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Protective effect of nimodipine against cerebral injury induced by subacute carbon monoxide intoxication in mice[1]

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KEY WORDS learning disorders; avoidance learning; carbon monoxide poisoning; monoamine oxidase; nimodipine

ABSTRACT

AIM: To study the effects of nimodipine on delayed cerebral injury in mice from subacute carbon monoxide (CO) exposure. **METHODS**: Mice were exposed to CO (100 mL/kg, ip) once a day, continuously for 7 d. After 7-d CO-exposure, mortality in mice, changes in learning ability and memory using passive avoidance test, the pathomorphologic observation of brain tissue slices, and changes of monoamine oxide (MAO)-B activities in cerebral tissue were studied. Nimodipine was administered 30 min before CO-exposure every time. **RESULTS**: The preadministration of nimodipine decreased the mortality in mice, almost reversed the impairment of learning and memory function, prevented the hippocampal neurons agaist delayed death and blunted the rise of MAO-B activity after subacute CO poisoning of mice. **CONCLUSION**: Pretreatment with nimodipine markedly prevented mice from delayed encephalopathy after CO poisoning.

INTRODUCTION

Fechter *et al*[1] established a cochlea impairment animal model induced by carbon monoxide (CO) ip in rats. In this study, carboxyhemoglobin (HbCO) rapidly reached peak levels (> 40 %) 30 min after CO ip (3.5 mL/kg) and cochlea impairment was obvious. Based on the model, Liu *et al*[2] investigated the protective effect of MK-801 against hearing loss induced by CO. Compared with CO-inhale poisoning model, this model, in which CO was injected ip, is simple, safe, and accurate. Recently, we have developed a delayed cerebral injury model by exposing mice to CO (100 mL/kg, ip)[3]. Exposure to CO produces relatively rapid impairment of learning and delayed amnesia. Histological studies showed that there was a marked neuronal death in the CA1 and/or CA2 and/or CA3 layers in hippocampus after CO-exposure. Nimodipine is a 1, 4-dihydropyridine calcium channel blocker. In previous studies, it was found that nimodipine had a protective effect against cerebral ischemic injury[4] and could reduce the damage in learning and memory induced by cerebral ischemia reperfusion or drugs, such as anisodine and sodium nitrite[5,6]. However, whether nimodipine has a protective effect on cerebral damage induced by CO is still unknown. We designed the present study to clarify the effects of nimodipine on cerebral injury in subacute CO poisoning mice.

MATERIALS AND METHODS

Animals Male mice of the I NIH strain (supplied by Chongqing University of Medical Sciences Laboratory Center, Grade Ⅱ, Certificate No 24301042) weighing 18 g - 22 g, aged 7 weeks, were used. They were kept in a regulated environment (23 ℃ ± 1 ℃, 50 % ± 2 % humidity), with 12 h light /dark cycle (light on 8:00 am-8:00 pm).

Protocol Mice were divided into 3 groups: (a) In air control group, mice were administrated ip air 100 mL/kg, once a day, continuously for 7 d; (b) In CO-exposure group, mice were injected ip CO 100 mL/kg, once a day, continuously for 7 d; (c) In nimodipine treated group, nimodipine 1 mg/kg was injected ip 30 min before CO exposure.

Mortality rate measure The mortality rate was recorded during the period of 7 d-CO-exposure and for another 7 d without CO exposure.

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Evaluation of learning ability and memory function After 7-d CO-exposure the step down type passive avoidance task was utilized as previously described[3]. Briefly, mice were trained to learn avoiding electric stimulus (ES) (36 V). Each mouse in test was placed on the grid floor with back against the platform; the intermittent ES was delivered to grid floor. If the mouse could immediately jump on platform to avoid the ES, it was considered that the mouse had learnt avoiding the ES. The number of trainings for mice to learn avoiding the ES was recorded as a standard to evaluate the ability of the study. The retention test was carried out 24 h after training. When each mouse was placed on the platform, intermittent ES was not applied to the grid floor. The step down latency (SDL) was measured. An upper cut-off time of 300 s was set.

Histology On the seventh day after CO-exposure, the method of Nabeshima *et al*[8] was followed. After anesthetizing with a solution of sodium pentobarbital (50 mg/kg, ip), mice were perfused transcardially with 100 mL of 0.9 % saline containing heparin (250 U) followed by 100 mL of a solution containing 3.5 % formaldehyde and 0.9 % saline in phosphate buffer 0.1 mol/L (pH 7.2). The brain were removed and kept in the same fixative solution for 2 – 7 d. Coronal sections 4 μm in thickness were selected from brain tissue. The sections were stained by hematoxylin and eosin stain (HE).

MAO-B activity On the seventh day after CO-exposure, the activities of MAO-B in cerebral tissue were tested by the method described by Chen *et al*[7]. The brain tissue was homogenized with 10 volumes (w: v = 1 g:10 mL) of ice-cold sucrose 0.32 mol/L buffered with sodium phosphate 10 mmol/L (pH 7.4). The homogenates were centrifuged at 600 × *g* for 10 min. The supernatant was again centrifuged at 15 000 × *g* for 10 min. The precipitate was suspended in the buffer solution as described above. Then the solution was again centrifuged at 15 000 × *g* for 10 min. The precipitate was diluted with 10 mmol/L phosphate buffer (pH 7.4) and incubated with benzylamine, the specific substrate for MAO-B, at 37 ℃ for 20 min in a total volume of 0.8 mL, and the reaction was terminated by adding 0.2 mL of HCl 1 mol/L. The metabolite, benzoldehyde, was extracted by vigorously shaking with 3 mL of cyclohexane for 1 min. The solution was stored at 4 ℃ for 24 h. The upper phase was measured at 242 nm, $\varepsilon = 14\,000\ L \cdot mol^{-1} \cdot cm^{-1}$.

Statistical analysis The data for the mortality rate of mice is expressed as percentage. Difference between the groups was evaluated using Chi square (χ^2) test; other data were expressed as $\bar{x} \pm s$, difference between groups was evaluated using *t*-test.

RESULTS

Mortality rate in mice No mice died in the air group during the 14-d-exposure. However, a high mortality rate (65.6 %) and a high total mortality rate (84.4 %) were observed in CO group during the first 7 d and 14 d, respectively. The administration of nimodipine 30 min before the CO-exposure reduced both the high mortality rate (25.0 %) and the high total mortality rate (43.8 %, Tab 1).

Effect of nimodipine on learning ability and memory disorder in subacute CO intoxicated mice Number of trainings to learn avoiding the ES was lower for nimodipine-treated mice than for CO-poisoning mice. The difference was not significant, compared with that of air control group (Tab 2). Similarly, the step down latency (SDL) of nimodipine-treated mice was obviously longer than that of CO-treated mice on the first 4 d after the last CO-exposure. The results showed that nimodipine pre-treatment 30 min before CO-exposure both relieved the impairment of learning ability and ameliorated the amnesia in mice induced by subacute CO intoxication (Tab 3).

Tab 1. Effect of nimodipine on mortality rate induced by CO in mice. $^cP < 0.01$ *vs* air group. $^eP < 0.05$ *vs* CO group.

	Mortality rate		
	1st – 7th day	8th – 15th day	1st – 15th day
Air	0 % (0/15)	0 % (0/15)	0 % (0/15)
CO	65.6 % (21/32)^c	18.9 % (6/32)	84.4 % (27/32)^c
CO + nimodipine	25.0 % (8/32)^e	18.8 % (6/32)	43.8 % (14/32)^e

Tab 2. Effect of nimodipine on learning function disability induced by CO poisoning. $\bar{x} \pm s$. $^cP < 0.01$ *vs* **air group.** $^fP < 0.01$ *vs* **CO group.**

	Number of training	*n*
Air	3.3 ± 1.3	13
CO	7.7 ± 1.4^c	9
CO + *nimodipine*	3.3 ± 1.9^f	9

Effect of nimodipine on death of neurons in the hippocampus On the seventh day after the last CO exposure, the deaths of neuronal pyramidal cells in hippocampal CA1, CA2, and CA3 subfields were marked in CO poisoning mice, and the layer of pyramidal cells in CA1, CA2, and CA3 subfields became thinner than that in the air-treated. However, the neuronal cell death in hippocampus was obviously decreased in nimodipine pretreated mice (Fig 1).

Effect of nimodipine on MAO-B activity in CO poisoning mice MAO-B activity obviously increased in subacute CO poisoning mice (8.5 ± 1.2) μmol · g^{-1} protein, compared with that of air-treated mice (3.8 ± 0.8) μmol · g^{-1} protein. Nimodipine pre-administration blunted the increase in MAO-B activity induced by subacute CO poisoning (Tab 4).

DISCUSSION

Carbon monoxide (CO) poisoning is still a frequent and serious casualty in the world. Until recently the hyperbaric oxygen (HBO) was one of the most important therapeutical means for management of CO poisoning. Generally, HBO is the first choice of treatment for CO poisoning to decrease mortality rate and to avoid occurrence of serious disorders and delayed sequel. However, the clinical application of HBO therapy is limited because of its expense, specific equipment including a multiplace hyperbaric chamber, and specific educational program and training for personnel employed in the clinical hyperbaric center. Furthermore, if the operation is not correct, serious side reactions of HBO such as oxygen poisoning, *etc* may occur.

Drugs, which can be used to effectively treat CO poisoning patients, are thus urgently required. Nimodipine is a good candidate among those drugs which have protective effects on brain damage because of its characteristics of neurotropism and safety. In this paper we tried to explore the effects of nimodipine on cerebral injury from subacute CO intoxication in mice.

Our experimental results showed that there was a high mortality rate and a high total mortality rate in subacute CO intoxicated mice. All survivors from CO poisoning, displayed a marked disturbance of learning ability and memory function, as well as marked neuronal cell death and loss in hippocampus. These pathological damages were asymmetrical in the hemispheres and could exist in either side of two hemispheres, and might take place in any area of involved hippocampus. Similar results have been reported by Nabeshima *et al*[8]. These results indicated that hippocampal neuronal death could be also induced by CO-exposure in human. However, the death of neuronal cells observed in white matter of human non-survivors from CO-poisoning was not noted in mice. The mechanism of this phenomenon is yet unknown, and, probably is due to the difference in species. However, regarding the impairment of learning and memory function, this model may be appreciated. It has been reported that monoaminenergic neurons and their transmitters are involved in behavioral learning and memory process in the hippocampus[9]. After CO-exposure, the MAO-B activity of brain tissue was observed to be greatly increased, compared with that in control mice. The changes in the MAO-B activity indicated that there existed damage of dopaminergic neurons induced by subacute CO poisoning, as MAO-B is responsible for metabolizing dopamine.

Tab 3. Effect of nimodipine on amnesia induced by subacute CO poisoning. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* **air group.** $^eP < 0.05$ *vs* **CO group.**

		Step down latency/s						
	n	d 1	d 2	d 3	d 4	d 5	d 6	d 7
Air	13	295 ± 17	295 ± 17	269 ± 71	156 ± 123	132 ± 112	140 ± 127	108 ± 124
CO	9	196 ± 132^b	123 ± 134^c	99 ± 135^c	77 ± 126	72 ± 129	74 ± 128	62 ± 181
CO + nimodipine	9	293 ± 21^e	244 ± 42^e	285 ± 97^e	229 ± 97^e	127 ± 160	130 ± 121	125 ± 137

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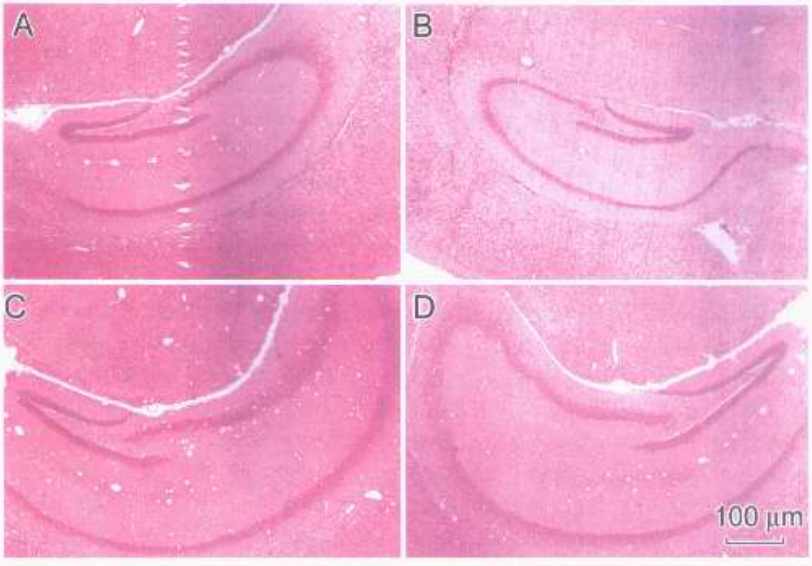

Fig 1. Effect of nimodipine on histological changes in the hippocampus after CO poisoning. (A) Air-treated group. (B) Delayed death of CA_1, CA_2, and CA_3 pyramidal cells in hippocampus 7 d after CO-exposure. No marked death of hippocampal neuronal cells were observed in nimodipine-treated group; (C) left hemisphere, (D) right hemisphere. Micrographs of 4-μm coronal sections of the HE-stained hippocampus. ×130.

Tab 4. Effect of nimodipine on MAO-B activity in subacute CO poisoning in mice. $\bar{x} \pm s$. $^cP < 0.01$ *vs* air group. $^aP < 0.05$ *vs* CO group.

	MAO-B activity/μmol·g^{-1} protein	*n*
Air	3.8 ± 0.8	9
CO	8.5 ± 1.2^c	7
CO + nimodipine	3.9 ± 1.5^a	8

Our experimental results also showed that nimodipine pre-administration markedly decreased the mortality rate and also the total mortality rate during CO-exposure. However, when nimodine was withdrawn the mortality rate did not decrease 7 d after CO-exposure. These results suggest that in order to keep a good therapeutic effect, nimodipine should be continuously administered for some days after CO poisoning. Nimodipine also improved the impairment of learning and memory function, prevented the delayed death in hippocampal neurons, and blunted the rise in MAO-B levels in subacute CO poisoned mice. The improvement in pathologic and biochemical parameters were consistent with the improvement observed in the learning and memory ability. These results indicate that nimodipine exerted distinctly protective effect on CO poisoning. It is well known that nimodipine can obviously increase the brain blood flow and reverse the intracellular calcium over loading, which may be attributing partly to its protective effect on CO poisoning.

In conclusion, nimodipine may be developed as an effective protective agent in management of CO poisoning.

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尼莫地平对亚急性一氧化碳中毒致小鼠脑损伤保护作用[1]

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关键词 学习障碍；回避学习；一氧化碳中毒；单胺氧化酶；尼莫地平

目的：观察尼莫地平对亚急性一氧化碳中毒(CO)致迟发性脑损伤保护作用. **方法**：小鼠腹腔注射 CO 100 mL/kg，每天1次，连续7天. 停止给予CO后，测定 CO 中毒小鼠死亡率，被动回避性学习记忆能力，进行脑组织病理学检查和测定单胺氧化酶-B 活性. 尼莫地平(1 mg/kg)在每次给予 CO 前 30 分钟腹腔注射. **结果**：尼莫地平预先给予能显著降低 CO 中毒小鼠死亡率，基本逆转小鼠 CO 中毒引起的学习记忆能力的损害；能防止海马神经元细胞延迟性死亡；并能阻遏 CO 中毒引起的单胺氧化酶-B 活性的升高. **结论**：尼莫地平对亚急性一氧化碳中毒致迟发性脑损伤有明显的保护作用

（责任编辑 吕 静）

A letter from author

Dear Editor:

The paper of our group, "Endogenous production of protoporphyrin IX induced by 5-aminolevulinic acid in leukemia cells" (Chen Ji-Yao, Mak Nai-Qi, Cheung Nai-Hao, Leung Rong-Neng, Peng Qian) was published in Acta Pharmacologica Sinica 2001; 22 (2): 163-168. Here the author's English names are from the pronunciation of the spelling of their Chinese names. However, some authors are from Hong Kong. They have their accustomed English name already. So, we make the correction here: Mak Nai-Qi is Mak NK and Leung Rong-Neng is Leung WN. Thanks.

JY Chen