学位論文

D-allose attenuates overexpression of inflammatory cytokines after cerebral ischemia/reperfusion injury in gerbil

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D-allose attenuates overexpression of inflammatory cytokines after cerebral ischemia/reperfusion injury in gerbil

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Highlights

- D-allose, a rare sugar, significantly reduced cerebral ischemia/reperfusion-induced overexpression of inflammatory cytokines.
- D-allose significantly attenuated ischemia-induced motor hyperactivity.
- The protection was associated with reduced oxidative damage, as well as less inflammation.

Abstract

The present study investigates the effects of D-allose, a rare sugar, on the inflammatory response after transient forebrain ischemia in the gerbil and whether it reduces oxidative stress (8-OHdG levels) and behavioral deficits. Transient forebrain ischemia was induced by occlusion of the bilateral common carotid arteries for 5 min. D-allose was intraperitoneal injected immediately after ischemia (400 mg/kg). Inflammatory cytokines and oxidative damage in the hippocampus, and behavioral deficits were examined three days after ischemia. D-allose administration reduced ischemia-induced cytokine production, oxidative stress and behavioral deficits (motor and memory related). The present results suggest that D-allose reduces brain injury after transient global ischemia by suppressing inflammation as well as by inhibiting oxidative stress.

Key Words: ischemia; reperfusion; D-allose; inflammation; cytokines; oxidation; hyperactivity

1. Introduction

Transient forebrain ischemia induces neurological deficits including learning and memory impairment. During the early period of ischemia/reperfusion injury, a transient inflammatory response is started by cytokines, such as interleukin (IL)-1β and tumor necrosis factor α (TNF-α), activated in response to ischemia [1]. That inflammatory response is thought to contribute to brain injury after global ischemia [2].

Rare sugars, such as D-allose, are monosaccharides which exist only rarely in nature. The biological effects of rare sugars remain largely unknown. Hossain et al. found that D-allose protects the liver during transplantation and during ischemia/reperfusion injury [3,4]. In kidney, Ueki et al. found that D-allose reduces ischemia/reperfusion injury in rats in part by inhibiting inflammation [5]. That group also found that D-allose inhibited lipopolysaccharide (LPS)-induced increases in serum and renal TNF-α, renal cytokine-induced neutrophil chemoattractant (CINC)-1 and myeloperoxidase concentrations, as well as the subsequent neutrophil-mediated renal injury [6].

In global cerebral ischemia, D-allose treatment started prior to ischemia protects against hippocampal cell death and behavioral deficits [7]. In focal cerebral ischemia, D-allose treatment started during ischemia, protects against brain injury and neurological deficits and it also suppressed inflammation as indicated by a reduced number of myeloperoxidase positive cells (e.g. neutrophils) in the brain after ischemia [8,9]. The present study investigated

whether D-allose given directly after global ischemia in the gerbil would protect against brain injury and suppress ischemia-induced overexpression of inflammatory cytokines as a potential mechanism underlying reduced inflammation.

2. Material and Methods

2.1. Animals

Animal protocols were approved by the Animal Committee of Kagawa University Faculty of Medicine. Male Mongolian gerbils (SLC, Hamamatsu, Japan), with a body weight of 60-80g, were used for all experiments. Food and water were available ad libitum.

2.2. Induction of ischemia

Transient global ischemia was induced by 5-min occlusion of bilateral common carotid arteries using micro-aneurysm clips (Sugita Clip; Mizuho, Nagoya, Japan) under sodium pentobarbital (30mg/kg i.p.) anesthesia. Rectal temperature was maintained at 36.5-37.5 degree using a feedback-controlled heating pad (CMA, Stockholm, Sweden). After recirculation, temperature was maintained at 37 degree for 60 min. Sham-operated control animals received the same operation except for the carotid artery occlusion. Blood glucose (samples from tail vein) and blood pressure (tail cuff method, Softron BP-98A, Tokyo, Japan) were measured before and 3h after ischemia as physiological parameters (n=6).

2.3. Experimental groups

In the first set of experiments, the effect of D-allose (Kagawa University Rare Sugar Research Center, 98% purity) on ischemia-induced functional deficits and spontaneous alternation deficits was examined three days after cerebral ischemia/reperfusion injury. Thirty gerbils were divided randomly into: sham, ischemia (vehicle:saline), and D-allose treatment at 200 mg/kg immediately after ischemia and 400 mg/kg immediately and 3h after ischemia. (n=6 per group). D-allose was injected intraperitoneally after ischemia. The second set of experiments examined the effect of D-allose on ischemia-induced expression of inflammatory cytokines. There were three groups of animals: sham, ischemia and 400 mg/kg D-allose treatment (n=3 per group). In the third set, the effect of D-allose on oxidative stress was examine three days after a sham operation or cerebral ischemia with and without D-allose treatment (n=3 per group).

2.4. Assessment for motor functional deficits

Locomotor activity was used to assess functional outcome. Gerbils were placed in a transparent cage and activity was assessed every hour over a period of 24 hours three days after brain ischemia using photobeam interruption sensors (LOCOMO LS-8, Melquest, Toyama, Japan). The number of beam breaks was evaluated as locomotor activity [8].

2.5. Assessment for spontaneous alternation deficits

Y maze Spontaneous Alternation is a behavioral test for measuring the willingness of rodents to explore new environments. Y maze was used to assess spontaneous alternation deficits 3days after brain ischemia. The number of arm entries and the number of triads were recorded in order to calculate the alternation percentage.

2.6. Enzyme immunoassay for cytokines

Gerbils were reanesthetized and decapitated 3 days after ischemia/reperfusion. Brains were removed and the bilateral hippocampi dissected and homogenized. The concentrations of the inflammatory cytokines, TNF-α, IL-1β and interleukin-6 (IL-6) were determined by ELISA kit (Thermo). Optical density was measured at 450 nm. The data, expressed as pg per ml, were calculated on the basis of linear calibrations curve generated with TNF-α, IL-1β and IL-6 standard solutions.

2.7. Enzyme immunoassay for DNA oxidation

DNA oxidative damage was examined by 8-hydroxyl-2'-deoxyguanosine (8-OHdG) assay. Hippocampi were homogenized for DNA extraction (Dojindo Molecular Technologies, Kumamoto, Japan). 8-OHdG levels were determined by ELISA kit (Japan Institute for

Control of Aging, Shizuoka, Japan). Optical density was measured at 450nm. The data, expressed as pg per μg of DNA, were calculated on the basis of linear calibration curve generated with 8-OHdG standard solutions [10].

2.8. Statistics

All data are express as mean±SD. The significance of differences was assessed with a one-way ANOVA followed by Turkey's post hoc test. Values of $p<0.05$ were considered statistically significant.

3. Results

3.1. Physiological parameters

All physiological parameters were measured immediately before and 30 min after ischemia and D-allose treatment (200mg/kg and 400mg/kg i.p. immediately after ischemia). Blood glucose and blood pressure were within the normal range (80-120mg/dl, 80-130mmHg, respectively). These variable were not influenced by D-allose administration (Table 1).

3.2. Effect of D-allose on ischemia-induced motor functional deficits

D-allose treatment attenuated behavioral deficits after cerebral ischemia. The activity counts (/day) in the ischemia + vehicle group was markedly increased (42478±4939)

compared with the sham-operated group (10440±1208, $p<0.001$), and there was no significant difference between vehicle and 200mg/kg D-allose immediately treated groups (37209±10208). However, 400mg/kg D-allose immediately and also 3h delayed treatment significantly decreased the behavioral deficit (immediately: 14512±4676, $p<0.001$ vs. vehicle group; 3h: 26870±12730, $p<0.05$ vs. vehicle group, Fig.1)

3.3. Effects of D-allose on ischemia-induced spontaneous alternation deficits

The Y-maze was used for assessment of spontaneous alternation for spatial working memory. The spontaneous alternation rates in the ischemia+vehicle group was smaller (52±6%) than in the sham group (71±4%, $p<0.001$), and there was no significant difference between vehicle and 200mg/kg D-allose treated groups (55±5%). Although 400mg/kg D-allose treatment increased the alternation rate (64±7%, $p<0.05$ vs. vehicle, Fig.2), there was no significant difference between vehicle and 400mg/kg D-allose 3h delayed treatment (54±10%).

3.4. Effects of D-allose on ischemia-induced hypercytokinemia

As the 200mg/kg D-allose treatment had no effect on motor function and spontaneous alternation deficits, cytokine and biomarkers were measured only after 400mg/kg D-allose treatment. The inflammatory cytokine, TNF-α, IL-6 and IL-1β was increased in the

hippocampus of ischemia animals (213±31, 146±21, 74±11 pg/ml, respectively) compared with sham group (97±21, 61±12, 32±7 pg/ml; $p<0.01$) at day 3. D-allose treatment significantly reduced TNF-α, IL-6 and IL-1β levels after ischemia (124±17, 76±11, 43±9 pg/ml; $p<0.01$, $p<0.01$, $p<0.05$ vs vehicle treatment, Fig.3, 4, 5).

3.5. Effect of D-allose on ischemia-induced DNA oxidative damage

The DNA oxidation biomarker, 8-OHdG, was increased in the hippocampus of ischemic animals compared to the sham-operated group (4.96±0.98 vs. 0.87±0.53 pg per μg of DNA; $p<0.01$). D-allose treatment significantly reduced 8-OHdG levels after ischemia ($p<0.01$, Fig.6).

4. Discussion

The current study demonstrates that D-allose causes a marked reduction in the cytokine response in a gerbil model of global ischemia with reperfusion. This was associated with improved behavioral outcomes and reduced oxidative stress, as assessed by 8-OHdG.

Whole-body ischemia/reperfusion in cardiac arrest with associated oxygen debt causes generalized activation of immunological and coagulation pathways, increasing the risk of multiple organ failure and infection. Thus, after cardiac arrest, there is a 'systemic inflammatory response syndrome' or 'sepsis-like syndrome' with alterations in multiple

cytokines in humans [11,12]. In brain, there are increases in multiple cytokines after global ischemia [13]. D-allose has inhibitory effects on activated leukocytes in various tissues and this has led to the hypothesis that it may inhibit brain injury after cerebral ischemia by suppressing inflammation [1]. This hypothesis was supported by findings in a rat focal ischemia/reperfusion model showing reduced numbers of myeloperoxidase cells (e.g. neutrophils) with D-allose treatment [9]. It is further supported by the current study showing that D-allose suppresses ischemia-induced upregulation of TNF-α, IL-1β and IL-6 in hippocampus, an area of brain particularly vulnerable to transient global ischemia. In human cerebral ischemia, IL-6 levels on admission are associated with early clinical deterioration [14].

Transient global cerebral ischemia in the gerbil causes uniform destruction of CA1 pyramidal neurons in the hippocampus, and this damage correlates with increased locomotor activity. We have previously shown that pretreatment with D-allose can ameliorate that hyperactivity [7]. In the present study, 400 mg/kg, but not 200 mg/kg, D-allose given after ischemia also reduced such ischemia-induced hyperactivity.

The hippocampus also plays an important role in learning, as a relay station for the acquisition of spatial recognition and the recall stage of spatial memory, the former role being more important [15]. In the current study, treatment with D-allose after cerebral ischemia also ameliorated the effects of ischemia on performance in the Y-maze spontaneous alternation test.

The underlying mechanisms by which D-allose ameliorates oxidative stress, neuroinflammation and neuronal death after global ischemia have not been fully elucidated. It may be related to the effects of D-allose on D-glucose metabolism/actions in the setting of ischemia and reperfusion. Ishihara et al. examined the effects of rotenone, an inhibitor of the electron chain in mitochondria, on the production of reactive oxygen species (ROS) in Neuro2A cells in vitro [16]. D-glucose enhanced the ROS production, an effect blocked by D-allose. ROS are involved in inducing both inflammation and cell death after cerebral ischemia [17]. In retina ischemia/reperfusion injury, D-allose suppressed the production of hydrogen peroxide during reperfusion [17]. In transient focal cerebral ischemia, D-allose given started during ischemia suppresses oxidative DNA injury markers as well brain injury after middle cerebral artery occlusion [8]. In global cerebral ischemia, pretreatment with D-allose also suppressed oxidative DNA injury [7]. In the current study, post ischemic treatment with D-allose also reduced hippocampal 8-OHdG levels after global cerebral ischemia. Infiltrating leukocytes are one source of free radical production and the effects of D-allose on inflammation may contribute to reduced oxidative stress.

5. Conclusions

The present experimental results extend findings indicating that D-allose has neuroprotective effects in cerebral ischemia/reperfusion injury. Given immediately after global cerebral ischemia, D-allose markedly reduces the levels of brain pro-inflammatory

cytokines and this may contribute to neuroprotection.

Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgments

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Figure Legend

Fig. 1. Outcome of locomotor activity tests. Gerbils underwent a sham operation, or transient forebrain ischemia with vehicle or D-allose treatment (200 mg/kg, D200, or 400 mg/kg, D400). Locomotor activity was measured on day 3. Ischemia caused locomotor

hyperactivity, an effect ameliorated with 400mg/kg D-allose treatment. Values are means±SD, n=6 per group; *p<0.001 vs. sham; #p<0.001 vs. vehicle.

Fig. 2. Outcome of alternation rates in Y-maze. Gerbils underwent a sham operation, or transient forebrain ischemia with vehicle or D-allose treatment (200 mg/kg, D200, or 400 mg/kg, D400). Transient forebrain ischemia caused a reduction in alternation rate compared to a sham operation. 400mg/kg D-allose treatment, but not 200 mg/kg, blocked this effect. Values are means±SD, n=6 per group; *p<0.001 vs. sham.

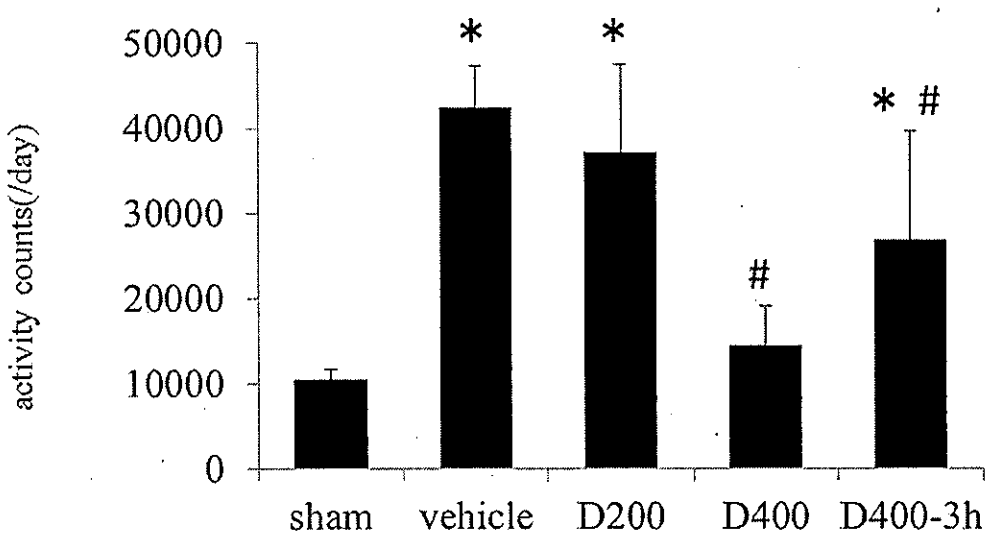
Fig. 3. Hippocampal TNF-α levels in gerbils that underwent a sham operation or transient forebrain ischemia with vehicle or 400mg/kg D-allose (D400) treatment. TNF-α levels were measured by ELISA at day 3. Ischemia increased hippocampal TNF-α levels, an effect ameliorated by D-allose. Values are means±SD, n=3 in each group; *p<0.01 compared with sham group; #p<0.01 compared with vehicle.

Fig. 4. Hippocampal IL-6 levels in gerbils that underwent a sham operation or transient forebrain ischemia with vehicle or 400mg/kg D-allose (D400) treatment. IL-6 levels were measured by ELISA at day 3. Ischemia increased hippocampal IL-6 levels, an effect ameliorated by D-allose. Values are means±SD, n=3 in each group; *p<0.01 compared

with sham group; #p<0.01 compared with vehicle.

Fig. 5. Hippocampal IL-1β levels in gerbils that underwent a sham operation or transient forebrain ischemia with vehicle or 400mg/kg D-allose (D400) treatment. IL-1β levels were measured by ELISA at day 3. Ischemia increased hippocampal IL-1β levels, an effect ameliorated by D-allose. Values are means±SD, n=3 in each group; *p<0.01 compared with sham group; #p<0.05 compared with vehicle.

Fig. 6. Hippocampal 8-OHdG levels at day 3 in gerbils that underwent a sham operation or transient forebrain ischemia with vehicle or 400mg/kg D-allose (D400) treatment. Ischemia markedly increased hippocampal 8-OHdG levels, an effect ameliorated by D-allose. Values are means±SD, n=3 in each group; *p<0.01 compared with sham group; #p<0.01 compared with vehicle.


activity counts(/day)
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D400-3h

Fig.1

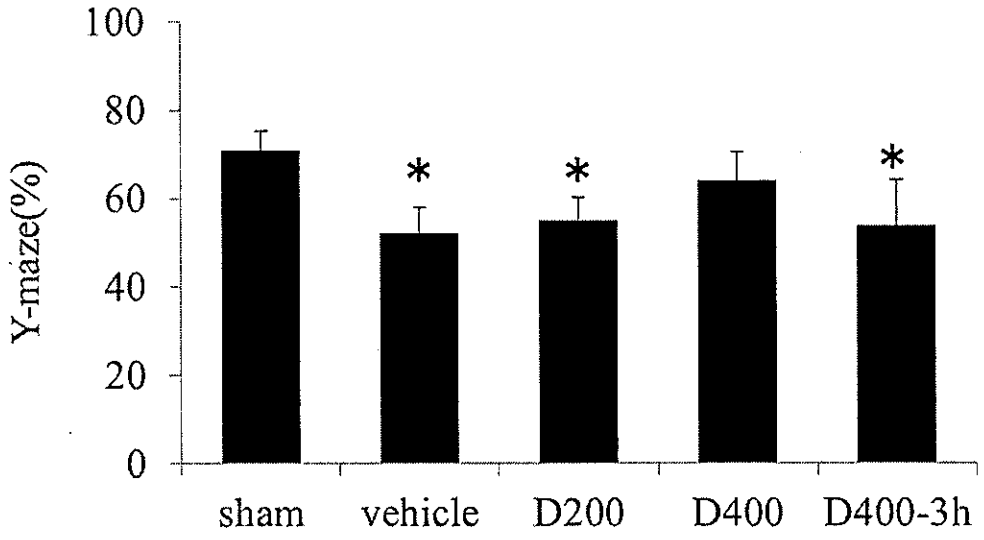


Fig.2

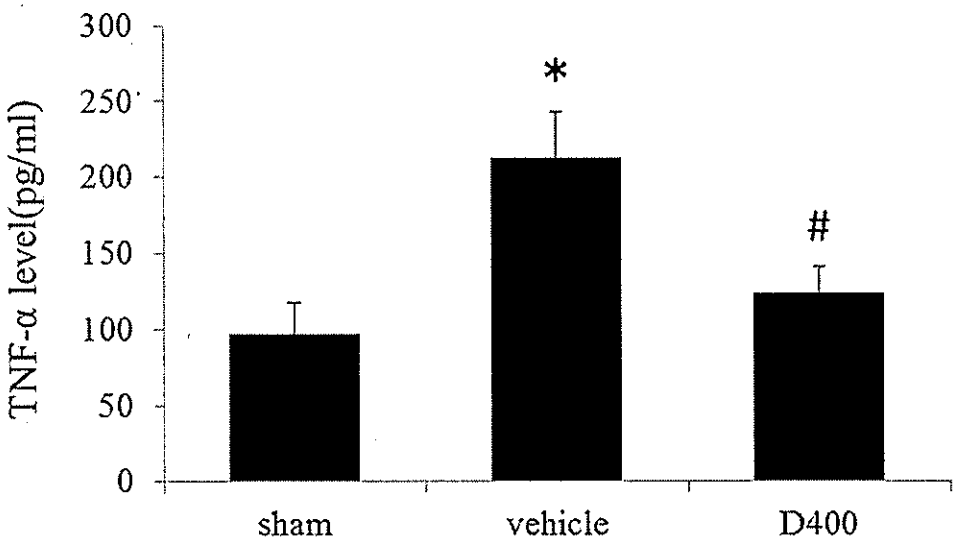
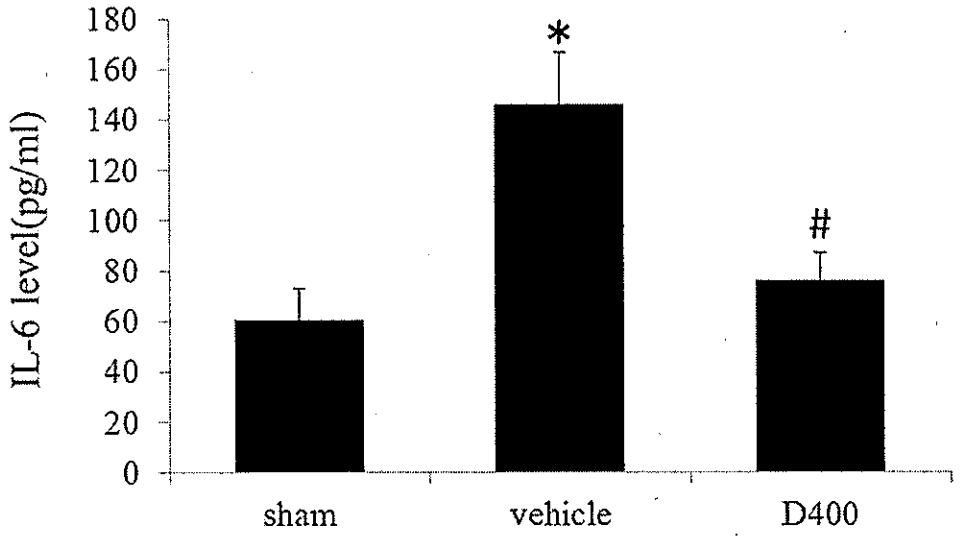


Fig.3


180
160
140
120
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IL-6 level(pg/ml)
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sham
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Fig.4

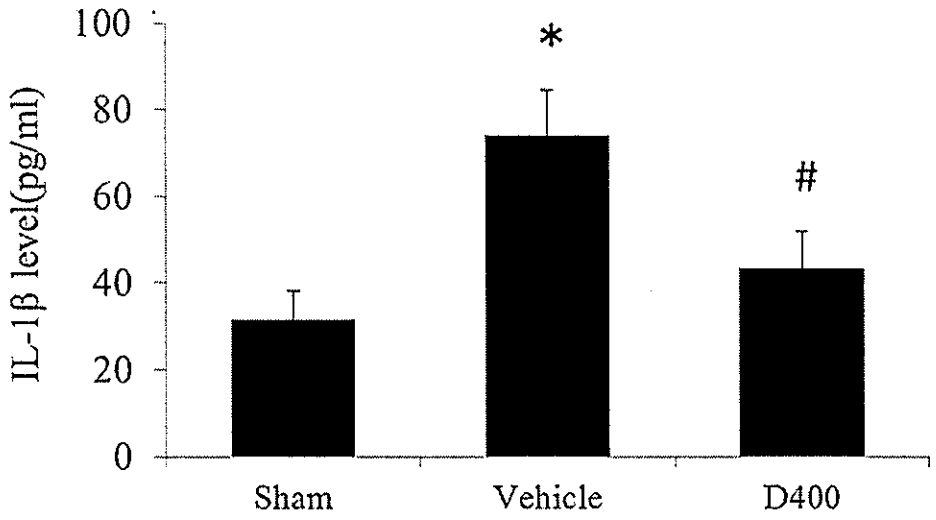

Fig.5

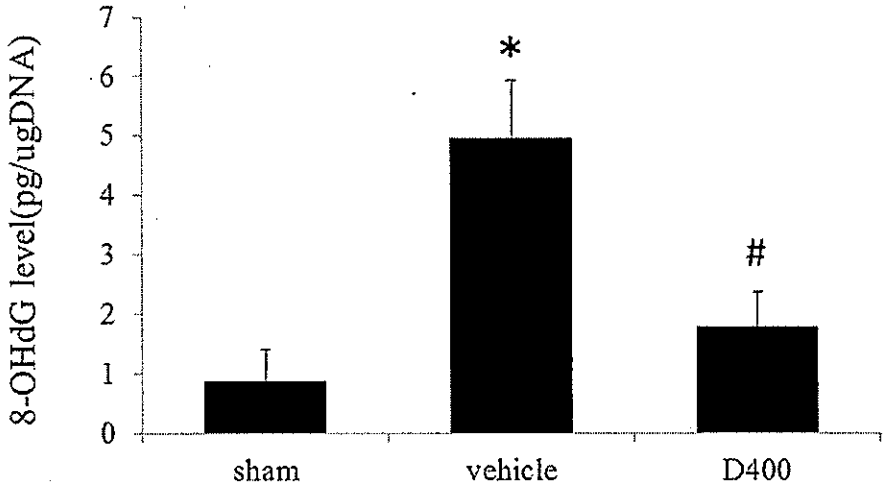

Fig.6