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Cardioprotective effect of fluvastatin on isoproterenol-induced myocardial infarction in rat

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ABSTRACT

The present study was designed to investigate whether fluvastatin, a 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor, would attenuate the acute myocardial infarction in isoproterenol-treated rat model via maintaining activities of endogenous antioxidant enzymes. Hemodynamic and electrocardiograph parameters were monitored and recorded continuously, cardiac marker enzymes and antioxidative parameters of plasma and heart tissues were measured, and histopathological examination of heart tissues was performed. Isoproterenol-treated rats showed lower of left-ventricular systolic pressure (LVSP), maximum ($LVdP/dt_{max}$) and minimum rate of developed left ventricular pressure ($LVdP/dt_{min}$), and higher of left ventricular end-diastolic pressure (LVEDP), in addition, a significant rise in ST-segment and increase in content of lactate dehydrogenase, glutamic oxalacetic transaminase, creatine kinase and malondialdehyde, as well as fall in activities of glutathione peroxidase, superoxide dismutase and catalase were observed. Oral administration of fluvastatin (5, 10 and 20 mg/kg, respectively) significantly prevented almost all the parameters of isoproterenol-induced heart failure and myocardial injury that mentioned above. The protective role of fluvastatin on isoproterenol-induced myocardial damage was further confirmed by histopathological examination. There was no significant change in heart rate in all experimental groups. Compared with control group, any indexes in sham rats treated with fluvastatin (20 mg/kg) alone were unaltered (all $P>0.05$). Our results suggest that fluvastatin has a significant effect on the protection of heart against isoproterenol-induced myocardial infarction through maintaining endogenous antioxidant enzyme activities.

1. Introduction

Although clinical care is improved, public awareness is raised and health innovations are widely used, myocardial infarction remains the leading cause of death worldwide (Aronow, 2006; Whellan, 2005). It is the acute condition of myocardial necrosis that occurs as a result of imbalance between coronary blood supply and myocardial demand. The patient may experience significant disability or die (Alla et al., 2007). Experimental and clinical studies have shown that there is increased generation of reactive oxygen species such as superoxide anion ($.O_2^-$) and hydroxyl radicals (.OH) in heart failure, which involved in the formation of lipid peroxides, damage of cell membrane, and destruction of antioxidative defense system (De Biase et al., 2003; Rajadurai and Stanely Mainzen Prince, 2007a,b). Therapeutic intervention via suppression of free radical generation and/or enhancement of endogenous antioxidant enzymes may limit the infarct size and attenuate myocardial dysfunction (Nakamura et al., 2000).

Fluvastatin, one of the clinically used 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor to reduce cholesterol biosynthesis, exerts additional beneficial pleiotropic effects independent of its lipid-lowering action, such as scavenging of free radicals (Yamamoto et al., 1998); anti-inflammatory, antithrombotic and antioxidant actions (Fischetti et al., 2004; Ferrara et al., 2003; Bandoh et al., 2003); and improvement impaired endothelial function (Murata et al., 2005). Recent research has demonstrated that treatment of fluvastatin can prevent ischemic heart diseases through its antioxidant property (Demyanets et al., 2006; Kowalski et al., 2004); ameliorate myocardial ischemic injury presumably by reducing the formation of free radicals (Nakamura et al., 2000; Obata et al., 2001; Obata, 2006); and improve ventricular function and clinical symptoms in patients with cardiac failure (Gürgün et al., 2008; Tiefenbacher et al., 2003). However, the effect of fluvastatin on endogenous antioxidant enzymes during myocardial injury remains unclear.

Isoproterenol, a synthetic β-adrenoceptor agonist, has been found to induce myocardial infarction in rat as a result of disturbance in physiological balance between production of free radicals and antioxidative defense system (Rathore et al., 1998; Srivastava et al., 2007). It is the acute condition of necrosis of the myocardium which accompanied by increased cardiac marker enzymes, alternated ischemic electrocardiograph, accumulated lipid peroxides, and damaged cardiac function (Rajadurai and Stanely Mainzen Prince, 2006, 2007a,b; Gupta et al.,

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2004). The pathophysiological and morphologic alterations in the heart of this non-coronary myocardial necrotic rat model are comparable with those taking place in human myocardial infarction (Rona, 1985; Anandan et al., 2007; Karthikeyan et al., 2007). In our study, this noninvasive myocardial infarction rat model was used to assess the efficacy of fluvastatin on protecting the heart against oxidative damage, and focus on the correlate functional, biochemical, and histopathological changes.

2. Materials and methods

2.1. Animals

Sprague–Dawley male rats, 250±20 g, were supplied by Ningxia Laboratory Animal Center (Yinchuan, China). The animals were housed individually in cages under hygienic conditions and placed in a controlled environment with a 12 h light–dark cycle (lights on 7:00–19:00) at 22±3 °C and 45±10% humidity for 7 days before the experiment. The animals were allowed a commercial standard rat cube diet and water *ad libitum*. The Animal center of Ningxia Medical College approved the study.

2.2. Chemical reagents

Fluvastatin was purchased from Beijing Nuohua Pharmaceutical Co. Ltd (Beijing, China). Superoxide dismutase, malondialdehyde, creatine kinase, catalase, glutamic oxalacetic transaminase, lactate dehydrogenase and glutathione peroxidase diagnostic agents were obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Isoproterenol was bought from Sigma Chemical Co. Other reagents were of commercial analytical grade.

2.3. Induction of myocardial injury

Isoproterenol was dissolved in normal saline and injected subcutaneously to rats (85 mg/kg) daily for 2 consecutive days to induce experimental myocardial infarction (Rajadurai and Stanely Mainzen Prince, 2007a).

2.4. Experimental protocols

Rats were randomly divided into 6 groups. Group1: rats oral administration of physical saline for 8 days as the normal control; Group 2: rats oral administration of physical saline for 8 days and at the 7th day subcutaneously injected with isoproterenol (85 mg/kg, subcutaneously injected, once at an interval of 24 h for 2 consecutive days); Group 3 to 5: rats pretreated with fluvastatin (5, 10 and 20 mg/kg; gastric gavages, respectively) for 8 days and at the 7th day subcutaneously injected with isoproterenol (85 mg/kg) for 2 consecutive days. Group 6: rats treated with fluvastatin (20 mg/kg, gastric gavages) for 8 days as the sham group. All rats fasted overnight but had free access to water at the last administration of the drug.

At the end of the experiment, the rats were anesthetized with urethane (1 g/kg, ip), needle electrodes were inserted under the skin for the limb lead at position II. Electrocardiograph was recorded continually, and ST-segment elevation or depression (expressed in mv) in normal and experiment animals were considered. To evaluate the cardiac left ventricular function, a catheter filled with heparin saline (500 U/ml) was inserted into the left ventricle, and left ventricular end-diastolic pressure (LVEDP), left-ventricular systolic pressure (LVSP), maximum and minimum rates of developed left ventricular pressure ($LVdP/dt_{max}$ and $LVdP/dt_{min}$) were measured by BL-420E+Biologic Function Experiment system (Chengdu, China). Blood was collected from right carotid after hemodynamic parameters were measured. After having clotted for 2 h at room temperature, serum was moved and stored at −20 °C for further biochemical assay.

Rats were killed and hearts were removed rapidly. The tissues of left ventricles were excised and washed with pre-chilled physical saline, homogenized with pre-chilled physical saline in tissue homogenizer, then centrifuged at 3000 ×*g* for 10 min at 4 °C. The supernatants were assayed immediately, and content of tissue protein was determined by Coomassie brilliant blue G250. Levels of creatine kinase, catalase, glutamic oxalacetic transaminase, lactate dehydrogenase, superoxide dismutase, malondialdehyde, and glutathione peroxidase in plasma and/or heart tissues were measured according to the manufacturer's instruction, and the absorbencies were measured by 721 Spectrophotometer (Shanghai, China).

2.5. Histopathological examination

The cardiac apex was excised and fixed in 4% buffered paraformaldehyde solution. Tissues were embedded in paraffin, sectioned at 4 μm and stained with hematoxylin and eosin (H&E). The sections were examined under light microscope, and then photomicrographs were taken.

2.6. Statistical analyses

All data was expressed as mean±S.D. and statistical analyses of observed values were performed using two-way ANOVA. A $P<0.05$ was accepted as statistically significant.

3. Results

3.1. Effect of fluvastatin on body weight and electrocardiograph parameters

The mean body weight gains of rats in all experimental groups had no significant change (Table 1). Normal rats showed a normal electrocardiograph-pattern. Normal rats treated with fluvastatin (20 mg/kg) alone did not show any change in electrocardiograph-pattern. Isoproterenol-treated rats showed a marked elevation in ST-segments. These changes were restored to near normal in fluvastatin (5, 10 and 20 mg/kg, respectively) pretreated isoproterenol-induced rats when compared to isoproterenol-alone induced rats (Fig. 1).

Table 1
Effect of fluvastatin on body weight and serum marker enzymes in myocardial infarction rats (mean±SD)

Groups	Body weight (g)		Serum		
(*N*=6–10)	Initial	Final	Lactate dehydrogenase (U/l)	Glutamic oxalacetic transaminase (U/l)	Creatine kinase (U/ml)
Control	269.9±25.81	269.9±25.81	5110.50±117.55	39.38±6.24	0.87±0.15
Isoproterenol (85 mg/kg)	258.6±16.75	262.6±19.82	7482.20±213.68**	74.08±11.99**	1.64±0.16**
Fluvastatin (5 mg/kg)+Isoproterenol	264.6±11.10	267.3±15.02	6130.22±226.50**,****	64.32±6.07**,***	1.40±0.11**,****
Fluvastatin (10 mg/kg)+Isoproterenol	252.6±11.90	261.1±13.94	5687.40±132.73**,****	56.83±4.29**,****	1.27±0.14**,****
Fluvastatin (20 mg/kg)+Isoproternol	243.2±18.61	256.1±12.49	5229.70±124.83****	44.39±5.57****	0.97±0.17****
Fluvastatin (20 mg/kg)	272.3±14.89	285.0±11.22	5113.17±111.88****	40.25±6.51****	0.89±0.05****

Compared with control: *$P<0.05$, **$P<0.01$. Compared with isoproterenol: ***$P<0.05$, ****$P<0.01$.

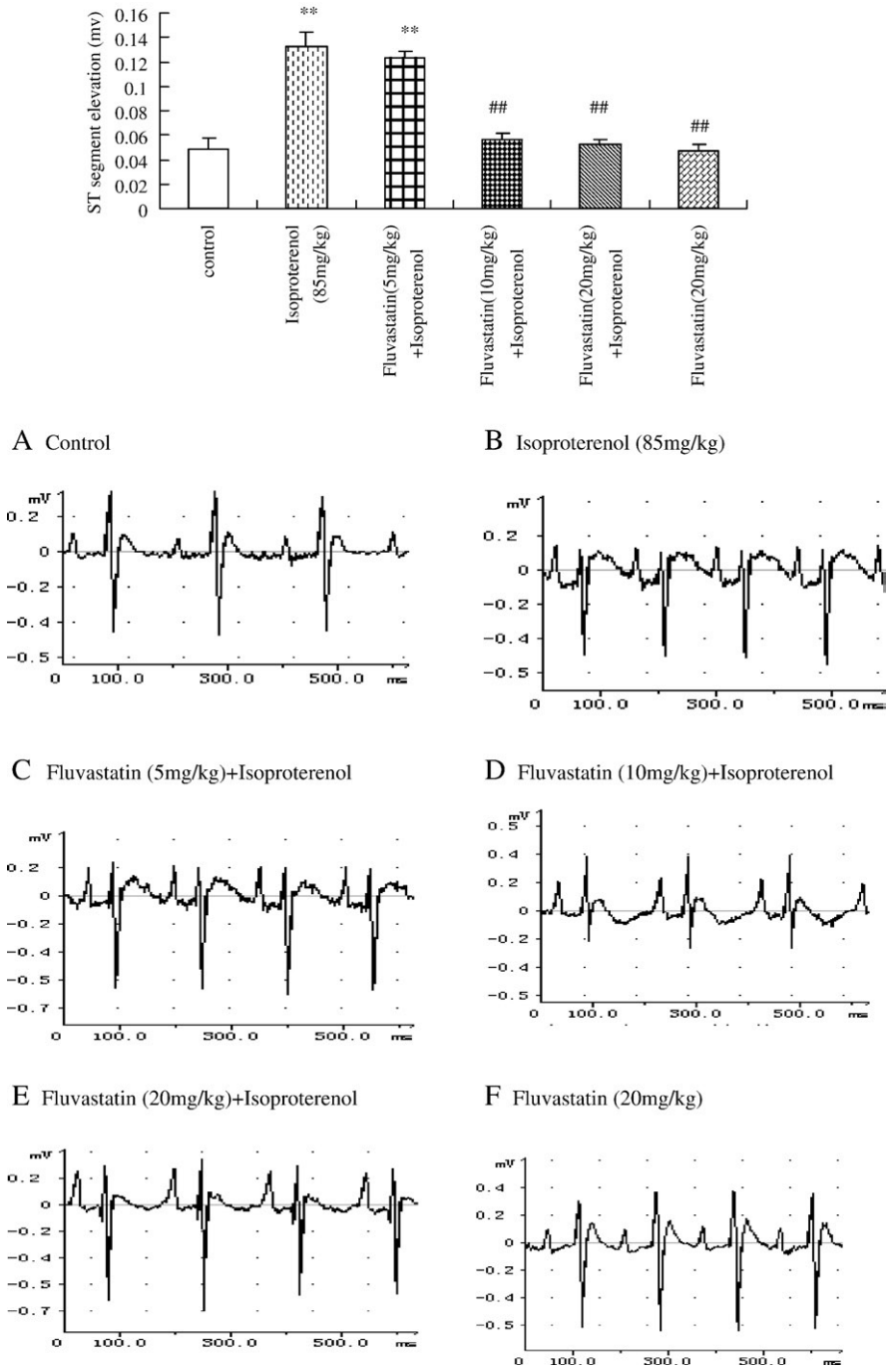

Fig. 1. Representative electrocardiogram tracings induced by isoproterenol (recorded from || limb leads with recorder speed 50 ms/div). Compared with control: $^{*}P<0.05$, $^{**}P<0.01$. Compared with isoproterenol: $^{\#}P<0.05$, $^{\#\#}P<0.01$.

3.2. Effect of fluvastatin on biochemical parameters and antioxidant enzymes

Isoproterenol induction caused significant increase in the activities of acute serum myocardial injury marker enzymes (lactate dehydrogenase, glutamic oxalacetic transaminase and creatine kinase) when compared with control rats. Prior treatment with fluvastatin (5, 10 and 20 mg/kg, respectively) almost restored all the isoproterenol-induced alterations of serum diagnostic maker enzymes to normal levels (Table 1).

A significant rise in the content of malondialdehyde, as well as a significant decline in the activity of superoxide dismutase, catalase and glutathione peroxidase was observed in the heart tissues and serum of isoproterenol-treated rats as compared with control. Administration of fluvastatin (5, 10 and 20 mg/kg, respectively) markedly prevented all the isoproterenol-induced alterations in antioxidant enzymes and maintained the rats at near normal status (Tables 2 and 3). Fluvastatin (20 mg/kg) alone did not demonstrate any significant changes in biochemical parameters and antioxidant enzymes when compared with control group indicating fluvastatin itself did not have any adverse effects (Tables 1–3).

3.3. Effect of fluvastatin on cardiac function

Compared with control group, isoproterenol-treated rats resulted in left ventricular dysfunction as indicated by a significant rise in

Table 2
Effect of fluvastatin on antioxidant parameters in serum of myocardial infarction rats (mean ± SD)

Groups (N=6–10)	Superoxide dismutase (U/ml)	Catalase (U/ml)	Glutathione peroxidase (U/ml)	Malondialdehyde (nmol/ml)
Control	218.91 ± 14.95	5.59 ± 0.66	9264.8 ± 776.1	6.98 ± 0.56
Isoproterenol (85 mg/kg)	170.12 ± 19.09**	2.52 ± 0.36**	5236.6 ± 789.1**	8.45 ± 1.19**
Fluvastatin (5 mg/kg)+Isoproterenol	195.46 ± 16.56**,****	3.49 ± 0.87**,***	7542.5 ± 620.9**,****	8.12 ± 0.68**
Fluvastatin (10 mg/kg)+Isoproterenol	202.07 ± 12.08*,****	4.40 ± 0.75**,****	8520.0 ± 383.7*,****	7.48 ± 0.79***
Fluvastatin (20 mg/kg)+Isoproterenol	209.29 ± 15.67****	5.09 ± 0.74****	9086.9 ± 617.3****	7.10 ± 0.87****
Fluvastatin (20 mg/kg)	219.42 ± 18.79****	5.69 ± 0.52****	9427.6 ± 377.3****	7.27 ± 0.53****

Compared with control: *P<0.05, **P<0.01. Compared with isoproterenol: ***P<0.05, ****P<0.01.

LVEDP, and a fall in values of LV$dP/dt_{max}$, LV$dP/dt_{min}$ and LVSP. Heart rate was not significantly altered (P>0.05) in isoproterenol-treated rat. Oral administration of fluvastatin (5, 10 and 20 mg/kg) improved cardiac function of isoproterenol-treated rat. Treatment with fluvastatin at dose of 20 mg/kg to normal rat did not have any significant effect on left ventricular function (Table 4).

3.4. Histopathological examination of cardiac tissues

On histopathological examination, heart tissues from isoproterenol-treated rats showed widespread myocardial structure disorder and subendocardial necrosis with capillary dilatation and leukocyte infiltration (Fig. 2B) as compared to control group (Fig. 2A). Pretreatment with fluvastatin (5, 10 and 20 mg/kg, respectively) demonstrated marked improvement in isoproterenol-induced subendocardial necrosis, capillary dilatation and leukocyte infiltration (Fig. 2C, D, E). Heart tissues from sham group did not have any histopathological changes (Fig. 2F).

4. Discussion

Supramaximal doses of isoproterenol induce subendocardial myocardial ischemia, hypoxia, necrosis, and finally fibroblastic hyperplasia with decreased myocardial compliance and inhibition of diastolic and systolic function, which closely resembles local myocardial infarction-like pathological changes seen in human myocardial infarction, so it is widely used as a model of evaluating cardioprotective drugs and studying myocardial consequences of ischemic disorders (Rona, 1985; `Karthick and Stanely Mainzen Prince, 2006). In the present study, we found that fluvastatin protected myocardium from isoproterenol-induced myocardial functional and structural injury via normalization levels of antioxidant enzymes.

Electrocardiograph-abnormalities are the main criteria generally used for the definite diagnosis of myocardial infarction. ST-segment elevation was observed either in patient with acute myocardial ischemia (Peacock et al., 2007) or in isoproterenol-induced myocardial infarction in rat (Rajadurai and Stanely Mainzen Prince, 2007a). These alterations could be due to the consecutive loss of cell membrane in injured myocardium (Holland and Brooks, 1977). In the present study, we observed an elevation of ST-segments in isoproterenol-induced rat, and pretreatment with fluvastatin markedly inhibited isoproterenol-induced ST-segment elevation suggestive of its cell membrane protecting effects.

Cytosolic enzymes of lactate dehydrogenase, glutamic oxalacetic transaminase and creatine kinase, which serve as the diagnostic markers of myocardial tissue damage, leak out from the damaged tissues to the blood stream when the cell membrane becomes permeable or rupture (Sabeena Farvin et al., 2004; Gürgün et al., 2008). The amount of these cellular enzymes presented in plasma reflects the alterations in plasma membrane integrity and/or permeability. Drug treatments such as naringin, Silibinin, and squalene evidenced by a decline in lactate dehydrogenase, glutamic oxalacetic transaminase and creatine kinase levels indicated their membrane stabilizing action (Rajadurai and Stanely Mainzen Prince, 2006; Zhou et al., 2006; Sabeena Farvin et al., 2004). Our results showed significant elevation in the levels of lactate dehydrogenase, glutamic oxalacetic transaminase and creatine kinase in plasma of isoproterenol-injected rats, which were in line with the previous reports (Sabeena Farvin et al., 2004; Gürgün et al., 2008) and were indication of isoproterenol-induced necrotic damage of the myocardial membrane. The prior administration of fluvastatin (5, 10 and 20 mg/kg) was found to significantly lower the isoproterenol-induced elevation in the activities of diagnostic marker enzymes (lactate dehydrogenase, glutamic oxalacetic transaminase and creatine kinase). This could be due to its action on maintaining membrane integrity thereby restricting the leakage of these enzymes.

Administration of high concentration of isoproterenol had been reported to induce severe oxidative stress and result in necrotic lesions in the myocardium of rats (Rona, 1985; Rathore et al., 1998). The increased generation of reactive oxygen species and/or depletion of the antioxidants in the defense system may contribute to oxidative stress and affect the pathogenesis of myocardial infarction (Sawyer et al., 2002). Free radical scavenging enzymes such as superoxide dismutase, catalase and glutathione peroxidase (Sawyer et al., 2002) are the first line cellular defense against oxidative stress, eliminating reactive oxygen radical such as superoxide ($.O_2^-$) and hydrogen

Table 3
Effect of fluvastatin on antioxidant parameters in heart of myocardial infarction rats (mean ± SD)

Groups (N=6–10)	Superoxide dismutase (U/mg protein)	Catalase (U/mg protein)	Glutathione peroxidase (U/mg protein)	Malondialdehyde (nmol/mg protein)
Control	1054.54 ± 222.10	15.32 ± 1.42	149.22 ± 7.12	6.76 ± 1.64
Isoproterenol (85 mg/kg)	767.01 ± 52.44**	7.72 ± 0.52**	61.78 ± 6.92**	9.95 ± 1.02**
Fluvastatin (5 mg/kg)+ Isoproterenol	877.70 ± 60.60**	9.64 ± 1.05**,****	94.67 ± 4.73**,****	8.82 ± 1.86**,***
Fluvastatin (10 mg/kg)+ Isoproterenol	945.96 ± 68.74****	10.23 ± 0.63**,****	106.49 ± 3.17**,****	7.94 ± 1.28****
Fluvastatin (20 mg/kg)+ Isoproterenol	1033.38 ± 140.86****	12.32 ± 0.84**,****	139.29 ± 10.79*,****	7.09 ± 1.84****
Fluvastatin (20 mg/kg)	1093.74 ± 74.88****	15.44 ± 0.65****	147.77 ± 5.91****	6.77 ± 1.32****

Compared with control: *P<0.05, **P<0.01. Compared with isoproterenol: ***P<0.05, ****P<0.01.

Table 4
Effects of fluvastatin on left ventricular function in myocardial infarction of rats (mean±SD)

Groups (N=6–10)	LVEDP (mm Hg)	LV$dP/dt_{max}$ (mm Hg/s)	LV$dP/dt_{min}$ (mm Hg/s)	LVSP (mm Hg)	Heart rate (bpm)
Control	−5.87±0.85	6092.15±1099.49	4984.73±1129.29	127.20±21.36	393.00±22.42
Isoproterenol (85 mg/kg)	1.88±1.02**	5096.18±676.92**	3729.35±717.43**	111.22±13.62**	395.78±33.76
Fluvastatin (5 mg/kg)+Isoproterenol	−3.60±0.77**,****	5639.29±538.99	4143.47±472.08*	125.80±10.37***	392.78±31.68
Fluvastatin (10 mg/kg)+Isoproterenol	−4.38±0.76**,****	5855.40±574.23***	4554.38±341.51****	129.60±7.23****	393.10±29.88
Fluvastatin (20 mg/kg)+Isoproterenol	−5.18±0.28****	5914.23±276.03****	4774.29±465.43****	130.87±8.14****	391.60±30.10
Fluvastatin (20 mg/kg)	−5.86±0.51****	5934.23±453.85***	4769.52±629.04****	128.13±5.58***	381.50±27.70

Compared with control: $^{*}P<0.05$, $^{**}P<0.01$. Compared with isoproterenol: $^{***}P<0.05$, $^{****}P<0.01$.

peroxide (H_2O_2), and preventing the formation of more reactive radical of hydroxyl radical (.OH). While Matsuki et al. (2006) reported that fluvastatin attenuates myocardial injury primarily through the nitric oxide pathway, not through its antioxidant property via affecting myocardial 8-hydroxydeoxyguanosine (a marker of oxidative DNA damage) content. Obata et al. (2001); Obata. (2006) reported that fluvastatin significantly decreased the formation of 2, 3-dihydroxybenzoic acid (the reactive products of salicylic acid and .OH) in myocardium of rats suggestive of its beneficial cardioprotective effect due to suppression of noradrenalin or copper-induced hydroxyl radical. This discrepancy in the results of different studies may be related to the different myocardial infraction animal protocols they were used. In the present study, we found that the decreased activities of superoxide dismutase, catalase and glutathione peroxidase in isoproterenol-injected rats were significantly elevated by treatment of animals with fluvastatin (Tables 2 and 3). These finding suggested that fluvastatin could considerably improve cellular antioxidative defense against oxidative stress.

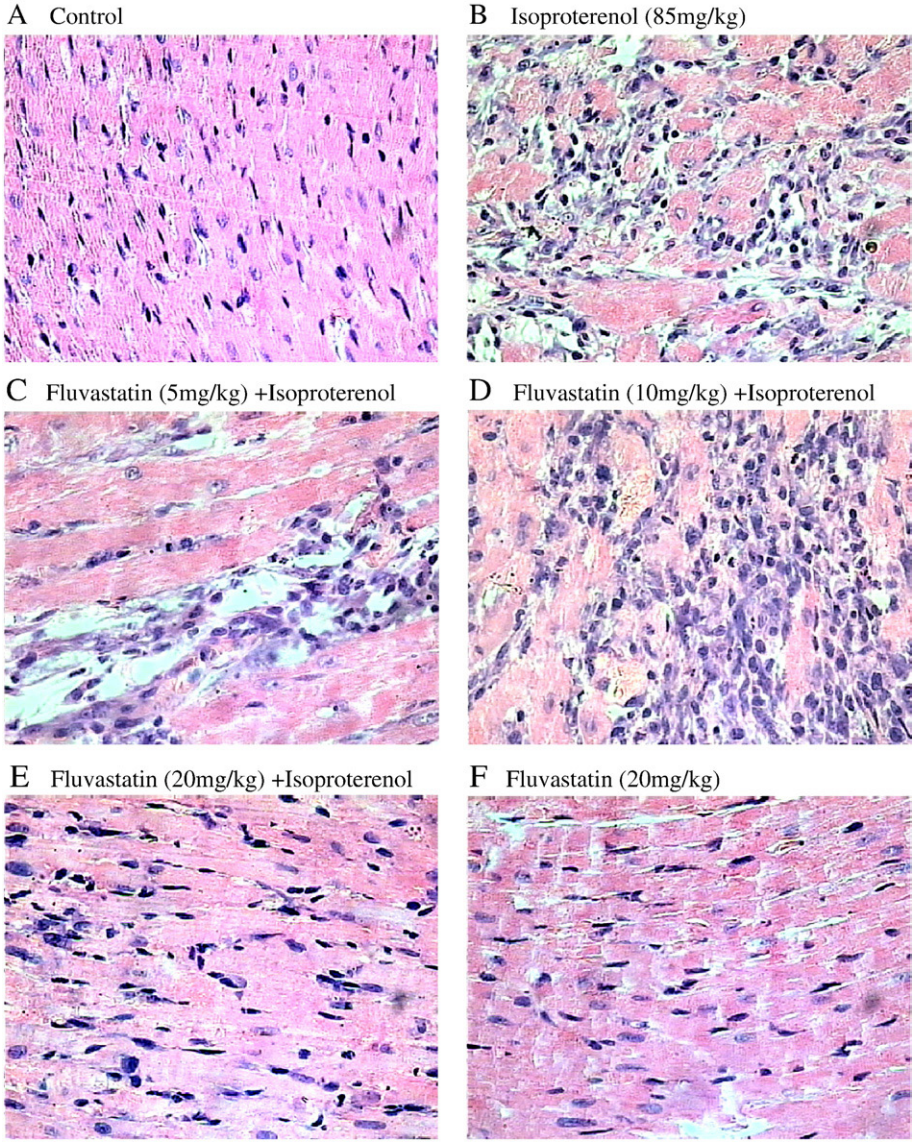

Fig. 2. Histopathological changes in rat cardiac apexes (H&E, 40×).

Lipid peroxidation, is an indication of the severity of isoproterenol-induced necrotic damage of the heart, and has been linked with altered membrane structure and enzyme inactivation (Karthikeyan et al., 2007). Malondialdehyde is a major lipid peroxidant end product; increased malondialdehyde content may contribute to increased generation of free radicals and/or decreased activities of antioxidant system (Zhou et al., 2006). Previous studies have reported (Zhou et al., 2006) that isoproterenol-induced myocardial infarction could be owing to the induction of free radical-mediated lipid peroxidation, as a result of stressed condition in rats. The results presented in this study indicated that fluvastatin (5, 10 and 20 mg/kg) pretreatment could decrease isoproterenol-induced malondialdehyde content elevation. The decreased level of malondialdehyde in heart tissues and plasma might be due to the enhanced activities in antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase). It was quite possible that the free radicals induced by isoproterenol were effectively neutralized and/or scavenged, resulting in cardioprotective effect of fluvastatin.

To understand the protective effect of fluvastatin on cardial dysfunction better, hemodynamic parameters were incorporated into the present experiment design. A growing body of evidence showed that supramaximal dosages of isoproterenol lead to heart failure characterized by increased end-diastolic volume, end-diastolic pressure, and left-ventricular-wall thickness (Gupta et al., 2004). The present study demonstrated that fluvastatin significantly prevented isoproterenol-induced left ventricular systolic dysfunction as evidenced by improvement in LV$dP/dt_{max}$ and LVSP, as well as left ventricular diastolic dysfunction as evidenced by improvement in LV$dP/dt_{min}$ and LVEDP. In a sense, our results suggested that fluvastatin offered protection to the myocardium by attenuated of ventricular dysfunction through maintaining the ECG-patterns, cardiac marker enzymes, and antioxidant enzymes to near normal status in isoproterenol-treated rats.

Histopathological finding of fluvastatin (5, 10 and 20 mg/kg, respectively) pretreated myocardium showed reversal of myonecrosis and leukocytic cells infiltration seen with isoproterenol-treatment. Fluvastatin (20 mg/kg)-treated normal rats had no toxic effects on cardiac architecture. These data further confirmed the cardioprotective action of oral administration of fluvastatin.

It is well known that isoproterenol-induced myocardial injury is mediated primarily via the β_1-adrenergic receptor. Acute β-adrenergic receptor stimulation not only rapidly generates reactive oxygen species, but also depresses total cellular antioxidant capacity, down-regulates copper–zinc superoxide dismutase enzyme activity, protein and mRNA, and reduces glutathione level, leading to the loss of membrane integrity and inducing heart contractile dysfunction and myocyte toxicity finally producing myocardial necrosis (Rathore et al., 1998; Srivastava et al., 2007). The data of the present study clearly showed fluvastatin dose-dependently modulated most of the hemodynamic, biochemical and histopathological parameters and maintained antioxidant enzymes to normal status in high-dose isoproterenol rats, suggesting the beneficial action of fluvastatin as a cardioprotective agent. There were a number of investigations showing fluvastatin acutely and chronically improved myocardial infarction via several mechanisms, such as scavenging of free radicals (Sumi et al., 2001) and/or depressing free radical generation (Obata et al., 2001; Obata 2006); anti-inflammatory, antithrombotic and antioxidant actions (Yang et al., 2006; Fischetti et al., 2004; Ferrara et al., 2003; Bandoh et al., 2003); and improvement impaired endothelial function (Murata et al., 2005; Tiefenbacher et al., 2003). Recent in vivo studies show that administration of fluvastatin for two weeks before ischemia, but not at the onset of myocardial ischemia or reperfusion, attenuates myocardial ischemia-reperfusion injury through the nitric oxide pathway rather than its antioxidant property (Matsuki et al., 2006). Our results demonstrated that 8 days fluvastatin oral administration also offered protection to the myocardium against isoproterenol-induced oxidative stress in rats, suggesting one mechanism of its beneficial effects in this setting may be primarily mediated through the nitric oxide pathway. Of cause, there remain other uncertainties, for instance, deteriorating myocardial status following isoproterenol administration is expected to affect heart rate (Rona, 1985). Increased heart rate leads to increase oxygen consumption and accelerate myocardial necrosis (Rona, 1985). In our study, there was no significant difference in heart rate among all experiment groups in anaesthetized animals; therefore we did not know the alterations of this parameter in conscious status during the ischemic period. On the other hand, we did not investigate the effect of nitric oxide pathway after treatment with fluvastatin; therefore further study is needed to confirm this mechanism.

In summary, the present study provided experimental evidence that fluvastatin maintained the antioxidant enzyme levels and improved cardiac performance following high-dose isoproterenol administration. This finding might be rational to understand the beneficial effects of fluvastatin on cardioprotection against myocardial injury, in which oxidative stress was long known to contribute to the pathogenesis.

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