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

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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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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 $\chi^2$ 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

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 levels in group A was the highest, followed by group C, the difference among groups was significant ($P<0.05$) (Table 2).

**Table 2**

Oxidative stress indicators of rats in each group (mean±SD).

| Groups | Number of cases | MDA (nmol/mgprot) | TSOD (U/mgprot) | NO (μ mol/L) |
|---|---|---|---|---|
| Group A | 10 | 2.35±0.13 | 135.4±4.2 | 55.3±3.9 |
| Group B | 10 | 5.00±0.17* | 88.9±2.6* | 17.5±3.0* |
| Group C | 10 | 3.54±0.09*# | 118.7±3.9*# | 40.6±3.1*# |
| Group D | 10 | 4.93±0.15*△ | 101.6±4.0*#△ | 19.2±2.1*△ |

Note: Compared with group A, * $P<0.05$; compared with group B, # $P<0.05$; compared with group C, △ $P<0.05$.

### 3.3. NT-proBNP determination

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 significantly ($P<0.05$)(Table 3).

**Table 3**

NT-proBNP determination of rats in each group (mean±SD).

| Groups | Number of cases | NT-proBNP (pg/mL) |
|---|---|---|
| Group A | 10 | 117.61±13.29 |
| Group B | 10 | 293.46±43.31* |
| Group C | 10 | 184.74±26.89*# |
| Group D | 10 | 242.25±27.44*#△ |

Note: Compared with group A, * $P<0.05$; compared with group B, # $P<0.05$; compared with group C, △ $P<0.05$.

## 4. Discussion

Oxidative stress refers to the imbalance of anti-oxidation and oxidation *in vivo*, which tends to oxidation. It can lead to the inflammatory infiltration of neutrophil, protease secretion increase, also produce a large number of intermediate products, such as free radicals, causing human diseases or aging[8]. SD rat occurs reperfusion injury(I/R) after myocardial ischemia-reperfusion. The pathological mechanism of I/R is still not clear in clinical. However, most scholars believe that it is closely related to the excessive production of oxygen free radicals after minutes of reperfusion and intracellular calcium overload or redistribution[9].During the first few minutes of reperfusion, the rich blood supply caused the outbreak of hydroxyl, superoxide anion, peroxynitrite and other oxygen free radical, which can occur reaction with various cellular components such as proteins, phospholipids, nucleic acids, and lead to cell structure damage and functional metabolism disorders[10].Once the free radicals are generated, intermediate metabolite can extend continuously and generate new free radicals and form a chain reaction. During early I/R, the release of free radicals and reperfusion-induced anti-oxidants decrease, leading to easily damaged myocardial cells. The protective effect of endogenous antioxidant system is difficult to remove a large number of OFR,meanwhile OFR can stimulate endothelial cells to release platelet-activating factor[11,12], and then attract the neutrophils, resulting in an increase of oxygen free radicals, aggravate the degree of reperfusion injury.

LDH and CK as serum enzyme indicators for reminder myocardial ischemia - reperfusion injury, its levels rise showed successful model[13]. In this study, the highest level of it is group B, group A has the lowest level, group C is closer to group A, which indicated the ischemia-reperfusion group have the most serious damage. Atorvastatin may inhibit ischemia-reperfusion injury, but after adding NO synthase inhibitor L-NNA, the level of group D is closer to group B, which showed atorvastatin can reduce I/R injury and that is related with increased NO.

Serum NO reduce is the sign of I/R injury[14,15]. NO is a very unstable biological radicals, the generation of which relies on the nitric oxide synthase.After NO synthesized into the blood, it can promote the body's blood circulation to protect vascular tissue. Meanwhile NO can effectively remove excess free radicals and inhibit oxidative stress[16] to maintain cardiovascular health. In this study, the lowest level of NO is group B, followed by group D, the highest level is in group A. Further prompted the reason atorvastatin can inhibit I/R injury is related with increased NO synthesis and inhibit oxidative stress.

MDA, TSOD are used as indicators of oxidative stress status[17-19]. MDA is the major end product of lipid metabolism after the biological membrane damage by free radicals[20], which may lead to biofilm polyunsaturated fatty acid peroxidation and induced cell damage and protein synthesis disorders[21]. Therefore detecting MDA may reflect the degree of lipid peroxidation and indirectly reflect the degree of cell damage. SOD is one of the most important antioxidant enzymes *in vivo* which can specifically clear superoxide anion and block the damage to cells caused by oxygen free radicals[22] and timely impaired cells[23]. In this study, atorvastatin group can reduce the MDA level and increased TSOD level of I/R rats. That prompted atorvastatin can improve the body's ability to eliminate oxygen free radicals and repair autologous cells, meanwhile inhibit lipid peroxidation.

NT-proBNP as the most sensitive and specific indicator of myocardial damage will be increase in a variety of pathological conditions[24,25]. In this study, ischemia-reperfusion group have the highest NT-proBNP, atorvastatin group is closer to the sham group which have the lowest NT-proBNP level, which prompted atorvastatin have a protective effect on myocardial cells.

In summary, atorvastatin has a protective effect on the myocardial injury in the myocardial ischemia and reperfusion rats. The mechanism may be related to 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.

## Conflict of interest statement

We declare that we have no conflict of interest.

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