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Effect of batroxobin against dog heart ischemia/reperfusion injury

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KEY WORDS reptilase; myocardial reperfusion injury; heart function tests; lactate dehydrogenase; creatine kinase; malondialdehyde; electron microscopy

ABSTRACT

AIM: To study the effect of batroxobin (Bat) on dog heart ischemia/reperfusion (I/R) injury. METHODS: Dog heart I/R injury was induced by occluding the left anterior descending coronary artery for 30 min and restoring blood perfusion for 90 min. Bat was intravenously injected before heart ischemia and 15 min before reperfusion. Plasma creatine kinase (CK), lactate dehydrogenase (LDH), and myocardial malondiadehyde (MDA) concentrations were measured. The pathologic changes of I/R myocardium were observed. RESULTS: Bat reduced the mortality rate of I/R dog (I/R group 65.0 % *vs* Bat-Ⅰ group 30.0 % and Bat-Ⅱ group 28.6 %, $P<0.05$). Myocytes of I/R heart showed intracellular edema, damaged mitochondria, and concentrated nucleus. Bat decreased these changes. In Bat-Ⅰ and Bat-Ⅱ group, plasma CK and LDH level were reduced, the $+dp/dt_{max}$ and $-dp/dt_{max}$ at 30 min after ischemia and 90 min after reperfusion were elevated, and left ventricular end dilation pressure (LVEDP) was lowered. The myocardial MDA contents were decreased by 42.3 % and 38.1 % ($P<0.01$) in Bat-Ⅰ and Bat-Ⅱ group, respectively. CONCLUSION: Bat may exert an apparent role against dog heart ischemia/reperfusion injury and improve myocardial function.

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INTRODUCTION

Ischemia/reperfusion (I/R) injury is often seen in clinics which pathogenesis has not been elucidated clearly. It is generally believed that its mechanism is related to lipid peroxide induced by oxygen free radical and to irreversible damage caused by intracellular calcium overload. To date, the treatment of ischemia-reperfusion injury has not been satisfactory clinically.

Batroxobin (Bat), a thrombin-like enzyme derived from *Bothrops atrox, moojeni venom*, consisted of 231 aminoacids[1]. In contrast to thrombin, which converts fibrinogen into fibrin by removing fibrinopeptides A and B, Bat only removes fibrinopeptide A. Parenteral administration of the enzyme causes the conversion of fibrinogen into a fibrin derivative which is rapidly degraded through a secondary fibrinolytic process and then eliminated *via* the urine. It is known that this factor has a series of biological effects. Besides fibrinogen lysis and t-PA release action, it lowers blood viscosity, prevents formation of thrombus[2,3], reduces I/R injury of rat brain[4], decreases periferal vascular resistance, and dilates coronary artery[5,6]. These beneficial actions of Bat suggest its important value theoretically and applicably, and indicate that this glycopeptide may have some other important biological effect which is worth further investigating. Here we aim to study the influence of Bat on ischemia-reperfusion injury of canine heart so as to further understand its cardiovascular effect.

MATERIALS AND METHODS

Experimental materials Dogs were provided by Experimental Animal Center of Beijing Medical University. Bat was manufactured by TOBISHI Pharmaceutical Co. LTD, Japan.

Preparation of heart ischemia/reperfusion in-

jury of dog[7] Forty-seven male dogs weighing (14.1 ± 2.6) kg were divided randomly into 4 groups: (1) ischemia/reperfusion group (I/R group). A left thoracotomy was performed from the third to fourth intercostal space, and the pericardium was opened to expose the heart. The left anterior descending coronary artery was ligated. Thirty min later, the snare of silk was released and reperfusion of myocardium was lasted for 90 min. This group was given no special treatment except intravenous infusion of normal saline at certain time point. (2) Bat-Ⅰ group. The operation of this group was the same as I/R group, and was iv injected Bat [1 Bu·kg^{-1}, Bu is the shorten name of Bat unit. When 0.1 mL Bat is added into 0.3 mL human-citric acid plasma at 37 ℃, and coagulation happens in (19.0 ± 0.2) s, the enzyme activity is defined as 2 Bu] before myocardial ischemia. (3) Bat-Ⅱ group. The operation of this group was the same as I/R group, and was iv injected Bat (1 Bu·kg^{-1}) 15 min after myocardial ischemia. (4) sham group. The left anterior descending coronary artery was surrounded by a silk but not ligated, and normal saline was iv infused at certain time point. Blood was collected before ischemia, 30 min after ischemia and 90 min after reperfusion.

Measurement of myocardial function[8] For myocardial function determination, an 8F Forgarty catheter was inserted into the left ventricular cavity via the carotid artery. The left ventricular dp/dt_{max} and left ventricular end dilation pressure (LVEDP) were measured by a physiological polygraphy system (EQ601G, Japan).

Pathomorphological observation of myocardium For histology, the left ventricular myocardium were collected and fixed in 10 % formalin for at least 3 d. Each fixed myocardium was embedded in paraffin. A few thin sections (4 μm) were cut from each paraffin block using a microtome and were stained with hematoxylin-eosin. For transmission electron microscopy, small pieces of left ventricular myocardium were collected and fixed in 2.5 % glutaraldehyde, postfixed with 2 % osmium tetroxide, dehydrated with the graded series of ethanol, passed through propylene oxide, and then embedded in EPON resin. Ultrathin sections were stained with uranyl acetate and lead citrate and observed with a transmission electron microscope (JEMCIX-100II, Hitachi, Japan).

Determination of myocardial MDA content Myocardial MDA content was determined by thiobarbituric acid method[9].

Measurement of plasma CK[10] and LDH[11] concentration Serum levels of CK and LDH were evaluated as indices of cardiac cellular damage by using commercial kit (Sigma).

Analysis of statistics Group values were presented as $\bar{x} \pm s$. The significance of group differences was determined by one way ANOVA and q test.

RESULTS

Mortality rate of dogs Thirteen, 3, and 2 dogs in I/R (20 in total), Bat-Ⅰ (10 in total), and Bat-Ⅱ group (7 in total) died after myocardial ischemia or reperfusion, respectively. The mortality rates were 65.0 %, 30.0 %, and 28.6 %, respectively, while the mortality rate of sham group was 20.0 % (2 out of 10). The death rate of Bat-Ⅰ and -Ⅱ group was lower than that of I/R group ($P < 0.05$).

Pathological changes Myocardial cells of I/R heart showed intracellular edema, swollen and damaged mitochodria, and concentrated nucleus. Bat decreased these kinds of injuries apparently (Fig 1, 2).

Myocardial function Compared with sham group, the left ventricular $+dp/dt_{max}$ 30 min after ischemia and 90 min after reperfusion lowered by 31.4 % and 30.6 %, $-dp/dt_{max}$ lowered by 29.0 % and 43.6 %, and LVEDP increased by 64.5 % and 80.0 %, respectively. Compared with I/R group, administration of Bat before ischemia increased $+dp/dt_{max}$ at 30 min after ischemia and 90 min after reperfusion by 38.5 % and 41.5 %, augmented $-dp/dt_{max}$ by 43.4 % and 27.5 %, and decreased LVEDP by 45.5 % and 175.0 %, respectively. In Bat-Ⅱ group, $+dp/dt_{max}$ and $-dp/dt_{max}$ at 90 min after reperfusion augmented by 36.2 % and 36.3 %, respectively. LVEDP lowered by 2.5-fold (Tab 1).

Myocardial MDA content Myocardial MDA content in I/R group was 1.2-fold higher than that of sham group ($P < 0.01$), compared with I/R group, myocardial MDA content in Bat-Ⅰ and Bat-Ⅱ group lowered by 42.3 % and 38.1 %, respectively ($P < 0.01$) (Tab 1).

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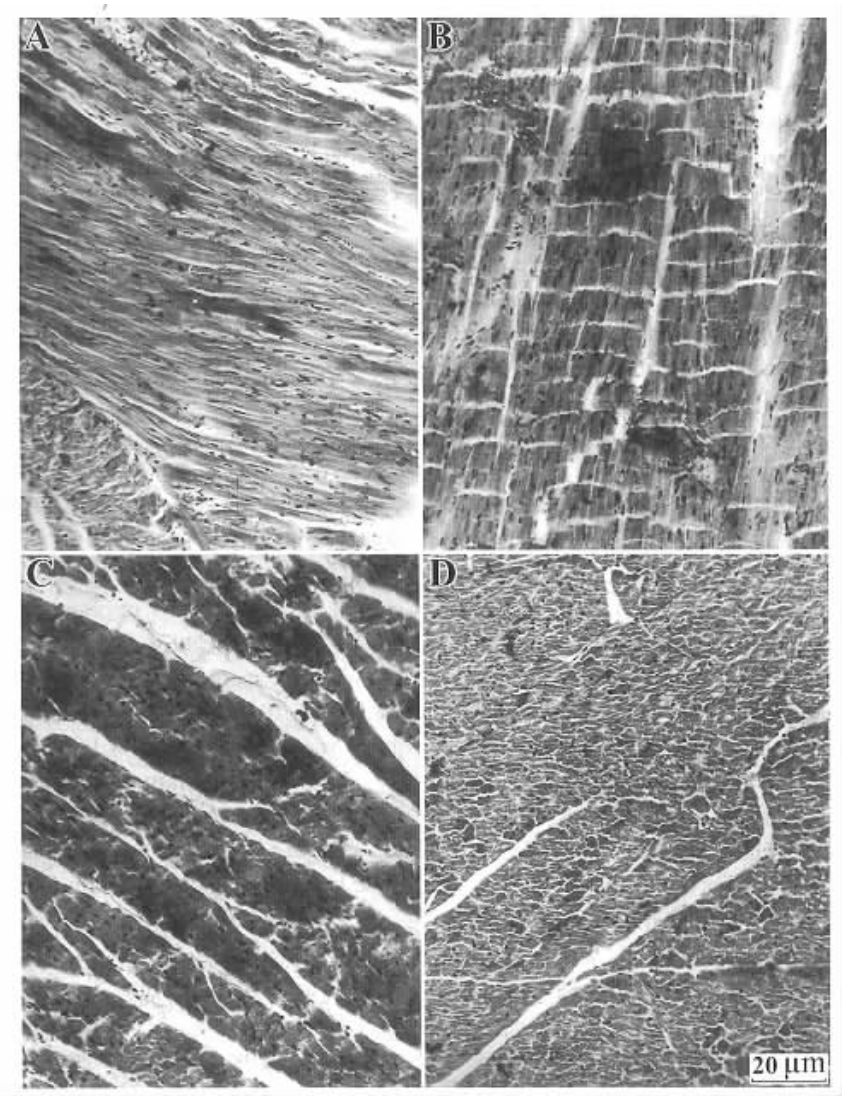

Fig 1. Dog myocardium. A) Sham group. B) Ischemia/reperfusion group. C) Bat-Ⅰ group. D) Bat-Ⅱ group. HE stain, ×400.

Plasma CK and LDH content Plasma CK levels at 30 min after ischemia and 90 min after reperfusion in I/R group were 1.1 and 2.3 times higher than those in sham group (Tab 1, $P<0.01$), while LDH concentration was increased by 75.6 % and 112.6 % (Tab 1, $P<0.01$), respectively. Compared with I/R group, plasma CK content at 30 min after ischemia and 90 min after reperfusion in Bat-Ⅰ group was lowered by 30.9 % ($P<0.05$) and 41.6 % ($P<0.01$), LDH content was lowered by 43.9 % and 37.9 % ($P<0.01$), respectively.

While in Bat-Ⅱ group, plasma CK content was reduced by 25.4 % and 30.8 % ($P<0.05$), LDH level was decreased by 34.7 % and 36.8 % ($P<0.01$), respectively (Tab 1).

DISCUSSION

We found that, on the whole, Bat apparently reduced animal mortality rate of I/R group.

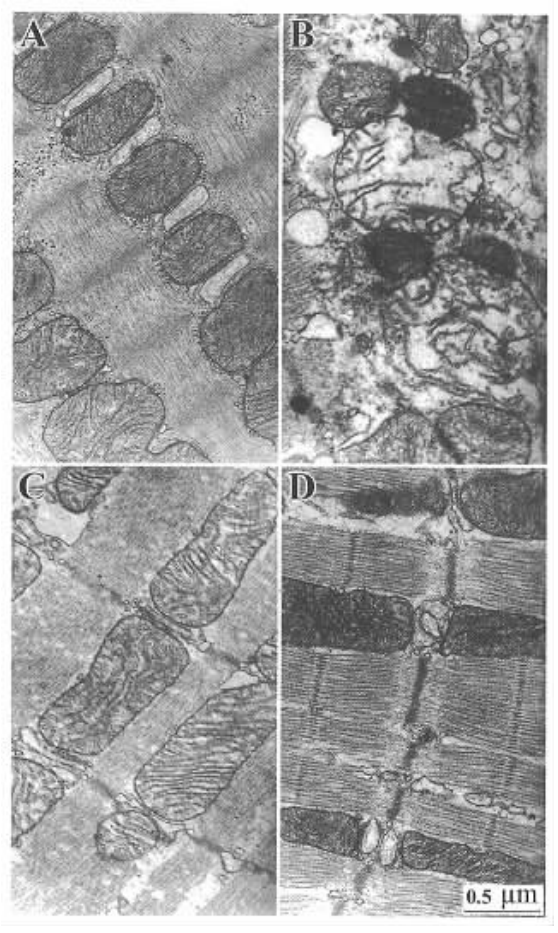

Fig 2. Transmission electron microscopy of dog myocardium. A) Sham group. B) Ischemia/reperfusion group. C) Bat-Ⅰ group. D) Bat-Ⅱ group. ×19000.

Pathomor- phological observation proved a modified myocardial damage induced by I/R in animals treated with Bat. Myocardial MDA content was decreased by more than one third. Meanwhile, plasma CK and LDH concen-trations were reduced significantly. Furthermore, our results showed that Bat apparently improved myocardial function injured by I/R, left ventricular dp/dt_{max} increased and LVEDP decreased significantly. It was worth noting that administration of Bat before and after myocardial ischemia had no significant difference not only in animal mortality rate and reduction of myocardial injury induced by I/R, but also in improvement of myocardial function and reduction of myocardial MDA production as well as plasma CK and LDH contents, which suggested a similar role against myocardial I/R injury by these two ways of Bat application. The mechanism of Bat against myocardial I/R injury and restoration of damaged myocardial function may be related to prevention of lipid peroxide in I/R myocardium, improvement of coronary dilatant function injured by I/R, and increase of coronary flow (data not show). Besides, the preventive role of Bat against thrombus formation may also have a beneficial effect on improvement of myocardial blood supply, and it may be involved in the role against myocardial I/R injury. A further study of Bat biological effect may show a new field of its application in the future.

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Tab 1. Effect of batroxobin on myocardial MDA, plasma CK, and LDH concentrations, $\pm dp/dt_{max}$, and LVEDP in ischemia/reperfusion heart of dog. $\bar{x} \pm s$. $^bP<0.05$, $^cP<0.01$ *vs* I/R group.

	Sham	I/R	Bat-Ⅰ	Bat-Ⅱ
Dogs	8	7	7	5
MDA (nmol/g protein)	1030 ± 45	2283 ± 91	1318 ± 41	1414 ± 56
CK ($U \cdot L^{-1}$)				
Before ISC	364 ± 44	374 ± 49	381 ± 30	392 ± 35
ISC 30 min	365 ± 40^c	769 ± 71	532 ± 62^b	574 ± 44^b
REP 90 min	396 ± 36^c	1323 ± 92	774 ± 72	916 ± 64^b
LDH ($U \cdot L^{-1}$)				
Before ISC	86 ± 8	95 ± 6	93 ± 16	90 ± 12
ISC 30 min	90 ± 9^c	159 ± 19	89 ± 4^c	104 ± 11^c
REP 90 min	94 ± 10^c	200 ± 25	124 ± 13^b	126 ± 14^b
$+dp/dt_{max}$ ($kPa \cdot s^{-1}$)				
Before ISC	745 ± 58	668 ± 65	745 ± 98	674 ± 62
ISC 30 min	771 ± 100^c	496 ± 15	758 ± 75^c	537 ± 35
REP 90 min	778 ± 90^c	352 ± 40	528 ± 38^c	452 ± 70^c
$-dp/dt_{max}$ ($kPa \cdot s^{-1}$)				
Before ISC	541 ± 81	563 ± 48	554 ± 56	489 ± 81
ISC 30 min	525 ± 76^c	355 ± 41	512 ± 59^c	381 ± 41^c
REP 90 min	496 ± 54	273 ± 32	362 ± 29	346 ± 27
LVEDP (kPa)				
Before ISC	−0.60 ± 0.17	−0.59 ± 0.17	−0.64 ± 0.12	−0.57 ± 0.07
ISC 30 min	−0.77 ± 0.13^c	−0.31 ± 0.07	−0.43 ± 0.05^b	−0.31 ± 0.07
REP 90 min	−0.76 ± 0.07^c	−0.20 ± 0.07	−0.44 ± 0.08^c	−0.31 ± 0.07^c

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巴曲酶对抗狗心脏缺血/再灌损伤

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关键词 蛇毒凝血酶；心肌再灌注损伤；心脏功能试验；乳酸脱氢酶；肌酸激酶；丙二醛；电子显微镜检查

目的：研究巴曲酶(Bat)对狗心脏缺血/再灌损伤的影响. **方法**：狗冠脉左前降枝结扎 30 min 后恢复血液灌注，于缺血前(Bat-Ⅰ组)或缺血后再灌前 15 min (Bat-Ⅱ组)静脉注射 Bat (1 $Bu \cdot kg^{-1}$). 测定 dp/dt_{max} 和 LVEDP 及血浆 CK 和 LDH 及心肌 MDA 含量，观察心肌病理形态学改变. **结果**：I/R 组动物缺血或再灌后死亡率高达65.0 %，心肌损伤明显. Bat-Ⅰ和 Bat-Ⅱ组动物的死亡率分别为30.0 %和 28.6 %，$P<0.05$，心肌损伤减轻；血浆 CK、LDH 含量，LVEDP 及心肌 MDA 含量降低；$+dp/dt_{max}$ 和 $-dp/dt_{max}$ 增加. **结论**：Bat 可明显拮抗狗心脏缺血/再灌注损伤，改善心功能.

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