

Delayed encephalopathy of acute carbon monoxide intoxication in rats: potential mechanism and intervention of dexamethasone

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Abstract: We aimed to investigate the potential mechanism (s) of delayed encephalopathy after acute carbon monoxide (CO) poisoning in rats, and the effect of dexamethasone on this process. A delayed encephalopathy animal model was generated by intraperitoneal injection of CO into Wistar rats. Normal rats were sent as a control group, and poisoning rats were randomly separated into two groups treated with vehicle and dexamethasone respectively. The rat behavior was evaluated by Morris water maze. The level of myelin basic protein (MBP), myeloperoxidase (MPO) expression in the serum and hippocampus of experimental rats was measured using enzyme-linked immunosorbent assay (ELISA) and immunohistochemistry. The latency to find the platform was significantly increased by dexamethasone treatment for rats after poisoning at day 7 and 14. MBP serum concentration in the vehicle treatment group was significantly higher than that in rats injected with dexamethasone following poisoning at 90min, 7d, 14d, 21d. Moreover, MPO concentration was higher at day 14 after poisoning as well. In addition, MBP expression was down regulated in the poisoning group, which was nearly reversed at control level in the dexamethasone group. Inflammation plays a key role in delayed encephalopathy of rats induced by acute CO intoxication, which could be attenuated by dexamethasone via protecting myelin from damage of inflammation response.

Keywords: Acute carbon monoxide poisoning, myelin basic protein, myeloperoxidase, dexamethasone, rat.

INTRODUCTION

Delayed encephalopathy after acute carbon monoxide poisoning (DEACMP) is a disease with poor knowledge of the pathogenesis and treatment strategy, which needs to be further investigated. The inflammation response could be involved in the pathogenesis of DEACMP, however, the underlying mechanism is still obscure. In order to understand the mechanism of DEACMP disease and develop a novel treatment strategy, we generated a DEACMP rat model and observed the effect of dexamethasone on the level of myelin basic protein (MBP), myeloperoxidase (MPO) expression in the serum and hippocampus of the rat model.

MATERIALS AND METHODS

Animals

Adult male Wistar rats (180-230g, Inner Mongolia University, China) were housed at room temperature (18-23°C), artificial daylight was on from 07.00 to 19.00 and the rats had free access to water and standard food. The Regional Ethics Committee approved the study.

Animal training trial

The rats were randomly placed into a water maze from four sites, and trained to learn how to find the escape platform for four times a day. If the rat did not find the platform within 120s, the operator led rat there and stayed for 10s. After 3 days, the rats, which escaped within 60s were chosen for experiment.

Animal model generation

The rat was intraperitoneally injected with CO at 150ml/Kg, according to Xiang *et al.* study (Wen *et al.*, 2013). After 30min poisoned rats were randomly injected with dexamethasone (30mg/Kg, n=40) and physiological saline (n=40) at a certain time.

Morris water maze

The study started on the first day of injection with CO and trial sessions for place navigation within the Morris water maze were carried out at 90min, day 7, 14 and 21 after poisoning. Each session contained four searches for the platform from different starting positions. Rats were placed in the pool facing the sidewall and had a maximum of 120 s to search for the platform. The performance of the rats was monitored with an overhead video camera connected to an image analyzer (HVS Image, Hampton, UK) and analyzed by the water maze software HVS Water 2020.

Sample preparation

After the water maze, the rats were rapidly decapitated and trunk blood (2-3ml) was collected for later analyses of MBP and MPO serum concentration. The brain was fixed with 4% para formaldehyde for 4h, followed by exposure to 15% and 30% sucrose solution, respectively, overnight. The tissue was cut into 8µM slides at -20°C, which were subsequently dried at 60°C for 4-6h. The immunohistochemistry assay was carried out using kit according to standard manufacturer protocol.

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STATISTICAL ANALYSIS

All data were represented as mean \pm SD. Statistical analysis was performed using one-way ANOVA of SPSS v17.0 software. Results were considered as statistically significant with $P < 0.05$.

RESULTS

Animal poisoning and water maze test

As expected, the injection of CO into rats resulted in intoxication symptoms. Compared to the control group, 15 rats died in the vehicle treatment group and 4 rats died in the dexamethasone group. In the water maze test, poisoning rats with the vehicle treatment took more time to escape compared with normal ones at day 7 and 14. However, dexamethasone treatment significantly shortened the time that poisoning rats took to find the platform (table 1).

MBP and MPO concentration in blood

There was no significant difference between control and dexamethasone group in terms of MBP and MPO concentration ($P > 0.05$). However, MBP level in the poisoning group was much higher than that in the control and dexamethasone groups as shown in table 2 ($P < 0.05$). Additionally, MPO level is also increased in the poisoning group at day 14 compared to the dexamethasone group (table 3).

As fig. 1 shows, the structure and cell shape of the hippocampus in the control group is normal, which is also found in the dexamethasone-treated poisoning rats. In contrast, there is neuron damage observed in the poisoning group, such as cell shrinkage, nuclear solid contraction, and structure mistiness.

Immunohistochemistry

Poisoning rat hippocampus showed less positive staining on MBP than that in normal rats in a time-dependent manner. However, dexamethasone treatment seemed to attenuate MBP down-regulation at day 7 and 14 after poisoning (fig. 2). MPO expression was not detectable in rat hippocampus of all three groups.

DISCUSSION

Although the detailed mechanism of DEACMP is still not fully understood, immune-mediated demyelinating reaction could cause damage of the central nervous system, and this has been supported by some animal and clinical studies (Ide *et al*, 2009). It has been shown that malondialdehyde resulting from lipid peroxidation after CO poisoning could modify MBP, which will then be attacked by macrophages, CD4⁺ lymphocytes and activated microglia, therefore leading to delayed encephalopathy (Thom *et al*, 2004). As the main

composition of myelin protein, MBP is a phosphatide protein around axons. The damage of brain white matter could cause the release of MBP into the cerebrospinal fluid and serum, which could last for 2 weeks (Shahsavand *et al*, 2012). In present experiments, MBP levels in the serum and hippocampus of rats was increased significantly 90min after poisoning, which is in agreement with Watanabe's study (Watanabe *et al*, 2010). Consistently, the latency for poisoning rats to find the platform is also higher than that in the control group at day 7 and 14. Thus, the central nervous system damage induced by demyelinating reaction could be involved in the process of DEACMP.

MPO is an important peroxidase from neutrophils, the level of which is significantly associated with neutrophils activation, and can be used as a marker of neutrophils function and activation (Prokopowicz *et al*, 2012). Our data showed that there was an increase in the level of serum MPO concentration after poisoning from 7 d to 14 d, compared to the control group. This suggests that inflammatory response might occur after 7 d from the acute CO poisoning, and aggravate over the following days. The activated neutrophils will produce chemokines and cytokines, which upregulate iNOS mRNA expression and activate the xanthine oxidase system, resulting in the augmentation of NO and reactive oxygen species (ROS) production, both of which could play roles in neural lesions (Lou *et al*, 2004). Meanwhile, the adhesion of leukocytes and vascular endothelial cells could initiate adhesion molecules, which lead to pro-inflammatory cells arresting on the endothelium and subsequent transendothelial migration to brain (Prandini *et al*, 2005). With the rearrangement of cytoskeleton and enlargement of intracellular space, blood-brain barrier will be broken, which causes the loss of MBP and initiates DEACMP.

In addition, we also found that dexamethasone treatment significantly reduced the escape latency compared with the vehicle treatment after poisoning for 7 and 14 days, suggesting that dexamethasone could improve the behavior of poisoned rats. These data provide basic evidence for the clinical application of dexamethasone on DEACMP therapy, although the underlying mechanism is still not clear. Based on our findings, dexamethasone seemed to suppress MPO expression, therefore blocking neutrophil activation and inflammatory response-mediated neural damage and eventually facilitating protecting rats from CO poisoning.

Taken together, acute CO intoxication could activate neutrophil and initiate inflammatory response-induced myelin protein loss, causing DEACMP. This process can be blocked by dexamethasone through the suppression of MPO expression, indicating dexamethasone might be a potential drug for DEACMP therapy.

Table 1: Poisoning treatment of the escape latency

Group	N	Escape latency			
		Before poisoning	Day 7	Day 14	Day 21
Control	12	26.2±3.2	25.7±1.3	24.1±2.6	23.3±1.4
Poisoning	40	26.3±7.0	104.5±9.0 [#]	63.5±2.5 [#]	24.0±3.0
Dexamethasone	40	25.6±5.0	23.0±3.0*	22.5±1.5*	21.7±3.2

Table 2: Poisoning treatment of MBP value in rat blood

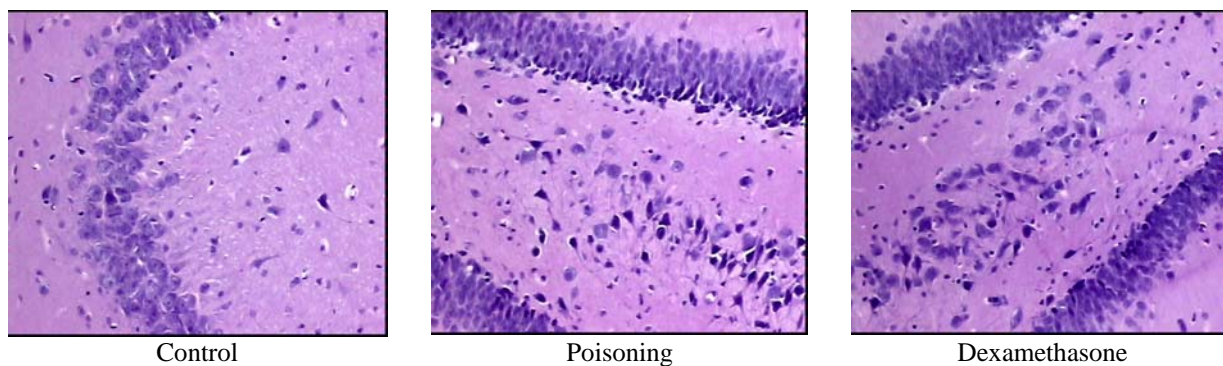
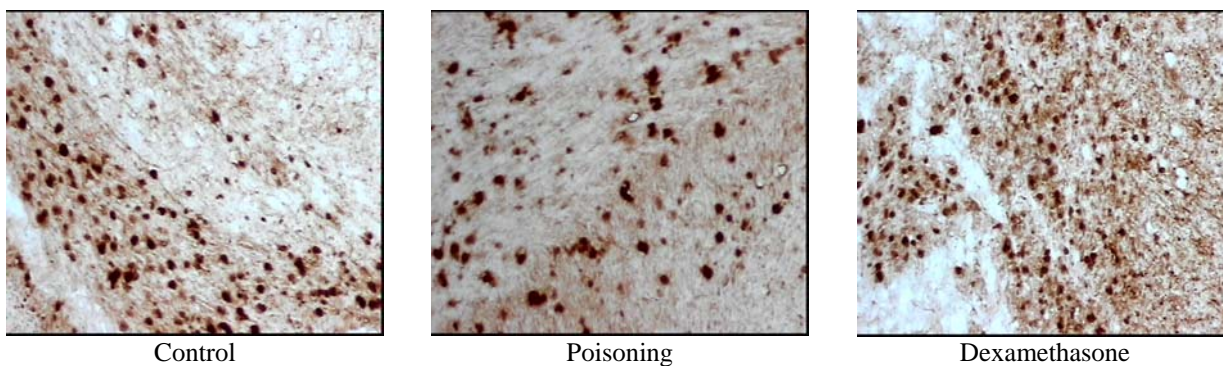
Group	N	Escape latency			
		90min	7d	14d	21d
Control	12	3.81±1.11	3.68±0.81	3.83±0.71	3.82±1.01
Poisoning	25	6.98±1.14 [#]	6.48±0.87 [#]	5.92±1.28 [#]	5.21±1.18 [#]
Dexamethasone	36	4.98±1.72*	4.56±0.70*	4.60±0.74*	4.12±0.68*

Table 3: Poisoning treatment of MPO value in rat blood

Group	N	Escape latency			
		90min	7d	14d	21d
Control	12	1.01±0.6	1.00±0.4	0.99±0.8	1.00±0.7
Poisoning	25	0.99±0.8	1.21±0.7	4.15±0.4 [#]	1.39±0.2
Dexamethasone	36	1.00±0.6	1.00±0.2	1.64±0.3*	1.22±0.4

[#] Indicates statistical significance as compared to control (P<0.05).

Number indicates statistical significance as compared to dexamethasone group* (P<0.05).

**Fig. 1:** Hematoxylin and eosin (H&E) staining (×100)**Fig. 2:** MBP immunohisto chemistry staining (×100)

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