Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading doi: 10.1016/S1995-7645(14)60064-X

Effect of atorvastatin on serum oxidative stress and N-terminal brain natriuretic peptide expression in rats

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ARTICLE INFO

Article history:
Received 10 December 2013
Received in revised form 15 January 2014
Accepted 15 March 2014
Available online 20 May 2014

Keywords:
Atorvastatin
Myocardial ischemia-reperfusion
Oxidative stress
n-terminal pro-brain natriuretic peptide

ABSTRACT

Objective: To investigate the effect of atorvastatin on serum oxidative stress and *N*-terminal brain natriuretic peptide expression in rats. **Methods:** A total of 40 healthy male SD rats were randomly divided into the sham group (Group A, *n*=10, saline 5 mL/d), ischemia-reperfusion group (Group B, *n*=10, saline 5 mL/d), atorvastatin group (Group C, *n*=10, atorvastatin 20 mg/kg•d), atorvastatin + N-amino-arginine group (Group D, *n*=10, atorvastatin 20 mg/kg•d + N-amino arginine 15 mg/kg). Myocardial ischemia-reperfusion rat model was established after 3 days of gavage. N-amino arginine 15 mg/kg was given by tail vein injection 15 min before ischemia. After reperfusion, enzymology indicators such us creatine kinase (CK) and lactate dehydrogenase and the oxidative stress parameters such as nitric oxide (NO), malondialdehyde (MDA) and total superoxide dismutase (TSOD), and *n*-terminal pro-brain natriuretic peptide (NT-proBNP) expression was detected by immunohistochemistry. **Results:** LDH and CK levels of group A were significantly lower than the other three groups, and group B was the highest. There was significant difference between group B and group C ($P<0.05$), and no significant difference between group B and group D ($P>0.05$). MDA levels in group B were significantly higher than the other three groups. The lowest was group A, followed by group C, the difference among groups was significantly ($P<0.05$). TSOD and NO levels in group B was the lowest, the level in group A was the highest, followed by group C, the difference among groups was significant ($P<0.05$). NT-proBNP level in group B was significantly higher than the other three groups, the lowest was group A, followed by group C, the difference among groups was significant ($P<0.05$). **Conclusions:** Atorvastatin has a protective effect on the myocardial injury in the myocardial ischemia and reperfusion rats. It can increase NO synthesis and decrease MDA content, increase serum TSOD activity and the oxidative stress effect, meanwhile protect myocardial cells and reduce myocardial injury.

1. Introduction

Statins are HMG coenzyme A reductase inhibitors[1],this kind of drug can competitively inhibit endogenous cholesterol synthesis rate-limiting enzyme reductase, block the intracellular mevalonate pathway, reduce intracellular cholesterol synthesis, then stimulate LDL more on cell surface[2],increase the activity and the number of receptors and serum cholesterol removal, which is widely used in clinical to regulate blood lipids. Recent studies have found that statins can not only regulate blood lipids, but also protect cardiovascular, such as delay atherosclerosis, inhibit endothelial inflammation, anti-inflammatory and anti-thrombosis[3]. This study established myocardial ischemia-reperfusion model to explore the impact of atorvastatin on serum oxidative stress and N-terminal brain natriuretic peptide expression.

2. Materials and methods

2.1. Animals, reagents and instruments

40 male SD (Spragu-Dawley) rats weighing 250-300 g

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Foundation project: It is supported by Science and Technology Department General Project in Hunan Province (2012SK3127).

were provided by Beijing Vital River Laboratory Animal Technology Co., Ltd. License No.: SCXK (Beijing) 2002-0003. Atorvastatin (Lipitor; specifications 20 mg; Zhunzi J20120049) was purchased from Pfizer. *N*-amino-arginine (L-NNA) was purchased from Shanghai XinRan research reagents and detection assays center. Hoechst was purchased from Shenyang Biyuntian Institute of Biotechnology. Malondialdehyde (MDA) test kit (HY-60003 KIT) was from Beijing Huaying Biotechnology Institute. Nitric oxides (NO), total superoxide dismutase (TSOD), lactate dehydrogenase (LDH), stimulated kinase (CK) kit were from Nanjing Jiancheng Bioengineering Institute. High-speed refrigerated centrifuge was from Beckman Allerga 25R. High-speed desktop centrifuge was from Sigma company. Fluorescent spectrophotometer (RF-5301PC) was from Shimadzu Corporation. NT-proBNP ELISA kit was from U.S. Rapidbio Biosource company. Ultra-low temperature freezer (-86 ℃) was from SSNYO Japan.

2.2. Grouping and modeling

(Group A, n=10, saline 5 mL/d), ischemia-reperfusion group (Group B, n=10, saline 5 mL/d), atorvastatin group (Group C, n=10, atorvastatin 20 mg/kg•d), atorvastatin + *N*-amino-arginine group (Group D, n=10, atorvastatin 20 mg/kg•d + *N*-amino arginine 15 mg/kg). Myocardial ischemia-reperfusion rat model was established after 3 days of gavage. *N*-amino arginine 15 mg/kg was given by tail vein injection 15 min before ischemia.

The rats were anesthetized with ether at supine position, fixed on the operating table. After endotracheal intubation, they were connected with ventilator (tidal volume 30 mL, frequency of 55 times/min), then connected with II-lead ECG for ECG recording[4],meanwhile connected with ECG Monitor to observe ECG changes. Chest was opened from the 3rd or 4th left intercostal to expose the heart. It was threaded at 2-3 mm of left anterior descending branch of coronary artery[5] between the left atrium and pulmonary artery cone. In sham group only threading was performed without ligation, while in the rest groups the coronary artery was immediately ligated with No. 0 thread after 15 min. After 30 min of ischemia, thread was cut to regain blood stream. Reperfusion was carried out for 120 min. Rats were sacrificed and the hearts were obtained. Successful criteria of ligation were as follows: ECG showed ST segment elevation or R wave rose; ligature distal myocardial was in cyanosis color. Successful criteria of reperfusion were as follows: the ligature was loosened to restore coronary blood, the color of myocardial ischemic area recover , ST segment elevation was reduced by more than 50%[6].

2.3. Index measurement

After reperfusion, serum enzyme indicators of rats such as serum LDH and CK, oxidative stress indicators such as NO, MDA and TSOD was measured, and *N*-terminal brain natriuretic peptide precursor expression was detected by immunohistochemical assay. After reperfusion for 120 min, 1 mL carotid artery blood was obtained from each group. After centrifugation, the supernatant was collected. CK and LDH level was measured according to kit instructions. After the hearts removing, the left ventricular anterior wall was obtained, the trace of blood was dried by filter paper. It was stored in a refrigerator (-70 ℃). After the experiment, the MDA was detected by thiobarbituric acid[7], xanthine oxidase method was used to detect TSOD, and Griess reagent was used to detect serum NO.

Blood samples were collected on the last day of the experiment, serum NT-proBNP levels were quantitatively determinated by chemiluminescence method.

2.4. Statistical analysis

Data were analyzed by SPSS 16.0 statistics software, t-test and χ^2 testwas adopted and the data were expressed as mean±SD values. Repeated measurement data was analyzed by variance F test, multiple comparison by LSD-t test. P<0.05 was regarded as statistical significant difference.

3. Results

3.1. Serum enzymes

LDH and CK levels of group A were significantly lower than the other three groups, and the level in group B was the highest. There was significant difference between group B and group C (P<0.05), and no significant difference between group B and group D (P>0.05) (Table 1).

Table 1
Serum enzymes indicators of rats in each group (mean±SD).

Groups	Number of cases	LDH (U/L)	CK (μ mol/L)
Group A	10	932.58±15.55	55.89±2.18
Group B	10	1 488.17±71.46*	95.57±1.82*
Group C	10	1 113.34±42.77$^{*\#}$	63.54±3.55$^{*\#}$
Group D	10	1 453.44±37.11$^{*\triangle}$	93.18±2.17$^{*\triangle}$

Note: Compared with group A, * P<0.05; compared with group B, # P<0.05; compared with group C, $^{\triangle}$ P<0.05.

3.2. Oxidative stress

MDA levels in group B were significantly higher than the