Combination Therapy with Cerivastatin and Nifedipine Improves Endothelial Dysfunction After Balloon Injury in Porcine Coronary Arteries

Yasuhiro Eto, MD, Hiroaki Shimokawa, MD,*† Yoshihiro Fukumoto, MD,* Yasuharu Matsumoto, MD,* Kunio Morishige, MD,* Ikuko Kunihiro,* Tadashi Kandabashi, MD,* and Akira Takeshita, MD**

Abstract: HMG-CoA reductase inhibitors and calcium channel blockers have antiatherogenic effects; however, their mechanisms remain to be elucidated. This study examined the effect of cerivastatin and/or nifedipine on the endothelial dysfunction in porcine balloon-injured coronary arteries. Normal male pigs were randomly divided into the following four groups: control, cerivastatin (1 mg/kg/d PO), nifedipine (4 mg/kg/d PO), and their combination (n = 10 each). We started the treatments 3 days before balloon injury in the proximal left coronary arteries and continued for 4 weeks after the procedure. Then, we examined endothelial vasodilator functions ex vivo in organ chambers and in vitro by Western blotting for eNOS expression. Endothelium-dependent relaxations to serotonin, but not those to bradykinin or the calcium ionophore A23187 or endothelium-independent relaxations to sodium nitroprusside, were significantly impaired by balloon injury. The monotherapy with cerivastatin or nifedipine partially improved, and their combination supernormalized the relaxations to serotonin without affecting those to bradykinin or A23187 or endothelium-independent relaxations to sodium nitroprusside. The expression of eNOS was significantly reduced by balloon injury and normalized by the combination therapy. These results indicate that the combination therapy improves endothelial dysfunction after balloon injury, in which the up-regulation of eNOS may be involved.

Key Words: endothelium-dependent relaxation, balloon injury, statin, calcium channel blockers

(*J Cardiovasc Pharmacol*™ 2005;46:1–6)

Percutaneous coronary intervention (PCI) is widely performed for the treatment of coronary artery disease[1]; however, endothelial injury of the coronary arteries is inevitable. We have previously demonstrated that endothelial vasodilator function is impaired in the regenerated state after balloon injury.[2,3] The healthy endothelium plays a key role in maintaining vascular homeostasis by synthesizing and releasing several vasodilators, including prostacyclin, nitric oxide (NO), and endothelium-derived hyperpolarizing factor (EDHF).[4,5] NO, which is synthesized from L-arginine by endothelial nitric oxide synthase (eNOS), exerts antiatherogenic effects, such as the suppression of proliferation/migration of vascular smooth muscle cells (VSMC) and aggregation/adhesion of platelets.[4,5] Thus, it is important to maintain endothelial function in the regenerated state to inhibit the progression of atherosclerosis in general and the restenosis after PCI in particular.[4,5]

Recent studies have shown that HMG-CoA reductase inhibitors (statins) reduce the incidence of coronary artery disease and cardiac death.[6,7] Indeed, statins improve endothelial vasodilator function,[8] up-regulate eNOS expression,[9] and stabilize atherosclerotic plaques.[10,11] Calcium channel blockers are also effective in patients with coronary artery disease,[1] especially in those with vasospastic angina, which can dilate vascular smooth muscle. The calcium channel blockers could also up-regulate eNOS expression in vitro[12] and improve endothelial dysfunction in hypercholesterolemic patients.[13]

However, it remains to be examined whether statins and/or calcium channel blockers improve vasodilator functions of regenerated endothelial cells after balloon injury, and if so, what mechanism is involved. Thus, the present study was designed to examine whether monotherapy with cerivastatin or nifedipine or their combination therapy improves endothelial dysfunction after balloon injury in porcine coronary arteries.

METHODS

This experiment was reviewed by the Ethics Committee on Animal Experiment at the Kyushu University and was carried out in accordance with the Guidelines for Animal Experiment at the Kyushu University and The Law (No. 105) and Notification (No. 6) of the Japanese Government.

Animal Preparation

Male domestic pigs (Nihon Crea Inc, Tokyo; 2 to 3 months old; weight 25 to 30 kg) were used. The animals were

Received for publication November 3, 2004; accepted February 18, 2005.
From the *Department of Cardiovascular Medicine, Kyushu University Graduate School of Medical Sciences, Fukuoka, Japan; and †21st Century COE Program of Lifestyle-Related Diseases, Kyushu University Graduate School of Medical Sciences, Fukuoka, Japan.
This work was supported in part by a grant from the 21st Century COE Program and grants-in–aid from the Japanese Ministry of Education, Culture, Sports, Science, and Technology (Nos. 12032215, 12470158, 12877114, 13307024, 13557068), Tokyo, Japan.
Reprints: Hiroaki Shimokawa, MD, PhD, Department of Cardiovascular Medicine, Kyushu University Graduate School of Medical Sciences, 3-1-1 Maidashi, Higashi-ku, Fukuoka, 812-8582, Japan (e-mail: shimo@cardiol.med.kyushu-u.ac.jp).

housed individually at a controlled room temperature. They were randomly divided into the following four groups: control (placebo), cerivastatin (1 mg/kg/d, PO), nifedipine (4 mg/kg/d, PO), and both of them (at the same dose as in the monotherapy). Because 3 days are needed to achieve the plateau plasma levels of the drugs, the drug treatments were started 3 days before the balloon injury and were continued for 4 weeks until the end of the experiments. In a preliminary study, we confirmed that the present drug treatments do not significantly lower blood pressure in normal pigs (data not shown). On the day of balloon injury, the animals were sedated with ketamine hydrochloride (12.5 mg/kg IM) and were then anesthetized with sodium pentobarbital (20 mg/kg IV).[14,15] The animals were then intubated and ventilated with room air, and oxygen was supplemented via a positive-pressure respirator (Shinano Inc).[14,15] Arterial pH, Po_2, and Pco_2 were kept within normal ranges. A catheter was inserted into the right carotid artery, and heparin (5000 U) was administered intravenously. Coronary arteriography was performed in a left anterior oblique view, using the Toshiba cineangiography system. ECGs along with mean arterial pressure and heart rate were continuously monitored. The diameter of the balloon was chosen so that it was ~1.3 times greater than that of the coronary segment.[15] The balloon was inflated at a pressure of 8 atm for 30 seconds 5 times.[15] The balloon injury was performed in the middle portion of the left anterior descending and the left circumflex coronary artery. We have previously confirmed that there is no difference in endothelium-dependent responses or responses to balloon endothelial injury among the coronary arteries.[2,3]

Organ Chamber Experiments

Four weeks after the endothelial injury, the coronary artery segments from the balloon-injured left coronary artery and the normal right coronary artery were isolated, cleaned of any perivascular tissue, and cut into rings 4 mm in length. In some rings, the endothelium was removed by gentle rubbing of the luminal surface with a cotton swab.[2,3] The rings were fixed vertically between hooks in an organ bath of 20 mL capacity containing Krebs solution of the following composition (mmol/L): NaCl 121, KCl 4.7, $NaHCO_3$ 24.7, $MgSO_4$ 12.2, $CaCl_2$ 2.5, KH_2PO_4 1.2, and glucose 5.8, which was maintained at 37°C and aerated with a mixture of 95% O_2-5% CO_2 in cold physiological salt solution (PSS). PSS was aerated with 95% O_2 and 5% CO_2. The hook anchoring the upper end of the strip was connected to the lever of a force transducer (Nihon-Kohden Kogyo). The resting tension was adjusted to 5 g. KCl solution (62 mmol/L) was applied every 15–20 minutes until the amplitude of the contraction reached a constant value. The developed tension was expressed as a percentage of the tension attained in the last precontraction with 62 mmol/L KCl, the extent of which was comparable among the treatment groups (data not shown). The presence or absence of the endothelium was confirmed by the presence or absence of the relaxation to bradykinin (10^{-7} mol/L) during a stable contraction evoked by prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (1–3 $\times$ 10^{-6} mol/L, adjusted to 50% of maximal contraction to KCl).[2,3] Endothelium-dependent relaxations to serotonin (10^{-9} to 10^{-6} mol/L), bradykinin (10^{-10} to 10^{-7} mol/L), and the calcium ionophore A23187 (10^{-9} to 10^{-6} mol/L) were examined in rings from the balloon-injured and control sites in parallel during a stable contraction evoked by $PGF_{2\alpha}$ (2×10^{-6} mol/L). Serotonin, bradykinin, and A23187 were chosen because they are G_i-protein-, G_q-protein-, and receptor-independent agonists, respectively.[2,3] The endothelium-dependent relaxations to serotonin were examined in the presence of ketanserin 10^{-6} mol/L, a $5HT_{2A}$-serotonergic receptor antagonist, to inhibit the direct vasocontracting effect of the monoamine on the vascular smooth muscle.[2,3] The endothelium-independent relaxation to sodium nitroprusside was also examined in rings without endothelium.

After the experiments, the rings were fixed with formalin. We have previously confirmed that the use for organ chamber experiment does not significantly change the histological characteristics of blood vessels.[2,3,20] Tissue samples were embedded in paraffin, sectioned into slices 5 μm thick, mounted on glass slides, and stained with hematoxylin and eosin (H&E) and Verhoeff-van Gieson elastin (VVG) stain.[15] The degree of the intimal thickening was analyzed as follows. The intimal area (Ai) was calculated by the formula Ai = Ae − Al, where Ae and Al are the areas within the internal elastic lamina and the internal border of the vessel, respectively. The degree of the neointimal formation was expressed as percentage of intimal area (Ai/Ae × 100).[15]

Blood Analysis

Blood samples were collected from each pig from the aorta with heparin before and 4 weeks after drug administration. The samples were centrifuged at 3000 rpm at 4°C for 15 minutes, and the plasma was frozen at −80°C. They were analyzed for lipid profiles, liver transaminases, and creatinine kinase in SRL Co Ltd (Tachikawa, Tokyo, Japan) to examine the potential side effects of the drug treatments.

Western Blotting

The expression of eNOS was measured by SDS-PAGE followed by electrophoretic transfer of the proteins to a nitrocellulose membrane.[16] The coronary segments from the balloon-injured site and the normal right coronary artery were used. The vessels were homogenized with 200 μL of SDS-PAGE sample buffer [Tris(hydroxymethyl) aminomethane 25.0 mM, glycine 192.0 mM, and SDS 288.4 mM]. The buffer was centrifuged at 15,000 rpm for 15 minutes, the supernatant was extracted, and its density was measured. An equal volume of sample was subjected to SDS-PAGE immunoblot analysis, using a specific antibody against eNOS[16] by an ECL Western blotting luminol reagent, and the density of the blots was measured by densitometry. The influence of the background density was excluded.

Drugs

The following drugs were used; ketamine hydrochloride, 5-hydroxytryptamine (serotonin), bradykinin, sodium nitroprusside, the calcium ionophore A23187, L-NNA (Sigma Chemical Co), $PGF_{2\alpha}$ (Ono Pharmaceutical Co), and antibodies to eNOS (N30020, Transduction Laboratory, Lexington, KY).

Data Analysis

All results are expressed as the mean ± SEM. Statistical analysis was performed by ANOVA followed by Fischer post-hoc test for multiple comparisons. A value of $P < 0.05$ was considered to be statistically significant.

RESULTS

Coronary Arteriography

When we performed coronary balloon injury, the balloon/artery ratio and percentage dilatation (the coronary diameter after balloon injury divided by that before the procedure) were comparable among the four groups, indicating that the coronary artery was equally balloon-injured. Four weeks after the balloon injury, coronary angiography demonstrated that mild stenotic lesion was developed at the previously injured site (15.2 ± 3.4%) but not at the uninjured site (0%). The combination therapy with cerivastatin and nifedipine significantly inhibited the development of the coronary stenotic lesion as compared with other three groups (control, 15.2 ± 3.4%; cerivastatin, 7.0 ± 2.1%; nifedipine, 15.9 ± 5.6%; and combination, 4.9 ± 1.9%) ($P < 0.05$ versus other three groups).

Organ Chamber Experiments

Endothelium-dependent relaxations to serotonin (G_i-protein-mediated) were significantly impaired at the balloon-injured site compared with the uninjured site (Fig. 1). The monotherapy with cerivastatin or nifedipine improved the impaired relaxations to serotonin at the balloon-injured site (Fig. 1). The combination therapy with cerivastatin and nifedipine markedly improved (supernormalized) the relaxations to serotonin (Fig. 1). By contrast, endothelium-dependent relaxations to bradykinin (G_q-protein-mediated) and A23187 (receptor-independent) were preserved at the balloon-injured site (Fig. 2). The endothelium-independent relaxations to sodium nitroprusside were not affected by balloon injury or any drug treatments (Fig. 3).

Histologic Examination

Histologic examination demonstrated that monolayer cells covered all inner surface of the vascular wall and that they were immunohistologically positive for eNOS (data not shown). The coronary vascular lesion formations after balloon injury (as expressed by pecentage intima) were not significantly different among the four groups; control, 55 ± 8%; cerivastatin, 44 ± 12%; nifedipine, 32 ± 12%; combination, 33 ± 15%. No intimal lesion was noted at the uninjured site.

Blood Sample Analysis

Blood sample analysis showed no significant differences in lipid profiles, liver transaminases, or creatinine kinase before and 4 weeks after the balloon injury in each group (data not shown). There were also no significant differences in those values among the four groups. The plasma concentrations of cerivastatin and nifedipine peak 1 hour after oral administration. The peak plasma concentrations of cerivastatin and nifedipine were 0.1 ± 0.06 μM and 2.7 ± 1.1 μM, respectively.

Western Blotting

The expression of eNOS was significantly reduced by balloon injury (Fig. 4). The monotherapy with cerivastatin or nifedipine tended to increase the eNOS expression, and the combination therapy with cerivastatin and nifedipine normalized the expression at balloon-injured site (Fig. 4).

DISCUSSION

The novel finding of the present study is that the combination treatment with cerivastatin and nifedipine improves endothelial dysfunction in the regenerated state after balloon injury. We have previously demonstrated that the regenerated endothelial cells have selective impaired endothelium-dependent responsiveness to serotonin but not to bradykinin or A23187,[2,3] which has been confirmed in the present study. The reduced expression and/or function of endothelial G_i protein may be one of the important molecular mechanisms for the dysfunction of regenerated endothelim.[3,4,17,18]

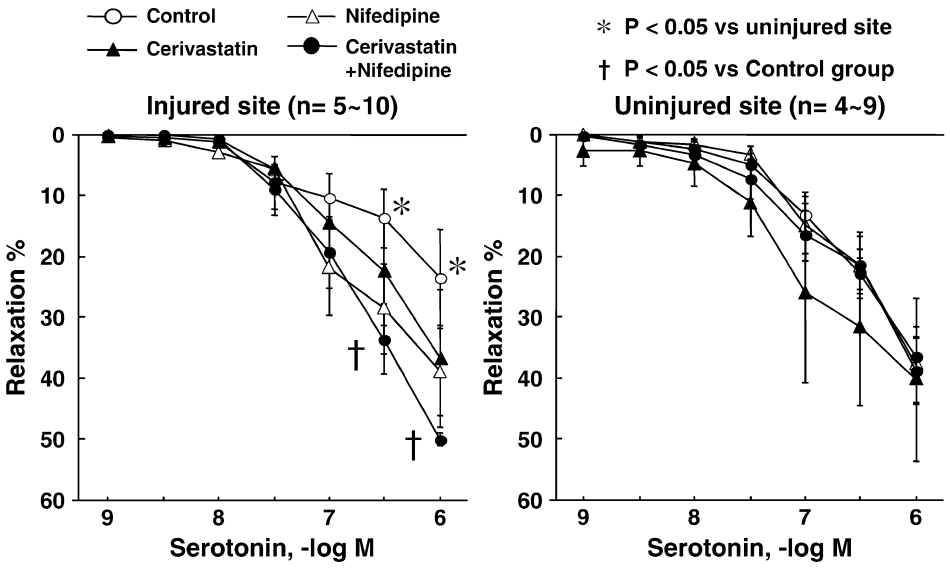

FIGURE 1. Endothelium-dependent relaxations to serotonin in porcine coronary arteries. The relaxations were significantly impaired at the balloon-injured site compared with the uninjured site. Although the treatment with cerivastatin or nifedipine alone improved the relaxations, the combined therapy with cerivastatin and nifedipine supernormalizes the responses.

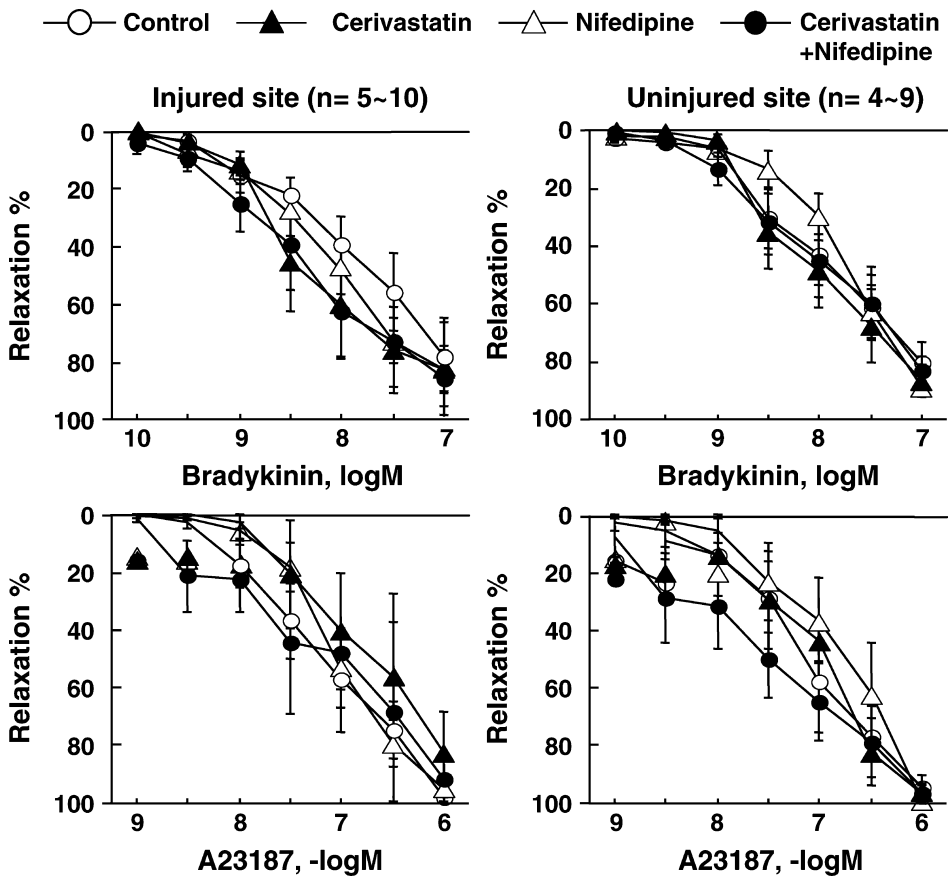

FIGURE 2. Endothelium-dependent relaxations to bradykinin and A23187 in porcine coronary arteries. The relaxations were not significantly impaired at balloon-injured site compared with the uninjured site. The treatments with cerivastatin or nifedipine alone, or their combination, did not significantly alter the responses.

It is important to maintain endothelial vasodilator function in the regenerated state. We have previously demonstrated that fish oil and its major component, eicosapentaenoic acid, improve endothelial dysfunction in animals and humans.[19–22] It also has been reported that lipid-lowering therapy by diet or statin improves eNOS expression in atherosclerotic rabbits.[23] Indeed, statins can up-regulate eNOS expression in vivo and in vitro, independent of lipid-lowering effects.[24–26] Calcium channel blockers could also enhance eNOS expression in vivo and in vitro, independent of blood pressure–lowering effects.[12,13,27–29]

There have been several reports regarding the beneficial effects of the combination therapy with statins and calcium channel blockers. The REGRESS trial was the first to

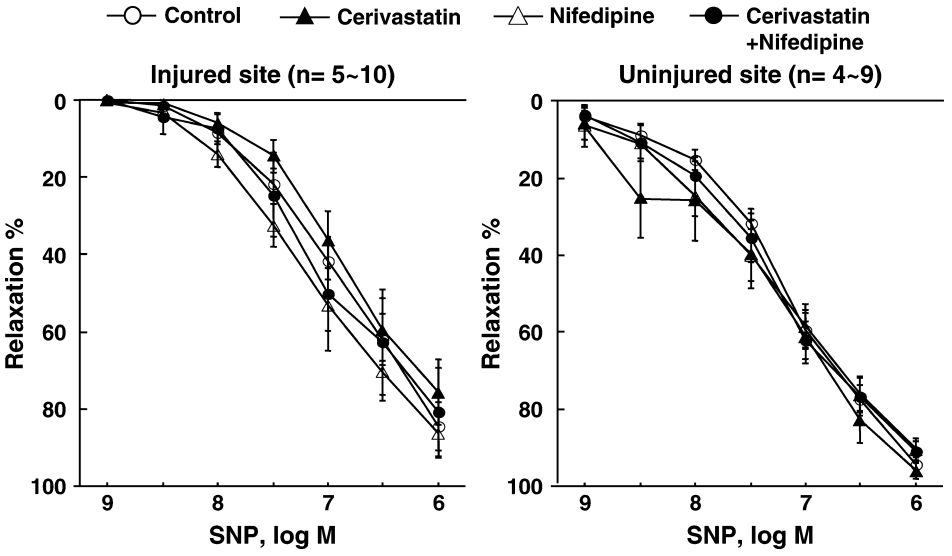

FIGURE 3. Endothelium-independent relaxations to sodium nitroprusside (SNP) in porcine coronary arteries. The relaxations were not impaired at the balloon-injured and uninjured sites. The treatment with cerivastatin or nifedipine alone, or their combination did not significantly alter the responses.

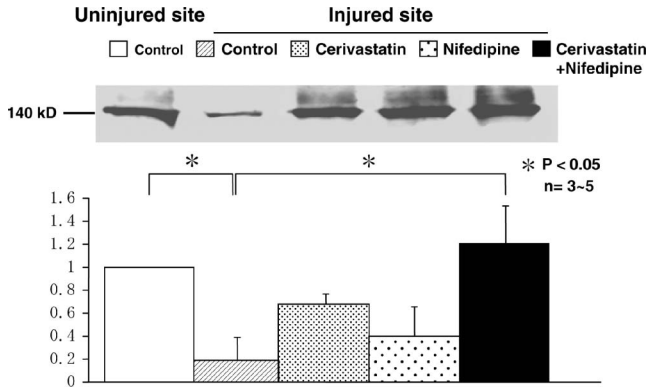

FIGURE 4. Western blotting analyses of eNOS protein expression in porcine coronary arteries. The balloon injury significantly reduced the eNOS expression, whereas the combination therapy with cerivastatin and nifedipine normalized its expression.

demonstrate the inhibitory effect of the combination therapy with pravastatin and calcium channel blockers on the progression of coronary atherosclerosis in humans.[30] The beneficial effects of the combination therapy have been confirmed in mouse models of atherosclerosis.[31,32] It was reported that the combination of amlodipine and lovastatin has a higher inhibitory effect on the atherogenicity of low-density lipoprotein as compared with amlodipine or lovastatin alone in cultured vascular smooth muscle cells.[33] Furthermore, amlodipine has been reported to improve arterial compliance and fibrinolytic balance in hypertensive and hyperlipidemic patients, while the addition of atorvastatin showed further beneficial effects than those with amlodipine alone.[34,35]

The ENCORE I study has recently examined the effects of cerivastatin or nifedipine GITS alone and their combination for 6 months on coronary endothelial functions in patients with coronary artery disease. The results demonstrated that nifedipine significantly improved coronary endothelial function in the most constricted segments, whereas the combination therapy with cerivastatin and nifedipine did not show additional benefits.[36] Recently, the ENCORE II study has further confirmed that nifedipine GITS for 2 years is useful to improve coronary endothelial function and suppress the progression of coronary atherosclerosis in patients with coronary artery disease.[37]

Our present study has demonstrated that the monotherapy with cerivastatin or nifedipine alone can only partially improve the impaired endothelium-dependent relaxation to serotonin and tends to improve eNOS protein expression, whereas their combination can supernormalize the relaxation responses and normalize the eNOS protein expression without affecting lipid profiles or blood pressure. Endothelium-dependent relaxations to serotonin are largely mediated by NO.[4,18] Thus, it is conceivable that the improvement of eNOS expression may be involved in the mechanisms for the improved endothelial vasodilator function achieved by the combination for the normalization of the relaxation responses to serotonin by the combination therapy remains to be elucidated. Although the extent of the coronary stenotic lesion at the previously injured site was very mild in all four groups, it was significantly less in the combination therapy group as compared with the other three groups. This result coincides with those of endothelial function and eNOS expression. We consider that the summed effects of cerivastatin and nifedipine are involved in the effects of the combination therapy.

Several limitations should be mentioned for the present study. First, although we evaluated functional involvement of NO in the endothelium-dependent relaxations and eNOS expression, we did not measure NO production from the endothelium or NO bioavailability (eg, NO_x and cGMP). However, the increase in functional contribution of NO associated with increased eNOS expression suggests that endothelial NO production is improved by the combination therapy with cerivastatin and nifedipine. Second, only one dose of each drug was used in this study. However, the present study includes a long-term treatment of large animals (pigs) with a relatively higher dose of cerivastatin (1 mg/kg/d or 25–30 mg/d/animal) and nifedipine (4 mg/kg/d or 100–120 mg/d/animal). Thus, we believe that we were able to examine the effect of the monotherapy with each drug.

In summary, we were able to demonstrate that the combination therapy with cerivastatin and nifedipine improves endothelial dysfunction in the regenerated state after balloon injury, independent of lipid profiles or blood pressure, suggesting a usefulness of the combination therapy for the treatment of coronary artery disease.

ACKNOWLEDGMENTS

The authors thank M. Sonoda and E. Gunshima for excellent technical assistance.

REFERENCES

1. Gibbons RJ, Chatterjee K, Daley J, et al. ACC/AHA/ACP-ASIM guidelines for the management of patients with chronic stable angina: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee on Management of Patients With Chronic Stable Angina). *J Am Coll Cardiol*. 1999; 33:2092–2197.
2. Shimokawa H, Aarhus LL, Vanhoutte PM. Porcine coronary arteries with regenerated endothelium have a reduced endothelium-dependent responsiveness to aggregating platelets and serotonin. *Circ Res*. 1987;61: 256–270.
3. Shimokawa H, Flavahan NA, Vanhoutte PM. Natural course of the impairment of endothelium-dependent relaxations after balloon endothelium removal in porcine coronary arteries. Possible dysfunction of a pertussis toxin-sensitive G protein. *Circ Res*. 1989;65:740–753.
4. Shimokawa H. Primary endothelial dysfunction: atherosclerosis. *J Mol Cell Cardiol*. 1999;31:23–37.
5. Luscher TF. Vascular protection: current possibilities and future perspectives. *Int J Clin Pract Suppl*. 2001;117:3–6.
6. 4S Group. Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). *Lancet*. 1994;344:1383–1389.
7. Shepherd J, Cobbe SM, Ford I, et al. Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. West of Scotland Coronary Prevention Study Group. *N Engl J Med*. 1995;333:1301–1307.
8. Treasure CB, Klein JL, Weintraub WS, et al. Beneficial effects of cholesterol-lowering therapy on the coronary endothelium in patients with coronary artery disease. *N Engl J Med*. 1995;332:481–487.
9. Laufs U, La Fata V, Plutzky J, et al. Upregulation of endothelial nitric oxide synthase by HMG CoA reductase inhibitors. *Circulation*. 1998;97: 1129–1135.

10. Aikawa M, Rabkin E, Sugiyama S, et al. An HMG-CoA reductase inhibitor, cerivastatin, suppresses growth of macrophages expressing matrix metalloproteinases and tissue factor in vivo and in vitro. *Circulation*. 2001;103: 276–283.
11. Fukumoto Y, Libby P, Rabkin E, et al. Statins alter smooth muscle cell accumulation and collagen content in established atheroma of watanabe heritable hyperlipidemic rabbits. *Circulation*. 2001;103:993–999.
12. Ding Y, Vaziri ND. Nifedipine and diltiazem but not verapamil upregulate endothelial nitric-oxide synthase expression. *J Pharmacol Exp Ther*. 2000;292:606–609.
13. Verhaar MC, Honing ML, van Dam T, et al. Nifedipine improves endothelial function in hypercholesterolemia, independently of an effect on blood pressure or plasma lipids. *Cardiovasc Res*. 1999;42:752–760.
14. Shimokawa H, Ito A, Fukumoto Y, et al. Chronic treatment with interleukin-1β induces coronary intimal lesions and vasospastic responses in pigs in vivo. The role of platelet-derived growth factor. *J Clin Invest*. 1996;97:769–776.
15. Fukumoto Y, Shimokawa H, Kozai T, et al. Tyrosine kinase inhibitor suppresses the (re)stenotic changes of the coronary artery after balloon injury in pigs. *Cardiovasc Res*. 1996;32:1131–1140.
16. Takemoto M, Sun J, Hiroki J, et al. Rho-kinase mediates hypoxia-induced downregulation of endothelial nitric oxide synthase. *Circulation*. 2002; 106:57–62.
17. Flavahan NA, Vanhoutte PM. G-proteins and endothelial responses. *Blood Vessels*. 1990;27:218–229.
18. Shimokawa H, Nakaike R, Takeshita A. Significance of defective endothelial signal transduction in impaired endothelium-dependent relaxation in atherosclerosis. *Gerontology*. 1995;41(Suppl 1):28–33.
19. Shimokawa H, Lam JY, Chesebro JH, et al. Effects of dietary supplementation with cod-liver oil on endothelium-dependent responses in porcine coronary arteries. *Circulation*. 1987;76:898–905.
20. Shimokawa H, Vanhoutte PM. Dietary cod-liver oil improves endothelium-dependent responses in hypercholesterolemic and atherosclerotic porcine coronary arteries. *Circulation*. 1988;78:1421–1430.
21. Tagawa H, Shimokawa H, Tagawa T, et al. Long-term treatment with eicosapentaenoic acid augments both nitric oxide-mediated and non-nitric oxide-mediated endothelium-dependent forearm vasodilatation in patients with coronary artery disease. *J Cardiovasc Pharmacol*. 1999;33:633–640.
22. Shimokawa H. Beneficial effects of eicosapentaenoic acid on endothelial vasodilator functions in animals and humans. *World Rev Nutr Diet*. 2001; 88:100–108.
23. Aikawa M, Sugiyama S, Hill CC, et al. Lipid lowering reduces oxidative stress and endothelial cell activation in rabbit atheroma. *Circulation*. 2002;106:1390–1396.
24. Laufs U, Liao JK. Post-transcriptional regulation of endothelial nitric oxide synthase mRNA stability by Rho GTPase. *J Biol Chem*. 1998;273: 24266–24271.
25. Mital S, Zhang X, Zhao G, et al. Simvastatin upregulates coronary vascular endothelial nitric oxide production in conscious dogs. *Am J Physiol Heart Circ Physiol*. 2000;279:H2649–H2657.
26. Amin-Hanjani S, Stagliano NE, Yamada M, et al. Mevastatin, an HMG-CoA reductase inhibitor, reduces stroke damage and upregulates endothelial nitric oxide synthase in mice. *Stroke*. 2001;32:980–986.
27. Ding Y, Vaziri ND. Calcium channel blockade enhances nitric oxide synthase expression by cultured endothelial cells. *Hypertension*. 1998;32: 718–723.
28. Yamashita T, Kawashima S, Ozaki M, et al. A calcium channel blocker, benidipine, inhibits intimal thickening in the carotid artery of mice by increasing nitric oxide production. *J Hypertens*. 2001;19:451–458.
29. Lenasi H, Kohlstedt K, Fichtlscherer B, et al. Amlodipine activates the endothelial nitric oxide synthase by altering phosphorylation on Ser^{1177} and Thr^{495}. *Cardiovasc Res*. 2003;59:844–853.
30. Jukema JW, Zwinderman AH, van Boven AJ, et al. Evidence for a synergistic effect of calcium channel blockers with lipid-lowering therapy in retarding progression of coronary atherosclerosis in symptomatic patients with normal to moderately raised cholesterol levels. The REGRESS Study Group. *Arterioscler Thromb Vasc Biol*. 1996;16:425–430.
31. van de Poll SW, Delsing DJ, Wouter Jukema J, et al. Effects of amlodipine, atorvastatin and combination of both on advanced atherosclerotic plaque in *APOE*3-Leiden* transgenic mice. *J Mol Cell Cardiol*. 2003;35:109–118.
32. Delsing DJ, Jukema JW, van de Wiel MA, et al. Differential effects of amlodipine and atorvastatin treatment and their combination on atherosclerosis in *ApoE*3-Leiden* transgenic mice. *J Cardiovasc Pharmacol*. 2003;42:63–70.
33. Orekhov AN, Tertov VV, Sobenin IA, et al. Antiatherosclerotic and antiatherogenic effects of a calcium antagonist plus statin combination: amlodipine and lovastatin. *Int J Cardiol*. 1997;62(Suppl 2):S67–S77.
34. Leibovitz E, Beniashvili M, Zimlichman R, et al. Treatment with amlodipine and atorvastatin have additive effect in improvement of arterial compliance in hypertensive hyperlipidemic patients. *Am J Hypertens*. 2003;16:715–718.
35. Fogari R, Derosa G, Lazzari P, et al. Effect of amlodipine–atorvastatin combination on fibrinolysis in hypertensive hypercholesterolemic patients with insulin resistance. *Am J Hypertens*. 2004;17:823–827.
36. The ENCORE Investigators. Effect of nifedipine and cerivastatin on coronary endothelial function in patients with coronary artery disease: the ENCORE I Study (Evaluation of Nifedipine and Cerivastatin On Recovery of coronary Endothelial function). *Circulation*. 2003;107:422–428.
37. Luscher TF. ENCORE II. The long-term effect of nifedipine on vasomotion and vessel morphology in patients with coronary artery disease: an intravascular ultrasound-assisted evaluation. *ESC Congress*. 2004;Hot Line Program No. 102.

