Hydrophilic statin suppresses vein graft intimal hyperplasia via endothelial cell-tropic Rho-kinase inhibition

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Background: **Recent studies suggest that statins can protect the vasculature in a manner that is independent of their lipid-lowering activity through inhibition of the small guanosine triphosphate-binding protein, Rho, and Rho-associated kinase. Little information is available on the inhibitory effect of statins on vein graft intimal hyperplasia, the main cause of late graft failure after bypass grafting. We therefore examined the effects of a hydrophilic statin on vein graft intimal hyperplasia in vivo and Rho-kinase activity in vitro.**

Methods: **In the first experiment, rabbits were randomized to a control group (n = 7) that was fed regular rabbit chow or to a pravastatin group (n = 7) that was fed regular rabbit chow supplemented with 10 mg/kg pravastatin sodium. The branches of the jugular vein were ligated and an approximately 3-cm segment of the jugular vein was taken for an autologous reversed-vein graft. The carotid artery was cut and replaced with the harvested autologous jugular vein. At 2 and 4 weeks after the operation, vein grafts in both groups were harvested, and intimal hyperplasia of the vein grafts was assessed. In the second experiment, human umbilical vein endothelial cells and vascular smooth muscle cells were cultured and then treated with 1 μmol/L and 30 μmol/L pravastatin for 24 hours and harvested. Immunoblotting was performed on the resulting precipitates. Quantitative evaluation of phosphorylated myosin binding subunit and endothelial nitric oxide synthase was performed by densitometric analysis.**

Results: **We demonstrated that oral administration of the hydrophilic statin pravastatin to normocholesterolemic rabbits inhibited intimal hyperplasia of carotid interposition-reversed jugular vein grafts 4 weeks after implantation (pravastatin group, 39.5 ± 3.5 μm vs control group, 64.0 ± 7.1 μm; n = 7; $P < .05$) and suppressed cell proliferation and apoptosis in the neointima 2 weeks after implantation. In addition, we found that pravastatin inhibited Rho-kinase activity and accelerated endothelial nitric oxide synthase expression in human umbilical vein endothelial cells but did not inhibit Rho-kinase activity in vascular smooth muscle cells.**

Conclusions: **These novel findings clearly demonstrate that a hydrophilic statin can suppress intimal hyperplasia of the vein graft in vivo and also show endothelial cell-tropic inhibition of Rho-kinase in vitro. Furthermore, these results strongly support the clinical use of hydrophilic statins to prevent intimal hyperplasia of the vein graft after bypass grafting. (J Vasc Surg 2005;42:757-64.)**

Clinical Relevance: **Late graft failure caused by neointimal hyperplasia limits the efficacy of vein grafting. Various treatments were examined to reduce neointimal hyperplasia, but a standard clinical treatment has not yet been established. We report here the inhibitory effect of pravastatin on the development of vein graft intimal hyperplasia. In addition, we demonstrate that pravastatin showed endothelial cell-tropic benefits through both the inhibition of Rho-kinase activity and acceleration of eNOS expression in vitro. Because the clinical benefits and safety of pravastatin have been established to a certain extent through long-term clinical usage, pravastatin may soon become standard treatment after vein bypass grafting.**

Arterial occlusive disease such as peripheral arterial occlusive disease and coronary artery disease is a clinical manifestation of atherosclerosis that is prevalent in industrialized societies. An autologous vein graft is the most suitable conduit for reconstruction at various sites of arterial occlusive disease and coronary artery bypass grafting.[1] However, late graft failure caused by either intimal hyperplasia or a progression of the underlying atherosclerotic disease limits the efficacy of vein grafting.[2,3]

The 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase inhibitors, also known as statins, block the rate-limiting step in cholesterol biosynthesis and are widely prescribed to lower cholesterol synthesis in hyperlipidemic patients.[4] Several large clinical trials have demonstrated the benefits of cholesterol lowering with these agents in the primary and secondary prevention of coronary heart disease.[5,6] Furthermore, subgroup analysis of the data from clinical trials suggests that statins may have some beneficial effects independent of their cholesterol-lowering effects.[6,7]

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Competition of interest: none.

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0741-5214/$30.00

doi:10.1016/j.jvs.2005.05.041

Experimental studies in vitro indicate that some of these cholesterol-independent (pleiotropic) effects of statins involve suppression of cell proliferation[8] and improvement or restoration of endothelial function through endothelial nitric oxide synthase (eNOS) activation.[9] These effects may reflect the ability of statins to block the synthesis of important isoprenoid intermediates like the small guanosine triphosphate (GTPase) protein, Rho, and Rho-associated kinase (Rho-kinase/ROK/ROCK) activity.[4,10]

Our previous work demonstrated a relationship between NO and intimal hyperplasia of vein grafts.[11-13] Other studies showed that long-term inhibition of Rho kinase reversed endothelium-dependent relaxation and caused regression of arterial intimal hyperplasia.[14] However, there is no information regarding the effects of statins on vein graft intimal hyperplasia.

Experimental studies indicate that some statins, but not pravastatin, inhibited intimal hyperplasia in balloon-injured arteries.[15,16] However, some studies reported that the clinical prescription of lipophilic statins was limited because lipophilic statins showed more side effects and drug interactions with cytochrome P450 (CYP) enzymes.[17]

The present study was therefore designed to determine if oral administration of the hydrophilic statin pravastatin suppresses intimal hyperplasia of the vein graft in vivo and, if so, to confirm the previously reported effects of pravastatin on cell proliferation and apoptosis.[12] To elucidate the vasculo-protective properties of a hydrophilic statin, we also examined the effect of pravastatin on Rho-kinase activity and eNOS expression in different types of extrahepatic cells in vitro.

METHODS

Experiment 1: In vivo effect of hydrophilic statin on vein graft intimal hyperplasia

Animals and chemicals. Pravastatin sodium was obtained from Sankyo Co, Ltd (Tokyo, Japan). Male Japanese white rabbits (Nippon SLC, Hamamatsu, Japan), each weighting 2.5 to 3.0 kg, were used in this study. The rabbits were housed individually at 20°C ± 3°C with free access to water. The animal experiments were fully reviewed by the Committee of Ethics on Animal Experiments from the Faculty of Medicine, Nagoya University and were carried out under the Guidelines for Animal Experiments in the Faculty of Medicine, Nagoya University. The study also was in conformance with the publication *Guiding Principles for the Care and Use of Laboratory Animals* (publication No. NIH 80-23, revised 1985).

Drug administration. Rabbits were randomly divided into a control group (n = 7) that was fed commercial rabbit chow (CR3) (CLEA Japan Inc, Tokyo, Japan) and a pravastatin group (n = 7) that received CR3 with 10-mg/kg pravastatin sodium, as described in previous reports.[15,16] Feeding was restricted to 120 g/day. Pravastatin treatment was started on the day of operation and continued until the end of the experiment. All the rabbits appeared to be healthy throughout the course of study.

Plasma lipid analysis. After an overnight fast, blood samples were obtained in each group from the marginal ear vein at the time of operation and 28 days after operation, and plasma was obtained by the addition of sodium-ethylenediamine tetraacetic acid . Total cholesterol, triglycerides, and low-density lipoprotein (LDL) in plasma were measured by enzymatic assays as previously reported.[18]

Vein graft implantation. Carotid interposition-reversed jugular vein grafts were performed as described previously.[12] Anesthesia was induced intramuscularly with ketamine hydrochloride (50 mg/kg) and xyradine (10 mg/kg) and maintained with the intravenous administration of ketamine hydrochloride (10 mg/kg) and xyradine (10 mg/kg). After a longitudinal neck incision, the left jugular vein and the left common carotid artery were exposed. The branches of the jugular vein were ligated and an approximately 3-cm segment of the jugular vein was taken for the autologous reversed-vein graft. Graft harvesting was completed with meticulous care to avoid injuring the graft wall. The harvested graft was kept moistened in heparinized saline (5 IU/mL) at room temperature.

The carotid artery was clamped at the proximal and the distal ends. The carotid artery was cut and replaced with the harvested autologous jugular vein. The vein graft was anastomosed in a reversed end-to-end fashion with interrupted 8-0 monofilament sutures under a surgical microscope. The wound was closed layer to layer.

Harvest of implanted grafts. At each time point after implantation of the autologous vein grafts, the vein grafts were harvested under general anesthesia as described previously.[12] In brief, the graft was isolated and harvested after systemic heparinization (200 IU/kg intravenously), and the rabbits were sacrificed by an overdose of pentobarbital. The harvested vein graft was fixed with 4% formaldehyde at a pressure of 100 mm Hg for 30 minutes. The perfused vein graft was immersed in the same fixative overnight at room temperature. The middle portion of the harvested graft was used for histologic study and immunochemistry. Each sample was embedded in paraffin and cut into 5-μm sections.

Assessment of intimal hyperplasia. Four weeks after the operation, vein grafts in both groups (n = 7, respectively) were harvested, and intimal hyperplasia of the vein graft of both groups was assessed as described previously.[12] In brief, each section harvested 4 weeks after implantation was deparaffinized in a xylene/ethanol series and stained with either hematoxylin and eosin or the elastica van Gieson method. Four sections were obtained from each vein graft. The intimal hyperplasia was measured by MACSCOPE (Mitani Co, Fukai, Japan) at eight randomly selected different views per sections. The average of eight values was considered to represent the intimal hyperplasia of the section, and the average of the four sections was considered to represent those of the graft. The cross-sectional area of the intimal hyperplasia was also calculated by MACSCOPE in the same manner. These values were then used for statistical analysis.

Immunohistochemical staining for proliferative cell nuclear antigen. Two weeks after implantation, vein grafts in both groups (n = 7, respectively) were harvested. For immunohistologic analysis, monoclonal mouse antibody against proliferative cell nuclear antigen (PCNA) (clone PC 10, 1:200 dilution) (DAKO Cytomation Inc, Carpenteria, Calif) was used as described in our previous report.[12] The formalin-fixed, paraffin-embedded tissue sections harvested 2 weeks after implantation were deparaffinized in a xylene/ethanol series and treated with 3% hydrogen peroxidase for 10 minutes at room temperature to inhibit endogenous peroxidase activity. To decrease nonspecific binding, the slides were preincubated with normal horse serum for 30 minutes.

After washing with phosphate-buffered saline (PBS), the sections were incubated with primary antibody for 60 minutes at room temperature. The sections were then treated with the DAKO Envision + System, following the manufacturer's instructions. Briefly, peroxidase-labeled polymer conjugated to goat antimouse immunoglobulins was applied to the sections for 30 minutes at room temperature. After washing with PBS, liquid 3,3′-diaminobenzidine (DAB) plus substrate-chromogen solution was applied to the sections, which were incubated for 5 to 10 minutes. Each section was then lightly counterstained with hematoxylin and brown-stained positive cells were counted.

To calculate the PCNA index, eight high-power fields (×400) per section were selected randomly and PCNA-positive cells and total cells were counted in the neointima in a blinded manner. The average of the eight values was considered to represent the number of cells in one section, and the average of the four sections was considered to represent those of one vein graft. The number of positive cells divided by the total number of cells of the vein graft was defined as the PCNA index, as described in our previous report.[12]

Terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick end labeling assay. Two weeks after implantation, vein grafts in both groups (n = 7, respectively) were harvested. To evaluate apoptotic activity, we used the terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling (TUNEL) method to detect in situ apoptosis. The assay was performed with a detection kit (Takara Bio, Shiga, Japan) in accordance with the manufacturer's instructions as well as a previous report.[19] The counterstaining was done lightly with hematoxylin. Eight high power fields (×400) per section were selected randomly, and TUNEL-positive cells and total cells were counted in the neointima in a blinded manner. Nonspecific cytoplasmic staining without nuclear involvement was considered negative. The number of positive cells divided by the total number of cells counted was defined as the TUNEL index of the vein graft.

Experiment 2: In vitro cell-selective effect of hydrophilic statin

Cell culture. Human umbilical vein endothelial cells (HUVECs) and vascular smooth muscle cells (VSMCs) pooled from several donors were purchased from Cascade Biologics, Inc (Portland, Ore) and grown in Humedia-EG, (Cascade Biologics, Inc) according to the manufacturer's instructions as described previously.[20] In all experiments, the cells were used between passages five and six.

Observation of myosin binding subunit phosphorylation and eNOS protein expression. Myosin binding subunit (MBS) phosphorylation and Rho/ Rho-kinase activity in HUVECs and VSMCs were examined. The cells were seeded on 60-mm collagen type-1 coated dishes (Iwaki, Tokyo, Japan) at a density of 8×10^5 cells per dish and cultured overnight. The cells were then treated with 1 μmol/L and 30 μmol/L pravastatin for 24 hours and harvested with 10% (wt/vol) trichloroacetic acid. The resulting precipitates were subjected to immunoblotting with antiphospho-MYPT-1 (Thr850) (Upstate, Waltham, Mass), anti-MBS antibody,[20] anti-RhoA (Cytoskeleton Inc, Denver, Colo), anti-ROCK I (C-19) (Santa Cruz Biotechnology, Inc, Santa Cruz, Calif), anti-ROCK II (C-20) (Santa Cruz Biotechnology, Inc), and anti-eNOS/NOS type III (BD Biosciences Inc, San Jose, Calif). The region containing MBS, ROCK I, ROCK II, RhoA, and eNOS was visualized using ECL Western blotting system (Amersham Biosciences, Piscataway, NJ). Quantitative evaluation of phosphorylated MBS and eNOS was performed by densitometric analysis using the ATTO Densitograph (ATTO, Tokyo, Japan), as described previously.[20]

Statistical analysis. All values were expressed as the mean ± SEM. The data were evaluated with Student's *t* test or compared by one-way analysis of variance. Differences between groups were considered to be statistically significant when the *P* value was $< .05$.

RESULTS

Experiment 1: In vivo effect of pravastatin on vein graft intimal hyperplasia

All animals survived, and all vein grafts (n = 28) were patent until the time of harvest.

Suppression of intimal hyperplasia of the vein graft by pravastatin. Intimal hyperplasia developed in both groups (n = 7, respectively), but the degree of intimal thickness in the pravastatin group (39.5 ± 3.5 μm) was significantly suppressed compared with the control group (64.0 ± 7.1 μm) ($P < .05$) (Fig 1, *A* and *B*). These findings showed the suppressive effect of pravastatin on the degree of the intimal hyperplasia of vein grafts implanted in the arterial circulation.

Plasma lipid profile levels after 4 weeks' administration of pravastatin. After 4 weeks, there was no significant change in plasma total cholesterol levels in the pravastatin group (21.4 ± 3.4 mg/dL) compared with the control group (26.6 ± 2.1 mg/dL), and no change in triglyceride levels in the pravastatin group (21.4 ± 3.4 mg/dL) compared with the control group (24.0 ± 2.6 mg/dL) (Fig 2, *A* and *B*). There was a significant reduction in plasma LDL levels in the pravastatin group (4.9 ± 0.3

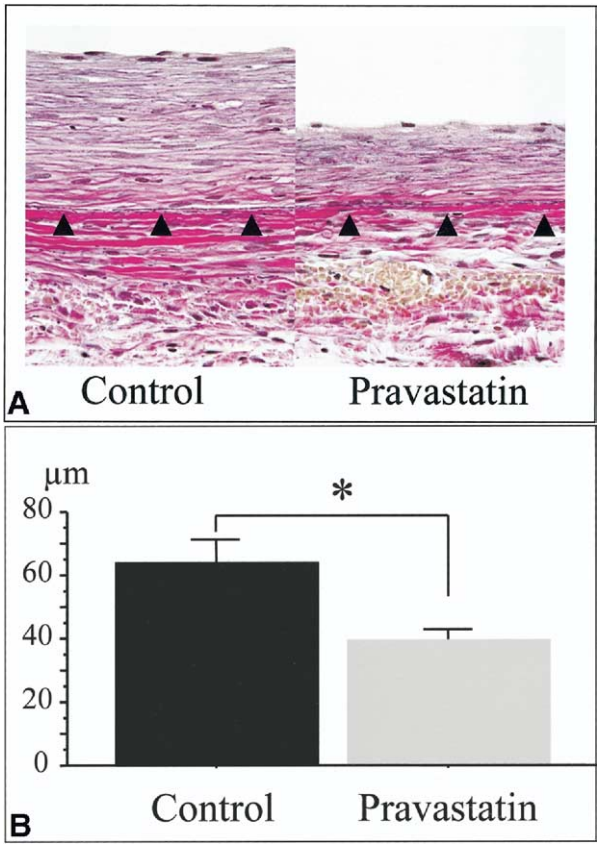

Fig 1. Effect of pravastatin on the development of intimal hyperplasia of the vein graft 4 weeks after operation. **A,** Microscopic findings of the middle portion of an autologous vein graft from the control group fed with normal rabbit chow and the pravastatin group fed daily with special chow containing 10 mg/kg pravastatin. *Arrowheads* indicate the internal elastic lamina (elastica van Gieson staining; original magnification ×400). **B,** Quantitative analysis of intimal hyperplasia of vein grafts by random eight-point measurement. The intimal thickness was significantly suppressed in the pravastatin group (n = 7) compared with the control group (n = 7). Results are expressed as the mean ± SEM. $^{*}P < .05$ vs control.

mg/dL) compared with the control group (6.3 ± 0.4 mg/dL) ($P < .05$) (Fig 2, *C*).

Suppression of cell proliferative activity by pravastatin. The PCNA index was measured by using vein grafts harvested 2 weeks after implantation (n = 7, respectively). The PCNA index of the neointimal lesion was significantly lower in the pravastatin group (16.2% ± .7%) compared with the control group (24.5% ± 0.5%) ($P < .05$) (Fig 3, *A* and *B*).

Cell apoptosis and vein graft intimal hyperplasia. To confirm the relationship between cell apoptosis and the inhibitory effect of pravastatin on intimal hyperplasia, a TUNEL assay was performed with vein grafts harvested 2 weeks after implantation (n = 7, respectively). TUNEL-positive cells in the neointima of the vein graft were signif-

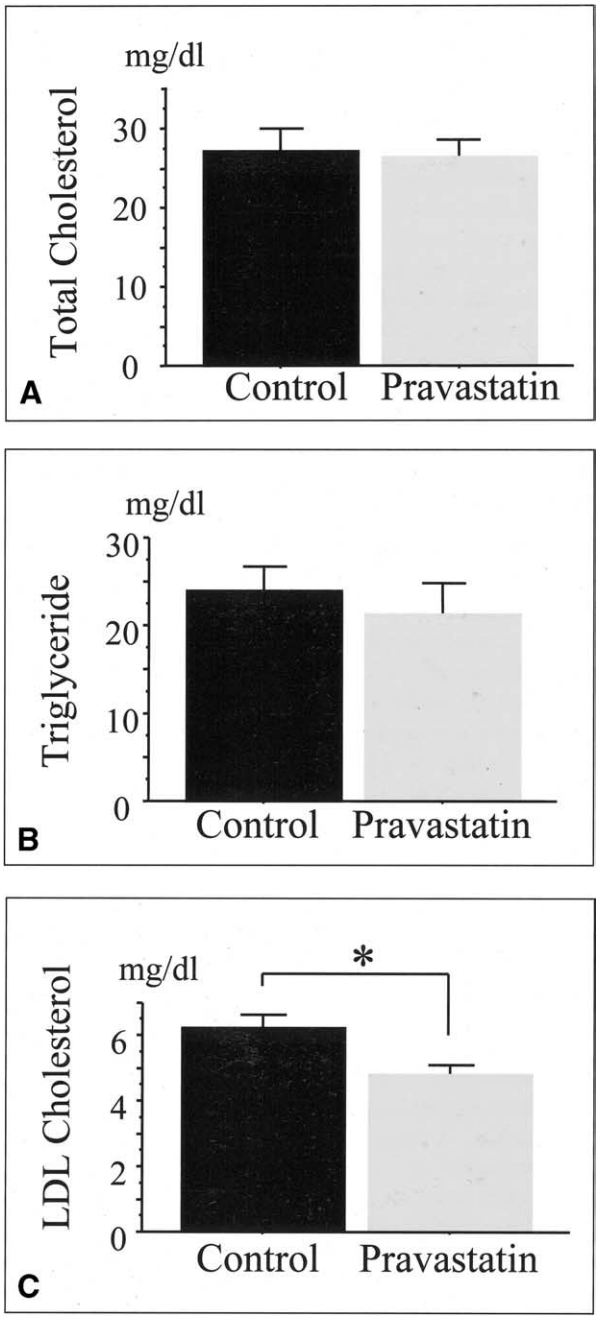

Fig 2. Lipid profile after oral administration of pravastatin. Plasma lipid profiles were measured after 4 weeks in the control group (n = 7) fed with normal rabbit chow and the pravastatin group (n = 7) fed daily with special chow containing 10 mg/kg of pravastatin. **A,** Total cholesterol levels and **(B)** plasma triglyceride levels in both groups showed no significant change. **C,** Plasma low-density lipoprotein *(LDL)* levels were slightly decreased in the pravastatin group, but the baseline LDL levels in both groups were very low. Results are expressed as the mean ± SEM. $^{*}P < .05$ vs control.

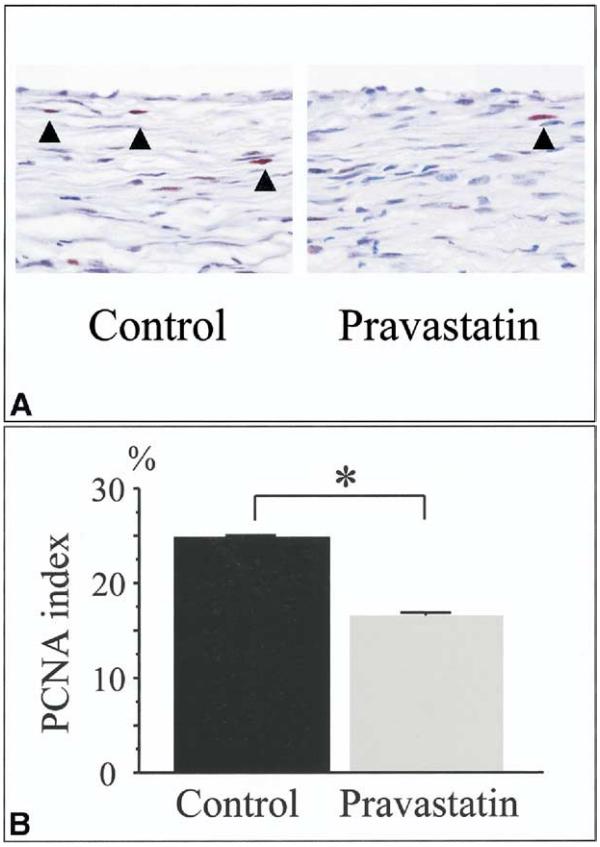

Fig 3. Effects of pravastatin on proliferative activity of the vein graft neointima. The proliferative cell nuclear antigen *(PCNA)* index was measured by using vein grafts harvested 2 weeks after implantation. Counterstaining was done lightly with hematoxylin. **A,** Microscopic findings with PCNA staining of the middle portion of an autologous vein graft from the control group and the pravastatin group. *Arrowheads* show PCNA-positive cells in the neointima (original magnification ×400). **B,** Quantitative analysis of the frequency of PCNA-positive cells. The PCNA index, the number of PCNA-positive cells divided by the number of total cells, was significantly suppressed in the pravastatin-treated group (n = 7) compared with the control group (n = 7). Results are expressed as the mean ± SEM. $^{*}P < .05$ vs control.

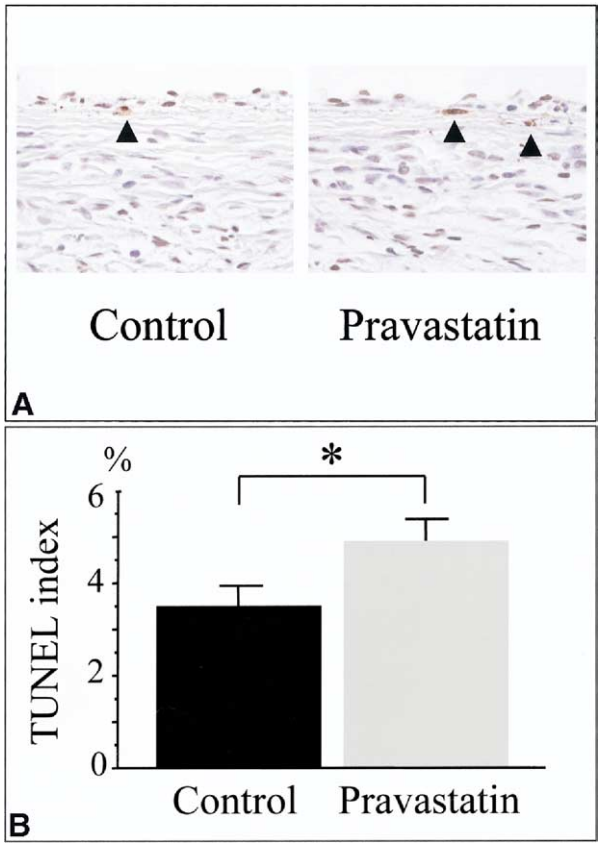

Fig 4. Effects of pravastatin on cell apoptotic activity in the vein graft neointima. The terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling (TUNEL) method was done by using vein grafts harvested 2 weeks after implantation. Counterstaining was done lightly with hematoxylin. **A,** Microscopic findings of the middle portion of vein grafts from the control group and the pravastatin group. *Arrowheads* show TUNEL-positive cells in the neointima (original magnification ×400). **B,** Quantitative analysis of the frequency of TUNEL-positive cells. The TUNEL index, the number of TUNEL-positive cells divided by the number of total cells, was significantly increased in the pravastatin group (n = 7) compared with the control group (n = 7). Results are expressed as the mean ± SEM. $^{*}P < .05$ vs control.

icantly elevated in the pravastatin group (10.6% ± 0.7%) compared with the control group (7.1% ± 0.7%) ($P < .05$) (Fig 4, *A* and *B*).

Experiment 2: In vitro cell-selective effect of pravastatin

MBS phosphorylation and Rho/ Rho-kinase activity in HUVECs and VSMCs. The expression levels of the Rho-kinase isoforms, RhoA, ROCK I, and ROCK II, were measured in HUVECs and VSMCs using Western blot analysis (n = 6, respectively). We also measured MBS, the target protein of Rho-kinase, and phospho-MBS, the inactive form of MBS (n = 6, respectively). Both types of cells were treated with 1 μmol/L and 30 μmol/L pravastatin or no pravastatin (control) for 24 hours. Phosphorylation of MBS in HUVECs was significantly suppressed by both concentrations of pravastatin compared with control (Fig 5), but expression levels of RhoA, ROCK I, and ROCK II were not affected (data not shown). In contrast, phosphorylation of MBS in VSMCs was not suppressed by pravastatin (Fig 6).

Effects of pravastatin on eNOS expression and phosphorylation in HUVECs. HUVECs were treated with 1 μmol/L and 30 μmol/L pravastatin or no pravastatin (control) for 24 hours, and protein levels of eNOS were examined (n = 6, respectively). The eNOS protein levels in the HUVECs were significantly higher even with 1 μmol/L of pravastatin compared with the control (Fig 5).

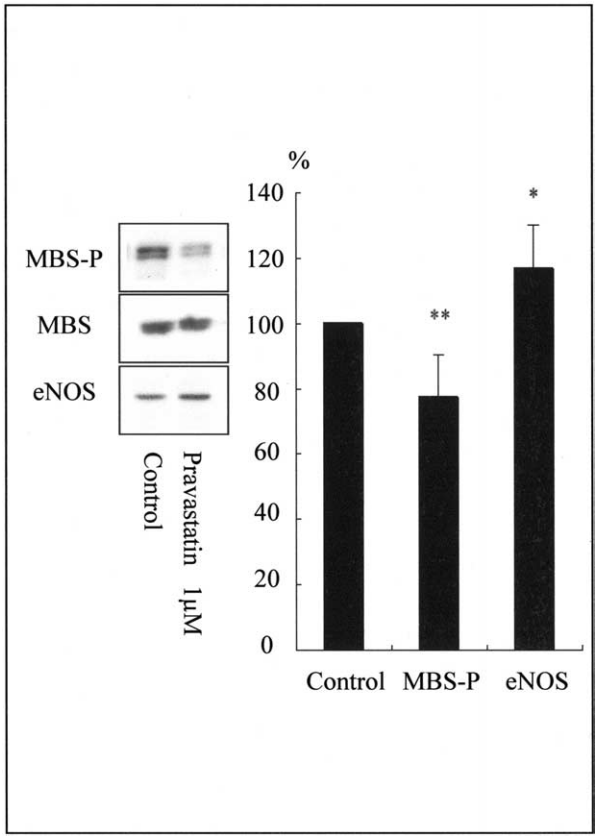

Fig 5. To evaluate the effect of pravastatin on Rho-kinase activity, myosin-binding subunit phosphorylation *(MBS-P)* and endothelial nitric oxide synthase *(eNOS)* expression were measured in human umbilical vein endothelial cells (HUVECs) (n = 6) by using Western blot analysis. HUVECs were treated with 1 μmol/L pravastatin or no pravastatin (control) for 24 hours. The total amount of eNOS was increased compared with the control cells, and phosphorylation level of MBS was decreased. The total amount of MBS was not affected by pravastatin. Results are expressed as the mean ± SEM. $^{*}P < .05$ vs control, $^{**}P < .01$ vs control.

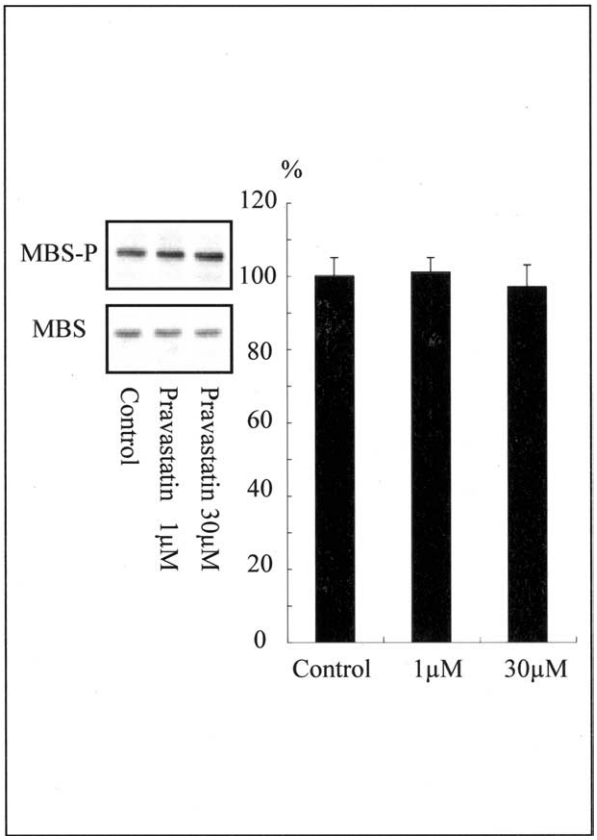

Fig 6. To evaluate the effect of pravastatin on Rho-kinase activity, myosin-binding subunit phosphorylation *(MBS-P)* was measured in vascular smooth muscle cells (VSMCs) (n = 6) by using Western blot analysis. VSMCs were treated with 1 μM and 30 μM pravastatin or no pravastatin (control) for 24 hours. Phosphorylation level of MBS in VSMCs was not suppressed by the pravastatin treatment at 1 μmol/L and 30 μmol/L. Results are expressed as the mean ± SEM.

DISCUSSION

We have demonstrated that the hydrophilic statin pravastatin inhibits vein graft intimal hyperplasia through suppression of the proliferative activity and acceleration of cell apoptosis in the neointima in the normocholesterolemic rabbit. We also found that pravastatin suppressed Rho-kinase activity in HUVECs, but not in VSMCs, and increased the expression levels of eNOS in HUVECs. To our knowledge, this is the first study reporting the endothelial cell-tropic suppression of Rho-kinase activity and a suppressive effect of hydrophilic statin treatment on vein graft intimal hyperplasia.

Vein graft intimal hyperplasia. Intimal thickening of autologous vein grafts is the major cause of late graft failure.[2,3] Implantation of a vein graft in the arterial circulation induces various changes that may lead to intimal hyperplasia, such as increased sheer stress, loss of endothelial cells, migration and invasion of inflammatory cells, and migration and proliferation of VSMCs.[21,22] We previously reported that pronounced intimal thickening of a vein graft was associated with impairment of endothelial responses and cell proliferative activity of the neointima.[11,23] Various treatments were examined to reduce intimal hyperplasia of vein grafts,[12,24] but standard clinical treatment has not yet been established.

We report here, for the first time, the inhibitory effect of pravastatin on the development of vein graft intimal hyperplasia in vivo. Our demonstrated suppression of cell proliferative activity and acceleration of cell apoptosis in the neointima of vein grafts by pravastatin confirms previous findings.[12]

Statins and Rho-kinase. Statins are among the most widely prescribed drugs, and several large clinical trials have demonstrated the benefits of cholesterol lowering with these agents in the primary and secondary prevention of

coronary heart disease.[5,6] Subgroup analysis of these trials showed that statins provided beneficial effects independent of lipid-lowering effects,[6,7] so called pleiotropic effects.[25] These effects reflect the ability of statins to reduce the formation of the important isoprenoid intermediates farnesyl pyrophosphate and geranylgeranylpyrophosphate.[4,10,26] Blockade of geranylgeranylation inhibits the small guanosine triphosphatase (GTPase) protein, Rho, and Rho-kinase/ROK/ROCK activity, the target protein of Rho.[26] The Rho/Rho-kinase pathway controls multiple cell functions such as adhesion, proliferation, migration, and the calcium sensitivity of the contractile proteins.[26,27] Previous studies also revealed that Rho-kinase inactivates myosin phosphatase through phosphorylation of MBS at thr-850, resulting in phosphorylation of myosin light chain and contraction of VSMCs,[28] and that phosphorylation level of MBS at thr-850 correlates with Rho-kinase activity specifically.

Recent studies revealed that inhibition of Rho-kinase activity protects endothelial cells. Some studies demonstrated that inhibition of myosin light chain stabilizes eNOS messenger RNA (mRNA) through suppression of actin stress fibers, which negatively regulates eNOS mRNA and increases eNOS protein expression.[29] Another study showed that active Rho or Rho-kinase decreased eNOS expression, and negative Rho or Rho-kinase abrogated downregulation of eNOS.[30] In addition, long-term inhibition of Rho-kinase reversed endothelium-dependent relaxation and regressed arterial intimal hyperplasia.[14]

Our study demonstrates, for the first time, the suppressive effect of therapeutic concentrations of pravastatin [31] on Rho-kinase activity confirmed by MBS phosphorylation in HUVECs. Interestingly, the tendency of this inhibition was recognized in HUVECs at 1 μmol/L of pravastatin but did not occur in VSMCs at concentrations of 1 μmol/L or 30 μmol/L.

Statins and eNOS. Endothelial-derived NO has been recognized as an anti-inflammatory and anti-arteriosclerotic molecule.[32,33] Inhibition of Rho-kinase activity by statins accelerates eNOS protein expression as described above.[9,34] Our previous studies demonstrated a relationship between reduced production of NO in experimental autologous vein grafts [11] and intimal hyperplasia. Furthermore, intimal hyperplasia of vein grafts was suppressed by activation of NO production through gene transfer of eNOS[12] and oral administration of the NO precursor L-arginine.[13] In this study, we demonstrated the tendency of increasing eNOS expression at 1 μm and 30 μm pravastatin in HUVECs.

The efficacy of hydrophilic statin. In the injured artery model, pravastatin did not suppress intimal hyperplasia in contrast to other statins like simvastatin and atrovastatin.[15,16] However, it is still controversial which statins—lipophilic or hydrophilic—have more clinical benefits, because many clinical trials suggest that hydrophilic statins have vasculo-protective effects similar to those of lipophilic statins.[5,6] Furthermore, some studies reported that hydrophilic statins were more effective in stabilizing plaques because thick fibromuscular caps, which consist of a large number of smooth muscle cells and collagen fibers, are thought to contribute to plaque stabilization.[35,36] In the present study, we showed the suppressive effect of a hydrophilic statin on intimal hyperplasia of vein grafts in vivo. We also demonstrated the suppression of Rho-kinase activity confirmed by MBS phosphorylation in HUVECs, but not in VSMCs, and the tendency of acceleration of eNOS in HUVECs at therapeutic pravastatin concentrations [37,38] in vitro.

Drug concentration and interaction. In the previous reports, the oral administration of pravastatin in hyperlipidemic rabbit decreased serum cholesterol at daily doses >10 mg/kg, almost the same dosage as in this study, and at doses >5 mg/day in primary hyperlipidemic patients. This indicates that the estimated therapeutic dose of pravastatin to reduce intimal hyperplasia was almost the same as the clinically approved doses in hypercholesterolemic patients.[39,40]

Because many drugs, including anticoagulants, antibiotics, antihypertensive and some lipophilic statins, are metabolized by CYP enzymes,[38] drug interactions between statins and other agents may be very important. For example, some anticoagulants were indispensable to postoperative patients who received vein bypass grafting of coronary or peripheral arteries, and important drug interactions occurred between statins and these agents. Hydrophilic statins like pravastatin are thought to have fewer drug interactions compared with other agents that are metabolized by CYP enzymes[17] and thus have fewer restrictions for use in postoperative patients.

CONCLUSION

Our present study clearly demonstrates the suppression of development of intimal hyperplasia of the vein graft through inhibition of cell proliferative activity and acceleration of cell apoptosis in vivo. In addition, we demonstrate for the first time that a hydrophilic statin showed endothelial cell-tropic benefits both through inhibition of Rho-kinase activity and acceleration of eNOS expression. This novel finding facilitates the clinical use of hydrophilic statins in postoperative patients to prevent intimal hyperplasia of the vein graft after vein bypass grafting.

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Submitted May 10, 2005; accepted May 18, 2005.