Original Research Article

Neuroprotective effects of delavatine A on LPS-induced activation of microglia *in vitro* and in a rat model of ischemia-reperfusion injury

Huijuan Xing, Qiang Sun*
Department of Neurology, Dalian Third People's Hospital, Dalian Medical University, Dalian, 116033, China

*For correspondence: **Email:** sunqiang811121@126.com*

Abstract

Purpose: To investigate the neuroprotective effects of deloratine A on liposaccharide (LPS)-induced microglia activation in vitro and in a rat model of ischemia-reperfusion injury.
Methods: LPS-induced microglial activation was successfully established in mouse microglia BV2 cell line. Then, the cells were randomly divided into model group, 2.5 µM delavatine A group, 5 µM delavatine A group, and 10 µM delavatine A group. The effect of delavatine A on the release of NO, TNF-α, IL-1β and IL-6 in BV2 cells was determined. In the vivo studies, 21 male Sprague Dawley (SD) rats were used to establish a rat model of ischemia-reperfusion injury, and effect of delavatine A on neural function and cerebral infarction area was determined.
Results: The NO content was significantly higher in LPS-induced microglial activation model than in blank control, but it was significantly lower in the 3 delavatine A groups than in model group (p < 0.05). The expression levels of TNF-α, IL-1β, and IL-6 were significantly higher in model group than in blank control group, but they were significantly and dose-dependently lower in delavatine A groups than in model group (p < 0.05). In the in vivo rat studies, neural function score and cerebral infarction area in the model group were significantly higher than those in the sham group, while cerebral infarction area in delavatine A groups were significantly lower than that in the model group (p < 0.05).
Conclusion: Delavatine A significantly reduces the inflammation associated with LPS-induced microglial activation, mitigates loss of neural function, and reduces cerebral infarction area in rats with ischemia-reperfusion injury. These findings may lead to the development of new neuroprotective drugs.

Keywords: Delavatine A, LPS, Microglia activation, Ischemia-reperfusion injury, Neural function, Cerebral infarction area

INTRODUCTION

Epidemiological survey of neurological diseases in China has revealed that there are more than 2 million new cerebrovascular diseases and more than 1.5 million associated deaths every year [1].

Cerebrovascular diseases seriously endanger the lives and health of middle-aged and elderly people in China, and they contribute significantly to disease-related death [2]. Ischemic stroke is a major cause of disability in Chinese population, accounting for about 80% of the total number of

strokes, with high degrees of morbidity, mortality and disability. Ischemic brain injury involves multiple links, and it is accompanied by a series of pathophysiological changes in nerve cells [3].

The pathogenesis of ischemic stroke is extremely complex. It involves excitatory toxicity and neuroinflammatory responses [4]. Although there are many treatment methods for ischemic stroke, there are no effective measures for brain protection. Some reports have confirmed that brain-protection drugs such as calcium antagonists produce unsatisfactory effects on patients with ischemic stroke [5]. Therefore, it is necessary to identify newer brain protective drugs for treating patients with ischemic stroke, and for reducing the mortality rate of patients with disability.

Delavatine A is extracted from the red polo flower of *Artemisia*. Clinical studies have shown that delavatine A inhibits astrocyte overactivation caused by brain injury, suggesting that it may effectively protect the nervous system [6]. Therefore, this study was carried out to investigate the neuroprotective effect of delavatine A on IPS-induced microglial activation *in vitro,* and its protective effect on a rat model of ischemia-reperfusion injury.

EXPERIMENTAL

Animals and cells

Mouse BV2 cells, and 28 male Sprague-Dawley (SD) rats [SCXK (Beijing) 2018-0028] were purchased from Chinese Academy of Sciences. The SD rats had a mean weight of 280 ± 30 g.

Ethical approval

This research was approved by the Animal Ethical Committee of Dalian Third People's Hospital (approval no. 20200083). And was performed according to "Principles of Laboratory Animal Care" (NIH publication no. 85-23, revised 1985) [7].

Main equipment and reagents

The instruments and reagents used, and their sources (in brackets) were: DMEM (Shenzhen Hongyilong Import and Export Co. Ltd), penicillin (Wuhan Xinxinjiali Biological Technology Co. Ltd), trypsin (Shaanxi Runfeng Biotechnology Co. Ltd), dimethyl sulfoxide (Xi 'an Taihua Pharmaceutical Technology Co. Ltd), CCK-8 detection kit (Qingdao Jieshikang Biotechnology Co. Ltd.), protease inhibitor (Shanghai Aiyan Biotechnology Co. Ltd), BCA protein quantitative kit (Shanghai Yihe Biotechnology Co. Ltd), PVDF membrane (Shanghai Bangjing Industrial Co. Ltd), RIPA cell lysis buffer (Shanghai Ziyi Reagent Factory), protein marker (Chengdu Huaxia Chemical Reagent Co. Ltd), and xylene (Jinan Trands Chemical Co. Ltd). The others were cell culture box (Xi 'an Qiyue Biotechnology Co. Ltd), fluorescence microscope (Shanghai Zhengxi Instrument Equipment Co. Ltd), low-temperature, high-speed centrifuge (Changzhou Haomai Drying Engineering Co. Ltd); 4ºC refrigerator (Haier Company), deionized water system (Shanghai Fenlinhuan Environmental Protection Technology Co. Ltd), shock heater (Beijing Dongmu Instrument Co. Ltd), and horizontal shaking bed (Wuxi Jiuping Instrument Co. Ltd).

Study design, treatments, and evaluation

Logarithmic growth-phase BV2 cells taken from mice were cultured for 12 h and treated with LPS to establish LPS-induced model of microglial activation. Thereafter, the cells were randomly divided into model group (untreated), 2.5 μM delavatine A group, 5 μM delavatine A group, and 10 μM delavatine A group. An untreated blank group served as control group. The effect of delavatine A on the release of NO in mouse BV2 cells were determined using chemical colorimetry, while its effect on expression levels of TNF-α, Il-6 and IL-1 β in BV2 cells was determined with ELISA. Twenty-one SD rats were selected and used to establish a mouse model of ischemia-reperfusion injury. Thereafter, the mice were randomly divided into model group, two groups treated with delavatine A at doses of 5 and 10 mg/kg, with 7 rats in each group. At the same time, 7 sham SD rats served as control.

The neurological function of SD rats was scored using LONGA's vascular thrombus method. Absence of neurological impairment was scored 0 point, inability to fully extend the contralateral forelimb was scored 1 point, while a score of 3 points indicated that the rats were weakly bent to one side. A score of 4 points indicated that the rats were strongly turned to one side, while a score of 5 indicated contralateral hemiplegia.

The area of cerebral infarction in the rats was determined using triphenyltetrazolium chloride staining.

Statistical analysis

Levels of inflammatory mediators and neurological function scores of rats are expressed as mean ± SD, and *t*-test was used

for two-group comparison, while repeatability measure ANOVA was used for multiple-group comparison. All data were analyzed using SPSS version 23 software. Differences were considered significant at $p < 0.05$.

RESULTS

Effect of delavatine A on NO release in mouse BV2 cells

The NO content of the model group was significantly higher than that of the blank control group, but NO contents of the delavatine A groups were significantly lower than that of the model group ($p < 0.05$). There were no significant differences in NO content among all delavatine A groups ($p > 0.05$). These results are shown in Table 1.

Table 1: Effect of delavatine A on NO release in BV2 cells of mice in each group (mean ± SEM)

Group	NO (%)
Blank control	100.00
Model	280.13±43.21[a]
2.5 µM Delavatine	175.89±32.45[b]
5 µM Delavatine A	165.23±30.52[b]
10 µM Delavatine A	170.56±27.23[b]

[a]$P < 0.05$, compared with blank control group; [b]$p < 0.05$, compared with LPS group

Effect of delavatine A on levels of TNF-α, IL-1β and IL-6 levels of BV2 cells

The contents of TNF-α, IL-1β and IL-6 in model group were significantly higher than those in blank control group ($p < 0.05$). However, the contents of TNF-α, IL-1β and IL-6 in the three delavatine A groups were significantly and concentration-dependently lower than those in model group ($p < 0.05$; Table 2).

Effect of delavatine A on neurological function of rats

The neurological function score and cerebral infarction area in model group were significantly higher than those in sham group, but they were significantly lower in the three delavatine A groups than in model group ($p < 0.05$). These data are presented in Table 3 and Figure 1.

DISCUSSION

Clinical studies have shown that delavatine A blocks the overactivation of astrocytes caused by craniocerebral injury, and it exerts anti-tumor effects [8]. However, not much is known on the effect of delavatine A on astrocytes.
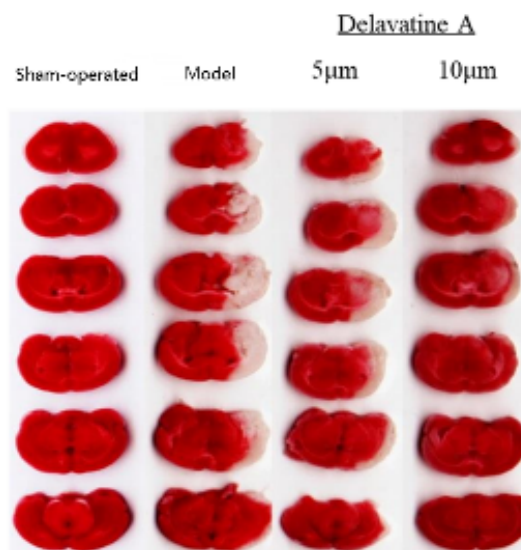
Figure 1: Cerebral infarction area of rats in each group

Table 2: Effect of delavatine A on levels of inflammatory mediators of BV2 cells in each group (mean ± SEM)

Group	TNF-α (pg/mL)	IL-1β (pg/mL)	IL-6 (pg/mL)
Blank control	200.00	100.00	1.01±0.34
Model	1213.56±156.56[a]	5889.36±213.58[a]	6.89±0.56[a]
2.5 µM delavatine A	750.98±82.23[b]	4456.52±156.23[b]	3.23±0.38[b]
5 µM delavatine A	605.36±103.89[b]	3856.58±145.41[b]	3.04±0.36[b]
10 µM delavatine A	315.25±78.56[b]	2800.47±189.78[b]	2.99±0.41[b]

[a]$P < 0.05$, compared with blank control group; [b]$p < 0.05$, compared with model group

Table 3: Effect of delavatine A on neurological function of rats in each group (mean ± SEM, n = 7)

Group	Neurological function score	Cerebral infarction area (%)
Sham-operated	0.00	0.00
Model	2.61±0.58[a]	29.77±2.94[a]
5 µM delavatine A	1.71±0.71[b]	21.13±5.57[b]
10 µM delavatine A	1.51±0.78[b]	15.69±5.14[b]

[a]$P < 0.05$, compared with blank control group; [b]$p < 0.05$, compared with model group

Therefore, this study was carried out to further investigate the effect of delavatine A on microglia activation in other diseases.

Microglias exist as innate immune organs in the central nervous system, and the first reaction of the body after central nervous system injury is manifested in microglias [9]. When microglia are overactivated, they secrete a variety of inflammatory mediators such as NO, TNF-α, IL-1β and IL-6, which lead to toxic effects on nerve cells, including apoptosis [10]. Nitric oxide (NO) plays an important role in neuroinflammatory response. Under normal circumstances, NO enhances the secretion of neurotransmitters and participates in reversible synaptic response. In addition, NO is crucial in immune response [11]. In pathological conditions, it acts in synergy with inflammatory factors, leading eventually to neuroinflammatory response [12].

In the present study, the content of NO in LPS group was significantly higher than that in blank control group, while NO levels in the three delavatine A groups were significantly lower than that in the untreated model group. These results indicate that delavatine A significantly inhibited NO release. Clinical studies have confirmed that TNF-α, IL-1β and IL-6 play major roles in inflammatory response, in which TNF-α appears first and accentuates the production and secretion of various cytokines. In addition, TNF-α has been used in combination with some anti-tumor drugs to exert anti-tumor effects [13]. The function of IL-1β is also extremely important: it is crucial in immune regulation and pathological injury [14,15].

In addition, the effect of delavatine A on levels of TNF-α, IL-1β and IL-6 in mouse BV-2 cells was determined. The results showed that the contents of TNF-α, IL-1β and IL-6 in model group were significantly higher than those in blank control group, while the levels of TNF-α, IL-1β and IL-6 in the three delavatine A groups were significantly and concentration-dependently lower than those in model group. These results suggest that delavatine A inhibited the release of inflammatory factors and reduced the neuroinflammatory response due to LPS-induced activation of mouse BV-2 cells. Thus, delavatine A played an important anti-inflammatory role.

The occurrence of ischemic stroke is often accompanied by excitatory neurotoxic reactions in which microglia and other cells are activated to release a large number of inflammatory factors, leading to further aggravation of tissue damage. Neurobehavioral scores can be used to evaluate the neurological function of experimental animals

with ischemia-reperfusion injury, and the scores are positively correlated with behavioral disorders. The neurological function score and cerebral infarction area in model group were significantly higher than those in sham-operated group, but they were significantly lower than those in model group. These results indicate that delavatine A effectively relieved ischemia/reperfusion injury in rats, and it exerted a significant protective effect on neural function.

Due to time limitation and other factors, this study investigated only the neuroprotective effect of delavatine A. Its mechanism of action will be investigated in subsequent studies.

CONCLUSION

Delavatine A significantly reduces the levels of inflammatory factors which are raised as a result of LPS-induced microglial activation. It also mitigates neurobehavioral deficit and reduces cerebral infarction area in rats with ischemia-reperfusion injury, resulting in significant neuroprotection. Thus, the findings of this study provide a lead for the development of new neuroprotective drugs.

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was performed by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Qiang Sun designed the study, supervised the data

collection, and analyzed the data. Huijuan Xing interpreted the data and prepared the manuscript for publication. Huijuan Xing supervised the data collection, analyzed the data and reviewed the draft of the manuscript.

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REFERENCES

1. *Vilas-Boas Mdo C, Cunha JP. Movement quantification in neurological diseases: Methods and applications. IEEE Rev Biomed Eng 2016; 9: 15-31.*

2. *Zang Y, Song X, Li C, Ma J, Chu S, Liu D, Ren Q, Li Y, Chen N, Zhang D. Pyrano[3,2-a]carbazole alkaloids as effective agents against ischemic stroke in vitro and in vivo. Eur J Med Chem 2018; 143: 438-448.*

3. *Jampathong N, Laopaiboon M, Rattanakanokchai S, Pattanittum P. Prognostic models for complete recovery in ischemic stroke: a systematic review and meta-analysis. BMC Neurol 2018; 18(1): 26.*

4. *Khoshnam SE, Winlow W, Farbood Y, Moghaddam HF, Farzaneh M. Emerging Roles of microRNAs in Ischemic Stroke: As Possible Therapeutic Agents. J Stroke 2017; 19(2): 166-187.*

5. *Schlemm L, Braemswig TB, Boutitie F, Vynckier J, Jensen M, Galinovic I, Simonsen CZ, Cheng B, Cho TH, Fiehler J, et al. Cerebral Microbleeds and Treatment Effect of Intravenous Thrombolysis in Acute Stroke: An Analysis of the WAKE-UP Randomized Clinical Trial. Neurology 2022; 98(3): e302-e314.*

6. *Zhang Z, Wang J, Li J, Yang F, Liu G, Tang W, He W, Fu JJ, Shen YH, Li A, et al. Total synthesis and stereochemical assignment of delavatine a:rh-catalyzed asymmetric hydrogenation of indene-type tetrasubstituted olefins and kinetic resolution through Pd-catalyzed triflamide-directed c-h olefination. J Am Chem Soc 2017; 139(15): 5558-5567.*

7. *World Health Organization. Principles of laboratory animal care. WHO Chron 1985; 39: 51-56.*

8. *Yu WW, Cao SN, Zang CX, Wang L, Yang HY, Bao XQ, Zhang D. Heat shock protein 70 suppresses neuroinflammation induced by α-synuclein in astrocytes. Mol Cell Neurosci 2018; 86: 58-64.*

9. *Raikwar SP, Thangavel R, Dubova I, Selvakumar GP, Ahmed ME, Kempuraj D, Zaheer SA, Iyer SS, Zaheer A. Targeted Gene Editing of Glia Maturation Factor in Microglia:a Novel Alzheimer's Disease Therapeutic Target. Mol Neurobiol 2019; 56(1): 378-393.*

10. *Huang PC, Tsai KL, Chen YW, Lin HT, Hung CH. Exercise combined with ultrasound attenuates neuropathic pain in rats associated with downregulation of IL-6 and TNF-α, but with upregulation of IL-10. Anesth Analg 2017; 124(6): 2038-2044.*

11. *Hulscher F, Farouki Y, Mine B, Bonnet T, Wang M, Elens S, Suarez JV, Jodaitis L, Ligot N, Naeije G, et al. Predictors of Good Clinical Outcome after Thrombectomy for Distal Medium Vessel Occlusions. World Neurosurg 2022: S1878-8750(22)00080-8.*

12. *Bystrom J, Clanchy FI, Taher TE, Al-Bogami MM, Muhammad HA, Alzabin S, Mangat P, Jawad AS, Williams RO, Mageed RA. Response to Treatment with TNFα Inhibitors in Rheumatoid Arthritis Is Associated with High Levels of GM-CSF and GM-CSF+ T Lymphocytes. Clin Rev Allergy Immunol 2017; 53(2): 265-276.*

13. *Wang Z, Zhang X, Zhang C, Li Y, Lu S, Mohanta S, Weber C, Habenicht A, Yin C. Combined Single-Cell RNA and Single-Cell α/β T Cell Receptor Sequencing of the Arterial Wall in Atherosclerosis. Methods Mol Biol 2022; 2419: 727-746.*

14. *Lim GB. Heart failure: Clonal haematopoiesis, IL-1β, and the NLRP3 inflammasome in HF. Nat Rev Cardiol 2018; 15(4): 198.*

15. *Lawlor KE, Feltham R, Yabal M, Conos SA, Chen KW, Ziehe S, Graß C, Zhan Y, Nguyen TA, Hall C, et al. XIAP Loss Triggers RIPK3- and Caspase-8-Driven IL-1β Activation and Cell Death as a Consequence of TLR-MyD88-Induced cIAP1-TRAF2 Degradation. Cell Rep 2017; 20(3): 668-682.*