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Early Administration of Fluvastatin, but not at the Onset of Ischemia or Reperfusion, Attenuates Myocardial Ischemia-Reperfusion Injury Through the Nitric Oxide Pathway Rather Than Its Antioxidant Property

Akira Matsuki, MD; Akihiko Igawa, MD; Takashi Nozawa, MD; Teruo Nakadate, MD; Norio Igarashi, MD; Makoto Nonomura, MD; Hiroshi Inoue, MD

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Background Three-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors (statins) are known to attenuate myocardial ischemia-reperfusion (IR) injury. Fluvastatin (FV) has a potent free radical scavenging action, but it is unclear whether the timing of FV administration could affect its cardioprotective effect or if the antioxidant property of FV might attenuate IR injury.

Methods and Results IR was induced in rats by left coronary artery occlusion for 30 min followed by 24-h reperfusion. The rats were divided into 4 groups: oral FV group (10 mg/kg per day for 2 weeks before ischemia); iv, FV group (10 mg/kg) before ischemia; iv, FV group (10 mg/kg) before reperfusion; and control group. Oxidative stress was evaluated by myocardial 8-hydroxydeoxyguanosine (8-OHdG) content. The area at risk did not different among the 4 groups. Pretreatment with FV for 2 weeks significantly reduced the infarct size by 28% as compared with the control group, but FV administered just before ischemia or reperfusion did not. Myocardial 8-OHdG content was not affected by FV. The infarct-sparing effect of FV was completely abolished by N^{ω}-nitro-L-arginine methyl ester or wortmannin.

Conclusions The present results indicate that pretreatment with FV, but not just before ischemia or reperfusion, attenuates IR injury primarily through the nitric oxide pathway, not through its antioxidant property. (*Circ J* 2006; **70:** 1643–1649)

Key Words: Fluvastatin; Myocardial ischemia-reperfusion injury; Nitric oxide; Oxidative stress

Early reperfusion of an occluded coronary artery preserves myocardial viability and function by limiting the size of the myocardial infarct[1,2] However, despite early reperfusion, myocardial ischemia-reperfusion (IR) injuries, including no reflow, stunning and reperfusion arrhythmias, sometimes occur, thereby attenuating the cardioprotective effect of reperfusion therapy[3] Recent studies in experimental animals have demonstrated that 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) attenuate IR injury independently of their lipid-lowering action[4–8] Statins have pleiotropic effects, including improvement of endothelial function by increased nitric oxide (NO) bioavailability[9] and antioxidant[10] and anti-inflammatory actions[11] which may explain their attenuation of IR injury. The experimental result of cardioprotection by statins has therapeutic implication for patients with acute coronary syndrome who will be treated with reperfusion therapy; however, the time at which statin treatment was administered before IR varied from hours to days, and the results are conflicting[5,12] It is not clear whether acute administration of statins at the onset of ischemia or reperfusion will prevent or attenuate the IR injury.

Oxidative stress plays an important role in IR injury, and antioxidants such as superoxide dismutase and catalase could limit the infarct size in IR[13] Statins are known to decrease free radical generation in the vascular wall[14,15] and myocardium[16] which suggests that statins may protect the ischemic myocardium from IR injury via suppression of oxygen-derived free radicals produced upon reperfusion. Among the statins, fluvastatin (FV) has a potent free radical scavenging property derived from its chemical structure[17] and the purpose of the present study was to elucidate the effects of acute administration of FV and the role of its antioxidant property on IR injury in rats.

Methods

The experimental procedures followed the approved guidelines for animal experimentation at the University of Toyama.

Myocardial IR

Male Wistar rats weighing 270–380 g (n=103) were intubated under ether anesthesia and ventilated using a rodent respirator. The heart was exposed by left thoracotomy and the left coronary artery was ligated 2–3 mm from its origin

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Second Department of Internal Medicine, Faculty of Medicine, University of Toyama, Toyama, Japan

Mailing address: Takashi Nozawa, MD, The 2nd Department of Internal Medicine, Faculty of Medicine, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan. E-mail: tnozawa@med.u-toyama.ac.jp

Table 1 Plasma Lipid Levels and Hemodynamics of Rats With Ischemia-Reperfusion Injury

	Sham (n=6)	*VEH (n=10)*	*FVPO (n=10)*	*FVIV (n=9)*	*FVREP (n=12)*
Body weight (g)	340±25	305±23*	317±36	306±8	303±9*
TC (mg/dl)	51±7	60±12	60±9	56±9	59±9
TG (mg/dl)	27±9	28±8	25±8	24±8	31±13
HR (beats/min)	394±25	410±38	403±44	413±26	428±39
MBP (mmHg)	87±10	77±14	91±16	80±6	87±10
LVSP (mmHg)	110±12	103±12	120±8*	112±5	111±7
LVEDP (mmHg)	3±2	10±3*	12±3*	9±4*	7±4
Peak +dP/dt ($\times 10^3$ mmHg/s)	10.8±2.3	7.7±1.5	9.8±2.4	9.2±1.1	8.7±2.8

Values are mean±SD.

Plasma levels of total cholesterol (TC) and triglyceride (TG), heart rate (HR), mean blood pressure (MBP), left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP) and peak positive value of rate of change in left ventricular pressure (+dP/dt) were measured 24 h after reperfusion.

Sham, sham operation; VEH, oral administration of vehicle (carboxymethyl cellulose) for 2 weeks before ischemia; FVPO, oral administration of fluvastatin (FV) for 2 weeks before ischemia; FVIV, intravenous administration of FV at 5 min before the onset of ischemia; FVREP, intravenous administration of FV at 5 min before reperfusion.

**$p<0.05$ vs Sham.*

with a 5-0 suture. After 30 min, the ligature was removed and reperfusion was visually confirmed. The chest wall was closed and the rat was allowed to recover for 24 h. Sham rats were operated similarly but without IR.

Pretreatment

The rats undergoing ligation were randomized into 4 groups before IR according to the study protocol: (1) FV (10 mg/kg per day, Tanabe Seiyaku, Saitama, Japan) was administered orally by gavage for 2 weeks before ischemia (FVPO group), (2) intravenously (10 mg/kg) 5 min before ischemia (FVIV group), (3) intravenously 5 min before reperfusion (FVREP group), and (4) vehicle (0.1% carboxymethyl cellulose) was administered orally for 2 weeks (VEH group).

Measurement of Hemodynamics and Myocardial Infarct Size

Hemodynamic study was performed 24 h after reperfusion. A 2Fr micromanometer-tipped catheter was inserted into the right carotid artery and advanced into the left ventricle (LV) to determine the pressure. With the rat anesthetized lightly with ether and breathing spontaneously, the signal of LV pressure was digitized online at 2 ms intervals and analyzed with a signal-processing computer system. After the hemodynamic study, blood samples were drawn from the carotid artery for analysis of plasma levels of total cholesterol (TC) and triglyceride (TG).

After an overdose of sodium pentobarbital (70 mg/kg ip) the chest was opened and the heart re-exposed. The left coronary artery was ligated at the same position, the heart was quickly excised and mounted on a Langendorff apparatus, and perfused with blue dye to stain perfused myocardium blue (ie, the area at risk would be unstained). The LV was sliced into 2-mm sections for incubation in triphenyl tetrazolium chloride for 10 min at 37°C to distinguish stained viable tissue from unstained infarcted area. Area at risk and infarct area were quantified using computer-assisted planimetry and the infarct size was determined by the following equation: infarct size (%) = (infarct area/area at risk) ×100.

Assessment of Myocardial Oxidative Damage

In a separate set of experiments, myocardial oxidative stress was assessed by 8-hydroxydeoxyguanosine (8-OHdG) content, a marker of oxidative DNA damage[18] After 30 min of reperfusion following 30 min coronary occlusion, the ischemic myocardium was dissected (approximately 300 mg), rapidly frozen in liquid N_2, and stored at –80°C until later analyses. The frozen tissue was homogenized and the DNA was extracted by NaI method using a commercially available kit (DNA extractor WB kit, Wako, Osaka, Japan). After the DNA pellet was dissolved in distilled water, 50 μg of DNA was digested with nuclease P_1 (Sigma-Aldrich, Tokyo, Japan) and alkaline phosphatase (Sigma-Aldrich), and then centrifuged at 14,000 g for 10 min through a 0.22-μm filter (Millipore, MA, USA) according to the manufacturer's instructions. The 8-OHdG content in the extracted DNA solution were determined by enzyme-linked immunosorbent assay (ELISA) method (Highly Sensitive 8-OHdG ELISA kit, Japan Institute for the Control of Aging, Shizuoka, Japan).

In the Langendorff-perfused hearts of rats, the amount of 8-OHdG is reported to increase significantly after 20 min of reperfusion following 30 min of ischemia[19] In our preliminary study, myocardial 8-OHdG content did not differ after 30 min or 2 h of reperfusion (data not shown). Accordingly, the myocardial 8-OHdG content was determined at 30 min of reperfusion in the present study.

Involvement of NO Pathway

To evaluate the contribution of the NO pathway to the cardioprotective effect of pretreatment with FV, N^{ω}-nitro-L-arginine methyl ester (L-NAME, 15 mg/kg, Sigma-Aldrich) was administered intravenously 10 min before ischemia in the FVPO and VEH groups. Hemodynamic changes caused by L-NAME treatment were measured before ischemia and the area at risk and infarct area were determined after 24-h reperfusion in both groups. In addition, to clarify the influence of FV on endothelial NO synthase (eNOS) activation via phosphatidylinositide (PI) 3-kinase/Akt pathway, wortmannin (15 μg/kg), a specific inhibitor of PI3-kinase, was administered intravenously 15 min before ischemia in the FVPO and VEH groups.

Statistical Analysis

All data are expressed as mean±SD. The differences between groups were tested with 1-way analysis of variance, followed by the Bonferroni test for multiple comparisons. A value of $p<0.05$ was considered statistically significant.

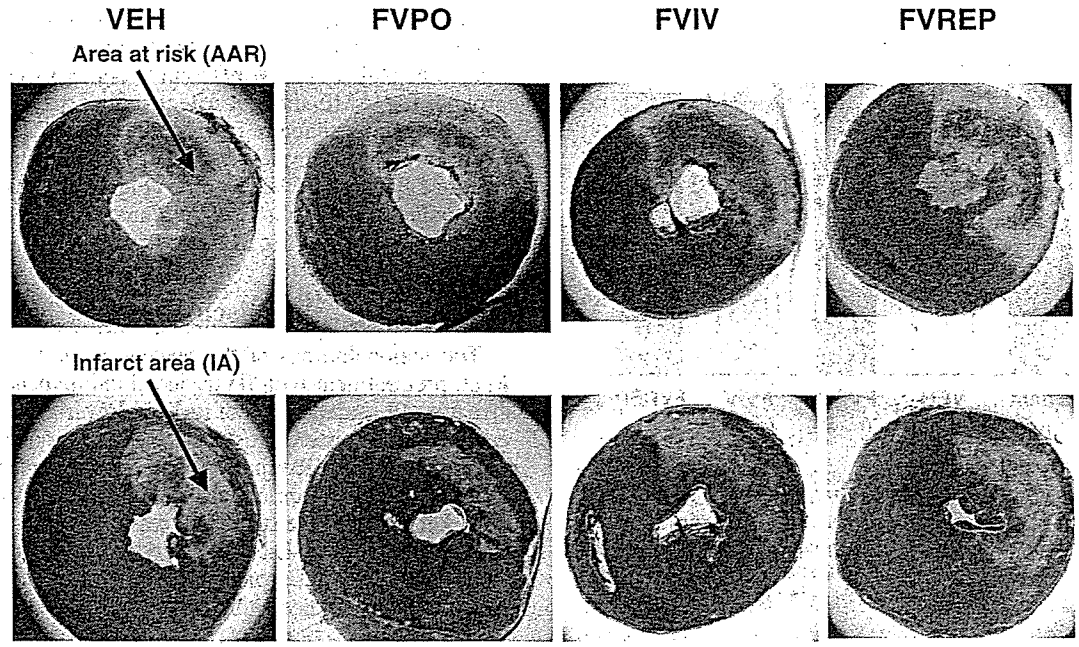

Fig 1. Representative examples of area at risk (AAR) and infarct area (IA) of vehicle (VEH) and fluvastatin (FV) treated rats with myocardial ischemia-reperfusion injury (IR). Infarct size defined by triphenyl tetrazolium chloride was smaller in the FVPO group than in the other 3 groups, although the AAR did not differ among the 4 groups. VEH, oral administration of vehicle for 2 weeks before ischemia; FVPO, oral administration of FV for 2 weeks before ischemia; FVIV, intravenous administration of FV at 5 min before the onset of ischemia; FVREP, intravenous administration of FV at 5 min before reperfusion.

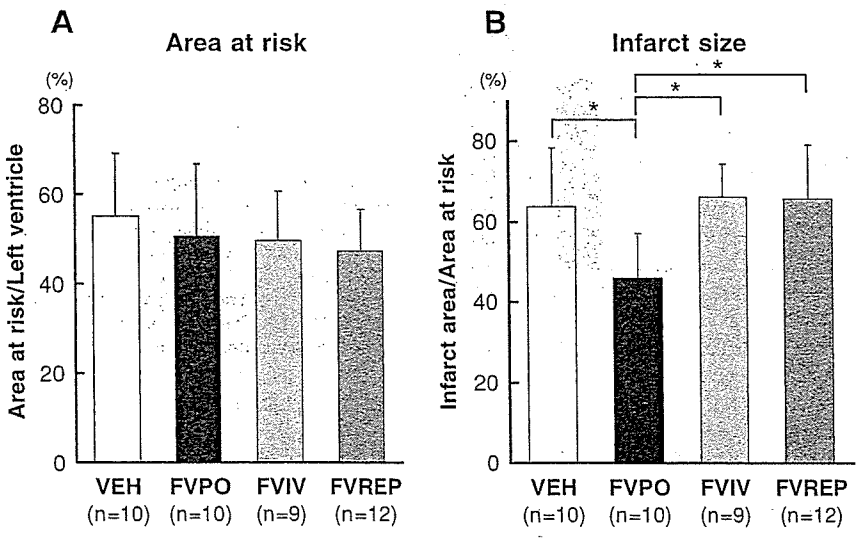

Fig 2. AAR and infarct size of VEH and FV treated rats with myocardial IR. There was no significant difference in the AAR among groups (A). Infarct size was significantly reduced in the FVPO group than in the other 3 groups (B). Data are means + SD. *$p<0.05$. Abbreviations as in Fig 1.

Results

Plasma Lipids and Hemodynamic Data (Table 1)

FV did not affect body weight, or the levels of TC and TG. In the VEH group, LV end-diastolic pressure was elevated and maximum values of the rate of change in LV pressure tended to decrease as compared with the sham rats. FV did not affect these indices significantly.

Area at Risk and Myocardial Infarct Size

Representative examples of the area at risk and infarct area of each group are shown in Fig 1. Pretreatment with FV (FVPO) significantly reduced infarct size by 28% as compared with VEH (46±11% vs 64±15%, $p<0.05$) despite a similar extent of area at risk (Fig 2). However, FV administered just before ischemia or reperfusion did not reduce the infarct size (FVIV, 66±8%; FVREP, 66±14%).

Myocardial 8-OHdG Content

Myocardial 8-OHdG content was significantly elevated in the reperfused myocardium as compared with the sham-operated rats. However, FV did not affect the myocardial 8-OHdG content after IR (Fig 3).

Influence of NO

L-NAME increased mean blood pressure (VEH, 97±10 to 151±17 mmHg, $p<0.05$; FVPO, 96±10 to 143±21 mmHg, $p<0.05$) and decreased heart rate (VEH, 383±41 to 332±

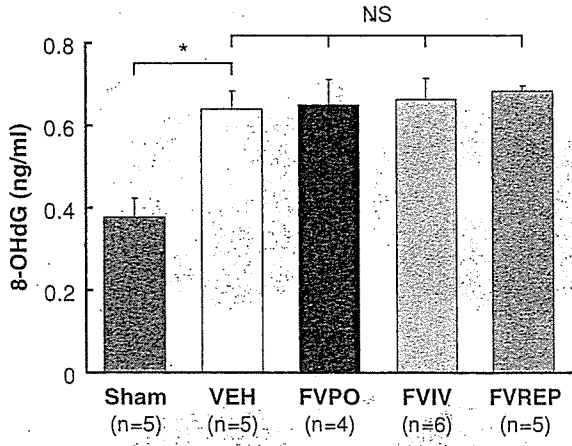

Fig 3. Myocardial 8-hydroxydeoxyguanosine (8-OHdG) content of sham-operated rats (Sham), VEH and FV treated rats with myocardial IR. In rats with IR, myocardial 8-OHdG content was significantly elevated as compared with the Sham group, although there was no significant difference in 8-OHdG content among the IR groups. Data are means+SD. *p<0.05. Abbreviations as in Fig 1.

24 beats/min, p<0.05; FVPO, 377±45 to 334±41 beats/min, p<0.05). Changes in mean blood pressure and heart rate after L-NAME were not different between VEH and FVPO groups. L-NAME did not affect infarct size in the VEH group, despite changes in the mean blood pressure and heart rate, but completely abolished the infarct-sparing effect of FV in the FVPO group (Fig 4). Pretreatment with wortmannin also abolished the infarct-sparing effect of FV in the FVPO group (Fig 5).

Discussion

The major findings of the present study are as follows. First, pretreatment with FV reduced the myocardial infarct size after IR, independent of its lipid-lowering action. However, FV administered just before ischemia or reperfusion did not attenuate IR injury. Second, pretreatment with FV did not affect myocardial 8-OHdG content, which served as a marker of oxidative stress and was elevated in reperfused myocardium. Third, L-NAME and wortmannin completely abolished the infarct-sparing effect of FV.

These results indicate that early administration of FV, be-

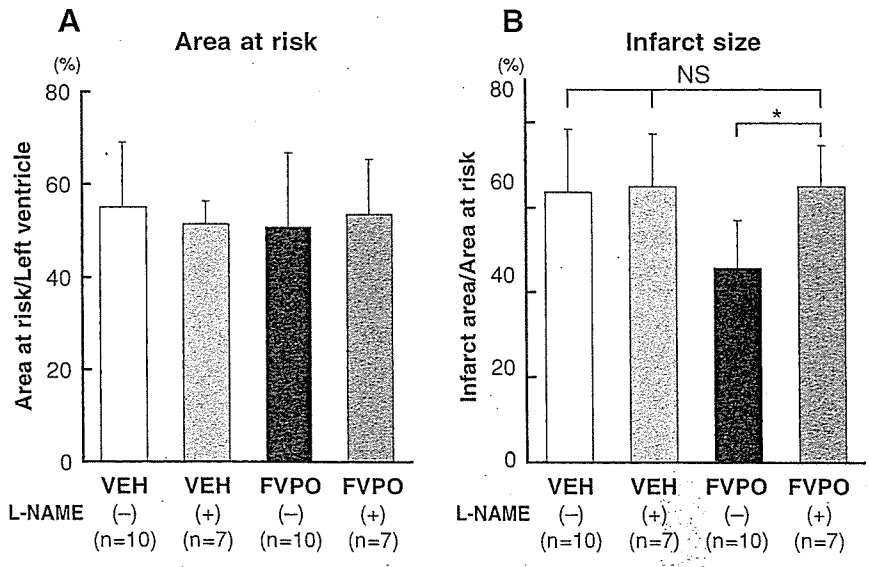

Fig 4. Effects of N^{ω}-nitro-L-arginine methyl ester (L-NAME) on AAR and infarct size in the VEH and FVPO groups. L-NAME did not affect AAR or infarct size in the VEH group (A). The infarct-sparing effect of FV was abolished by pretreatment with L-NAME (B). Data are means+SD. *p< 0.05. Abbreviations as in Fig 1.

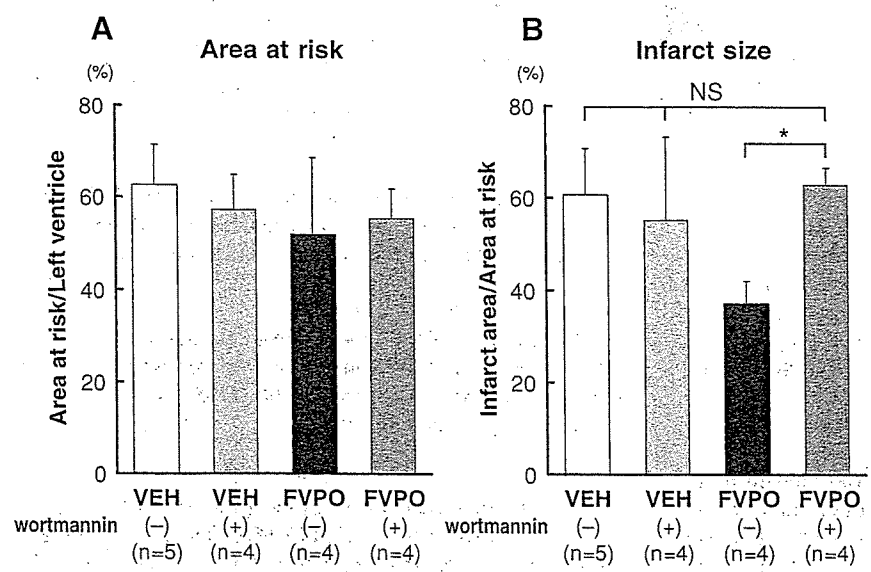

Fig 5. Effects of wortmannin on AAR and infarct size in the VEH and FVPO groups. Wortmannin did not affect AAR or infarct size in the VEH group (A). The infarct-sparing effect of FV was abolished by wortmannin (B). Data are means+SD. *p<0.05. Abbreviations as in Fig 1.

fore ischemia, is required for attenuation of IR injury and the cardioprotective effect of FV is primarily mediated through increased NO bioavailability via the PI3-kinase/Akt pathway, not through its antioxidant property.

Antioxidant Property of FV

There were few in vivo studies that have determined quantitatively the antioxidant effect of statins in IR injury. Production of oxygen-derived free radicals is considered to be a determinant of IR injury,[20] and of these, hydroxyl radical (·OH) is highly reactive and plays a critical role in post-ischemic myocardial damage during reperfusion.[21] The antioxidant action of statins is derived not only from the reduction of oxidized low-density lipoprotein, but from inhibition of NADPH oxidase, which generates superoxide anion ($\cdot O_2^-$) in the vascular wall.[22] FV has a potent antioxidant property as a free radical scavenger, because of its unique chemical structure,[17] but in the present study, however, FV did not reduce the myocardial content of 8-OHdG, which is produced by hydroxylation at the C-8 position of deoxyguanosine by ·OH and is currently used as a stable biomarker of oxidative DNA damage.[18,23] Formation of 8-OHdG in the heart with IR progressively increases after reperfusion and is completely blocked by co-treatment with a free radical scavenger.[19] A large amount of oxygen-derived free radicals are released from mitochondoria, the vessel walls, and leukocytes during reperfusion and therefore the antioxidant action of FV might be insufficient for cardioprotection from IR injury. Hayasaki et al reported that a reduction in myocardial oxidative stress was associated with attenuation of IR injury in mice deficient in the CC chemokine receptor 2.[24] The discrepancy between the study by Hayasaki et al and the present study regarding the influence of oxidative stress on IR injury may derive from differences in the animal model used or the timing for the evaluation of oxidative stress. Nonomura et al, however, showed no significant relation between oxidative stress and IR injury in rats treated with cardiac sympathetic denervation.[25] Thus, the role of oxidative stress in IR injury remains to be elucidated.

In the present study, the dose of FV was relatively high, but within the range commonly used in rat and mouse experiments.[15,26,27] Suzumura et al reported that FV showed dose-dependent free radical scavenging action in vitro.[28] To elucidate the antioxidative effect of FV we therefore used a high dose (10 mg/kg).

Improvement of NO Bioavailability by Statins

Earlier studies reported that statins had cardioprotective effects on IR injury because of increased release of NO via eNOS bioavailability.[4,6,7,29–31] Those results were supported by data that the infarct-sparing effect of statins was not observed in eNOS-deficient mice[6] or with pretreatment with L-NAME,[7] a finding consistent with the present result. The mechanisms by which statins increase eNOS activity are related to their ability to stabilize eNOS mRNA[32] and/or to activate the PI3-kinase/Akt pathway.[33] Jones et al reported that simvastatin did not attenuate the infarct size in the mouse heart when given less than 3 h before myocardial ischemia,[5] which is consistent with the present result that administration of FV just before ischemia or reperfusion did not reduce the infarct size. By contrast, the concentration of Akt phosphorylation by statins peaked at approximately 1 h and declined by 3 h after exposure in a cell-culture system.[33] The bolus administration of FV in the present study might be insufficient for maintaining eNOS activation via the PI 3-kinase/Akt pathway during reperfusion. Sanada et al also reported that statins had an acute cardioprotective effect via activation of PI3-kinase/Akt in a dog model of IR.[34] They suggested that there was an optimal dose for each statin for the infarct-sparing effect. Therefore, the intravenous injection of FV in the present study may not have been the optimal dose as compared with its oral administration for 14 days.

Mechanism of Cardioprotection by Statins

There are several possible mechanisms for the cardioprotective effect of FV in the present study. First, enhancement of the NO level by statins potentiates a vasodilatory effect of resistant vessels, leading to preservation of tissue perfusion after IR.[8] Second, statins inhibit leukocyte–endothelial cell interactions and reduce the leukocyte extravasation,[4] which suppresses the release of proinflammatory cytokines and chemically active compounds that induce tissue inflammation and injury. Third, the cardioprotective effect of statins may be mediated through an alteration of autonomic nerve function. Chronic treatment with simvastatin reduced sympathetic overactivity and plasma norepinephrine levels in animals with chronic heart failure.[35] The suppression of sympathetic activity during IR by statins may protect the ischemic myocardium from norepinephrine-induced myocyte injury.

Effect of FV on Plasma Cholesterol Level in Normocholesterolemic Rats

Pretreatment with FV did not reduce the level of plasma cholesterol in our study using normocholesterolemic rats and it is well known that plasma lipids do not change in normocholesterolemic rats treated with statins.[15,27] Although statins effectively inhibit HMG-CoA reductase in the rat, the plasma cholesterol level does not change, or even increases, with statin therapy because hepatic expression of the enzymes involved in its biosynthesis is markedly upregulated.[36,37]

Clinical Implications

Some clinical reports have revealed the cardioprotective effect of statins in IR injury. Patients on statins prior to the onset of acute myocardial infarction had smaller myocardial infarcts.[38] Wong et al showed that statin use within 2 weeks of the onset of acute myocardial infarction improved myocardial perfusion after thrombolysis.[39] However, it has not been elucidated whether acute statin treatment given at the onset of acute myocardial infarction might also reduce the myocardial damage. Our results did not show a cardioprotective effect of FV when administered at the onset of myocardial ischemia or reperfusion; however, our study suggests that pretreatment with statins would possibly reduce the extent of myocardial injury during a coronary event.

Study Limitations

First, we did not determine the dose–response effect of FV for protection against IR injury. Recent studies show that higher doses of statins do not reduce the infarct size and suggest that there could be optimal doses for each statin to elicit the acute cardioprotective effect in IR injury.[31,34] Second, we did not elucidate the time period required for statin treatment before IR to produce the cardioprotective effect. The optimal timing of statin administration in IR

injury needs to be studied in further investigations. Third, hemodynamic alterations by L-NAME might have affected infarct size. However, there was no significant difference in infarct size between the VEH and VEH+L-NAME groups (Fig 4). An earlier study reported that 15 mg/kg of L-NAME did not cause a significant change in infarct size as compared with control animals.[40] Therefore, the hemodynamic changes after L-NAME treatment might not have influenced infarct size in our study. Finally, we did not determine myocardial eNOS protein or plasma NO levels after treatment with FV. However, the present results using L-NAME and wortmannin strongly suggest an infarct-sparing effect of FV through the NO-dependent pathway.

Conclusions

Pretreatment with FV before ischemia, but not at the onset of ischemia or reperfusion, limited myocardial necrosis in IR injury. This cardioprotective effect is primarily mediated through a NO-dependent action, not through an antioxidant action. Earlier administration of statins before the onset of ischemia to augment NO production might be required for the cardioprotective effect to occur.

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Pharmacological Cardioversion of Persistent Atrial Fibrillation With and Without a History of Drug-Resistant Paroxysmal Atrial Fibrillation

Akira Fujiki, MD; Tamotsu Sakamoto, MD; Jotaro Iwamoto, MD;
Kunihiro Nishida, MD; Hidehiko Nagasawa, MD;
Koichi Mizumaki, MD; Hiroshi Inoue, MD

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Background Suppression by antiarrhythmic drugs of the maintenance mechanisms could convert persistent atrial fibrillation (AF) to sinus rhythm (SR). Whether a history of drug-resistant paroxysmal AF (PAF) would affect the outcome of pharmacological conversion of persistent AF by bepridil or in combination with aprindine was evaluated in the present study.

Methods and Results The study group comprised 51 consecutive patients (24 men, 61±8 years) undergoing pharmacological conversion of persistent AF lasting >1 month. Drug-resistant PAF was defined as AF and at least 2 ineffective antiarrhythmic drugs for suppression of AF recurrence. Fast Fourier transform analysis of fibrillation waves was used to measure fibrillation cycle length (FCL) from the peak frequency. Fifteen patients had a history of drug-resistant PAF (Group I), and the remaining 36 did not (Group II) before diagnosis of persistent AF. Ten patients (67%) in Group I and 26 patients (72%) in Group II were restored to SR by bepridil alone or in combination with aprindine after 29±15 days of drug administration. Before conversion to SR, bepridil increased the FCL more in Group II than in Group I. During a 12-month follow-up period, 4 of 10 patients in Group I and 2 of 26 patients in Group II ($p<0.01$) had recurrence of persistent AF with bepridil alone or in combination with aprindine.

Conclusions A history of drug-resistant PAF does not affect the efficacy of pharmacological conversion by bepridil or in combination with aprindine. However, recurrence of AF was significantly higher in patients with such a history. (*Circ J* 2006; **70:** 1138–1141)

Key Words: Antiarrhythmic drug; Aprindine; Atrial fibrillation; Bepridil; Defibrillation

Pharmacological termination of atrial fibrillation (AF) lasting more than 1 week is usually considered difficult[1] but we have demonstrated that oral administration of bepridil alone or in combination with aprindine can restore sinus rhythm (SR) in nearly 70% patients with long-lasting persistent AF (AF >1 month)[2] because bepridil suppresses the mechanisms of maintaining persistent AF[3]

Some cases of persistent AF may be preceded by episodes of paroxysmal AF (PAF)[4,5] If the mechanisms that initiate PAF are resistant to antiarrhythmic drug therapy, pharmacological cardioversion of persistent AF would be difficult because of the presence of a drug-resistant substrate for AF. However, whether a history of drug-resistant PAF would affect the outcome of pharmacological conversion is unclear, so we compared the efficacy of pharmacological cardioversion in patients with long-lasting persistent AF with and without a history of drug-resistant PAF.

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The Second Department of Internal Medicine, Faculty of Medicine, University of Toyama, Toyama, Japan

Mailing address: Akira Fujiki, MD, The Second Department of Internal Medicine, Faculty of Medicine, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan. E-mail: fujiki@med.u-toyama.ac.jp

Methods

Subjects

All 51 patients had had persistent AF lasting at least 1 month and were referred to Toyama University Hospital for elective cardioversion of AF between April 2003 and December 2005. Duration of AF was quantified with ECG recordings, and the median was 12 months (range 1–168 months). All patients underwent physical examination, 12-lead ECG, echocardiography, and biochemical and hematological testing. Excluded were women of childbearing age and patients with myocardial infarction or revascularization within 3 months, left ventricular ejection fraction <0.35, QTc >0.46 s, serum K <3.8 mmol/L or a history of sick sinus syndrome. Anticoagulation therapy with warfarin (international normalized ratio ≈2.0) had been given to all patients for at least 3 weeks before pharmacological conversion was attempted. Concurrent control of the ventricular rate with calcium antagonists or β-blocking drugs was permitted. All patients gave their written informed consent to inclusion in the study. The protocol was approved by the hospital Ethics Committee.

Study Protocol

Patients were divided into 2 groups: Group I consisted of patients with persistent AF that had been preceded by episodes of drug-resistant PAF, and Group II consisted of patients who had no history of drug-resistant AF before

diagnosis of persistent AF. Drug-resistant PAF was arbitrarily defined at least 2 ineffective antiarrhythmic drugs in suppressing AF recurrence.

The details of the pharmacological conversion protocol have been published previously[2] Briefly, after giving information about the risks and benefits of bepridil and aprindine therapy for AF termination, patients received oral bepridil (200 mg/day) and were followed for 2–4 weeks. If bepridil failed to restore SR and the QTc interval was not prolonged markedly, oral aprindine (40 or 60 mg/day) was added to bepridil and the patients were followed for another 4 weeks. After pharmacological conversion, the treatment was continued at the same dosage unless marked QT prolongation (QTc >0.50 or >25% increase) or sinus bradycardia occurred. During SR and AF, the QT interval was corrected by dividing the measured QT interval by the square root of the preceding RR interval that showed the minimal difference between the average values of RR intervals (QTc).

Fibrillation Wave Analysis

Spectral analyses of the fibrillation waves were performed before conversion to SR in both groups as in our previous study[5] Surface ECG lead V_1 was digitally stored on-line on a microcomputer (Value Star NX, NEC, Tokyo, Japan), and the QRST complexes were subtracted using a template-matching algorithm. ECG-segments were digitized at a sampling rate of 1 kHz. Frequency analysis of the subtracted electrograms involved 3 steps: (1) bandpass filtering, (2) application of a Hamming window and (3) 4,096-point fast Fourier transformation. A 50% overlap of adjacent spectral analyses allowed the use of averages of 20 epochs of analyses within a single 44-s data set.

Power spectra were quantified by measuring the peak frequency signal with the maximum magnitude derived from each epoch. The peak frequency of the spectrum in the 3–12 Hz range was converted to a cycle length (in ms = 1,000/frequency), named as fibrillation cycle length (FCL), and averaged from 20 epochs.

Statistical Analysis

All data are expressed as mean±SD. Mann-Whitney U

Table 1 Baseline Clinical Characteristics of the Patients With AF

	Group I (n=15)	*Group II (n=36)*
Age (years)	*61.5±11.4*	*60.9±10.3*
Gender (M/F)	*9/6*	*26/10*
Duration of AF (months)		
Mean	*46.7±48.0*	*33.7±50.1*
Median (range)	*36 (2–168)*	*9.5 (1–168)*
Underlying heart disease		
Valvular disease	*1*	*9*
Congenital disease	*0*	*2*
HCM	*1*	*3*
Hypertension	*6*	*12*
None	*7*	*10*
LA diameter (mm)	*43.8±5.3*	*44.0±7.9*
LVEF	*0.61±0.10*	*0.65±0.10*

Data are mean±SD, unless otherwise indicated.
AF, atrial fibrillation; Group I, patients with a history of drug-resistant paroxysmal AF; Group II, patients without a history of drug-resistant paroxysmal AF; HCM, hypertrophic cardiomyopathy; LA, left atrium; LVEF, left ventricular ejection fraction.

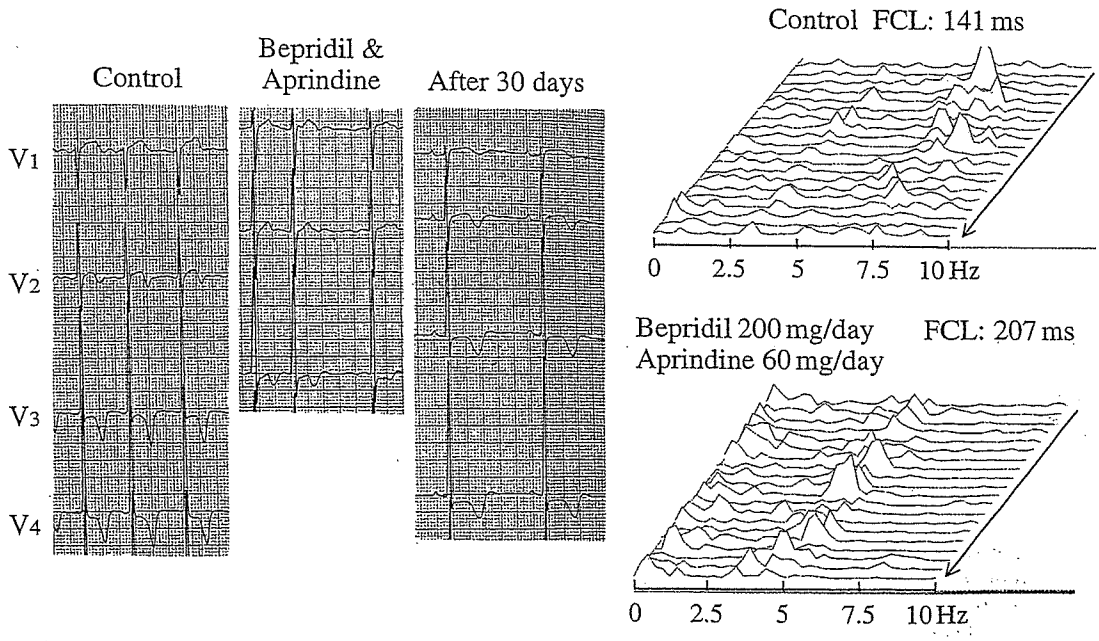

Fig 1. ECGs and spectral analyses of fibrillation waves in a representative patient with conversion of atrial fibrillation (AF) by bepridil and aprindine. This 61-year-old male had persistent AF lasting 36 months preceded by drug-resistant paroxysmal AF (18 months). He had apical type of hypertrophic cardiomyopathy and enlarged left atrium (48 mm). Before bepridil administration QTc was 0.44 and fine fibrillation waves were observed in V_1. After a combination of bepridil and aprindine QTc was 0.43, and fibrillation waves became coarser in V_1. After 30 days of bepridil therapy sinus rhythm was restored. During sinus rhythm QTc was 0.44. Mean fibrillation cycle length (FCL) calculated from the peak frequency was 141 ms before bepridil (Right-upper) and increased to 207 ms after bepridil and aprindine therapy (Right-lower). The peak frequency shifted to the left after a combination of bepridil and aprindine had been started.

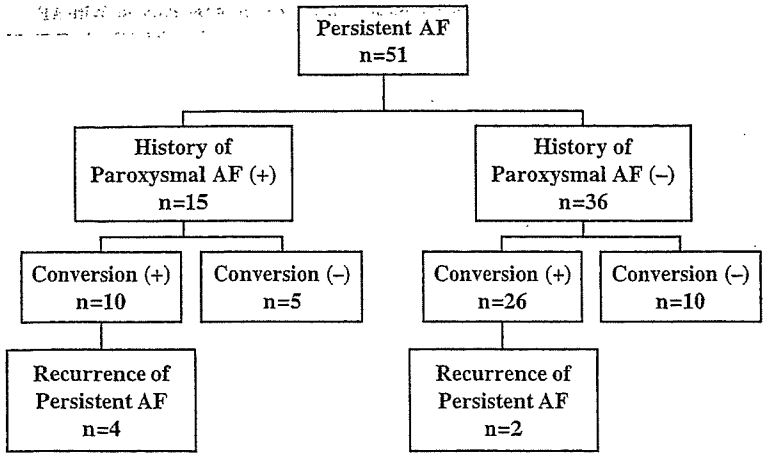

Fig 2. Outcome of pharmacological cardioversion of persistent atrial fibrillation (AF) with and without drug-resistant paroxysmal AF. Numbers in the bottom of each box indicate number of patients. Ten patients (67%) with a history of drug-resistant paroxysmal AF (Group I) and 26 patients (72%) without (Group II) had sinus rhythm restored pharmacologically.

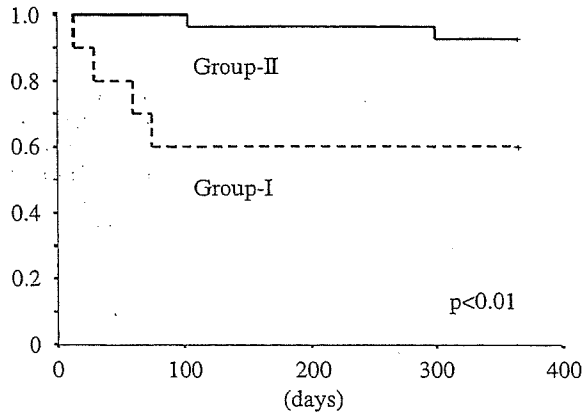

Fig 3. Kaplan-Meier curves for probability of remaining in sinus rhythm in persistent atrial fibrillation (AF) with (Group I) and without (Group II) a history of drug-resistant AF. At the 12-month follow-up examination, 60% of patients in Group I were free of AF recurrence as compared with 92% of patients in Group II ($p<0.01$).

and Wilcoxon tests were used for comparison of the 2 groups of results. Univariate analyses using chi-square tests evaluated categorical data. A Kaplan-Meier analysis with the log-rank test was used to compare the probability of freedom from recurrence of AF. Results were considered to be statistically significant at $p<0.05$. All statistical analyses were performed with the Statview for Windows program (Abacus Concepts, Berkeley, CA, USA).

Results

Baseline Characteristics

Group I comprised 15 patients and Group II had 36 patients. In Group I, antiarrhythmic drugs administered before transition to persistent AF were flecainide in 11, cibenzoline in 10, disopyramide in 8 and pirmenol in 1. Duration of persistent AF and echocardiographic variables were not different between the groups (Table 1).

Conversion to SR

A representative patient is shown in Fig 1. Ten patients (67%) in Group I and 26 (72%) in Group II had SR restored pharmacologically (Fig 2). In Group I, SR was restored by bepridil alone in 2 patients and a combination of bepridil and aprindine in 8 patients after 27±14 days of bepridil administration. In Group II 15 patients had SR restored by bepridil alone, and the remaining 11 patients by a combination of bepridil and aprindine after 30±15 days of bepridil administration. The proportion of patients restored to SR with bepridil alone was significantly greater in Group II than in Group I ($p<0.05$). Before bepridil administration, the FCL did not differ between Group I (140±14 ms) and Group II (152±26 ms). Bepridil increased the FCL more in Group II (196±38 ms) than in Group I (166±13 ms, $p<0.05$).

During a 12-month follow-up period, 4 of 10 patients in Group I and 2 of 26 patients in Group II had recurrence of persistent AF (Figs 2,3). The maintenance of SR was achieved more frequently in Group II than in Group I ($p<0.01$). After restoration of SR, the QTc interval increased to 0.46±0.04 s or by 18±14%. No adverse effects necessitating drug discontinuation occurred, apart from 1 patient who discontinued bepridil administration because of marked QTc prolongation (0.60 s) after restoration of SR.

Discussion

Of 51 patients with long-lasting persistent AF, 15 had had preceding drug-resistant PAF. Pharmacological conversion with bepridil alone or in combination with aprindine was achieved in 67% of patients with a history of drug-refractory AF and SR could be maintained in 60% of converters after a 12-month follow-up. A history of drug-resistant recurrent PAF did not affect pharmacological conversion after it became persistent. However, maintenance of SR over the 12-month period was significantly lower in patients with a history of drug-resistant PAF than in those without.

In the present study, we focused on a history of drug-refractory PAF in patients with established persistent AF. We did not find a history of recurrent PAF as a prelude in two-thirds of the patients with persistent AF. Because patients in Group II had not been treated with antiarrhythmic drugs for SR restoration before the diagnosis of persistent AF, it is possible that the group included patients who had a drug-resistant substrate for PAF. Patients with mitral stenosis who have a larger left atrium, for instance, usually maintain SR until they have triggering events of AF.[6] Once the initiating premature atrial beats appear, induced AF has a tendency not to terminate spontaneously because of the

presence of an established substrate for maintenance of AF. This could lead to an absence of recurrent episodes of PAF before the diagnosis of persistent AF in Group II.

In patients with frequent episodes of PAF, atrial electrical remodeling is associated with marked shortening of the action potential duration (APD) and loss of rate adaptation of the APD, leading to shortening of the FCL[7,8] A shortened FCL will favor transition from paroxysmal to persistent AF, which is the final common pathway, irrespective of various initiating mechanisms of AF[9] After bepridil administration, the patients in Group I had a shorter FCL than those in Group II and as shown in Fig 1, they needed a combination of bepridil and aprindine more frequently for pharmacological conversion, probably because they had a drug resistant substrate for AF[10]

Generally, patients with persistent AF who had a history of drug-refractory PAF attempt pharmacological conversion once and then accept rate-control therapy. In the present study, patients with a history of drug-resistant AF had almost the same pharmacological conversion rate as those without a history. Hence, pharmacological restoration of SR could become a therapeutic option for patients with persistent AF irrespective of a history of drug-refractory PAF. Bepridil is a class IV antiarrhythmic drug, but it has strong class III activity and careful observation is necessary to prevent excessive QT prolongation and torsades de pointes[11] Aprindine is classified as a class Ib drug and is expected not to prolong the QT interval; however, the response of the QT interval to the additional administration of aprindine is variable and should be monitored carefully.

Study Limitations

First, not all patients had antiarrhythmic drug therapy before they were established as having persistent AF and it is possible that some patients in Group II had drug-resistant PAF. From the clinical viewpoint, we arbitrarily classified patients according to the presence of a history of drug-refractory PAF. Second, patients with preserved left ventricular ejection fraction >0.35 were included. Persistent AF with a history of drug-resistant PAF in patients with left ventricular dysfunction might have responded differently. Further studies are needed to clarify clinical efficacy and the safety of bepridil and aprindine in such patients. Third, although patients with persistent AF preceded by drug-refractory AF had SR restored pharmacologically, recurrence of persistent AF occurred in 40% of them during the 12-month follow-up period. Additional treatment for initiation mechanisms may be needed.

Conclusions

The present study revealed the usefulness of pharmacological cardioversion using bepridil alone or in combination with aprindine in cases of persistent AF with and without a history of drug-resistant PAF. However, recurrence of AF was higher in patients with such a history.

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