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Protective effects of fructose-1，6-diphosphate against cerebral injury induced by subacute carbon monoxide intoxication in mice

YANG Jun-Qing，ZHOU Qi-Xin
(*Department of Biochemical Pharmacology，Chongqing University of Medical Sciences，Chongqing 400016，China*)

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

ABSTRACT

AIM：To study the effects of fructose-1，6-diphosphate (FDP) 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，continously for 7 d. After 7-d CO-exposure，mortality of mice，changes in learning ability and memory using passive avoidence test，the pathomorphologic observation of brain tissue slices，and changes in monoamine oxide (MAO)-B activities in cerebral tissue were studied. FDP was administrated 30 min before CO-exposure every time. **RESULTS**：the preadministration of FDP markedly decreased the mortality of mice，almost reversed the impairment of learning and memory function，prevented the cells from delayed death in hippocampal neurons and blunted the rise in MAO-B activity after subacute CO poisoning of mice. **CONCLUSION**：FDP pretreatment markedly prevented mice from delayed encephalophathy after CO poisoning.

INTRODUCTION

Fechter *et al*[1] established a cochlea impairment animal model induced by carbon monoxide (CO) ip in rats. In his research，carboxyhemoglobin (HbCO) rapidly reached peak levels (> 40 %) 30 min after CO ip (35 mL/kg) and cochlea impairment was obvious. Based on the model，Liu Y *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 produced relatively rapid impairment of learning and delayed amnesia. Histological studies showed that there was significant neuronal death in the CA1，CA2，or CA3 layer in hippocampus after CO-exposure.

Fructose-1，6-diphosphate (FDP) is an allosteric effector that modulates the activities of several enzymes taking part in glucose metabolism. In previous studies，FDP had a protective effect against cerebral ischemic injury[4]. However，whether FDP has protective effects against cerebral damage induced by CO is still unknown. We designed the present study to clarify the effects of FDP on cerebral injury in subacute CO poisoning in mice.

MATERIALS AND METHODS

Animals Male mice of the I NIH strain (supplied by Laboratory Center，Chongqing University of Medical Sciences，Certificate № ：24301042) with weight 18 – 22 g，aged 7 wk，were used. They were kept in a regulated enviroment (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，continously 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 FDP treated group，FDP 300 mg/kg was injected ip 30 min before CO-exposure.

Mortality measurement The mortality was recorded during the period of 7-d CO-administration and another 7 d without CO ip.

Evaluation of learning ability and memory function The step down type passive avoidence task was utilized as previously described[3]. Briefly，mice were trained to learn avoiding electric stimulus (ES) (36 V). Each mouse was placed on the grid floor with its

[1] Corresponding to Mr YANG Jun-Qing.
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back against the platform；the intermittent ES was delivered to grid floor. If the mouse could immediately jump on the platform to avoid the ES when delivered, we considered that the mouse had learnt avoiding the ES. The number of trainings required for mice to learn to avoid the ES was recorded as a standard to evaluate ability of 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[6] After being anesthetized with 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 a solution 100 mL containing 3.5 % formaldehyde and 0.9 % saline in phosphate buffer 0.1 mol/L (pH 7.2). The brains were removed and kept in the same fixative solution for 2 – 7 d. Coronal sections of 4 μm in thickness from brain tissue were selected. The sections were stained by HE.

MAO-B activities of brain tissue[5] The brain tissue was homogenized with 10 volumes (vol∶wt = 10∶1) 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 1500 × g for 10 min. The precipitate was suspended in buffer solution as described above. Then the solution was again centrifuged at 15000 × g for 10 min. The precipitate was diluted with phosphate buffer 10 mmol/L (pH 7.4) and incubated with benzylamine, the specific substrate for MAO-B, at 37 ℃ for 20 min in total volume of 0.8 mL, the reaction was terminated by adding 0.2 mL of HCl 1 mol/L. The metabolite, benzyaldehyde, 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, ε = 14000 L·mol^{-1}·cm^{-1}.

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

RESULTS

Mortality in mice No mice died in air group during the 14-d exposure. However, a high mortality (65.6 %) and a high total mortality (84.4 %) were observed in CO group during d 7 and d 14, respectively. The administration of FDP 30 min before CO-exposure reduced both the mortality (25.0 % and 50.0 %) at d 7 and d 14 respectively (Tab 1).

Tab 1. Effects of FDP on mortality induced by CO in mice. $^cP < 0.01$ *vs* air group, $^eP < 0.05$ *vs* CO group.

	Mortality		
	d 7	d 8 – d 15	Sum of 15 days
Air	0 % (0/15)	0 % (0/15)	0 % (0/15)
CO	65.6 % (21/32)^c	18.8 % (6/32)	84.4 % (27/32)^c
FDP	25.0 % (8/32)^e	25.0 % (8/32)	50.0 % (16/32)^e

Effects of FDP on subacute learning ability and memory disorder in CO intoxicated mice Number of trainings required to learn to avoid the ES of FDP-treated mice was lower than that of CO-poisoned mice. The difference was not significant, compared with air control group (Tab 2). Similarly, the step down latency (SDL) of CO + FDP mice was obviously longer than that of CO-treated mice on the first 6 days after last CO-exposure. The results showed that FDP pre-treatment 30 min before CO-exposure both relieved the impairment of learning ability and ameliorated the amnesia of mice induced by subacute CO intoxication (Tab 3).

Tab 2. Effects of FDP on deficiency in learning function 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 + FDP	4.1 ± 1.2^f	9

Effects of FDP on death of neuronal cells in hippocampus On d 7 after CO exposure, the deaths of neuronal pyramidal cells in hippocampal CA1, CA2, and CA3 subfields were significant in CO poisoning mice, and the layer of pyramidal cells in CA1, CA2, and CA3 subfields became thinner than that in non-CO-treated group. However, the neuronal cell death and loss in hippocampus were obviously decreased in FDP pre-treated group (Fig 1).

Effects of FDP on changes of MAO-B activities in CO poisoning mice Activities of MAO-B from subacute CO poisoning mice (8.5 ± 1.2) obviously

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Tab 3. Effects of FDP on amnesia induced by subacute CO poisoning. $\bar{x} \pm s$. $^{b}P < 0.05$, $^{c}P < 0.01$ *vs* **air group.** $^{f}P < 0.01$ *vs* **CO group.**

	n	Step down latency/s						
		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	7	196 ± 132^{b}	123 ± 134^{c}	99 ± 135^{c}	77 ± 126	72 ± 129	74 ± 128	62 ± 181
CO + FDP	8	300 ± 0^{f}	296 ± 26^{f}	299 ± 98^{f}	300 ± 0^{f}	259 ± 136^{f}	251 ± 110^{f}	255 ± 110

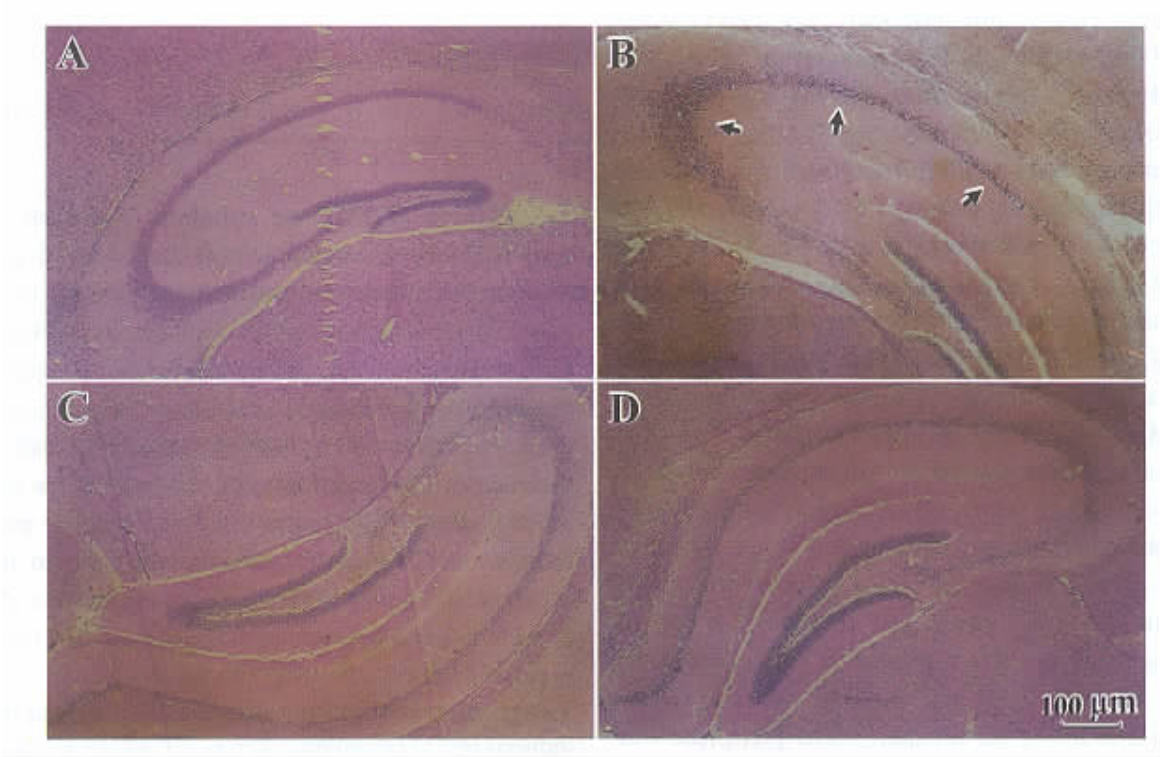

Fig 1. Effects of FDP on histological changes after CO poisoning. Air-treated group (A). Delayed death of CA1, CA2 and CA3 pyramidal cells in hippocampus 7 d after CO-exposure (B). There is no death of hippocampal neuronal cells in FDP-treated group: left hemisphere (C). Right hemisphere (D). Micrographs of 4-μm coronal sections of the HE-stained hippocampus, ×130.

increased, compared with that of air-treated mice (3.8 ± 0.8). FDP pre-administration obviously blunted the increase of MAO-B activities induced by subacute CO poisoning (Tab 4).

Tab 4. Effects of FDP on MAO-B activity in subacute CO poisoning in mice. $\bar{x} \pm s$. $^{c}P < 0.01$ *vs* **air group,** $^{e}P < 0.05$ *vs* **CO group.**

	MAO-B activity, μmol/g protein	*n*
Air	3.8 ± 0.8	9
CO	8.5 ± 1.2^{c}	7
CO + FDP	5.3 ± 0.5^{e}	6

DISCUSSION

Carbon monoxide (CO) poisoning still represents a frequent and serious casualty in the world. Until recently the hyperbaric oxygen (HBO) was one of the most important therapeutical intervention for CO poisoning. Generally HBO is the first choice of treatment for CO poisoning to decrease mortality and avoid occurrence of serious disorders and delayed sequel. However, the clinical application of HBO therapy is limited because of its expense, need of specific equipment including a multiplace hyperbaric chamber, specific educational program, and training for person employed in the clinical hyperbaric

center. Furthermore, if the operation is not correct, serious side reactions of HBO such as oxygen poisonging, *etc* may happen.

In this paper, we established a CO-poisoning model in mice and tried to find a useful drug to treat CO poisoning. Our experimental results showed that there was a high mortality and high total mortality in subacute CO intoxicated mice. Subacute CO poisoning could result in learning ability and memory disorders. The neuronal cell loss and death in hippocampus from CO-poisoning mice was significant. After CO-exposure, the MAO-B activity of brain tissue significantly increased, compared to air-treated mice. These results exhibit that the experimental model developed by our method is successful and reliable. Compared to CO inhalation, our experimental model is simple, safe, and reliable. It can be an effective model to illustrate the mechanisms of delayed injury caused by CO poisoning and help to find pharmacological interventions to control it.

Our experimental results also show that FDP pre-administration decreased the mortality, improved the impairment of learning and memory function, prevented hippocampal neurons from death and blunted the rising of MAO-B activities from subacute CO poisoning. These results indicate that FDP exerts a significant protective effect against CO poisoning. The mechanism of these effects of FDP is still unknown. It is well known that although exogenous administration of FDP does not appear to cross the cell membrane, however available evidences indicate that its main mode of action is via an interaction with the cell membrane and stimulation of phosphofructokinase activity followed by increase in ATP content. In conclusion, FDP can be developed as an effective protective agent in CO poisoning.

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1,6-二磷酸果糖对亚急性一氧化碳中毒致小鼠脑损伤的防护作用

杨俊卿，周岐新 （重庆医科大学生化药理研究室，重庆 400016，中国）

关键词 学习障碍；回避学习；一氧化碳中毒；单胺氧化酶；果糖二磷酸类

目的：观察 1,6-二磷酸果糖对亚急性一氧化碳中毒(CO)致迟发性脑损伤的防护作用. **方法**：小鼠腹腔注射 CO 100 mL/kg，每天一次，连续 7 天. 停止给予 CO 后，观察小鼠死亡率，学习记忆能力改变，脑组织病理学和单胺氧化酶-B 活性的改变；FDP 在每次给予 CO 前 30 min 腹腔注射. **结果**：FDP 预先给予 CO 中毒小鼠能显著降低死亡率，显著改善学习记忆能力；防止海马细胞延迟性死亡；并能阻遏单胺类氧化酶-B 活性的升高. **结论**：1,6-二磷酸果糖预防性给药对亚急性一氧化碳中毒致迟发性脑损伤有明显的防护作用.

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