Full Paper

Pravastatin Prevents Arrhythmias Induced by Coronary Artery Ischemia/Reperfusion in Anesthetized Normocholesterolemic Rats

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Abstract. HMG-CoA reductase inhibitors (statins) have been shown to decrease cardiovascular mortality. Since ventricular tachyarrhythmias are closely related to cardiovascular mortality, we tested effects of the hydrophilic statin pravastatin and the lipophilic statin fluvastatin in a rat arrhythmia model of ischemia/reperfusion and simultaneously measured serum total cholesterol level. Anesthetized rats were subjected to 5-min ischemia and 10-min reperfusion after chronic administration of oral pravastatin (0.02, 0.2, or 2 mg/kg), fluvastatin (0.2, 2, or 4 mg/kg), or vehicle for 22 days, once daily. The acute effect of pravastatin (0.2 or 2 mg/kg, once orally) was also observed. Chronically administrated pravastatin significantly reduced the incidence of ischemia-induced ventricular tachycardia (VT) from 70% (control) to 9% at 2 mg/kg, and it reduced the incidence of reperfusion-induced lethal ventricular fibrillation (VF) from 90% (control) to 20% at 0.2 mg/kg. Acute pravastatin and chronically administrated fluvastatin had no significant effect on these arrhythmias. There were no significant changes in blood pressure, heart rate, QT interval, and serum cholesterol among pravastatin-, fluvastatin-, and vehicle-treated groups. Hydrophilic pravastatin prevented reperfusion-induced lethal VF in anesthetized rats by chronic administration independent of its cholesterol lowering effect. This may be a new beneficial role of pravastatin in decreasing cardiovascular mortality.

Keywords: antiarrhythmic effect, cholesterol, ischemia, reperfusion, statin

Introduction

Statins inhibit cholesterol synthesis in the liver and reduce serum cholesterol level both in humans and most animal species (1, 2). Because of the well-established association between a high cholesterol level and adverse cardiovascular risks, such as angina, myocardial infarction (3), and even death (4), the reduction in cardiovascular morbidity and mortality by statins has been considered to be related to their cholesterol-lowering effect (5–7). However, recent research has shown additional beneficial pleiotropic effects independent of the simple cholesterol lowering effect (8–12). These

include effects on vessel endothelial tissue to decrease low-density lipoprotein oxidation and inflammation, to stabilize and regress atherosclerotic plaques, to promote smooth-muscle growth and then strengthen atherosclerotic plaques, to inhibit platelet aggregation and stimulation of fibrinolytic factors resulting in antithrombotic effects, to improve blood viscosity and flow (8), and to inhibit neutrophils (9–12).

Clinically, ventricular tachyarrhythmias, including ventricular tachycardia (VT) and ventricular fibrillation (VF), are the major direct causes of sudden cardiac death in patients with coronary artery disease (13), and sudden cardiac death is a leading cause in increasing cardiovascular mortality (14). However there is little information regarding the effect of statins on the ventricular tachyarrhythmias. One paper showed that fluvastatin combined with propranolol suppressed coronary reper-

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fusion-induced VT/VF in rats (15). Lefer et al. (11) showed that simvastatin and pravastatin were potent and effective cardioprotective agents that inhibited leukocyte-endothelial cell interactions and preserved cardiac contractile function and coronary perfusion after myocardial ischemia and reperfusion. Based on these clinical and experimental studies, we hypothesized that statins may have a beneficial effect on ischemia/reperfusion-induced arrhythmias. We examined and compared effects of hydrophilic pravastatin and lipophilic fluvastatin in normocholesterolemic rats. We also measured the serum cholesterol level to clarify whether or not it is involved.

Materials and Methods

Induction of coronary artery ischemia/reperfusion injury in rats

As reported earlier (16), male Sprague-Dawley rats (350 to 450 g) were anaesthetized 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. The standard limb lead I ECG was continuously recorded, together with arterial pressure, on a recorder (Nihon Kohden RM 6200; 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 replaced back in the chest, and the rat was allowed to stabilize.

Transient 5-min 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 10 min.

Ischemia and reperfusion were confirmed as described previously (17). 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. Successful reperfusion was

confirmed by the return of the arterial blood pressure to the pre-ischemic value.

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 our university 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 (18). Ectopic ventricular activity was categorized as a single premature ventricular contraction (PVC), ventricular tachycardia (VT, 4 or more consecutive PVC), or ventricular fibrillation (VF, inability to distinguish individual QRS complexes and to measure the rate). Complex forms (e.g., bigeminy) were included in the count of PVC and were not analyzed separately. 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 incidences of PVC, 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 5 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, VT continuing until reperfusion or VF during 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 one rats were used in this study. Three of them were excluded for low blood pressure before occlusion, two for consecutive VT,

and five for VF during coronary artery occlusion. There was no difference in the distribution among experiment groups of these seven animals excluded for VT or VF.

Cholesterol measurement

After 10 min of reperfusion, blood was taken from the heart. The serum was separated at room temperature by centrifugation and stored at -20°C until measurement. The serum cholesterol was measured by using a Fujifilm Dri-Chem 3000V (Fuji Mechanical Industry Co., Ltd., Tokyo).

Experimental protocols

Two protocols were employed. In protocol I (chronic administration), pravastatin (0.02, 0.2, and 2 mg/kg); fluvastatin (0.2, 2, and 4 mg/kg); or vehicle (distilled water) were orally administered daily for 22 consecutive days. The doses of pravastatin and fluvastatin were determined by referring to our previous experiment using 4 mg/kg per day on rat liver ischemia/reperfusion and preliminary experiment on the rat model of coronary artery ischemia/reperfusion. In rat experiments, usually higher doses, 50 to 250 mg/kg, were used for lowering cholesterol and suppressing inflammation and arteriosclerosis (19, 20). In humans, 10 to 40 mg/day is the usual dose for pravastatin and a higher dose of fluvastatin, 20 to 40 mg/day, for lowering plasma cholesterol levels (21). Two hours after the last administration, the rats were anesthetized with pentobarbital sodium intraperitoneally and subjected to 5-min left coronary artery ligation followed by 10-min reperfusion as described earlier. Immediately after reperfusion, the blood was taken for the measurement of serum total cholesterol. In protocol II (acute administration), only a single dose of pravastatin (0.2 or 2 mg/kg) was administered orally, and 2 h later, the same procedure as protocol I was performed. The blood sample was not taken in protocol II.

Drugs

Pravastatin and fluvastatin powder were kindly supplied by Sankyo Company, Ltd. (Tokyo). Pentobarbital sodium was purchased from Tokyo Kasei Kogyo (Tokyo). TCHO-P11 slides for the measurement of serum total cholesterol level was purchased from Fujifilm Medical Company, Ltd. (Tokyo). Pravastatin and fluvastatin were dissolved in distilled water on the day of administration.

Statistics

Heart rate, blood pressure, and QT interval 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 ischemia-induced arrhythmias (Fig. 1)

During 5-min ischemia, PVC, VT, and/or VF occurred in some rats. We excluded rats with VF or VT (continuing until reperfusion). The incidence of PVC and VT was high (PVC, 80% and VT, 70%) in the vehicle control group. In protocol I using chronically administrated rats (once daily orally for 22 consecutive days), pravastatin (0.02, 0.2, and 2 mg/kg) dose-dependently reduced the incidence of PVC (70%, 50%, and 36%, respectively) and VT (70%, 40%, and 9%, respectively), but fluvastatin did not show any significant effect on both PVC and VT even at the highest dose of 4 mg/kg. In protocol II, we examined two pravastatin groups of 0.2 mg/kg and 2 mg/kg by acute administration (one dose given 2 h before reperfusion). The dose of 0.02 mg/kg was not included, since in protocol I this dose of pravastatin had no effect on the arrhythmias. By acute administration, pravastatin did not change the incidence of PVC and VT.

Effects of pravastatin and fluvastatin on reperfusion-induced arrhythmias (Fig. 2)

During the reperfusion period, the incidence of VT, total VF (including non-lethal VF and lethal VF), and lethal VF in the control group were 100%, 90%, and 90%, respectively. In protocol I, chronic pravastatin (0.02, 0.2, and 2 mg/kg) did not significantly change the incidence of VT (100%, 80%, and 100%, respectively) and total VF (60%, 40%, and 82%, respectively), but reduced the incidence of lethal VF significantly ($P < 0.05$) to 20% at 0.2 mg/kg. Acute pravastatin in protocol II and chronic fluvastatin in protocol I did not change the incidence of the arrhythmias. In reperfusion-induced arrhythmias, all VF developed from VT, but not all VT developed VF.

Effects of pravastatin and fluvastatin on serum total cholesterol level (Fig. 3)

After 22 days of consecutive administration of pravastatin and fluvastatin, the serum total cholesterol level in the pravastatin- and fluvastatin-treated rats showed no significant changes when compared with those in the vehicle-treated rats, with 53 ± 4 mg/dl for the control group; 53 ± 3 , 54 ± 3 , and 54 ± 4 mg/dl for pravastatin (0.02, 0.2, and 2 mg/kg) groups; 58 ± 2 ,

Fig. 1. Effects of pravastatin and fluvastatin on ischemia-induced premature ventricular contraction (PVC) and ventricular tachycardia (VT) in anesthetized rats. Chronic administration (chronic ad.) of pravastatin (0.02, 0.2, and 2 mg/kg, once daily orally for 22 consecutive days) dose-dependently reduced the incidences of PVC and VT; and at the dose of 2 mg/kg, it produced a significant ($P < 0.05$) reduction in the incidence of VT. Acute administration (acute ad.) of pravastatin (0.2 and 2 mg/kg, once) and chronic administration of fluvastatin (0.2, 2, and 4 mg/kg) did not show any significant changes. Numbers above the bars indicate the incidences of arrhythmias.

Fig. 2. Effects of pravastatin and fluvastatin on reperfusion-induced ventricular tachycardia (VT), total ventricular fibrillation (VF), and lethal VF in anesthetized rats. Chronic administration (chronic ad.) of pravastatin (once daily orally for 22 consecutive days) at 0.2 mg/kg significantly ($P < 0.05$) reduced the incidences of lethal VF. Acute administration (acute ad.) of pravastatin (0.2 and 2 mg/kg, once) and chronic administration of fluvastatin (0.2, 2, and 4 mg/kg, once daily orally for 22 consecutive days) did not show any significant changes. Numbers above the bars indicate the incidences of arrhythmias.

56 ± 6 , and 56 ± 3 mg/dl for fluvastatin (0.2, 2, and 4 mg/kg) groups, respectively.

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

The heart rates, blood pressure, and QT intervals

showed no significant changes among 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), but the heart rate was not significantly changed.

Fig. 3. Effects of pravastatin and fluvastatin on serum total cholesterol level in rats. After chronic administration (once daily orally for 22 consecutive days) of pravastatin (0.02, 0.2, and 2 mg/kg) and fluvastatin (0.2, 2, and 4 mg/kg), the serum total cholesterol level was measured. There was no significant difference between drug-treated and control groups. The data are shown as means $\pm$ S.E.M.

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 = 10)	chronic	460 ± 12	421 ± 19	433 ± 29	128 ± 5	93 ± 7	58 ± 9**	53.6 ± 0.9
Pravastatin 0.02 mg/kg	(n = 10)	chronic	465 ± 7	419 ± 19	408 ± 18	134 ± 6	92 ± 8	56 ± 8**	52.9 ± 1.3
Pravastatin 0.2 mg/kg	(n = 10)	chronic	456 ± 17	415 ± 25	398 ± 28	125 ± 5	85 ± 6	50 ± 7**	52.5 ± 1.4
Pravastatin 2 mg/kg	(n = 11)	chronic	436 ± 12	408 ± 15	385 ± 17	119 ± 9	85 ± 4	43 ± 6**	52.7 ± 1.1
Pravastatin 0.2 mg/kg	(n = 10)	acute	474 ± 9	424 ± 14	418 ± 14	141 ± 3	91 ± 4	63 ± 6**	51.5 ± 0.8
Pravastatin 2 mg/kg	(n = 10)	acute	452 ± 12	401 ± 15	414 ± 29	135 ± 7	86 ± 5	46 ± 6**	52.9 ± 1.2
Fluvastatin 0.2 mg/kg	(n = 10)	chronic	437 ± 10	407 ± 11	436 ± 15	117 ± 6	87 ± 3	75 ± 7*	51.6 ± 0.9
Fluvastatin 2 mg/kg	(n = 10)	chronic	450 ± 13	412 ± 19	397 ± 20	127 ± 6	94 ± 7	59 ± 8**	52.8 ± 1.0
Fluvastatin 4 mg/kg	(n = 10)	chronic	443 ± 16	407 ± 12	421 ± 19	109 ± 7	86 ± 6	63 ± 8*	51.8 ± 0.7

Chronic: chronic administration, once daily orally for 22 consecutive days. Acute: acute administration, one dose given 2 h before reperfusion. Values are each a mean $\pm$ S.E.M. * P <0.05, compared with just before occlusion. ** P <0.01, compared with just before occlusion.

Discussion

This is the first study showing that pravastatin is associated with an inhibition of arrhythmias, especially lethal VF, induced by coronary ischemia/reperfusion in rats. Hydrophilic pravastatin when chronically administered significantly reduced the incidence of ischemia-induced VT and reperfusion-induced lethal VF in normocholesterolemic rats. However, chronically administered lipophilic fluvastatin and acute administration of pravastatin did not show such effects. Furthermore, both pravastatin and fluvastatin did not decrease the serum total cholesterol level after chronic administration.

It has been known that pravastatin and other statins, by lowering the cholesterol level, were expected to reduce relative cardiovascular events (22–24). However, recent studies have revealed that statins partly contribute to their cardiovascular benefits through non-lipid effects or so called pleiotropic effects (8–12, 25). Clinically, ventricular tachyarrhythmia is a major cause of sudden

cardiac death, which accounts for 50% of all cardiovascular mortality (14); therefore, it is important to investigate possible antiarrhythmic effects of statins. The present study was designed to examine effects of pravastatin on ventricular tachyarrhythmias induced by ligation/reperfusion in a rat model.

In clinical trials, it is difficult to separate the benefits of statins from their cholesterol-lowering effect. However, the rats used in our study may be suitable for differentiating the effects of statins from cholesterol-lowering effect because the cholesterol level in Sprague-Dawley rats (the present study) and also in Wistar rats are low and pravastatin lacks hypocholesterolemic effects (19).

As for the antiarrhythmic mechanism of pravastatin, we excluded the possible effects through direct actions on cardiac ionic channels, because both pravastatin and fluvastatin did not significantly change the heart rate and ECG parameters, and consistently, no paper has shown a direct effect of statins on cardiac ionic channels. Therefore, its pleiotropic effects, including stabilization

of vulnerable plaque, amelioration of endothelial dysfunction, reduction of platelet reactivity (26), and inhibition of neutrophils (27, 28), may provide some explanations. Among them, neutrophils may play more important roles in triggering arrhythmia. When neutrophils infiltrate into myocardial tissue with reperfusion, they produce toxic oxygen species, PAF (platelet-aggregating factor), arachidonic acid metabolites, and protease that may contribute to cardiac injuries (29–31), including arrhythmia (28). One clinical study showed that removing the neutrophils in patients with myocardial infarction decreased the frequency of ventricular premature beats during the first 30 min after reperfusion (27). Pravastatin has been proved to inhibit the neutrophils by inhibiting their interaction with endothelium through attenuating endothelial adhesion expression (32), infiltration, accumulation (9, 32), and superoxide generation (10). Therefore, we speculate that pravastatin may exert its antiarrhythmic effect through the inhibition of neutrophils to a certain extent, but we could not measure myeloperoxidase activity of neutrophils in the reperfused myocardium in this study.

In our experiments, we compared hydrophilic pravastatin with lipophilic fluvastatin. It is interesting that pravastatin, not fluvastatin, showed the antiarrhythmic effects, even though they share a cholesterol-lowering effect, reduction of cardiovascular mortalities (5, 7, 33, 34), and even some pleiotropic effects (35, 36). Lipophilic fluvastatin can enter the myocardial cells more easily and inhibit HMG-CoA reductase there, which is a key enzyme not only in cholesterol biosynthesis but also in ubiquinone biosynthesis (37). Since ubiquinones contribute to the energy generation in the mitochondrial chain, its reduction will thus deteriorate the performance of the myocardium, particularly under pathophysiological conditions. Many studies reported that lipophilic statins, simvastatin (38, 39) and fluvastatin (40, 41), decreased the myocardial level of coenzyme Q₁₀ and adenosine triphosphate (ATP) generation in mitochondria and increased contractile dysfunction of the myocardium after ischemia and reperfusion. It is convincing that, in the same experiments, hydrophilic pravastatin did not show these deleterious effects (38–41). We agree with the proposal by Satoh et al. (41) that the favorable, hypocholesterolemic effects of lipophilic statins on ischemic heart disease or coronary heart disease may be attenuated by the unfavorable effects of the statins on the myocardial energy-generating system and contractile function. Pravastatin has the advantage of its hydrophilicity, which hinders easy entry to myocardial cells. However, in the present experiment, pravastatin at 2 mg/kg was needed to suppress VT during ischemia, while a smaller dose 0.2 mg/kg was

enough to suppress VF during reperfusion and a higher dose lost its efficacy. We cannot explain this phenomenon, but some detrimental effects at higher doses must have hindered its beneficial effects.

The different incidences of VT and VF in our study indicate that the arrhythmia is more severe during reperfusion than during ischemia. Pravastatin lessened the severity of arrhythmia during ischemia and reperfusion as well, suppressing VT during ischemia and lethal VF, but not VT, during reperfusion. This preferential effectiveness on reperfusion VF rather than VT was also observed in our previous studies (42–45) using the same rat arrhythmia model with Na⁺/H⁺ exchange inhibitors, such as cariporide and FR168888, and a class III antiarrhythmic drug, MS-551. However, by recording only the ECG in the present experiment, it is difficult to speculate the mechanism involved.

There are some limitations in the present study. First, because of the species differences, the results of rats in our study might not necessarily predict the clinical outcome. Second, the short time (5 min) coronary occlusion cannot produce a prominently recognizable myocardial infarction; and rapid occurrence of lethal VF after reperfusion, which stops functional myocardial blood perfusion, makes it difficult to observe the filtration of neutrophils. Third, this study did not show hemodynamic and electrophysiological influences.

Based on our experimental results, we drew following conclusions: (1) Hydrophilic pravastatin reduces the incidence of arrhythmias, especially lethal VF, induced by coronary artery ischemia/reperfusion in rats, but lipophilic fluvastatin does not. (2) The inhibition of arrhythmias is independent of the cholesterol-lowering effect. (3) This effect is produced by a chronic administration, but not by an acute administration. (4) The effects of pravastatin might not be attributable to its hemodynamic effects because blood pressure and heart rate were not altered. Accordingly, pravastatin, through the antiarrhythmic effects, may contribute to reducing cardiovascular mortality. Our findings expand the pleiotropic spectrum of the statins' favorable effects on cardiovascular diseases.

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