ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2001 Dec; 22 (12): 1130 – 1134
http://www.chinaphar.com

Effect of phenolic alkaloids from *Menispermum dauricum* on myocardial-cerebral ischemia-reperfusion injury in rabbits

WANG Fang[1], QU Ling, LÜ Qing, GUO Lian-Jun (*Department of Pharmacology, Tongji Medical College of Huazhong University of Science and Technology, Wuhan 430030, China*)

KEY WORDS alkaloids; *Menispermum dauricum*; reperfusion injury; malondialdehyde; superoxide dismutase

ABSTRACT

AIM: To explore the mechanism underlying the effect of phenolic alkaloids from *Menispermum dauricum* (PA*Md*) on simultaneous myocardial-cerebral ischemia-reperfusion injury in rabbits. **METHODS**: Both left anterior descending coronary artery and bilateral carotid arteries were occluded to induce myocardial-cerebral ischemia-reperfusion injury in rabbits. At 30 min after ischemia, the occlusion was removed and shed blood was rapidly reinfused. Two mL of blood was taken from femoral artery at 10 min before ischemia, 1, 10, and 30 min after ischemia, and 1, 10, 30, 60, 120, 180, and 240 min after reperfusion. Each rabbit was sacrificed at the end of reperfusion, and left ventricle, hippocampus, cortex, and cerebellum were taken out. Malondialdehyde (MDA) content and superoxide dismutase (SOD) activity were determined. **RESULTS**: At 10 min after reperfusion, MDA content in serum was significantly higher and SOD activity was lower in ischemia-reperfusion (I-R) group than those of control group ($P < 0.05$). After administration of PA*Md*, MDA content was lower and SOD activity was higher in serum than those of I-R group ($P < 0.05$). Both MDA content and SOD activity in tissues had the similar results with those in serum. **CONCLUSION**: PA*Md* could attenuate the injury induced by lipid peroxidation and enhance the activity of SOD, thus PA*Md* might play a protective role in simultaneous myocardial-cerebral ischemia-reperfusion injury.

[1] Correspondence to Dr WANG Fang. Phn 86-27-8364-4206.
E-mail wangfang0322@yahoo.com.cn
Received 2001-03-06 Accepted 2001-09-21

INTRODUCTION

Cerebral ischemia and myocardial ischemia, which are common diseases and harmful for human health, come on either separately or simultaneously and make their etiology and therapy more complex. Phenolic alkaloids from *Menispermum dauricum* (PA*Md*) are isolated from the rhizome of this plant, which contain mainly dauricine (Dau) and daurisoline (Ds)[1]. The pharmacological effects of Dau and Ds have been studied extensively. The recent studies showed Dau had antiarrhythmia and antiplatelet aggregation effects in animal studies[2-4], and Ds can protect against cerebral ischemia injury in cell culture[5]. Since the technological process of extracting PA*Md* is simpler and the product is more, if PA*Md* is exploited into product, the cost of production will be reduced. But the study on PA*Md* has been rarely reported. The primary research on PA*Md* in our laboratory showed that PA*Md* could antagonize contraction of isolated peripheral and cerebral vascular of rabbit that caused by several agonists (ie, KCl, histamine, methoxamedrine). This study was undertaken to investigate the effects of PA*Md* on malondialdehyde (MDA) and superoxide dismutase (SOD) in myocardial-cerebral ischemia-reperfusion injury to explore the mechanism of PA*Md* on anti-ischemic injury, which may have important clinical significance and social benefits.

MATERIALS AND METHODS

Drugs and reagents PA*Md* (purity: 95 %), extracted by Kunming Institute of Botany, was dissolved in HCl 1 mol/L and its pH value was adjusted to 6.5 ± 0.1 with NaOH, then diluted to 3.5 g/L by normal saline (NS). MDA and SOD reagents were purchased from Nanjing Jiancheng Biological Company.

Grouping New Zealand white rabbits (♂ ♀, 2.0 kg ± 1.0 kg, Grade II, Certificate № 19-023, provided by Experimental Animal Center of Huazhong University

of Science and Technology) were randomly divided into 4 groups: control, sham-operation, ischemia-reperfusion (I-R), and PA*Md*. The PA*Md* group was given iv PA*Md* 3.5 mg/kg, 10 min prior to ischemia.

Model I-R group: anesthesia was induced with 3 % pentobarbital sodium 1.0 mL/kg, iv. The right external carotid vein was cannulated for injecting drugs. The femoral artery was cannulated for sampling blood. Thereafter, the rabbit was carried out artificial respiration. A thoracotomy was made in the left 4 intercostal space, and the heart was suspended in a pericardial cradle. Suture was positioned around the left anterior descending coronary artery and the end of suture was threaded through a small plastic tube[6]. At the same time, bilateral common carotid arteries were occluded using atraumatic arterial clips. Reperfusion after 30 min ischemia was performed for 240 min. Sham-operation group: the left anterior descending coronary artery was surrounded by silk but not ligated, and NS was infused at certain time point. Control group: only the femoral artery was cannulated for sampling blood.

Experimental protocols Two mL of blood was collected at 10 min of preischemia, 1, 10, and 30 min after ischemia, and 1, 10, 30, 60, 120, 180, and 240 min after reperfusion, respectively. At the end, the heart and brain were quickly removed. Then left ventricle, hippocampus, cortex, and cerebellum were removed respectively and put into homogenated medium [sucrose 0.25 mol/L; hydroxymethyl amino-methane (Tris-HCl) 0.005 mol/L; edetic acid 0.001 mol/L; pH 7.5] to make a 10 % homogenate at 0 ℃. MDA content was determined by thiobarbituric acid method[7]. SOD activity was determined by inhibition of pyrogallol antioxidation[8].

Statistical analysis Data were expressed as $\bar{x} \pm s$, and analyzed by t test with ANOVA.

RESULTS

Effect of PA*Md* on MDA content in serum

In I-R group, the content of MDA after 10 min of reperfusion was higher than that at preischemia 10 min and ischemia 1 min; at 120 min after reperfusion, MDA content achieved the zenith. Compared with control and sham-operation group, 10 min after reperfusion, MDA content was higher in I-R group ($P < 0.05$). In PA*Md* group, MDA value reached the zenith at 60 min after reperfusion, then decreased gradually. Nevertheless, MDA content after 10 min of reperfusion in PA*Md* group was markedly lower than that in I-R group ($P < 0.05$) (Tab 1).

Effect of PA*Md* on SOD activity in serum

SOD activity had no change in sham-operation group. In I-R group, SOD activity after 10 min of reperfusion was lower than that at preischemia 10 min and ischemia 1 min; at 120 min after reperfusion, SOD activity achieved the minimum, and then elevated, but still lower than preischemia ($P < 0.05$). Compared with control and sham-operation group, 10 min after reperfusion, SOD activity was significantly lower in I-R group ($P < 0.05$). In PA*Md* group, SOD activity reached the minimum at 120 min after reperfusion, then increased

Tab 1. The effect of PA*Md* on MDA content (μmol/L) in serum in myocardial-cerebral ischemia-reperfusion injury of rabbit. $n = 4$. $\bar{x} \pm s$. $^bP < 0.05$ *vs* control. $^cP < 0.05$ *vs* I-R. $^hP < 0.05$ *vs* ischemia 1 min. $^kP < 0.05$ *vs* preischemia 10 min.

Time/min	Control	Sham-operation	I-R	PA*Md*
Preischemia 10	2.8±0.3	2.8±0.4	2.8±0.4	2.8±0.4
Ischemia 1	2.8±0.4	2.8±0.4	3.2±0.4	2.9±0.4
Ischemia 10	2.6±0.4	2.8±0.3	3.3±0.5	3.0±0.6
Ischemia 30	2.8±0.3	2.8±0.4	3.3±0.5	3.0±0.3
Reperfusion 1	2.8±0.4	2.9±0.4	3.4±0.4	3.1±0.4
Reperfusion 10	2.80±0.27	2.87±0.28	4.3±0.6^{bhk}	3.1±0.5^c
Reperfusion 30	2.8±0.3	2.9±0.4	4.4±0.6^{bhk}	3.2±0.5^c
Reperfusion 60	2.7±0.4	3.0±0.4	4.5±0.4^{bhk}	3.37±0.25^c
Reperfusion 120	2.6±0.5	3.0±0.4	4.9±0.9^{bhk}	3.2±0.4^c
Reperfusion 180	2.7±0.4	2.9±0.3	4.9±0.8^{bhk}	3.1±0.4^c
Reperfusion 240	2.8±0.5	3.0±0.4	4.8±0.5^{bhk}	3.1±0.6^c

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2001 Dec; 22 (12)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

gradually. Nevertheless, SOD activity after 30 min of reperfusion in PA*Md* group was markedly higher than that in I-R group ($P<0.05$) (Tab 2).

Effect of PA*Md* on MDA content in myocardial and brain tissues Briefly, compared with control group, the content of MDA in I-R group was increased by 64.4 % in cerebellum ($P<0.05$), 76.8 % in cortex ($P<0.05$), 78.5 % in hippocampus ($P<0.05$), and 83.5 % in left ventricle ($P<0.05$), respectively. Compared with I-R group, pretreated with PA*Md* could reduce the content of MDA by 28.0 % in cerebellum ($P<0.05$), 29.1 % in cortex ($P<0.05$), 32.9 % in hippocampus ($P<0.05$) and 34.7 % in left ventricle ($P<0.05$), respectively (Tab 3).

Effect of PA*Md* on SOD activity in myocardial and brain tissues Contrast to the changes of MDA content in myocardial and brain tissues, the activity of SOD in I-R group was decreased by 18.8 % in cerebellum ($P<0.05$), 32.1 % in cortex ($P<0.05$), 46.5 % in hippocampus ($P<0.05$), and 45.2 % in left ventricle ($P<0.05$), respectively (*vs* control group). Administration of PA*Md* can increase the activity of SOD by 16.5 % in cerebellum ($P<0.05$), 44.3 % in cortex ($P<0.05$), 80.0 % in hippocampus ($P<0.05$), and 75.3 % in left ventricle ($P<0.05$), respectively (*vs* I-R group) (Tab 4).

Tab 2. The effect of PA*Md* on SOD activity (kU/L) in serum in myocardial-cerebral ischemia-reperfusion injury of rabbit. $n=4$. $\bar{x}\pm s$. $^bP<0.05$ *vs* control. $^eP<0.05$ *vs* I-R. $^hP<0.05$ *vs* ischemia 1 min. $^kP<0.05$ *vs* preischemia 10 min.

Time/min	Control	Sham-operation	I-R	PA*Md*
Preischemia 10	476 ± 40	481 ± 30	480 ± 31	477 ± 31
Ischemia 1	477 ± 28	476 ± 33	481 ± 34	474 ± 33
Ischemia 10	469 ± 29	481 ± 30	469 ± 31	468 ± 30
Ischemia 30	479 ± 32	476 ± 33	460 ± 33	463 ± 38
Reperfusion 1	480 ± 36	474 ± 30	448 ± 30	457 ± 31
Reperfusion 10	475 ± 31	485 ± 30	422 ± 38^{bhk}	443 ± 30
Reperfusion 30	485 ± 29	480 ± 33	398 ± 28^{bhk}	436 ± 32^e
Reperfusion 60	484 ± 36	481 ± 30	338 ± 22^{bhk}	437 ± 29^e
Reperfusion 120	479 ± 33	476 ± 32	314 ± 21^{bhk}	433 ± 28^e
Reperfusion 180	481 ± 30	484 ± 29	326 ± 27^{bhk}	445 ± 31^e
Reperfusion 240	476 ± 33	479 ± 30	351 ± 30^{bhk}	467 ± 31^e

Tab 3. The effect of PA*Md* on MDA content ($\mu mol \cdot g^{-1}$ protein) in myocardial and brain tissues after ischemia-reperfusion injury of rabbit. $n=4$. $\bar{x}\pm s$. $^bP<0.05$ *vs* control. $^eP<0.05$ *vs* I-R.

Time/min	Control	Sham-operation	I-R	PA*Md*
Cerebellum	87 ± 10	90 ± 9	143 ± 13^b	103 ± 9^e
Cortex	86 ± 9	89 ± 9	152 ± 14^b	107 ± 11^e
Hippocampus	93 ± 8	97 ± 9	166 ± 16^b	112 ± 11^e
Left ventricle	91 ± 8	95 ± 8	167 ± 16^b	109 ± 9^e

Tab 4. The effect of PA*Md* on SOD activity ($kU \cdot g^{-1}$protein) in myocardial and brain tissues after ischemia-reperfusion injury of rabbit. $n=4$. $\bar{x}\pm s$. $^bP<0.05$ *vs* control. $^eP<0.05$ *vs* I-R.

Time/min	Control	Sham-operation	I-R	PA*Md*
Cerebellum	76 ± 6	83 ± 8	62 ± 6^b	72 ± 6^e
Cortex	76 ± 7	75 ± 6	51 ± 3^b	74 ± 6^e
Hippocampus	82 ± 8	79 ± 7	44 ± 4^b	79 ± 7^e
Left ventricle	76 ± 8	76 ± 7	42 ± 3^b	73 ± 6^e

DISCUSSION

In this experiment, the changes of MDA content and SOD activity in serum during myocardial-cerebral ischemia-reperfusion were observed in detail. The results showed that MDA content in serum hoiked after 10 min of reperfusion and reached the zenith at 120 min after reperfusion. SOD activity in serum declined markedly after 10 min of reperfusion and reached the minimum at 120 min after reperfusion. Previous reports had showed that in single cerebral ischemia-reperfusion of rabbit, MDA content in serum was elevated markedly after 60 min of reperfusion and achieved the zenith at 180 min of reperfusion; while SOD activity in serum had inverse change at the same time point[9]. Compared with these results, the changes of MDA content and SOD activity are relatively quick, and the time of peak value appears earlier in our experiment. Other reports showed that in single myocardial ischemia-reperfusion of rabbit, MDA content in serum was elevated gradually after reperfusion, and achieved the zenith at 120 min of reperfusion; SOD activity in serum was reduced markedly after 30 min of reperfusion and reduced to the minimum at 120 min of reperfusion[10]. Compared with those results, the time of peak value of MDA content and SOD activity were same, but the time when MDA content increased and SOD activity decreased was relatively quick in our experiment. This result illuminated that the changes of MDA content and SOD activity were more prominent during myocardial-cerebral ischemia-reperfusion than single myocardial or cerebral ischemia-reperfusion, which suggested that lipid peroxidation was more severe during simultaneous myocardial-cerebral ischemia-reperfusion. After 240 min of reperfusion, MDA content in tissues was elevated significantly, and SOD activity was reduced markedly, especially in left ventricle and hippocampus.

After administration of PA*Md*, MDA content was lower and SOD activity was higher in serum and tissues than those of I-R group. It suggested that during simultaneous myocardial-cerebral ischemia-reperfusion, PA*Md* could alleviate cell injury induced by lipid peroxidation, as well as enhance the elimination of oxygen free radicals during myocardial-cerebral ischemia-reperfusion, thereafter the injury of myocardial-cerebral induced by simultaneous ischemia-reperfusion was effectively attenuated. Liu *et al* showed that NMDA (100 μmol/L) induced widespread cell injury which was substantially attenuated by adding Ds (0.01 – 1 μmol/L)[11,12]. Our result is consistent with these reports. PA*Md* had no effect at 10 and 30 min after ischemia. We speculated that only after about 60 min of administration, PA*Md* could reach the efficient concentration in heart and brain to exert protective effect.

This study indicated that PA*Md* could protect cell from lipid peroxidation and enhance the activity of SOD in experimental animals, then it could modulate the balance of lipid peroxidation and anti-peroxidation effect *in vivo*. Thus PA*Md* might play a protective role in simumtaneous myocardial-cerebral ischemia-reperfusion injury.

ACKNOWLEDGEMENT To Prof PAN Xi-Ping (Kunming Institute of Botany, China) for supplying PA*Md*.

REFERENCES

1 Gong PL, Du ZH, Zeng FD, Hu CJ. Effect of phenolic alkaloids of *Menispermum dauricum* on antiarrhythmia. Tradit Chin Drug Res Clin Pharmacol 1995; 6: 13 – 6.
2 Li GR. Antagonistic effect of dauricine on experimental arrhythmias. J Tradit Chin Med 1984; 4: 2 – 5.
3 Guo T, Zhang YZ, Liu DX, Zhou P. Dauricine inhibits redistribution of platelet membrane glycoprotein IV and release of intracellular α-granule thrombospondin induced by thrombin. Acta Pharmacol Sin 1999; 20: 533 – 6.
4 Ding YX, Zhou J, Ye X, Ye XM, Gu SF, Zeng FD. Study of the effect of dauricine on platelet aggregation and its mechanism. Chin Pharmacol Bull 1991; 7: 271 – 4.
5 Dawson VL. Nitric oxide mediates glutamate neurotoxicity in primary cortical cultures. Proc Natl Acad Sci USA 1991; 81: 6368 – 70.
6 Yao XJ, Cui Y, Yu BR, Tan YH, Guo ZR. Protection by anisodamine against postistchemic reperfusion arrhythmia and lipid peroxidation in anesthetized rats. J Fourth Mil Med Univ 1992; 13: 349 – 52.
7 Asakawa T, Matsushita S. Thiobarbituric acid test for detecting lipid peroxides. Lipids 1979; 14: 401 – 6.
8 Marklund S, Marklund G. Involvement of the superoxide anion radical in the antioxidation of pyrogallol and a convenient assay for SOD. Eur J Biochem 1974; 47: 469 – 74.
9 Jian DL, Yu JP, Huang HB, Yang G, Xiong GX. The protective effect of *Panax notoginseng* on brain damage after acute cerebral ischemia. Chin Crit Care Med 1999; 11: 145 – 7.
10 Guan F. The effect of PA*Md* on myocardial ischemia-reperfusion. Res Tradit Chin Med 2000; 16: 46 – 7.
11 Wang T, Liu GQ, Zhu XZ. Daurisoline reduced neurotoxicity by inhibiting NMDA induced intracellular free calcium level. Chin Pharm J 1999; 34: 139 – 42.
12 Liu JG, Ben C, Li R. (–)-S · R-daurisoline protects

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2001 Dec; 22 (12)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

cultured cortex neurons against NMDA neurotoxicity. Chin J Pharmacol Toxicol 1998; 12: 308-9.

蝙蝠葛酚性碱抗兔心脑缺血再灌注损伤作用

王 芳[1], 曲 玲, 吕 青, 郭莲军
(华中科技大学同济医学院基础药理教研室, 武汉 430030, 中国)

关键词 生物碱类; 蝙蝠葛; 再灌注损伤; 丙二醛; 超氧化物歧化酶

目的: 探讨蝙蝠葛酚性碱抗家兔心脑缺血再灌注损伤的作用及其机制. **方法**: 结扎家兔左冠状动脉前降支及双侧颈总动脉, 30 min 后复灌, 制成心脑缺血再灌模型, 分别于缺血前 10 min, 缺血 1、10、30 min, 再灌 1、10、30、60、120、180、240 min 时经股动脉取血 2 mL, 再灌 240 min 后立即取出左心室、海马、皮层、小脑. 测定血清及各组织中丙二醛(MDA)含量和超氧化物歧化酶(SOD)活性. **结果**: 与对照组相比, 缺血再灌组(I-R)复灌 10 min 后血清 MDA 含量显著升高, 而 SOD 活性显著降低($P<0.05$). 与 I-R 组相比, 应用 PA*Md* 后, 血清 MDA 含量降低, 而 SOD 活性升高($P<0.05$). 各组织中 MDA 含量与 SOD 活性变化与血清中相似. **结论**: 蝙蝠葛酚性碱通过减轻脂质过氧化所造成的损伤及提高 SOD 活性, 对心脑缺血再灌注损伤具有一定的保护作用.

(责任编辑 吕 静)