Pharmacology of atorvastatin on myocardial ischemia-reperfusion in rats and drug effect analysis

Bingfeng Cao[1], Guofeng Bi[2] and Yufeng Wang[1]*
[1]Department of Cardiology, Weihai Central Hospital, Weihai, China
[2]Department of Electrophysiological Examination, Weihai Central Hospital, Weihai, China

Abstract: Statins are the most important drugs in the treatment of atherosclerosis. In this paper, the authors analyze the protective effect of atorvastatin on myocardial ischemia-reperfusion in rats and its drug effect. The results showed that the ratio of myocardial infarction area to total ischemia area in atorvastatin group was smaller than that in ischemia reperfusion group (P<0.01). Compared with the ischemia-reperfusion group, the MDA (16.23±4.05), TNF alpha (41.84 ±5.61) and MPO (17.54±2.81) were decreased in atorvastatin group. The results showed that atorvastatin could improve many hemodynamic indexes including SBP, DBP, LVSP, LVEDP and so on. To sum up, atorvastatin can affect infarct size, improve hemodynamics and left ventricular function after myocardial ischemia-reperfusion injury.

Keywords: Atorvastatin, myocardial ischemia-reperfusion, apoptosis, oxidative stress, lipid regulating drugs.

INTRODUCTION

With the improvement of living standards, cardiovascular diseases have become the main killer of human health and life. Long-term myocardial ischemia can cause tissue damage and even cell death (Balasubramaniam *et al.*, 2014). The key to solving ischemic injury is coronary artery recanalization. However, reperfusion after long-term myocardial ischemia often leads to more serious injury (Chang *et al.*, 2016). This phenomenon is called ischemia/reperfusion (I/R). Ischemia reperfusion injury is an important complication after coronary artery recanalization, such as thrombolysis, PTCA or bypass. How to not only restore the blood perfusion of ischemic tissue, but also reduce the occurrence of I/R injury has become a hot issue of current research. In 1986, Murry proposed the concept of ischemic preconditioning (IPC), i.e. ischemic preconditioning (IPC), in which the myocardium undergoes several transient ischemia prior to long-term ischemia, and the myocardium can tolerate longer ischemia (Dinicolantonio *et al.,* 2014). Statins are the most important drugs in the treatment of atherosclerosis. Studies have shown that long-term use of statins can not only improve long-term prognosis (Dalal *et al.,* 2017), but also reduce the elevation of myocardial injury markers after percutaneous transluminal coronary intervention (PCI). Randomized controlled trials over the past two years have further shown that a higher dose of statins (whether statins are being used or not) 24 hours before PCI can significantly reduce the elevation of myocardial injury markers after PCI (Dan *et al.*, 2015; Orivaldo *et al.*, 2018).

Early recovery of myocardial perfusion through thrombolysis or percutaneous coronary intervention (PCI) after myocardial infarction is an important means to reduce the size of myocardial infarction and improve clinical prognosis (Fuu *et al.*, 2017; Navaro, 2018). However, the recovery of ischemic myocardial blood flow may lead to ischemia reperfusion injury (IRI), which may reduce the efficacy of myocardial reperfusion. Statins drugs commonly used to regulate blood lipids and reduce plasma cholesterol and lipoprotein levels (Gaopeng *et al.*, 2015; Shi *et al.,* 2018). Recent studies have shown that these drugs have multiple cardiovascular protective functions (Ghoneum *et al.,* 2015). In this study, the myocardial ischemia-reperfusion model of rats was used to study the protective effect of atorv astatin on myocardium. This research could be useful for further study and clinical drug use.

MATERIALS AND METHODS

Research object

60 clean SD rats aged 6-8 weeks were selected and provided by Animal studies department. They were 50% male and 50% female, weighing 180-250g. All 60 rats were randomly divided into 3 different groups as atorvastatin group (n=20), ischemia-reperfusion group (n =20) and control group (n=20): Atorvastatin group: 1 week before operation, 15mg/kg of Atorvastatin and 2 ml of normal saline were administered orally; ischemia-reperfusion group and control group: 2ml of normal saline were administered orally once a day. All rats were fasted for 12h before operation. The control group was set up to eliminate the influence of feeding conditions, saline irrigation and routine operation on the experimental results.

The animal experiment program has been approved by the experimental animal ethics committee, No.DCWCHP/ 16ST.

**Corresponding author:* e-mail: bgf70@163.com

Preparation of myocardial ischemia reperfusion model

Intraperitoneal injection of pentobarbital sodium 60 mg/kg anesthetized rats, and fixed the head and limbs on the operating table. Connect the electrocardiograph with standard lead. The left anterior descending coronary artery (LAD) was separated and ligated at the cone of pulmonary artery and 2-3 mm below the left atrial appendage between left ventricle and left ventricle in atorvastatin group and ischemia-reperfusion group. Bradycardia, dark red myocardium, elevation of ST segment and/or elevation of T wave in ECG were observed. The heart was homed and timed 30min, the heart was removed again, the ligature was removed and the reperfusion was 3 hours.

Measurement of infarct size

Three hours after reperfusion, 10 rats in each group were taken, 20 rats in the Atorvastatin group and the ischemia-reperfusion group were ligated in situ and 3mL Evans blue (10g/L) was injected into the apex of the heart for staining. Rats were sacrificed and their hearts were quickly removed. Atrial, right ventricle and large blood vessels were washed out with normal saline. Five thin slices of 2 mm were cut along the long axis and immersed in TTC phosphate buffer solution (0.5mg/mL, pH 7.4). 30 min was incubated at 37^{o}C for dyeing. After TTC and Evans blue staining, myocardial tissue was divided into three parts: The blue staining part (Evans blue staining part) was normal myocardial tissue, the red staining part (TTC staining part) was ischemic myocardial tissue without infarction and the gray-white part was ischemic myocardial tissue. The photos were taken and calculated the area of each part with image analysis software.

Determination of malondialde Hyde (MDA), tumor necrosis factor alpha (TNF alpha) and myeloperoxidase (MPO) in myocardium of ischemic non-infarct area

The myocardial tissue of rats with left ventricular ischemia and non-infarction was taken from 0.7 to 1.0g (the same position as the control group), mixed with lysate and normal saline. The 10% tissue homogenate was prepared by Omni Prep auto-tissue homogenizer. The supernatant was centrifuged for 10 minutes with a low-temperature and low-speed centrifuge for 3000 r/min. The content of MDA was determined by thiobarbituric acid (TBA) and the contents of TNF_a and MPO were determined by enzyme_linked immuno sorbent assay (ELISA). All operations were carried out strictly according to the instructions of the kit.

Detection of related indicators

The indexes of ventricular remodeling were measured. The rats were sacrificed after measuring body mass and the hearts were separated by thoracotomy. The relative mass of left and right ventricles (relative mass of left and right ventricles = mass / mass of left and right ventricles) was measured and the thickness of ventricular septum was measured.

STATISTICAL ANALYSIS

SPSS 17 statistical software was used, and all data has carried on the statistical tests. The measurement data were expressed by X± s. The comparison between the 2 groups was conducted by two independent sample t-test. Chi square test was used to compare the difference between counting data and constituent ratios. And $P<0.05$ means statistically significant.

RESULTS

Myocardial infarction area measurement results

The area of myocardial infarction in the Atorvastatin group was smaller than that in the ischemia-reperfusion group and the area of ischemia-reperfusion group was larger than that in the ischemia-reperfusion group ($P< 0.05$). The total area of ischemia in the Atorvastatin group was smaller than that in the ischemia-reperfusion group ($P >0.05$). The ratio of myocardial infarction area to total ischemia area in atorvastatin group was smaller than that in ischemia reperfusion group ($P<0.01$), as shown in table 1. Myocardial HE staining in each group was shown in fig. 1.

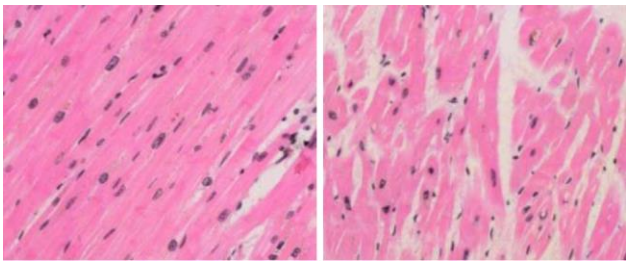

Fig. 1: HE staining of myocardium in rats of each group

Determination results of MDA, TNF-α and MPO contents

Compared with the control group, MDA, TNF-a and MPO were increased in the ischemia-reperfusion group and atorvastatin group ($P<0.05$). Compared with ischemia-reperfusion group, MDA, TNF-a and MPO in atorvastatin group were decreased ($P<0.05$) (table 2).

Hemodynamic measurements

Compared with the control group, HR increased, SBP, DBP and LVSP decreased, LVEDP increased in the ischemia reperfusion group ($P<0.05$). Compared with ischemia-reperfusion group, HR decreased, SBP, DBP, LVSP increased, LVEDP and LVEDP decreased in atorvastatin group ($P<0.05$) (table 3).

Measurement results of ventricular remodeling

There was no significant difference in body mass between the two groups ($P>0.05$). Compared with the control group, the relative weight of left and right ventricles and the thickness of interventricular septum in the ischemia-reperfusion group were larger ($P<0.05$). Compared with

Table 1: Comparison of myocardial infarction area

Grouping	Myocardial infarct ratio (%)	Ischemic not myocardial infarct ratio (%)	Ischemic myocardium ratio (%)	Myocardial infarct size/ischemic myocardium size (%)
Ischemia reperfusion group	14.27±1.58	32.16±5.14	55.21±8.47	28.14±6.51
Atorvastatin group	22.65±1.76	21.08±3.48	42.74±5.12	55.18±12.08

Table 2: Comparison of the results of MDA, TNF-α and MPO in rats of each group

Grouping	MDA(μmol/L)	TNF-α(μg/L)	MPO(μ g/L)
Control group	4.29±1.56	15.27±1.89	4.23±0.78
Ischemia reperfusion group	16.23±4.05	41.84±5.61	17.54±2.81
Atorvastatin group	9.54±2.78	31.54±3.48	11.04±1.24

Table 3: Comparison of hemodynamic results in rats of each group

Grouping	HR (BPM)	SBP (mmHg)	DBP (mmHg)	LVSP (mmHg)	LVEDP (mmHg)
control group	315.18±32.05	175.12±7.45	159.46±5.87	164.20±5.84	26.2±1.94
Ischemia reperfusion group	465.72±43.21	134.49±11.28	126.91±5.13	127.56±12.74	31.25±8.49
Atorvastatin group	412.64±31.48	158.26±6.54	138.29±8.36	152.18±12.40	17.42±6.18

Table 4: Comparison of ventricular remodeling in rats of each group

Grouping	BW(g)	LVRW(mg/g)	RVRW(mg/g)	IVST(mm)
Control group	367.31±38.12	1.59±0.35	0.57±0.08	1.46±0.34
Ischemia reperfusion group	334.27±59.26	2.48±0.41	0.71±0.25	2.81±0.46
Atorvastatin group	342.59±62.47	1.84±0.24	0.49±0.12	2.16±0.49

the ischemia-reperfusion group, the relative mass of left and right ventricles in atorvastatin group was smaller and the thickness of ventricular septum was narrower ($P< 0.05$), as shown in table 4.

DISCUSSION

Statins are widely used lipid-lowering drugs. Studies have shown that statins can reduce the level of myocardial injury after myocardial ischemia reperfusion and improve long-term prognosis (Hausenloy *et al.*, 2015; Herrera *et al.*, 2018). The results showed that there was no difference in total ischemic area between the two groups, but the myocardial infarction area was smaller than that of the ischemia-reperfusion group, the area without ischemia-reperfusion group was larger than that of the ischemia-reperfusion group and the proportion of myocardial infarction area to total ischemia-reperfusion group was smaller than that of the ischemia-reperfusion group (Hsuan *et al.*, 2016; Samano *et al.*, 2018). The significance of research is consistent with previous studies. These results suggest that atorvastatin may reduce the area of myocardial infarction, thereby reducing the injury caused by reperfusion, but not by reducing the area of ischemia to play a protective role (Li *et al.*, 2014; Alejandrina *et al.*, 2018).

The significant increase of free radicals is closely related to the injury after myocardial ischemia and reperfusion (Wang, 2016; Zhu *et al.*, 2015). MDA as the end product of lipid peroxide, its content can reflect the degree of oxygen free radical damage (Luo, 2015). The results of this study showed that compared with the control group, MDA in the ischemia-reperfusion group and atorvastatin group increased, but the increase in atorvastatin group was less than that in the ischemia-reperfusion group (Meybohm *et al.*, 2015). TNF-a is an important inflammatory cytokine, which can aggravate IRI by stimulating the production of oxygen free radicals and activating cardiomyocyte apoptosis (Xiong *et al.*, 2015; Peterzan *et al.*, 2017). It was found that the content of TNF-a increased significantly after myocardial ischemia-reperfusion in rats, which was consistent with the results of this study, but also showed that the effect of atorvastatin on TNF-a increased slightly in rats (Oka *et al.*, 2014). Activation of neutrophils plays an important role in IRI. MPO, as a marker, is closely related to oxidative stress during inflammatory reaction and tissue damage leading to IRI (Sheng *et al.*, 2015).

The results of this study showed that MPO increased in both ischemia reperfusion group and atorvastatin group, but the increase in MPO in atorvastatin group was less

than that in ischemia reperfusion group (Pistevou *et al.*, 2015). The results showed that atorvastatin could improve many hemodynamic parameters including SBP, DBP, LVSP, LVEDP, the maximum rate of left ventricular pressure rise and the maximum rate of left ventricular pressure drop (Reber *et al.*, 2013; Wei *et al.*, 2017). The results of measurement of ventricular remodeling showed that atorvastatin could effectively inhibit left ventricular dilatation after ischemia-reperfusion, improve left ventricular function and alleviate myocardial injury caused by reperfusion (Stephen *et al.*, 2016). Other animal studies have shown that the protective effect of atorvastatin may be related to the repair of damaged endothelial cells, the promotion of mitochondrial ATP-sensitive potassium channel opening and the regulation of nitric oxide production, but the mechanism remains to be further studied (Schmidt *et al.*, 2013).

CONCLUSION

Reperfusion therapy of ischemic myocardium is one of the main treatments for ischemic heart disease, and the accompanying ischemic reperfusion injury has become an important factor affecting the curative effect. Statins can reduce myocardial ischemia reperfusion injury and play a protective role in cardiovascular system. To sum up, atorvastatin can affect the infarct size, improve hemodynamics and left ventricular function after myocardial ischemia reperfusion injury, which provides an important theoretical basis for further study of statins in the treatment of myocardial IRI.

REFERENCES

Alejandrina V, Carmen VM, Elia H, Martinez M and Alma G (2018). Pomegranate juice consumption (*Punica granatum*) and its effect on glycemia, lipid profile and histology of the pancreas in a model of induced hyperglycemia by streptozotocin, *Arch. Latino. Nutr.,* **68**(1): 29-40.

Balasubramaniam A, Kuperstein E (2014). Update on oral herpes virus infections. *Dent. Clin. N. Am.*, **58**: 265-280.

Chang SY, Deu JM, Yoonae K and Ji M (2016). Identification of pyrrolo[3,2-c]pyridin-4-amine compounds as a new class of entry inhibitors against influenza viruses *in vitro*. *Bioche. Biophy. Res. Comm.*, **478**(4): 1594-1601.

Dinicolantonio JJ, Niazi AK and McCarty M (2014). L-carnitine for the treatment of acute myocardial infarction. *Rev. Cardio. Med.*, **15**: 52-62.

Dalal JJ and Mishra S (2017). Modulation of myocardial energetics: An important category of agents in the multimodal treatment of coronary artery disease and heart failure. *Indi. Hea. J.*, **69**: 393-401.

Dan L, Jie W, Li L, Ying W, Ling L and Xiao H (2015). Cytotoxic triterpenoid glycosides (saikosaponins) from the roots of *Bupleurum chinense*. *Bioor. & Medi. Chem. Lett.*, **25**(18): 3887-3892.

Fuu T, Tsung H, Chi C, Xiang Lu and Hsinyi T (2017). Effect of Chinese herbal medicine on stroke patients with type 2 diabetes. *J. Ethnop.*, **200**: 31-44.

Gaopeng S, Xintian S, Sumei L, Yibin L, Yunpeng L and Yushan Z (2015). Structure–activity relationships of 3-O-β-chacotriosyl ursolic acid derivatives as novel H5N1 entry inhibitors. *Euro. J. Medi. Chem*., **93**: 431-442.

Ghoneum M, Felo N, Nwaogu OM, Fayanju IY, Jeffe JA and Margenthaler DB (2015). Clinical Trials in Surgical Oncology. *Asian Pac. J. Surg. Oncol*., **1**(2): 73-82.

Herrera-Fontana M and Caicedo-Borras R (2018). Analysis of the strengths and weaknesses of the Program Policies and Practices of Infant Feeding in Ecuador, *Arch. Latino. Nutr.,* **68**(1): 5-16.

Hausenloy DJ and Yellon D (2015). Targeting myocardial reperfusion injury - The search continues. *N. Engl. J. Med.,* **373**: 1073-1075.

Hsuan C, Shu M, Dino T, Yuh C and Li W (2016). Oral Ulcers as an Initial Presentation of Juvenile Pemphigus: A Case Report. *Pedia. & Neon.,* **57**(4): 338-342.

Li P, Guo X and Lei P (2014). PI3K/Akt/uncoupling protein 2 signaling pathway may be involved in cell senescence and apoptosis induced by angiotensin II in human vascular endothelial cells. *Mol. Biol. Rep.*, **241**: 6931-6937.

Luo X (2015). Clinical research of azithromycin in treatment of acute enteritis. *Guangzhou Medi. J.*, **22**(03): 80-81.

Meybohm P, Bein B, Brosteanu O, Cremer J, Gruenewald M, Stoppe C and Coburn M (2015). A multicenter trial of remote ischemic preconditioning for heart surgery. *N. Engl. J. Med.*, **373**: 1397-1407.

Navaro M (2018). Tests and treatments for Malaria, *Bole. Mala. Salu. Amb.*, **58**(3): 33-36.

Oka T, Akazawa H and Naito AT (2014). Angiogenesis and cardiac hypertrophy: Maintenance of cardiac function and causative roles in heart failure. *Circ. Res.*, **114**: 565-571.

Orivaldo B and Campello S (2018). A case study on dengue fever and pregnancy, *Bole. Mala. Salu. Amb*., **58**(4): 49-54.

Peterzan MA, Lygate CA and Neubauer S (2017). Metabolic remodelling in hypertrophied and failing myocardium: a review. *Am. J. Physiol. Heart. Circ. Physiol.,* **313**(3): H597-H616.

Pistevou K, Zygogianni A and Kantzou I (2015). Splenic irradiation as palliative treatment for symptomatic splenomegaly due to secondary myelofibrosis: A multi-institutional experience. *J. BUON.*, **20** (4): 1132-1136.

Reber A and Katz J (2013). Immunological assessment of influenza vaccines and immune correlates of protection. *Expert Rev. Vaccines,* **12**(2): 519-536.

Stephen C, Lee I, Min Y, Chun L and Chiang L (2016). Anti-melanoma activity of *Bupleurum chinense*, Bupleurum kaoi and nanoparticle formulation of their major bioactive compound saikosaponin-d. *J. Ethnop*., **179**: 432-442.

Schmidt A, Xu A, Khan T, 'Donnell S and Khurana L (2013). Preconfiguration of the antigen-binding site during affinity maturation of a broadly neutralizing influenza virus antibody. *Proc. Natl. Acad. Sci. USA*, **110**(13): 264-269.

Samano R, Chico B and Gabriela A (2018). Barriers and facilitators of exclusive breastfeeding practice in mothers from Mexico City, *Arch. Latino. Nutr.*, **68**(1): 41-50.

Sheng W, Zhang B, Chen W, Gu D and Gao W (2015). Laparoscopic colectomy for transverse colon cancer: comparative analysis of short- and long-term outcomes. *Int. J. Clin. Exp. Med.*, **8**(9): 16029-16035.

Shi X, Shang J and Zhu M (2018). Public Health, Antibiotics Application and the Farmers' Decreased Willingness of Using Chemical Fertilizers around the Wetland in Zhaodong City, *Bole. Mala. Salu. Amb.*, **58**(3): 50-63.

Wang K (2016). Comparison of clinical efficacy of azithromycin and pefloxacin in treatment of acute enteritis. *Anti-Infection Phar.*, **12**(02): 371-374.

Wei F, Yin Y and Bao G (2017). Anti-influenza triterpenoid saponins (saikosaponins) from the roots of Bupleurum marginatum var. stenophyllum. *Bioorg. & Medic. Chem. Lett.*, **27**(8): 1654-1659.

Xiong X, Corti D, Liu J, Pinna D, Foglierini M, Calder R and Martin Y (2015). Structures of complexes formed by H5 influenza hemagglutinin with a potent broadly neutralizing human monoclonal antibody. *Proc. Natl. Acad. Sci. USA*, **112**: 9430-9435.

Zhu H and Zhai D (2015). Clinical efficacy of azithromycin in treatment of acute enteritis. *Medical J. Chi. Peop. Hea.*, **24**(20): 17-18.