The protection effects of different dosages of ulinastatin on myocardial

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Abstract

The aim is to explore the protection effect of ulinastatin (UTI) on myocardial in different dosages and its mechanism. From May to August 2014, in the animal laboratory of the 309th hospital of PLA, the experiment was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals for the National Institutes of Health. The animal protocol was approved by the Institutional Animal Care Committee of the 309th Hospital of PLA (Permit Number: 13-269). 120 SD rats were randomly divided into control group (treated with normal saline), UTI low-dose group (30000 IU/kg), UTI moderate-dose group (60000 IU/kg) and high-dose group (90000 IU/kg). All rats were treated with UTI for 2 weeks via vein. Then left ventricular end diastolic diameter (LVEDD), left ventricular endsystolic dimension (LVESD) and left ventricular ejection fraction (LVEF) were employed to evaluate heart function. Hyp determination with chloramine-T oxidation method was employed to detect the myocardial fibrosis degree, while brain natriuretic peptide (BNP) was used to assess heart function. By comparison among groups, the results of the high-dose group were better than that of any other group in LVEDD, LVESD, LVEF, BNP content, as well as in heart function.

It is better for myocardial protection with high-dose of ulinastatin, the possible mechanism is relevant to inhibit myocardial interstitial fibrosis and ventricular remodeling.

Introduction

As the advantages of urinary protease inhibitors, ulinastatin (UTI), such as improving organs ischemia/reperfusion, and anti-inflammatory effects, it is widely used in cardiac protection under the conditions of cardiopulmonary resuscitation, heart failure and the others [1-5]. However, clinically, the myocardial protective effect of UTI is not obvious at low dosage level. Therefore, we supposed that its therapeutic effect may be dose-related. In this study, we explored myocardial protective effect of UTI in different dosages and its mechanism.

Materials and methods

From May to August 2014, in the animal laboratory of the 309th hospital of PLA, the experiment was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals for the National Institutes of Health. The animal protocol was approved by the Institutional Animal Care Committee of the 309th Hospital of PLA (Permit Number: 13-269). We used doxorubicin-induced cardiomyopathy as the model of this study. In 11 weeks, the rats were treated every week by intraperitoneal injection with 2.8 mg/kg doxorubicin. In the 12th week, without intraperitoneal injection, the models were observed to confirm [6]. 120 SD rats were randomly divided into control group (treated with normal saline), UTI low-dose group (30000 IU/kg), UTI moderate-dose group (60000 IU/kg) and high-dose group (90000 IU/kg) [7-8]. All rats were treated with UTI for 2 weeks by tail vein injection with digoxin, by nasogastric tube with valsartan , and by vein with UTI.

Left ventricular end diastolic diameter (LVEDD), left ventricular endsystolic dimension (LVESD) and left ventricular ejection fraction (LVEF) were employed to evaluate heart function by echocardiography before the experiment and two weeks after.

The measurement of Hyp (hydroxyproline) was based onchloramine-T oxidation method. Left ventricular myocardia (30~100 mg wet tissue) were placed in tubes, then mixed with 1 ml hydrolysis liquid at 95°C for 20 mins. After cooled, sample solutions were added with indicator agent, then adjusted to pH 6~6.8, mixed with activated carbon, and centrifuged at 3500 r/min for 10 minutes. Supernatant fluid was collected with added with agent A, B, C (Hyp Elisa Kit, Puyuan Company, China) 0.5 ml each for 5 mins. The supernatant fluid was detected at 550 nm by UV-Vis spectrophotometer.

Before treatment and after two weeks, blood samples, after overnight fasting, were prepared by collecting venous blood. Plasma Brain natriuretic peptide (BNP) levels were determined with rapid fluorescence immunoassay.

Results

Compared with the control group, after applied UTI, the heart function of SD rats in any group was significantly improved (LVEDD and LVESD decreased, while LVEF and FS improved), and moreover

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Table 1. The results of evaluation of cardiac function.

Item	Control		Low-dose		Moderate-dose		High-dose	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
LVEDD/mm	1.29 ± 0.01	1.53 ± 0.13	1.29 ± 0.06	1.21 ± 0.02[a]	1.35 ± 0.14	1.21 ± 0.09[a]	1.33 ± 0.23	1.01 ± 0.01[a,b]
LVESD/mm	1.09 ± 0.02	1.23 ± 0.01	1.09 ± 0.03	0.98 ± 0.04[a]	1.03 ± 0.17	0.98 ± 0.12[a]	1.07 ± 0.19	0.99 ± 0.03[a,b]
FS/%	37.51 ± 1.21	13.8 ± 0.36	27.43 ± 2.11	27.38 ± 3.12[a]	23.68 ± 1.25	26.98 ± 2.02[a]	20.62 ± 1.37	32.12 ± 2.14[a,b]
LVEF/%	52.58 ± 2.13	32.49 ± 1.29	49.78 ± 1.29	53.22 ± 2.19[a]	51.32 ± 2.19	56.91 ± 1.87[a]	54.28 ± 1.20	59.96 ± 2.10[a,b]

[a]: compared to the control group, $p<0.05$; [b]: compared to the other three groups, $p<0.05$

Table 2. The results of BNP before and after treatment (ng/L).

Group	Before treatment	After treatment
Control	4917.51 ± 85.82	3783.82 ± 89.17[a,b]
Low-dose	4878.84 ± 89.21	3272.91 ± 73.41[a,b]
Moderate-dose	4983.94 ± 93.33	2884.62 ± 70.94[a,b]
High-dose	4894.17 ± 99.45	1689.33 ± 74.86[a,b]

[a]:compared to the control group, $p<0.05$; [b]: compared to the high-dose group, $p<0.05$

high-dose group of UTI was better than the other three groups in heart function and left ventricular internal diameter improvement (Table 1).

The Hyp content of the control group, low-dose, moderate-dose and high-dose group is (40.16 ± 2.31) mg/g, (34.24 ± 1.98) mg/g, (22.79 ± 1.69) mg/g, and (19.84 ± 1.47) mg/g, respectively. Compared to the control, in three applied UTI groups, the difference of Hyp before and after treatment increased with the increase of UTI.

Before treatment, plasma BNP levels of four groups were higher than normal, consisting with cardiac dysfunction characteristic. After applied UTI, the value of the four groups was significantly decreased, especially in lager-dose group (Table 2).

Discussion

Many researchers have proved the myocardial protective effect of UTI. However, the association between the clinical efficacy and dosage of UTI has not been confirmed. Moreover, the optimal dosage is also uncertain. In this study, we explored and analyzed therapeutic effect of UTI at different dosage, and its probable mechanism.

In any group after treatment with UTI, LVEDD, LECSD, and plasma BNP decreased. However, LVEF was improved significantly. The most obvious change appeared in the high dose group, so high dose group have a stronger protective effect on myocardial, which suggested clinical effect should be dose-dependent within a certain range. There are the following probable reasons: (i) Inhibiting ventricular remodeling. Applying UTI may make inside diameter of ventricular smaller, while the fractional shortening of left ventricular increases. It was indicated that UTI may inhibit ventricular remodeling after heart failure, and the effect is most obvious under the condition of high dosage [9-11]. (ii) Inhibiting myocardial interstitial fibrosis. Fibrillar collagen types I and III are the major components of the myocardial collagen matrix. Collagen type I has been found to represent nearly 80% of the total collagen protein, while type III collagen is present in lower proportions (approximately 11%). Hydroxyproline, whose content determines myocardial interstitial fibrosis, is the main component constituting the collagen fibers. In the high-dose group, myocardial had the lowest content of Hyp, and the interstitial myocardial fibrosis was in low level, which was prompted that myocardial collagen fiber synthesis was inhibited. So we can conclude that a high dose of UTI can inhibit myocardial interstitial fibrosis, thus effectively improving cardiac function [12,13]. (iii) Antagonizing myocardial oxygen radicals.

Myocardial lipid peroxidation damage is an important pathogenic mechanism of doxorubicin-induced dilated myocardiopathy. One of the pharmacological effects of UTI are inhibiting the inflammatory response and reducing the release of oxygen free radicals, thereby reducing the consumption of serum SOD in order to increase SOD activity. High dose of UTI can effectively reduce SOD consumption and clear superoxide anion radicals in order to protect myocardial from damage ultimately [14,15].

This study has confirmed the protective effect on myocardial cells is dose-related and there is a dose-dependent relationship between the overall effect of UTI and the dosage in a certain range. As a supplementary measure of heart failure drug therapy, it is worth being applied in clinical. Moreover, for the optimal dosage, further exploration is needed.

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