Original Research Article

Quaternary amines exert anti-myocardial ischemia effects via regulation of energy metabolism and oxygen free radicals in myocardial cells in acute myocardial infarction rats

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Abstract

Purpose: To investigate the effect of quaternary amines on myocardial cells of a rat model of cardiac arrest, with respect to energy generation potential and oxygen free radicals.
Methods: Forty-five Sprague-Dawley (SD) rats were assigned to sham, model and quaternary amine groups (each with 15 rats). After their corresponding treatments, lectrocardiogram (ECG) monitoring of the rats in the three groups at various time periods was carried out. Serum levels of myocardial enzymes, thromboxane B2 (TXB2), prostacyclin I2 (PGI2), serum carbon monoxide (CO), and changes in endothelial carbon monoxide synthase (eNOS) and endothelin (ET), were determined.
Results: The levels of NO and eNOS were significantly reduced in model rats, relative to sham operation rats, while ET was significantly elevated in sham rats (p < 0.05). There were higher levels of NO and eNOS in the quaternary amine group than in model rats, but ET was higher in quaternary amine group than in model rats. Thromboxane B2 (TXB2) concentration was higher in model rats than in sham rats (p < 0.05). While PGI2 was markedly lower in quaternary group than in sham operation rats. TXB2 was lower in the quaternary amine group than in model rats, while PGI2 was significantly higher in quaternary amine group, relative to model rats (p < 0.05).
Conclusion: Quaternary amines exert anti-myocardial effects by regulating energy metabolism and oxygen free radicals in myocardial cells of congestive heart failure rats, and thus are potentially useful for the management of acute myocardial infarction.

Keywords: Quaternary amine, Acute myocardial infarction, Electrocardiogram, Serum myocardial enzymes, Myocardial cells

INTRODUCTION

Congestive heart failure is a life-threatening complication of coronary heart disease. Statistics have shown that about one million patients die from acute myocardial infarction every year in China [1,2]. In recent years, due to improvements in living standards and changes in

lifestyle, the incidence of acute myocardial infarction has been on the rise. In the pathogenesis of this disease, continuous ischemia and hypoxia of coronary artery leads to myocardial necrosis and eventually to acute myocardial infarction. The main clinical manifestations are severe retrosternal pain or compressive pain in the precardiac area, which lasts for a long time. The symptoms are not alleviated after rest and oral administration of nitrate drugs. In severe cases, arrhythmias and heart failure may occur, which seriously endanger patients' lives [3]. Infarct size, treatment methods and complications have great impacts on the end result. The main treatment strategies for patients with congestive heart failure involve the use of percutaneous coronary intervention (PCI) and thrombolytic therapy. However, these two treatments have many limitations. For example, for patients with liver and kidney dysfunction, the use of contrast media before PIC may lead to acute kidney injury and angiographic nephropathy. Thrombolytic therapy is not suitable for individuals who had stroke or cardiac arrest over 3 h [4]. Studies have found that quaternary amines, being bioactive compounds in *yanhusol*, produced significant therapeutic effect on atrial premature beats and borderline premature beats in patients, with effects similar to those of ethamiodarone [5]. There are many clinical studies on the treatment of myocardial ischemia using corydalis, but there are limited studies on the effect of the bioactive quaternary amines on myocardial ischemia [7]. Therefore, the purpose of this study was to investigate the effect of quaternary amines on myocardial ischemia in rats, and the mechanisms involved.

EXPERIMENTAL

Drugs and instruments

The drugs and instruments used were: quaternary amine base (40 mg/kg, Dalian Institute of Chemical Physics, Chinese Academy of Sciences); Agilent 1260 HPLC (Agilent Company, USA); XD-7100 electrocardiograph (Shanghai Medical Electronic Instrument Co Ltd); Ab204-N electronic balance (METELER, Germany); Diamonsil Plus C18 column (Dikma, USA); Ultrasonic cleaning instrument (Jining Fengxin Ultrasonic Equipment Co Ltd); MicrotoF-Q III High performance electrospray-four rod-time of flight MS/MS tandem mass Spectrometer (Shenzhen Hongyong Jing Instrument Technology Co Ltd), and animal ventilator (Harvard Instrument Company, USA).

Establishment of rat model of AMI and treatment methods

Eighty SPF male SD rats were provided by Beijing Charles River Experimental Animal Co. Ltd., and they were assigned to 3 groups (sham group, quaternary amine group and model group). All rats were anesthetized with 10 % chloral hydrate, and their limbs and head were fixed in a supine position. Endotracheal intubation was used to maintain the breathing rate of the rats at 80 times/min. Electrocardiogram of the rats after anesthetics was determined, and the surgical site was depilated and disinfected. In the sham group, only thoracotomy was performed, without coronary ligation. Acute myocardial infarction was simulated by coronary ligation in model group and quaternary amine group, and the range of myocardial infarction was determined with TTG staining. Rats in the quaternary amine group were given quaternary amine (40 mg/kg) solution at a dose of 1 ml/100 g via gavage one week before operation, and operation was performed 30 min after the last drug administration. This study received approval from the Ethical Authority of Shenyang Medical College (approval no. 2021032), and was conducted according to "Principles of Laboratory Animal Care" (NIH publication no. 85-23, revised 1985) [8].

Evaluation of parameters/indices

The ECG of rats was recorded periodically at 5, 15, 30 and 45 min, and 1, 1.5, 2, 3, and 24 h after surgery. Infarct area was determined using TTC staining method. Frozen heart tissue slices were stained with TTC for 15 min (after the ECG monitoring of the rats, the rat hearts were excised and placed in the refrigerator at -20 °C for about 30 min, and then removed and sectioned). Digital imaging was used to analyze the infarct area and to calculate the degree of myocardial infarction. In the determination of serum myocardial enzymes, rat abdominal aortic blood was centrifuged and the serum was assayed for AST, LDH and CK-MB with their respective kits. Serum from rat abdominal aortic blood was assayed for levels of endothelin (ET), eNOS and NO. Thromboxane B2 (TXB2) in rat heart samples was determined using biotin double antibody sandwich enzyme immunosorbent assay.

Statistical analysis

The SPSS 19.0 software package was applied for statistical processing of the results. Measurement data are presented as mean ±

Table 1: Comparison of ECG results at each time period after surgery

Group	Time after surgery	Sham operation (n=15)	Model (n=15)	Quaternary amine (n=15)	F	P-value
ECG monitoring time	0 min	0.027±0.006	0.023±0.006	0.026±0.014	0.73	0.489
	5 min	0.026±0.005	0.135±0.029[a]	0.115±0.029[b]	88.76	<0.001
	15 min	0.026±0.009	0.156±0.032 [a]	0.129±0.012[b]	169.56	<0.001
	30 min	0.023±0.008	0.159±0.049 [a]	0.124±0.023[b]	74.95	<0.001
	45 min	0.029±0.012	0.160±0.053 [a]	0.120±0.035[b]	48.54	<0.001
	1 h	0.032±0.012	0.136±0.020 [a]	0.101±0.061[b]	29.55	<0.001
	1.5 h	0.028±0.007	0.143±0.034 [a]	0.102±0.069[b]	25.62	<0.001
	2 h	0.032±0.012	0.140±0.040 [a]	0.105±0.042[b]	38.95	<0.001
	3 h	0.029±0.009	0.135±0.023 [a]	0.109±0.028[b]	98.52	<0.001
	24 h	0.025±0.008	0.134±0.034 [a]	0.111±0.025[b]	80.51	<0.001

[a]$P < 0.05$, vs sham rats; [b]$p < 0.05$, vs model rats

standard deviation (SD), and multi-group comparison was done with ANOVA, while paired comparison was done with LSD *t*-test. Enumeration results are presented as (n (%)), and χ^2 test was used for inter-group comparison. Values of $p < 0.05$ were considered indicative of statistically significant differences.

RESULTS

ECG at different time points before and after surgery

There were no significant differences in ST segment elevation amongst the three groups before operation ($p > 0.05$), and no significant differences were found in ST segment elevation amongst the quaternary amine, sham operation and model groups 0 min after operation ($p > 0.05$). Electrocardiogram results at each time point after operation were comparable in the quaternary amine group and model rats. These results are presented in Table 1.

Serum levels of myocardial enzymes in the three groups

Table 2 indicates higher activities of CK-MB, LDH and AST in model rats than in sham rats, but the activities of these enzymes were lower in the quaternary amine group than in model rats ($p < 0.05$).

Serum NO, eNOS and ET levels in the three groups

There were markedly lower levels of NO and eNOS in the model group than in the sham group, but ET was significantly higher in sham-operated rats than in rats with congestive heart failure (model). In comparison, NO and eNOS levels in the quaternary amine group were significantly higher than the corresponding levels in the model group, while ET was significantly

higher in model rats. These data are shown in Table 3.

Table 2: Serum myocardial enzyme activities in the three groups of rats (U/L)

Group	n	CK-MB	LDH	AST
Sham operation	15	472.42±12.13	476.48±13.42	415.73±15.98
Model	15	630.19±12.47 [a]	795.42±25.01 [a]	612.27±12.04 [a]
Quaternary amine	15	551.63±7.53 [b]	635.18±19.27 [b]	583.47±22.06 [b]
F		779.29	972.35	571.56
P-value		<0.001	<0.001	<0.001

[a]$P < 0.05$, compared with sham operation rats; [b]$p < 0.05$, vs model rats

Table 3: Serum levels of NO, eNOS and ET in the three groups of rats

Group	n	NO (μmol/L)	eNOS (U/L)	ET (pg/ml)
Sham operation	15	24.03±2.49	0.073±0.009	55.21±2.19
Model	15	9.23±1.10	0.041±0.011	86.92±2.92
Quaternary amine	15	18.01±1.72	0.052±0.010	70.18±1.06
F		240.42	39.39	783.87
P-value		<0.001	<0.001	<0.001

[a]$P < 0.05$, compared with sham operation rats, [b]$p < 0.05$; compared with model group

Levels of TXB2 and PGI2 of rats in the three groups

Table 4 depicts markedly enhanced level of TXB2 in model rats, relative to sham rats, but there was lower PGI2 level in model rats than in sham rats ($p < 0.05$). In contrast, TXB2 level in the quaternary amine group was lower than that

in the model group, while PG12 was markedly higher.

Table 4: Comparison of TXB2 and PGI2 levels amongst the three groups of rats

Group	n	TXB2 (ng/mL)	PGI$_2$ (ng/mL)
Sham operation	15	36.21±3.42	84.21±2.72
Model	15	58.10±1.34 [a]	49.25±2.19 [a]
Quaternary amine	15	44.98±1.99 [b]	72.96±2.10 [b]
F		312.95	863.14
P-value		<0.001	<0.001

[a]$P < 0.05$, vs sham; [b]$p < 0.05$, vs model

DISCUSSION

Recent studies have shown that quaternary amines, which are bioactive compounds in *Rhizoma corydatidis*, possess good water-soluble properties [9]. In clinical use, they exert anti-myocardial ischemia effect by reducing myocardial oxygen consumption, and they protect myocardial cells. The effects of quaternary amine on endogenous blood vessels and myocardial sites result in regulation of metabolism and ultimate enhancement of the tolerance of the myocardium to ischemia and hypoxia [10]. Quaternary amines reduce blood pressure by lowering heart rate and dilating blood vessels, and they prolong Q-T interval and T-wave with efficacy similar to that of ethamiodarone [11]. The results of this study showed that, on the premise that there was no obvious ST segment height in the three groups before surgery, ECG was comparable amongst the quaternary amine group, sham operation group and model group at 0 min after surgery. However, ECG values at 5, 15, 30 and 45 min, and 1, 1.5, 2, 3 and 24 h after surgery differed statistically between the quaternary amine and model groups. Electrocardiogram is the most effective method for measuring the area of congestive heart failure. It was applied for studying the efficacy of treatments on rats with congestive heart failure by monitoring ST segment elevation before and after treatment.

Myocardial tissues specifically contain CK-MB isoenzyme. Thus, measurement of blood CK-MB activity serves as an index for early diagnosis of AMI, and it is of great significance in judging the extent of myocardial infarction and post-infarction reperfusion [12]. Changes in LDH content in serum are used as important and sensitive markers of myocardial damage, and the selectivity of serum LDH diagnosis is second only to those of CK-MB [13]. The serum activities of CK-MB, LDH and AST in model group were higher than the corresponding sham group levels, but their serum activities in the quaternary amine group were markedly reduced, relative to the levels in model rats. Quaternary amines regulate the metabolism of cardiomyocytes and reduce their apoptosis, thereby minimizing the degree of damage to cardiomyocytes [14]. Nitric oxide (NO) exerts a variety of biological effects such as inhibition of platelet accumulation, reduction of leukocyte adsorption, relaxation of coronary arteries, and protection of myocardial cells. In the present investigation, 7 days after treatment, there were lower NO and eNOS levels in model rats than in sham operation rats, but ET in model rats was elevated, relative to sham group. However, NO and eNOS levels in quaternary amine group were markedly higher than model group values, and ET was markedly elevated, relative to model rats. A key enzyme in synthesis of NO is eNOS. Quaternary amine base enhances the level of eNOS and makes it synthesize more NO so as to protect the cardiomyocytes and reduce blood pressure [15]. Thromboxane A2 produced by platelets is metabolized to TXB2 which exerts a contractile effect on vascular smooth muscle. Vascular endothelial cells produce PG12 through synthesis or release, and it inhibits the proliferation of smooth muscle cells via anticoagulation [16]. In pharmacology, quaternary amine inhibits myocardial lipid peroxidation due to its antioxidant properties, thereby reducing oxygen free radical damage to myocardial cells. It was found in this study that 7 days after administration, the level of TXB2 in model rats was higher than that in sham operation rats, but PGI2 level was significantly lower than that in the sham group. However, TXB2 level in quaternary amine group was reduced, relative to model rats, while PG12 was markedly elevated, relative to model rats. These results showed that the quaternary amine increased PGI2 by decreasing the level of TXB2, thereby reducing platelet accumulation and thrombosis.

CONCLUSION

Quaternary amine mitigates myocardial ischemia by regulating energy metabolism and levels of oxygen free radicals in rats with acute myocardial infarction. It would be necessary to carry out further investigations to ascertain its usefulness in clinical practice.

DECLARATIONS

Funding

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 is 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. Ying Wang designed the study, supervised the data collection, and analyzed the data. Zhong Zhang interpreted the data and prepared the manuscript for publication. Min Zhang supervised the data collection, analyzed the data and reviewed the draft of the manuscript. Ying Wang and Zhong Zhang contributed equally to this work and should considered as co-first authors.

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