

Effects of Puerarin Derivative P on Learning, Memory and MPO Activity in Vascular Dementia Model Mice

Pei Jiang^{1,2} and Yubin Ji^{1,2}

¹Research Center on Life Sciences and Environmental Sciences, Harbin University of Commerce, Harbin 150076, China;

²Engineering Research Center of Natural Anticancer Drugs, Ministry of Education, Harbin 150076, China

Abstract. Pharmacological experiments confirmed that Puerarin could not only expand the arterial vessels, increase the local blood flow, but also could protect the myocardium, improve the blood supply of ischemic tissue, which was used to treat the cerebrovascular dementia disease in clinical practice. Its own structure particularity caused the lower bioavailability and poorer solubility. In order to improve the deficiency, the related pharmacological experiments of the newly synthesized puerarin derivative (P), so as to expect the better curative effect than puerarin in the process of treating vascular dementia. The vascular dementia model was established by permanently ligating the common carotid artery in mice. The effects of puerarin derivative (P) on learning and memory in mice by water maze, Y maze and new object discrimination methods; The myeloperoxidase activity in the ischemic cerebral cortex was evaluated in mice by biochemical method. The experimental results showed that the mice spontaneous alternation response accuracy in Y maze could be obviously improved in 100mg/kg puerarin derivative group; In water maze, the swimming time to the safe platform would be significantly decreased in puerarin derivative group. Meanwhile, the MPO activity in the cerebral cortex of dementia mice was significantly decreased in 100mg/kg puerarin derivative group by permanently ligating the unilateral common carotid artery.

Vascular dementia (VD) is a kind of intellectual disability disease caused by a variety of cerebrovascular diseases (ischemic and hemorrhagic cerebrovascular disease, chronic and acute hypoxic cerebrovascular disease, etc.). It seriously damages the brain parenchyma, hinders the patients' learning ability and memory function to a certain extent, and then affects the life and health of patients [1]. At present, VD is the second common dementia disease worldwide except Alzheimer's Disease (AD) and showing a rising trend in some countries and regions year by year. With the continuous improvement of people's living standards, the morbidity of VD will rise perpendicularly with the increase of the age [2], [3]. Ischemic and hypoxic hypoperfusion was considered to be one of the VD pathogenesis [4].

In general, the white blood cells do not exist in the brain tissue. The inflammatory cells infiltration only occurs at time of cerebral ischemia or hypoxia [5], [6]. Among them, the activity of myeloperoxidase (MPO) representing the marker of neutrophil infiltration is also increasing gradually at time of cerebral ischemia, which also proves "inflammatory response theory" in cerebral injury due to cerebral ischemia.

Puerarin is white powdery substance extracted from the root of *Radix Puerariae*. Its chemical name is 7, 4'-dihydroxy- β -C-D-pyranoidisoflavone. Puerarin has wide pharmacological actions, which is mainly used to treat

the cardiovascular and cerebrovascular diseases [7]. Studies showed that [8], [9], puerarin could alleviate the brain edema, remove lipid peroxidation, enhance antioxidant capacity, improve the antioxidant activity of brain tissue and alleviate focal cerebral ischemic injury degree in cerebral ischemia reperfusion injury rats. However, the development of new drugs to treat central nervous system diseases was restricted by many factors. One of the main factors was how to effectively pass through the blood brain barrier [10]. Puerarin application was limited due to its lower water solubility and lipid solubility. The Puerarin structure was modified aiming to its defects in the paper and the novel puerarin derivative P was designed and synthesized. Its lipophilicity (log_p value was increased) was increased. The effects of the new compound on learning memory functions and MPO activity were investigated preliminarily in vascular dementia mice, so as to improve the permeability of blood brain barrier, enhance its pharmacological activity, which could provide the reference for further developing and utilizing puerarin and its derivatives in the treatment of vascular dementia.

1 Experimental

1.1 Reagents and Instruments

MS spectra were obtained with an Agilent G6300 Series (HPLC-MS) LC-MS analyzer. ¹H NMR spectra were obtained with a Bruker DMX600MHz / 300MHz

NMR spectrometer(DMSO-d₆ as solvent, tetramethylsilane as internal standard-). AutoDockVina molecular docking software was used. Puerarin pharmaceutical raw materials ($\geq 98\%$) were purchased from Xi'an Frierson Biotechnology Co., Ltd. All reagents and solvents were of analytical grade. The silica gel used for column chromatography (reagent grade, 200 to 300 mesh) was purchased from Qingdao Haiyang Chemical Co., Ltd.

1.2 Experimental animals

Male Kunming mice (SCXK (Army) 2013-006, weighing 25 ~ 30g) were used in this study.

1.3 Synthesis of P

Puerarin (15g, 36mmol) was dissolved in anhydrous DMF (200ml) to which K₂CO₃ (29.8g, 216.3mmol) was then added at room temperature. After stirring for 90min, ethyl bromoacetate (35.8g, 216mmol) was added and the mixture was stirred at room temperature for an additional 4h. The mixture was filtered to remove insoluble solids and then solvents were removed using a rotovapor until the mixture was in an oily state; the oily solute was then solubilized with anhydrous ether. After again rotovapping the ether solution, the remains were spin-dried then dissolved in anhydrous methanol and isolated by silica gel column chromatography.

P(ethyl 2-((3-(4-hydroxyphenyl)-4-oxo-8-((2S,3R,4R,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)-4H-chromen-7-yl)oxy)acetate) : logP-0.23; ESI-MS: m/z503.1 [M+H]⁺, ¹H-NMR (300MHz, DMSO-d₆) δ _H 8.43 (1H, s, H-2), 8.07 (1H, d, J=9.0 Hz, H-5), 7.18 (1H, d, J=9.0 Hz, H-6), 7.43 (1H, d, J=8.7 Hz, H-2', 6'), 6.81 (1H, d, J=8.7 Hz, H-3', 5'), 4.88 (2H, s, H-1''), 4.22 (2H, m, H-3'''), 1.25 (3H, m, H-4'''), 4.91 (1H, d, J=9.6 Hz, H-1'''), 3.95 (1H, m, H-2'''), 3.69 (1H, m, H-3'''), 3.47 (1H, m, H-4'''), 3.30 (1H, m, H-5'''), 3.43 (1H, m, H-6a'''), 3.65 (1H, m, H-6b'''), 9.55 (1H, s, -OH).

1.4 Preparation, grouping and drug administration scheme of animal model

The healthy male Kunming mice were intraperitoneally injected with 2mg/kg atropine. 3.5% chloralhydrate was intraperitoneally injected 5min later to anesthetize the mice. After the fixation and disinfection, the aseptic surgery was performed. The cervical median incision was made, the unilateral common carotid artery was separated (not including the vagus nerve), permanently ligated and sutured with the operative thread. The mice occurred to convulsion, slow deep breathing and accelerating heart beat. 0.3 ml blood was discharged 1 cm way from the tail tip and stopped by compression. The drug was infused in the stomach 48h later. The distilled water was infused in the model group and sham operation group.

The mice were randomly divided into 5 groups, 12/group, including the sham operation group, model group, 100mg/kg puerarin positive group; 100mg/kg puerarin derivative P group and 25mg/kg puerarin derivative P group. All the mice were treated in the postoperative 48h. The distilled water was given in the sham operation group and model group.

1.5 Measurement of MPO activity in the cerebral cortex in mice

The mice were rapidly decapitated. The brain was removed, placed on the watch glass with ice blocks. The surface blood was sucked up. The bilateral hippocampal cortexes were separated, weighed and frozen at -80 °C for standby. The biochemical indexes were detected. 4 °C saline was added according to 1:9 weight/volume ratio. 10% homogenate of brain tissue was made and centrifuged by 3000 r/min at low temperature for 15 min. The supernatant was removed and MPO activity was detected by UV-2102 Ultraviolet and visible spectrophotometer according to the kit.

1.6 Statistics processing

The experimental data were expressed using $\bar{x} \pm SD$. The one-way^[11] analysis of variance was performed using SPSS 18.0 statistical software. P < 0.05 indicated that the difference had the statistical significance.

2 Results

2.1 Water maze test

The experimental results showed that compared with the sham operation group, the swimming time to the safe platform was significantly prolonged in the mice of the experimental group from the first day to the fifth day; Compared with the model group, the swimming time was shortened significantly in varying degrees in all administration groups and positive administration group. And the swimming time to the safe platform was also decreased significantly in the fifth day compared with that of the first day.

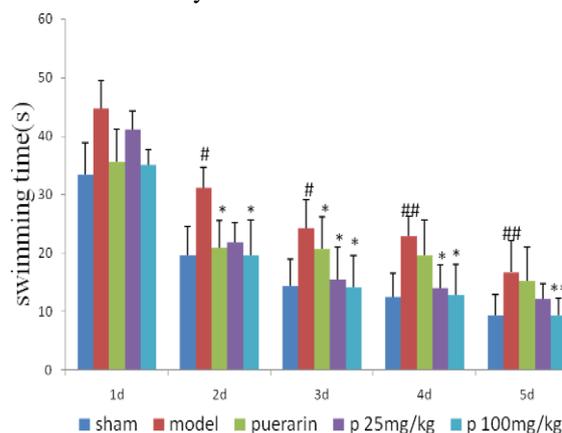


Figure 2-1. Effect of P on swimming time in water maze test in mice with Permanent ligation of the left common carotid artery (n=12, $\bar{X} \pm SD$)
[#]P <0.05, ^{##}P < 0.01 vs. sham group; *P < 0.05, **P < 0.01 vs. model group

2.2 Y maze test

The experimental results showed that compared with the sham operation group, the spontaneous alternating response accuracy was decreased significantly in the model group; Compared with the model group, the mice spontaneous alternate response accuracy was increased in varying degrees in high dose P group, low dose P group and positive drug administration group.

Table 2-2. Effect of P on the spontaneous alternation behavior in the Y-maze test in the mice with Permanent ligation of the left common carotid artery (n=12, $\bar{X} \pm SD$)

Group	Dose(mg/kg)	Alternation Behavior(%)
Sham	--	71.76 ± 10.31
Model	--	41.27 ± 8.77 ^{##}
Puerarin	100	65.21 ± 11.44*
P	25	61.37 ± 13.87
	100	70.36 ± 10.11**

^{##}P <0.01 vs. sham group; *P <0.05, **P <0.01 vs. model group.

2.3 New object discrimination experiment

The experimental results showed that compared with the sham operation group, the priority index and discrimination coefficient of the mice in the model group were decreased significantly in two training periods (1h and 24h); Compared with the model group, the priority index and discrimination coefficient of the mice in high dose P group and positive drug administration group were significantly enhanced in two periods (1h and 24h).

Table 2-3. Effect of P on discrimination index for the new object of 1h and 24h in the novel object recognition in the mice with Permanent ligation of the left common carotid artery (n=12, $\bar{X} \pm SD$)

Group	Dose(mg/kg)	Discrimination index(1h)	Preferential index(24h)
Sham	--	0.47 ± 0.08	0.86 ± 0.08
Model	--	0.36 ± 0.09 ^{##}	0.46 ± 0.06 ^{##}
Puerarin	100	0.46 ± 0.07*	0.76 ± 0.09*
P	25	0.38 ± 0.05	0.66 ± 0.06
	100	0.45 ± 0.09*	0.81 ± 0.11*

^{##}P <0.01 vs. sham group; *P <0.05, **P <0.01 vs. model group.

2.4 Effects of puerarin derivative P on MPO activity in VD mice cerebral cortex

The results were shown in Table 2-4. Compared with the sham operation, the MPO activity in the mice cortex was significantly increased in the model group and the inflammatory reaction was obvious; Compared with the model group, the MPO activity was decreased in all P administration groups and positive drug administration group by ligating the unilateral common carotid artery. Among them, the MPO activity was significantly decreased in the high dose P administration group and there was significantly different.

Table 2-4. Effect of P on the activity of MPO in the mice with Permanent ligation of the left common carotid artery (n=12, $\bar{X} \pm SD$)

Group	Dose(mg/kg)	MPO activity(U/g)
Sham	--	0.26 ± 0.16
Model	--	0.66 ± 0.21 ^{##}
Puerarin	100	0.38 ± 0.08*
P	25	0.42 ± 0.10
	100	0.36 ± 0.15*

^{##}P <0.01 vs. sham group; *P <0.05 vs. model group.

3 Discussion

In this paper, learning and memory obstacle model was established by permanently ligating the unilateral common carotid artery. The principle of this model was to ligate the common carotid artery to cause cerebral ischemia, insufficient cerebral blood supply and oxygen supply and ultimate cerebral injury. The experiments showed that the cerebral injury symptoms of the animal model were clear and definite, could cause the mice memory obstacle, could simulate the pathogenesis of VD and then could targetedly investigate the anti-vascular dementia activity of puerarin derivative.

Y maze and water maze were the common behavioral approach to study the neurodegenerative diseases. The working memory and spatial memory were investigated respectively. New object discrimination experiment was a learning and memory test method based on the principle of exploratory tendency [12], [13]. The learning and memory tests could be performed in mice under free state, and could more approximately simulate the behaviors of human learning and memory. Meanwhile, the experiment could test the long-term or short-term memory mechanism and evaluate the memory in particular stages by flexible conversion of the new object (shape and size), which was widely used in the learning, memory, new drug evaluation and other fields. In this paper, the behavioral experiment results showed that 100mg/kg puerarin derivative P could improve the learning and memory abilities of model mice in varying degrees, suggesting that it could improve the memory impairment caused by VD.

Studies [14], [15] showed that myeloperoxidase was a very important and special peroxidase secreted in neutrophil (PMN), most of which was located in PMN. The brain tissue hypoxia and ischemia would lead to the

inflammatory response of PMN infiltration. The myeloperoxidase (MPO) activity could indirectly reflect the PMN infiltration in cerebral ischemic areas. The cerebral cortex was the most sensitive to ischemia and was the important part of the human brain system. So the MPO activity in the cerebral cortex could indirectly reflect the degree of inflammatory damage. The experimental results showed that the MPO activity of the ischemic brain cortex was increased significantly in the model group, proved that PMN could indeed be related to a series of processes of the ischemic brain injury once again; The myeloperoxidase activity in the mice cerebral cortex could significantly decrease in high dose puerarin derivative group, so as to alleviate the inflammatory response and to improve the learning and memory abilities.

4 Conclusions

The structural modification of puerarin was conducted aiming to the solubility defects of puerarin, so as to obtain better lipophilic Puerarin derivatives. P. 100mg/kg puerarin derivative P showed better improving the learning, memory and alleviating the inflammatory response, which could provide the experimental basis to study the vascular dementia pathogenesis and therapeutic effects for natural drug structural modification.

References

- Roman GC. Vascular dementia revisited: diagnosis, pathogenesis, treatment, and prevention. *Med Clin North Am*, **86**(3): 477- 499(2002).
- TorreillesF, et al[J]. *Brain Res*, **30**: 153-163(1999)
- LongaZ, WeinsteinPR, CarsonSeta.l Reversible middle cerebral artery without craniotomy in rats. *Stroke*, **20**: 84- 91(1989)
- Dohmen C,Kumura E,Rosner G,et al. Extracellular correlates of glutamate toxicity in short-term cerebral ischemia and reperfusion:a direct in vivo comparison between white and gray matter.*Brain Res*, **1037**(1-2): 43-51(2005).
- Poornima Venkat,Michael Chopp, Jieli Chen.*Experimental Neurology*.**272**:97-108(2005)
- Xu Chen,Rong Li,Tao Liang,Kefeng Zhang,Ya Gao,Lingyuan Xu.*Phytomedicine*.**20**:849-852(2013)
- Mohammad Abul Kaisar,Shika Prasad, Luca Cucullo.*Brain Research*.**19**(1627):90-100(2015)
- Fatma Emel Kocak,Aysegul Kucuk,Filiz Ozyigit,Murat Tosun,Cengiz Kocak, et al.*Journal of Surgical Research*.**199**:393-401(2015)
- Uzakov S,Frey JU ,KorzV. Reinforcement of rat hippocampal LTP by hole board training. *Learn -Mem*.**12**:165-171(2005).
- Kano T,Shimizu-Sasamata M,Huang PL,et al.Effects of nitric oxide synthase gene knockout on neuro transmitter release in vivo.*Neuro science*.**86**(3):695-699(2011).
- Rosenberg GA. Ischemic brain edema. *Prog Cardiovasc Dis*, **42**(3): 209-216(1999).
- Luo J, Wang YH, YasudaRP, et al. The expression of N2methyl2D-2aspartate receptor complex in the cerebral cortex contain at least three different subunits (NR1 /NR2A /NR2B)[J] *MolPharma-col*,**51** (1) :79-86(1997).
- Nathan C. Nitric oxide as a secretory product of mammalian cells.*Faseb J*. **6**(12): 3051(1992)
- Roman Gustavo C.Cholinergic dysfunction in vascular dementia. *Curt Psychiat, Rep*, **7**:18-26(2005).
- HuT,Fu Q,et al.Increased acetylcholinesterase and caspase-3 expression in the brain and peripheral immune system of focal cerebral ischemia. *Neuroimmunol*, **211** (1-2):84-91(2009).