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Protective Effect of Methylamine Irisolidone, a Novel Compound, on Acute Myocardial Ischemia in Anesthetized Dogs

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ABSTRACT

Kakkalide, a compound obtained from a traditional Chinese medicine Puerariae flos, has been shown significant anti-ischemia activity in animal experiments recently. However, the solubility of the compound need to be enhanced with hydroxypropyl-β-cyclodextrin, which is a strong controversial solution adjuvant via intravenous administration. Methylamine irisolidone is a structurally modified kakkalide with good solubility in the water. In this study, the effect of this new compound on acute myocardial ischemia in dogs was investigated. The results showed that methylamine irisolidone (80 mg/kg, i.v) could reduce the myocardial infarct areas in dogs with myocardial infarction. The serum lactate dehydrogenase (LDH) activity and MB isoenzyme of creatine kinase (CK-MB) were suppressed by methylamine irisolidone after 240 min of administration. These results suggested that methylamine irisolidone exerts the protective effects on myocardial ischemia injury, which may be due to its function of inhibiting LDH and CK-MB releasing, stabilizing myocardial cell membrane and improving myocardial microcirculation and metabolism.

Key words: Methylamine irisolidone, myocardial ischemia, infarct area, myocardial zymogram

INTRODUCTION

Puerariae flos is the dried flower bud of *Pueraria lobata* (Wild.) Ohwi, which belongs to the leguminosae family. It is commonly employed to relieve fever and dysentery, promote the production of body fluid, and reduce stiffness and pain of the nape. Previous phytochemistry studies on Puerariae flos reported a number of bioactive isoflavones, such as kakkalide, kakkalidone, irisolidone and so on. Puerariae flos is usually used for counteracting symptoms associated with alcohol drinking, liver injury, and menopause in traditional medicine clinic[(1-5)]. Yamazaki *et al.*[(6)] reported that intraperitoneally administered kakkalide reduced mortality and serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities associated with administration of ethanol. Lee *et al.*[(7)] and Han *et al.*[(8)] reported that kakkalide is metabolized to irisolidone by human intestinal microflora to show hepatoprotective activity. However, the cardiovascular effects of Puerariae flos and its constituents have not been thoroughly investigated.

The plenitudinous ground studies on pueraria radix have been made in our lab and then much more research concerning the constituents of Puerariae flos on cardiovascular activity has been progressed on this ground[(9-11)]. There is large amount of kakkalide in Puerariae flos and our recent research proved that kakkalide had significant anti-ischemia activity in the whole animal experiments. However, the compound need to be dissolved with help of hydroxypropyl-β-cyclodextrin. Hydroxypropyl-β-cyclodextrin is a strong controversial solution adjuvant via intravenous administration. So kakkalide was modified, the saccharide group hydrolyzed with 4′, 5, 7 powerful active groups on, and alkalinity group insertion at 8 position, so that the water-solubility was boosted. A new compound was obtain, 8-methylene-methylamine-irisolidone. It could combine a weak acid to form a water-soluble salt, making the preparation without any auxiliary solvent. The purity of this new compound is above 99.9%. The present study was aimed to investigate the protective effect of the new compound on the acute myocardial ischemia in anesthetized dogs.

MATERIALS AND METHODS

I. *Animals*

Twenty four male and female beagle dogs weighed

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between 8.0 and 12.0 kg were purchased from the Shanghai Laboratory Animal Multiplying Field. The animals were all individually housed in stainless steel cages in a temperature and light controlled room and fed with standard laboratory diet for 7 days after arrival. The dogs were given free access to food and distilled water.

II. *Preparation of Puerariae flos Extracts and Methylamine Irisolidone*

Kakkalide was extracted from the Puerariae flos with ethanol and distilled ethanol, and then was hydrolyzed into irisolidone. One gram irisolidone, 1 mL formaldehyde, 2 mL methylamine 30 mL ethanol were mixed, incubated for 4 hours at 70°C, cool down and filtered, and 1.3 g of methylamine irisolidone was produced.

III. *Preperation of Myocardial Infarction Model in Dogs*

The Animal and Ethics Review Committee at Shandong University evaluated and approved the protocol used in this study and the research was adhered to the "Principle of Laboratory Animal Care" (NIH publication #85-23, revised in 1985). All the experiments were performed on healthy beagle dogs which were anesthetized with 2.5% continal (1 mL/kg, i.v.). The surgical procedure was performed as reported[12,13]. In brief, the trachea was incised longitudinally and cannulated. The chest was opened under ventilation with room air by left thoracotomy. After the pericardium was opened, the left anterior descending (LAD) coronary artery was ligated near its origin by a 6.0 prolene suture according to Harris's two-step ligation method and the epicardium electrocardiogram was recorded.

IV. *Treatment*

Twenty four dogs were randomly divided into four groups. The control group consisted of dogs treated with normal saline. The three treatment groups consisted of dogs treated with methylamine irisolidone at the doses of 80 and 40 mg/kg and 100 mg/kg puerarin (as positive control) i.v, respectively. All dogs were sacrificed after 4 hours administration and hearts were removed and treated as described below.

V. *Measurement of Epicardium Electrocardiogram and Myocardial Zymogram*

XD-2-epicardial lead (Xiyuan Hospital of Beijing Traditional Chinese Medicine Academy) was stitched on epicardium and connected with BioPAC multiplying channel physiological signal collection analytical system (BioPAC, USA) with waver, and the epicardial electrogram was recorded. After operation, normal saline was infused to the supplement body fluid. Thirty minutes after coronary artery ligation, epicardial electrogram was recorded to calculate the total-value of 30 leads ST shift (Σ-ST) and lead numbers of ST shift (N-ST) over 2 mv as basic value before drug administration. The speed of intervenous infusion is 40 drop/min and the volume is 10.0 mL/kg. Myocardial ischemia control group received the isochoric normal saline. In order to calculateΣ-ST, N-ST and its change ratio, epicardial electrogram was recorded at 15, 30, 60, 90, 120, 180 and 240 minutes after drug administration. At the time intervals of 0, 2 and 4 hours after drug administration, myocardial zymograms including serum lactate dehydrogenase (LDH), creatine kinase (CK), MB isoenzyme of creatine kinase (CK-MB) and aspartate aminotransferase (AST) were determined using KNOEPRO automatic biochemistry analyzer (Finland Kangyi Instrument Company).

VI. *Measurement of Ischemia and Infarct Area*

Both ischemia and infarct areas were measured in five horizontal sections between the point of ligation and the apex. The non-ischemia and ischemia or non-infarction and infarction areas were demarcated after incubation with black ink from auricula sinistra region and stained with 0.5% nitroblue tetrazolium（N-BT）in phosphate buffered solution (pH 7.4) at 37°C for 15 min. With the digital imaging program from Meta Imaging Series 6.0, the sizes of ischemia and infarct myocardium were calculated and infarct size was reported as a percentage of ischemia area.

VII. *Statistical Analysis*

Data are expressed as mean ± SD. Statistical significance among the groups was assessed by ANOVA followed by Dunnett's test. A level of $P < 0.05$ was considered statistically significant.

RESULTS

I. *Effect of Methylamine Irisolidone on Myocardial Ischemia Degree (∑-ST) in Acute Myocardial Ischemia Dogs*

After 240 minutes of administration, methylamine irisolidone (80 mg/kg) can lessen the degree of myocardial ischemia obviously. In contrast with myocardial ischemia control group, the difference is significant ($P < 0.05$). Puerarin obviously mitigated the degree of myocardial ischemia of acute myocardial infarction in anesthetized dogs, too ($P < 0.05$). There is no manifest significance in low dose of methylamine irisolidone group (40 mg/kg). Results are shown in Table 1.

II. *Effect of Methylamine Irisolidone on the Range of Myocardial Ischemia (N-ST) in Acute Myocardial Ischemia Dogs*

Journal of Food and Drug Analysis, Vol. 17, No. 1, 2009

After 240 minutes of administration, methylamine irisolidone (80 mg/kg) can manifestly reduce the range of myocardial ischemia (N-ST) in contrast with the myocardial ischemia control group ($P < 0.05$). With puerarin injection, the range of myocardial ischemia N-ST was reduced obviously ($P < 0.05$). There is no manifest significance in 40 mg/kg methylamine irisolidone group. Results are shown in Table 2.

III. *Effect of Methylamine Irisolidone on the Infarct Area in Acute Myocardial Ischemia Dogs*

Myocardial infarct area displayed by the N-BT staining is roughly similar to the results measured by the epicardial electrogram. Methylamine irisolidone (80 mg/kg) exhibited obvious effects of lessening injury in myocardial ischemia, and the infarct area is conspicuously decreased as compared with the ischemia control group ($P < 0.05$). The effect of puerarin is comparable with that of methylamine irisolidone (80 mg/kg), but not with that of low dose methylamine irisolidone group. Results are shown in Table 3.

IV. *Effect of Methylamine Irisolidone on Myocardial Zymogram in Acute Myocardial Ischemia Dogs*

Under ischemia and hypoxia conditions, the physiological function and biochemical metabolism of cardiac muscle will change accordingly. Serum LDH levels and CK and CK-MB activity increased significantly, which may also reflect the extent of myocardial ischemia. After 2 hours of methylamine irisolidone (80 mg/kg) administration, methylamine irisolidone obviously decreased blood serum LDH and CK-MB activity. There is no statistic

Table 1. Effect of methylamine irisolidone on the myocardial ischemia degree (∑-ST change ratio %)

Time (min)	Group			
	A	B	C	D
0	—	—	—	—
15	2.78 ± 8.04	-5.68 ± 4.86	-7.76 ± 13.65	3.15 ± 6.46
30	4.67 ± 13.75	-5.59 ± 8.43	4.52 ± 27.42	7.07 ± 11.96
60	11.52 ± 17.90	-5.74 ± 13.57	1.15 ± 31.56	6.19 ± 17.05
90	9.59 ± 17.87	-5.65 ± 18.31	-2.20 ± 24.69	3.38 ± 19.13
120	9.06 ± 17.55	-15.04 ± 17.46	-8.97 ± 19.79	-1.64 ± 23.42
180	9.98 ± 26.91	-27.23 ± 24.32	-27.60 ± 17.28	-15.99 ± 20.87
240	4.97 ± 24.66	-40.06 ± 20.77*	-26.61 ± 12.64	-30.75 ± 16.36*

Note : data are expressed by mean ± SD, same as the follows.
* $P < 0.05$ for compared with ischemia control group analyzed by one way ANOVA followed by Dunnett's test.
A: ischemia control group received vehicle, i.v
B: ischemia group received methylamine irisolidone 80 mg/kg, i.v
C: ischemia group received methylamine irisolidone 40 mg/kg, i.v
D: ischemia group received puerarin 100 mg/kg, i.v

Table 2. Effect of methylamine irisolidone on the range of myocardial ischemia (N-ST change ratio %)

Time (min)	Group			
	A	B	C	D
0	—	—	—	—
15	-5.54 ± 15.29	-4.44 ± 4.82	- 4.09 ± 12.60	-2.50 ± 5.59
30	-9.00 ± 15.05	-2.11 ± 2.88	- 2.41 ± 24.43	-2.38 ± 3.67
60	3.30 ± 5.22	-5.05 ± 6.87	- 1.14 ± 24.06	-4.64 ± 3.27
90	2.02 ± 11.90	5.44 ± 6.81	- 0.21 ± 29.85	-2.98 ± 4.69
120	0.17 ± 12.22	0.77 ± 5.70	-11.12 ± 14.81	-6.95 ± 5.01
180	15.94 ± 22.46	-5.26 ± 12.69	-14.09 ± 16.02	-7.16 ± 7.35
240	22.67 ± 33.36	-12.60 ± 6.66*	-4.72 ± 6.14	-13.31 ± 9.80*

Table 3. Effect of methylamine irisolidone on the infarct size in acute myocardial ischemia dogs (n = 6)

Group	Ischemia area /Left ventricle area (%)	Infarct area /Left ventricle area (%)	Infarct area / Ischemia area (%)
A	13.56 ± 7.60	9.47 ± 5.26	71.51 ± 7.19
B	12.02 ± 3.79	6.83 ± 1.67	57.90 ± 8.70*
C	13.34 ± 4.61	8.42 ± 2.48	63.12 ± 7.59
D	13.57 ± 5.27	7.46 ± 3.83	53.35 ± 15.91*

difference for CK and AST, though with declining tendency. Results are shown in Table 4.

DISCUSSION

Methylamine irisolidone is a new structurally modified compound, which has the main structure of flavonoid. Flavonoids are widely distributed in food and drinks and act as antioxidants and iron chelators[14]. Lines of evidence indicated that flavonoid intake correlates inversely with coronary heart disease risk. Consumption of flavonoid-rich foods and beverages is thought to reduce the risk of cardiovascular diseases[15]. Puerarin, as one of flavonoid, has been used to treat patients with coronary artery diseases[16]. The action mechanism of puerarin may be due to coronary artery dilatation, lower oxygen consumption of myocardium, microcirculation improvement, and serum nitric oxide level elevation of rat with myocardial infarction[17]. Zhang *et al*. found that puerarin induced angiogenesis in myocardium of rat with myocardial infarction[18].

Under ischemia and hypoxia conditions, the physiological function and biochemical metabolism of cardiac muscle will change accordingly. ST segment deviation from the baseline and the elevation degree are closely related to myocardial blood flow and oxygen tension depression under acute myocardial ischemia conditions[19]. ST elevation represented the degree of myocardial ischemia infarction scope and the extent of myocardial necrosis[20]. Harris's two-step ligation of anterior descending coronary artery is an effective method to evaluate the pharmacologic effects of drug on myocardial ischemia. Results of this study showed that methylamine irisolidone (80 mg/kg) can significantly reduce the total deviation value of ST-segment of epicardial electrogram in coronary artery ligation anesthetized dogs, which suggests that methylamine irisolidone has apparent

Table 4. Effect of methylamine irisolidone on myocardial zymogram in acute myocardial ischemia dogs (n = 6)

Group	Index (U/L)	0h	2h	4h
A	CK-MB	24.40 ± 11.28	138.20 ± 23.18	190.40 ± 37.34
	CK	122.00 ± 75.41	669.80 ± 224.54	1025.20 ± 300.65
	AST	45.20 ± 20.32	80.40 ± 26.31	111.80 ± 47.81
	LDH	40.40 ± 22.01	88.80 ± 15.32	105.40 ± 18.96
B	CK-MB	26.80 ± 11.61	82.40 ± 34.32*	97.00 ± 34.63**
	CK	202.60 ± 151.53	510.40 ± 261.11	659.40 ± 287.58
	AST	43.00 ± 10.65	52.60 ± 16.88	57.00 ± 20.55
	LDH	40.20 ± 18.83	53.00 ± 21.76*	60.60 ± 21.81*
C	CK-MB	25.15 ± 10.25	112.23 ± 21.02	130.23 ± 45.25*
	CK	189.56 ± 166.34	600.25 ± 146.65	725.23 ± 167.35
	AST	44.25 ± 15.23	65.23 ± 20.32	60.23 ± 26.31
	LDH	40.15 ± 16.23	70.26 ± 12.48	75.23 ± 22.58
D	CK-MB	45.00 ± 15.02	101.40 ± 22.17	100.23 ± 23.94**
	CK	240.80 ± 105.48	724.80 ± 185.12	1253.60 ± 474.61
	AST	46.00 ± 6.89	82.20 ± 24.18	104.60 ± 16.65
	LDH	34.40 ± 14.08	55.20 ± 21.48*	70.00 ± 13.56*

protective effect on acute myocardial ischemia dogs. Biochemical analysis showed that methylamine irisolidone significantly reduces the serum CK-MB activity, and improves acute myocardial ischemia and myocardial infarction in coronary occlusion dogs. Administration of methylamine irisolidone can significantly reduce the extent of myocardial ischemia (∑-ST, N-ST), as measured by the epicardial electrogram mapping. Serum LDH levels, CK and CK-MB activity increased significantly, which may also reflect the extent of myocardial ischemia. After 2 hours of methylamine irisolidone (80 mg/kg) administration, methylamine irisolidone obviously decreased the blood serum LDH and CK-MB activity. The action mechanisms may be owing to that methylamine irisolidone could increase myocardial blood flow, promote the establishment of collateral circulation, increase myocardial oxygen supply, improve myocardial hypoxia tolerance, dilate peripheral vessels, reduce the cardiac pre- and afterload, and reduce myocardial oxygen consumption, thereby improving the imbalance state of oxygen / aerobic in myocardial ischemia, stabilizing myocardial membrane, reducing the enzyme leakage ratio and improving myocardial microcirculation and metabolism. The morphologic results showed that the infarct size was reduced by puerarin injection, which is comparable with that of methylamine irisolidone. The results mentioned above show that methylamine-irisolidone has protective effect on ligation anterior descending coronary artery induced acute myocardial ischemia in anesthetized dogs and may be developed into an effective new drug to treat cardiovascular diseases.

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