Full Paper

Pravastatin Inhibits Arrhythmias Induced by Coronary Artery Ischemia in Anesthetized Rats

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Received October 3, 2006; Accepted January 25, 2007

Abstract. We have reported that chronically administered pravastatin prevented coronary artery reperfusion-induced lethal ventricular fibrillation (VF) in anesthetized rats without lowering the serum cholesterol level. The present study was undertaken to evaluate whether pravastatin prevents ischemia-induced lethal VF, simultaneously examining myeloperoxidase (MPO) activity in ischemic myocardial tissues. Anesthetized rats were subjected to 30-min ischemia and 60-min reperfusion after chronic administration of pravastatin (0.02, 0.2, and 2 mg/kg), fluvastatin (2 and 4 mg/kg), or vehicle for 22 days, orally, once daily. ECG and blood pressure were continually recorded, and MPO was measured by a spectrophotometer. Pravastatin and fluvastatin significantly ($P < 0.05$) decreased MPO activities, but only pravastatin decreased the incidence of ischemia-induced lethal VF. Both statins had no significant effects on body weight, blood pressure, heart rate, and QT interval as we reported earlier. Our results prove further that pravastatin has benefits to decrease cardiovascular mortality beyond its cholesterol-lowering effect. Pravastatin is more potent than fluvastatin in prevention of arrhythmias. A decrease in the neutrophil infiltration may be partly involved in the inhibitory effect of pravastatin on the ischemia-induced VF.

Keywords: pravastatin, fluvastatin, ischemia, arrhythmia, myeloperoxidase

Introduction

Statins have been widely used in medical practice (1) and shown to reduce cardiovascular morbidity and mortality in a number of large clinical trials (2–5). Because there is a well-established association between a high cholesterol level and adverse cardiovascular risks, such as angina, myocardial infarction (6), and even death (7), it may be reasonable to relate these beneficial effects to the cholesterol-lowering effects (2, 3, 8). However, many researchers showed that statins contribute to the clinical benefits directly or indirectly through their broad spectrum of cholesterol-independent cardioprotective effects (9–17) such as plaque stabilization;

preservation of endothelial function; scavenging of free radicals; and antiproliferative, antiinflammatory, and antiapoptotic effects, and so on. To our best knowledge, few studies examined the effects of statins on ventricular arrhythmias, especially, ventricular tachycardia (VT) and ventricular fibrillation (VF), which are the major direct causes of sudden cardiac death (18, 19). Our previous experiment proved that pravastatin could prevent coronary artery reperfusion-induced VF in anesthetized rats without lowering the serum cholesterol level (20). Another clinical study also reported that pravastatin reduced the incidence of ventricular arrhythmias in the patients with acute myocardial infarction (21). Therefore, this indicates that pravastatin may contribute to its cardioprotective effects in clinical practice through its antiarrhythmic effects. Among many pleiotropic effects of statins, the suppression of neutrophils may be related to the antiarrhythmic action because

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Published online in J-STAGE: March 7, 2007
doi: 10.1254/jphs.FP0061235

increased adhesion and extravasation of neutrophils are the major pathophysiological mechanisms leading to the development of cardiac reperfusion injury including arrhythmias (22, 23), and statins have coincidentally a strong effect to decrease neutrophil infiltration (24, 25).

Based on our previous studies, we hypothesize that pravastatin also has an inhibitory effect on arrhythmia induced by long period of ischemia and the effect may be related to the infiltration of neutrophils. The aim of our study, therefore, was to examine our hypothesis by using the 30-min ischemia and 60-min reperfusion rat model. We also compared hydrophilic pravastatin with lipophilic fluvastatin.

Materials and Methods

Induction of coronary artery ischemia/reperfusion injury in rats

As reported earlier (26), male Sprague-Dawley rats (350–450 g) were anesthetized with pentobarbital sodium (60 mg/kg, intraperitoneally) and the trachea was cannulated for artificial ventilation. Systemic blood pressure was monitored via a catheter inserted into the carotid artery. A standard limb lead I ECG was continuously recorded, together with arterial pressure, on a recorder (RM 6200; Nihon Kohden, Tokyo). Artificial ventilation was started with room air, using a tidal volume of 1.5 ml/100 g and at a rate of 54 strokes/min in order to maintain arterial blood gases and pH within the normal range. The chest was opened by a left thoracotomy, followed by sectioning of the 4th and 5th ribs, approximately 2 mm to the left of the sternum. After incising the pericardium, the heart was exteriorized by using gentle pressure on the rib cage. A 5/0 nylon suture attached to a 14-mm micropoint reverse cutting needle was placed under the left coronary artery. The heart was put back in the chest, and the rat was allowed to stabilize.

Thirty-minute regional myocardial ischemia was induced by pulling the two ends of the suture through a small plastic tube and pressing the tube against the surface of the myocardium and then clamping the tube together with the suture. Then, reperfusion was initiated by declamping and removing the tube. After reperfusion, the responses were observed for 60 min.

Ischemia and reperfusion were confirmed as described previously (27). In brief, successful occlusion was confirmed by the increase of the amplitude of the R wave of lead I during the first few seconds of each occlusion, which has been demonstrated to be more useful than lead II or other ECG leads in rats, and a 20%–30% decline in the arterial blood pressure compared to the pre-ischemic values.

Animals were obtained through the Animal Laboratory for Research of University of Yamanashi. The rats were housed in stainless steel hanging cages (3 rats per cage) under normal lighting conditions (lights on from 7 a.m. to 7 p.m.). Diet and water were given ad libitum during the entire experimental period. All experiments were conducted in accordance with the Guideline for Animal Experiments at University of Yamanashi and conformed with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Definition of arrhythmias and ECG analysis of QT interval

Definition of arrhythmias was based on the description of the Lambeth Conventions (28). Ectopic ventricular activity was categorized as a single premature ventricular contraction (PVC), ventricular tachycardia (VT, 4 or more consecutive PVC), or VF (inability to distinguish individual QRS complexes and to measure the rate). Reference was made to the blood pressure tracings to confirm which type of ectopic activity was occurring, particularly to distinguish between the polymorphic VT and VF. When the former occurred, the blood pressure was usually still pulsatile, whereas with VF the blood pressure fell rapidly towards zero and was no longer pulsatile. VF may be sustained (lethal VF) or may revert (non-lethal VF) spontaneously to a normal sinus rhythm in the rat. In all experiments, the onset time of VT and the incidences of VT and lethal VF (due to terminal VF sustained for 3 min or more) were noted.

The heart rate and QT interval were measured from the ECG lead I. The heart rate was measured just before the chest opening, just before occlusion, and 30 min after occlusion, whereas the QT was only measured just before the chest opening.

Exclusion criteria

Experiments were terminated or excluded from the final data analysis if any of the following occurred: arrhythmias prior to coronary artery occlusion, mean arterial blood pressure less than 60 mmHg prior to ligation, and atrioventricular block during the first 5 min of ischemia (probably caused by ligature occluding the septal branch of the left coronary artery). One hundred and six rats were used in this study. Three of them were excluded for low blood pressure before occlusion, four for consecutive VT, and four for VF before coronary artery occlusion. There was no difference in the distribution among experiment groups of these eight animals excluded for VT or VF.

Determination of tissue myeloperoxidase (MPO) activity

At the end of the experiment, tissue samples weighing 0.1 g were taken from the non-ischemic (right ventricle) and ischemic (left ventricle) tissues for analysis of MPO activity. MPO is specific for neutrophil accumulation in myocardium. The samples were frozen and stored at -70°C until assayed. The samples were homogenized in 0.5% hexadecyltrimethyl ammonium bromide and dissolved in 50 mM potassium phosphate buffer (pH 6) with a glass homogenizer for 2 min. The homogenate were centrifuged at $12,500 \times g$ and 4°C for 30 min. The supernatants were then collected and reacted with 0.167 mg/ml of *o*-dianisidine dihydrochloride and 0.0005% hydrogen peroxide in 50 mM phosphate buffer (pH 6). The activity of MPO was measured spectrophotometrically (spectrophotometer DU640; Beckman, USA) at 460 nm and expressed as arbitrary units.

Experimental protocols

Pravastatin (0.02, 0.2, and 2 mg/kg), fluvastatin (0.2, 2, and 4 mg/kg), or vehicle (distilled water) was orally administered daily for 22 consecutive days. The doses of pravastatin and fluvastatin were determined by referring to our previous experiment on the rat model of coronary artery ischemia/reperfusion. At 2 h after the last administration, the rats were anesthetized with pentobarbital sodium intraperitoneally and subjected to 30-min left coronary artery ligation followed by 60-min reperfusion. During the 30-min coronary ligation period, ECG lead I and blood pressure were continually recorded. Analysis of arrhythmias was performed on the basis of ECG. Immediately after reperfusion, the hearts were taken for the determination of tissue MPO activity.

Drugs

Pravastatin and fluvastatin powder were kindly supplied by Sankyo Co., Ltd. (Tokyo). Pentobarbital

sodium was purchased from Tokyo Kasei Kogyo (Tokyo). Pravastatin and fluvastatin were dissolved in distilled water on the day of administration.

Statistics

All data are expressed as means $\pm$ S.E.M. Student's *t*-test was used to test drug effects on hemodynamic parameters and QT interval. Differences in the incidence of arrhythmias among groups were analyzed by Fisher's exact probability test. A *P* value of less than 0.05 was considered statistically significant.

Results

Effects of pravastatin and fluvastatin on the incidences of VF during ischemic period (Fig. 1)

During 30-min ischemia, VF occurred in some rats. All VF followed VT, but not all VT triggered VF. During the ischemic period, the incidence of total VF (including non-lethal VF and lethal VF) and lethal VF in the control group were 81% and 52%, respectively. Pravastatin (0.02, 0.2, and 2 mg/kg) dose-dependently reduced the incidences of total VF (87%, 67%, and 47%) and lethal VF (40%, 17%, and 20%), with significant reduction in lethal VF at 0.2 and 2 mg/kg ($P < 0.05$). However, fluvastatin did not show any significant effect on the incidences of total VF (81% and 88%), but showed a dose-dependent reduction in the incidences of lethal VF (44% and 31%) without significances.

Effects of pravastatin and fluvastatin on MPO activity (Fig. 2)

In all groups, MPO activities (arbitrary unit) in tissues from the ischemic area were much higher than those in tissues from nonischemic tissues (right ventricles) showing extravasation of more neutrophils into ischemic tissues. As shown in Fig. 2, pravastatin and fluvastatin

Fig. 1. Effects of pravastatin and fluvastatin on ischemia-induced ventricular fibrillation (VF) in anesthetized rats. Pravastatin (0.02, 0.2, and 2 mg/kg, once daily orally for 22 consecutive days) dose-dependently reduced the incidences of total VF and lethal VF with significant reduction in lethal VF at 0.2 and 2 mg/kg ($P < 0.05$). Whereas fluvastatin did not show any significant effect on the incidences of total VF and lethal VF. Numbers above the bars indicate the incidences of arrhythmias.

dose-dependently reduced MPO activities in ischemic tissues compared with the control group (control: 5.64 ± 0.33 ; pravastatin, 0.02 mg/kg: 4.12 ± 0.51 , $P < 0.05$; pravastatin, 0.2 mg/kg: 2.59 ± 0.16 , $P < 0.001$; pravastatin, 2 mg/kg: 2.50 ± 0.42 , $P < 0.001$; fluvastatin, 2 mg/kg: 3.82 ± 0.32 , $P < 0.05$; fluvastatin, 4 mg/kg: 2.49 ± 0.28 , $P = 0.001$), but they did not change MPO activities in nonischemic tissues.

Effects of pravastatin on hemodynamics and QT intervals (Table 1)

The heart rates, blood pressure, and QT intervals showed no significant changes between pravastatin-, fluvastatin-, and vehicle-treated groups. After ischemia, the blood pressure was significantly decreased in each group when compared with its own baseline (just before ligation). The heart rate was not significantly changed.

Discussion

Extending our first report (20) showing that pravastatin inhibited arrhythmias, especially lethal VF, induced by coronary reperfusion in rats without lowering the serum cholesterol level, the present study showed that pravastatin also inhibits arrhythmias induced by coronary ischemia. Furthermore, in this study, we also found that hydrophilic pravastatin was more potent than lipophilic fluvastatin in suppressing arrhythmias even though they had the similar effect on the reduction of MPO in ischemic tissues.

Ischemia and reperfusion both are the important risk factors for the lethal ventricular arrhythmias. We have reported that pravastatin can reduce the incidence of lethal VF induced by reperfusion. Therefore, this time, we designed experiments particularly suited to examine

Fig. 2. Effects of pravastatin and fluvastatin on MPO activities (arbitrary units) in ischemic tissues (left ventricle) and nonischemic tissues (right ventricles). Pravastatin and fluvastatin dose-dependently reduced MPO activities in ischemic tissues significantly compared with the control group with $P < 0.05$ or $P < 0.01$. However, they did not change MPO activities in nonischemic tissues. The numbers inside the bars indicate the samples used.

Table 1. Effects of pravastatin and fluvastatin on heart rate, blood pressure, and QT interval in anesthetized rats

Groups		Heart rate (beats/min)			Mean blood pressure (mmHg)			QT interval (ms)
		just before open chest	just before occlusion	5 min after occlusion	just before open chest	just before occlusion	5 min after occlusion	just before open chest
Control	(n = 21)	424 ± 8	403 ± 9	409 ± 11	124 ± 3	89 ± 2	70 ± 4**	51.9 ± 0.4
Pravastatin 0.02 mg/kg	(n = 15)	431 ± 12	397 ± 15	419 ± 17	128 ± 5	88 ± 3	76 ± 5*	51.7 ± 0.5
Pravastatin 0.2 mg/kg	(n = 12)	411 ± 6	388 ± 16	413 ± 26	121 ± 4	93 ± 6	63 ± 4**	52.7 ± 0.4
Pravastatin 2 mg/kg	(n = 15)	422 ± 12	369 ± 14	382 ± 15	121 ± 7	83 ± 3	70 ± 5**	52.5 ± 0.7
Fluvastatin 2 mg/kg	(n = 16)	405 ± 13	406 ± 13	404 ± 12	116 ± 4	93 ± 4	67 ± 4**	51.4 ± 0.3
Fluvastatin 4 mg/kg	(n = 16)	401 ± 8	376 ± 15	375 ± 13	116 ± 4	86 ± 3	62 ± 4**	51.0 ± 0.4

Values are each a mean ± S.E.M. * $P < 0.05$, ** $P < 0.01$, compared with just before occlusion.

the effects of pravastatin on VF induced by ischemia in a rat model.

In our ischemia-reperfusion model in rats, five minutes of ischemia is the key time for triggering arrhythmias. If reperfusion was performed immediately after 5 min of ischemia, the lethal VF occurred in most of the rats (80%–100%). However, if reperfusion was performed after 30 min of ischemia, nearly no arrhythmia occurred after reperfusion. During 30 min of ischemia, most VF occurred between 4 and 8 min. This arrhythmogenicity is similar to that reported by others using a similar rat model of ischemia/reperfusion with 30 min of ischemia (29). It is easily to understand that the ischemia-injured myocardium undergoes a period vulnerable to arrhythmogenic factors. Mildly injured myocardium can resist the triggering of arrhythmias, but severely injured myocardium loses its ability to respond the triggering of arrhythmias. This vulnerable period or “arrhythmia triggering window” is not only for reperfusion, but also for ischemia. The difference between reperfusion-induced VF following 5 min of ischemia and ischemia-induced VF is that the former is more severe with more lethal VF.

As for the antiarrhythmic mechanism of pravastatin, the cholesterol lowering effect can be also excluded because both pravastatin and fluvastatin have no effect on serum cholesterol level in this rat model (20). We have postulated in our previous paper that neutrophils may play important roles in triggering arrhythmia because the substances such as toxic oxygen species, platelet-aggregating factor, arachidonic acid metabolites, and protease released by neutrophils contribute to cardiac injuries (30, 31) including arrhythmia (32, 33). The rush of neutrophils with reperfusion to the ischemic vulnerable myocardium will trigger severe arrhythmia, whereas the gradual accumulation of neutrophils at the borders of ischemic and non-ischemic regions also triggers arrhythmias of the vulnerable myocardium. Several studies have shown that statins inhibit the neutrophils by inhibiting their interaction with endothelium through attenuating endothelial adhesion expression (34), infiltration, accumulation (34, 35), and superoxide generation (14, 36). In the present study, we measured MPO activities in ischemic and non-ischemic tissues to determine the effect of the drugs on the neutrophils. Both pravastatin and fluvastatin have a similar significant effect in the reduction of MPO in ischemic tissue, but not in non-ischemic tissue. Therefore, it is reasonable to correlate the antiarrhythmic effect of pravastatin with its reduction of MPO in ischemic tissue or possibly in the border regions. However, why does not fluvastatin show an antiarrhythmic effect even with similar lowering effect on MPO? In our previous study,

we obtained the same results. Pravastatin reduced the incidence of arrhythmias, especially lethal VF, induced by coronary artery reperfusion in rats, but fluvastatin did not. We may explain this in the same way as before. In brief, the favorable effects of lipophilic statins on ischemic heart disease may be attenuated by their unfavorable effects on the myocardial energy-generating system and contractile function (37, 38). Pravastatin has the advantage of its hydrophilicity, which hinders easy entry to myocardial cells. Other potential explanations remain to be revealed with further studies.

Even though this experimental result might not necessarily predict the clinical outcome, there are two recent clinical studies showing that early use of pravastatin reduces the incidence of in-hospital ventricular arrhythmias (5, 39). Our studies, without the clinical complicating factors, clearly show the antiarrhythmic effects.

In conclusion, the present study extends the inhibitory effect of pravastatin on arrhythmias from reperfusion to ischemia, and this effect may be partly related to its suppression of neutrophil infiltration.

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