, J.C., Guiot, Y., Rahier, J., Henquin, J.C., 2003. Haeme-oxygenase 1 expression in rat pancreatic beta cells is stimulated by supraphysiological glucose concentrations and by cyclic AMP. Diabetologia 46, 1234–1244.

Kawahara, M., 2005. Effects of aluminum on the nervous system and its possible link with neurodegenerative diseases. J. Alzheimer's Dis. 8, 171–182.

Ke, Y., Qian, Z.M., 2007. Brain iron metabolism: neurobiology and neurochemistry. Prog. Neurobiol. 83, 149–173.

Li, Q., Li, J., Zhang, L., Wang, B., Xiong, L., 2007. Preconditioning with hyperbaric oxygen induces tolerance against oxidative injury via increased expression of heme oxygenase-1 in primary cultured spinal cord neurons. Life Sci. 80, 1087–1093.

Ma, B., Zhang, J., 2006. Nimodipine treatment to assess a modified mouse model of intracerebral hemorrhage. Brain Res. 1078, 182–188.

Maines, M.D., 1988. Heme oxygenase: function, multiplicity, regulatory mechanisms and clinical applications. FASEB J. 2, 2557–2568.

Maines, M.D., Kappas, A., 1974. Cobalt induction of hepatic heme oxygenase: with evidence that Cyt P450 is not essential for this enzyme activity. Proc. Natl. Acad. Sci. U. S. A. 71, 4293–4297.

Maines, M.D., Trakshel, G.M., Kutty, R.K., 1986. Characterization of two constitutive forms of rat liver microsomal heme oxygenase: only one molecular species of the enzyme is inducible. J. Biol. Chem. 261, 411–419.

Maines, M.D., Eke, B.C., Zhao, X.D., 1996. Corticosterone promotes increased HO-2 protein and transcript expression in the newborn rat brain. Brain Res. 722, 83–94.

McCoubrey, W.K., Huang, T.J., Maines, M.D., 1997. Isolation and characterization of a cDNA from the rat brain that encodes hemoprotein heme oxygenase-3. Eur. J. Biochem. 247, 725–732.

Pantopoulos, K., Mueller, S., Atzberger, A., Ansorge, W., Stremmeli, W., Hentze, M.W., 1997. Differences in the regulation of iron regulatory protein-1 (IRP-1) by extra- and intracellular oxidative stress. J. Biol. Chem. 272, 9802–9808.

Pratico, D., Uryu, K., Sung, S., Tang, S., Trojanowski, J.Q., Lee, V.M., 2002. Aluminum modulates brain amyloidosis through oxidative stress in APP transgenic mice. FASEB J. 16, 1138–1140.

Radi, R., Beckman, J.S., Bush, K.M., Freeman, B.A., 1991. Peroxynitrite induced membrane lipid peroxidetion: the cytotoxic potential of superoxide and nitric oxide. Arch. Biochem. Biophys. 288, 481–487.

Salgo, M.G., Bermudez, E., Squadrito, G.L., Pryor, W.A., 1995. Peroxynitrite causes DNA damage and oxidation of thiols in rat thymocytes. Arch. Biochem. Biophys. 322, 500–505.

Savage, L.M., Buzzetti, R.A., Ramirez, D.R., 2004. The effects of hippocampal lesions on learning, memory, and reward expectancies. Neurobiol. Learn. Mem. 82, 109–119.

Scapagnini, G., D'Agata, V., Calabrese, V., Pascale, A., Colombrita, C., Alkon, D., Cavallaro, S., 2002. Gene expression profiles of heme oxygenase isoforms in the rat brain. Brain Res. 954, 51–59.

Takeda, A., Onodera, H., Sugimoto, A., Itoyama, Y., Kogure, K., Shibahara, S., 1994. Increased expression of heme oxygenase mRNA in rat brain following transient forebrain ischemia. Brain Res. 666, 120–124.

Toyoda, M., Satio, H., Matsuki, N., 1996. Nitric oxide but not carbon monoxide is involved in spatial learning of mice. Jpn. J. Pharmacol. 71, 205–211.

Trakshel, G.M., Kutty, R.K., Maines, M.D., 1986. Purification and characterization of the major constitutive form of testicular heme oxygenase: the non-inducible isoform. J. Biol. Chem. 261, 11131–11137.

Veng, L.M., Mesches, M.H., Browning, M.D., 2003. Aged-related working memory impairment is correlated with increases in the L-type calcium channel protein alpha1D (Cav1.3) in area CA1 of the hippocampus and both are ameliorated by chronic nimodipine treatment. Brain Res. Mol. Brain Res. 110, 193–202.

Wang, W., Wang, L.N., Zhang, X.H., Ma, L., Li, D.J., 2006. A nimodipine interventional study of patients with mild cognitive impairment. Zhonghua Neike Zazhi 45, 274–276.

Ward, R.J., Zhang, Y., Crichton, R.R., 2001. Aluminum toxicity and iron homeostasis. J. Inorg. Biochem. 87, 9–14.

Wu, L., 2005. The pro-oxidant role of methylglyoxal in mesenteric artery smooth muscle cells. Can. J. Physiol. Pharmacol. 83, 63–68.

Yamanaka, K., Minato, N., Iwai, K., 1999. Stabilization of iron regulatory protein 2, IRP2, by aluminum. FEBS Lett. 462, 216–220.

Yoshida, T., Takahashi, S., Kikuchi, G., 1974. Partial purification and reconstruction of the heme oxygenase system from pig spleen microsomes. J. Biochem. (Tokyo) 75, 1187–1191.