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Inhibitory effect of fluvastatin at doses insufficient to lower serum lipids on the catheter-induced thickening of intima in rabbit femoral artery

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Abstract

The anti-atherosclerotic effect of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors at doses insufficient to lower serum cholesterol was investigated in rabbit femoral artery denuded by balloon catheter. Fluvastatin and pravastatin were given orally at doses of 4 and 8 mg/kg per day, respectively, for 2 weeks after the catheterization. There was little change in serum cholesterol, triglyceride and phospholipid by chronic treatment with the drugs. The cross-sectional area of the intima, expressed as relative values to media (I/M ratio), was increased by the catheterization, showing intimal thickening in the denuded arteries. The I/M ratio was reduced by fluvastatin but not pravastatin: 0.327 ± 0.060 for control, 0.116 ± 0.035 for 4 mg/kg fluvastatin, 0.088 ± 0.027 for 8 mg/kg fluvastatin and 0.22 ± 0.069 for 8 mg/kg pravastatin. Fluvastatin (8 mg/kg)-induced effect on the I/M ratio, was prevented by the combined administration with 40 mg/kg per day mevalonate, a metabolite in the HMG-CoA reductase pathway. These results suggest that fluvastatin inhibits intimal thickening after catheterization-induced injury through percutaneous transluminal coronary angioplasty (PTCA) and that the inhibition is presumably attributed to reduced migration and proliferation of smooth muscle cells but not secondarily to a lowering of serum lipid.

Keywords: Balloon catheterization; Hyperplasia; HMG-CoA reductase inhibitor

1. Introduction

Coronary heart disease remains a leading cause of death. The risk of the disease is closely associated with elevated plasma cholesterol, particularly low-density lipoprotein (LDL) cholesterol (Hjermann et al., 1981; Lipid Research Clinics Program, 1984; Oliver et al., 1984; Stamler et al., 1986; Frick et al., 1987), which brings an increased awareness for the need of nutritional intervention.

Fluvastatin, a potential lipid-lowering agent, is a competitive inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (Parker et al., 1990; Shaw et al., 1990; Prous and Castaner, 1991; Paterniti, 1992; Hayashi et al., 1993; Taylor, 1993) which converts HMG-CoA to mevalonic acid and is the rate limiting enzyme for the biosynthesis of cholesterol (Quesney-Huneeus et al., 1979, 1983; Goldstein and Brown, 1990). The lipid-lowering effect of fluvastatin has been established in hypercholesterolemic patients (Prous and Castaner, 1991; Taylor, 1993).

Besides the hypocholesterolemic action of fluvastatin, Corsini et al. (1993) have demonstrated that fluvastatin suppresses the proliferation of vascular smooth muscle cells in vitro. The intimal migration and proliferation of the smooth muscle cells are assumed to be involved in the progression of atherosclerosis. In percutaneous transluminal coronary angioplasty (PTCA), intimal hyperplasia accompanied by excessive growth of smooth muscle cells takes place, resulting in subsequent restenosis. It is of interest whether or not the intimal thickening following the angioplasty is inhibited by HMG-CoA reductase inhibitors at doses insufficient to elicit the primary action of lipid lowering.

The present study was performed to clarify whether intimal thickening is attenuated by low doses of HMG-CoA

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reductase inhibitors without significant change in serum lipids. The vascular action of fluvastatin is further explored with mevalonate.

2. Materials and methods

2.1. Materials

Fluvastatin was supplied by Sandoz Pharmaceuticals (Switzerland). Pravastatin was obtained from Sankyo (Japan) (extracted from mevalotin). Monoclonal antibody against α-smooth muscle actin (HHF-35) was obtained from Enzo (USA) and avidin-biotin peroxidase system from Vector Labs. (USA).

2.2. Balloon catheter-induced denudation

Male New Zealand white rabbits (Kitayama Raves, Japan) of 8 weeks old were used. Under anesthesia with pentobarbital sodium (Nembutal, Dainippon Pharmaceutical, Japan) at an i.v. dose of 20 mg/kg, a balloon catheter (2 Fr arterial embolectomy catheter; Baxter, USA) was inserted retrogradely in a distance of 120 mm upwards into the lower part of the abdominal aorta through the external common iliac artery and right femoral artery from the incision site of right saphenous artery. Subsequently, the balloon was inflated sufficiently to stop bleeding at the incision site. The intimal surface was forcibly denuded once by rubbing with the inserted balloon at an internal pressure of 0.5–1.5 kg/cm^2. After the catheterization, the saphenous artery at the incision site was ligated with silk thread and heparin was given i.v. via the auricular vein.

2.3. Animal groups and drug treatment

All animals had free access to food (120 g/day) and water, and were allowed to acclimatize for 1 week before starting the experiment. 49 rabbits were divided randomly into 5 groups of 7–11 animals each: (1) control, (2) 4 mg/kg fluvastatin, (3) 8 mg/kg fluvastatin, (4) 8 mg/kg pravastatin, (5) 8 mg/kg fluvastatin and 40 mg/kg mevalonate. HMG-CoA reductase inhibitors were given orally in the evening (17 : 00–18 : 00 h) once a day. Mevalonate was administered twice a day in the morning and evening at a daily dose of 40 mg/kg. The control group was given distilled water. Each drug was given for 2 weeks, starting on the day of balloon catheterization.

2.4. Histological analysis

Rabbits were killed by an overdose of pentobarbital sodium. The rabbit arteries were fixed by perfusion with 70% ethanol after washout of the blood with saline for about 20 min in order to avoid potential vasoconstriction. The femoral arteries were isolated and the connective tissue was removed. The vessels were immersed in the same fixative for at least 24 h. The arterial rings were embedded in paraffin for histological study. Cross-sections at 5 μm thick were prepared and stained according to the Elastica Van Gieson method. The cross-sectional area of the intima, expressed as relative values to media (I/M ratio) was presented as the mean of 5 cross-sections every 5 mm distant from the branch point of the iliac artery. Adjacent sections were used for the determination of α-smooth muscle actin with anti-mouse immunoglobulin.

The area were assessed quantitatively by using an image-analysis system composed of an RPC-6000 computer (IBM, USA), TPX01 video camera (Canon, Japan), BTC-01 color monitor, MX-10 microscope (Canon, Japan) and Optimetric (Ortex, USA) software.

2.5. Serum lipids analysis

Blood samples of animals after overnight fasting were collected on the day of sacrifice after 14 days of treatment. Total cholesterol, triglycerides and phospholipids in serum were measured with cholesterol oxidase-3,5-dimethoxy-*N*-ethyl (2-hydroxy-3-sulfopropyl)-aniline sodium (DAOS), glycerol-3-phosphate oxidase-DAOS and choline oxidase-DAOS, respectively, using commercial kits (Wako Chemical, Japan).

2.6. Statistical analysis

The data are expressed as the mean ± S.E.M. Statistical analysis of data was performed using one-way analysis of variance, followed by Tukey's multiple comparison test. Statistical significance was accepted at $P < 0.05$.

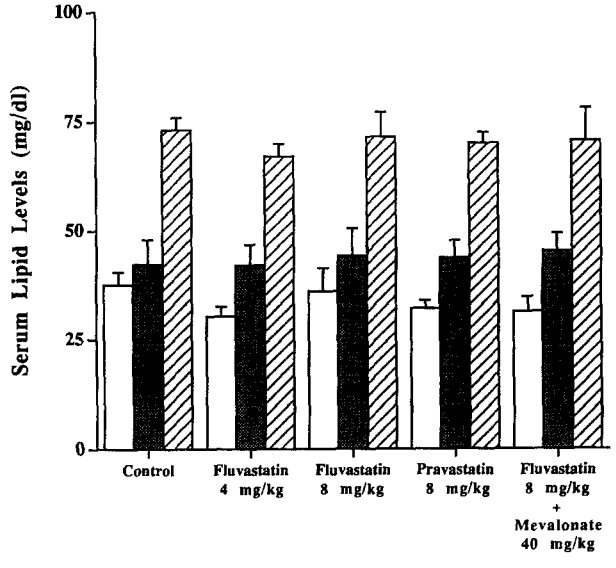

Fig. 1. Effects of vastatins on serum lipid levels. Fluvastatin and pravastatin were given orally at doses of 4 and 8 mg/kg per day, respectively, for 2 weeks after the balloon catheterization. Mevalonate was administered orally twice a day at a daily dose of 40 mg/kg. Total cholesterol (open columns), triglyceride (stippled columns) and phospholipid (hatched columns) were measured using enzymatic assay. Each column shows the mean ± S.E.M.

3. Results

3.1. Serum lipids after administration of HMG-CoA reductase inhibitors

The total cholesterol, triglyceride and phospholipid in serum were unchanged by the 2 weeks of treatment with each drug (Fig. 1).

3.2. Histological analysis

Balloon catheter-induced injury elicited a marked intimal thickening (Fig. 2A). Immunohistochemical staining revealed that the intimal thickening after the injury was composed of α-smooth muscle actin-positive cells (Fig. 2B).

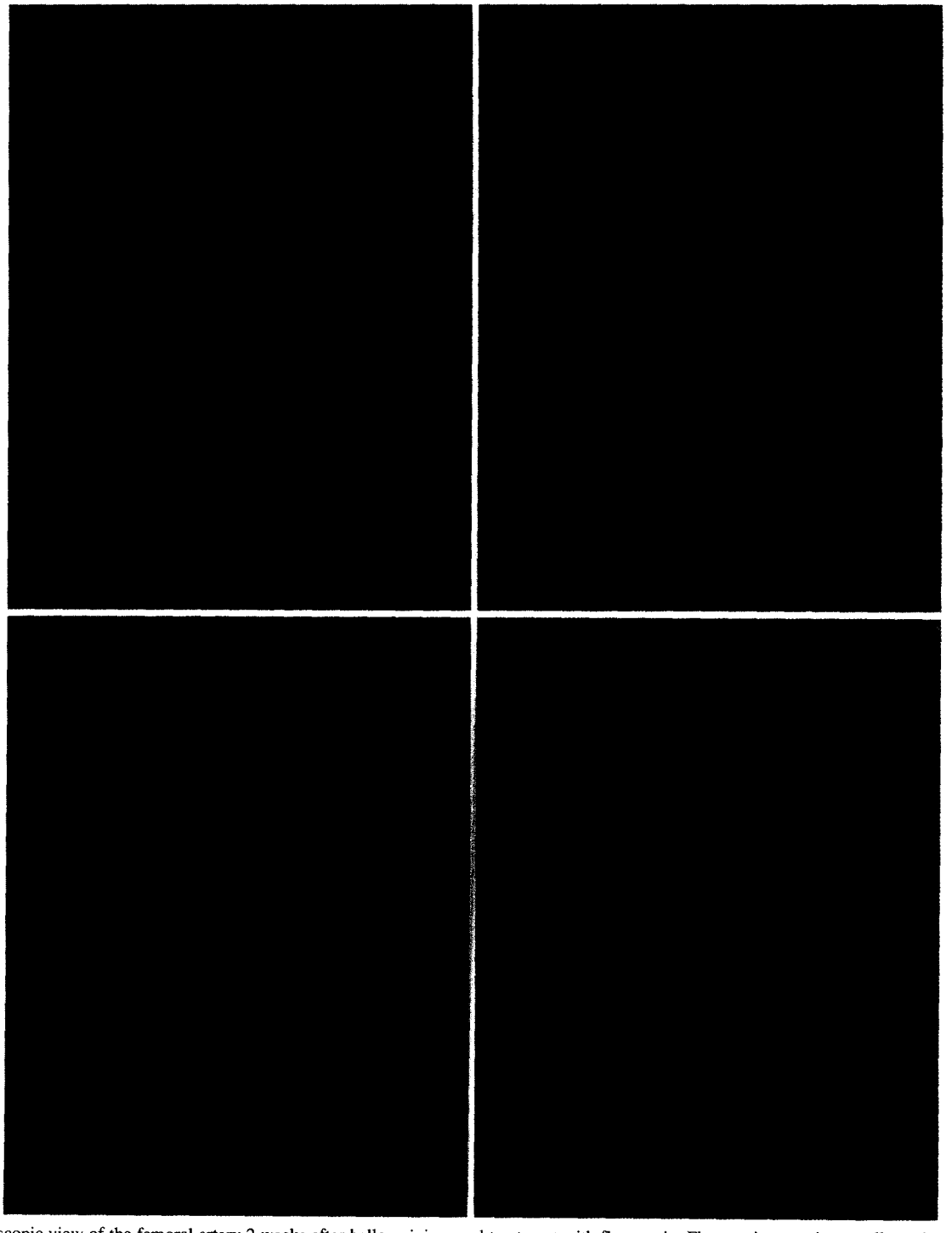

Fig. 2. Microscopic view of the femoral artery 2 weeks after balloon injury and treatment with fluvastatin. Fluvastatin was given orally at doses of 4 and 8 mg/kg per day after the balloon catheterization. (A) Femoral artery isolated from rabbits with balloon catheterization (control). (B) α-Smooth muscle actin antibody immunostaining of the femoral artery isolated from rabbits with balloon catheterization (control). (C,D) Femoral artery isolated from rabbits treated with balloon catheterization, and subsequently 4 (C) or 8 mg/kg (D) of fluvastatin.

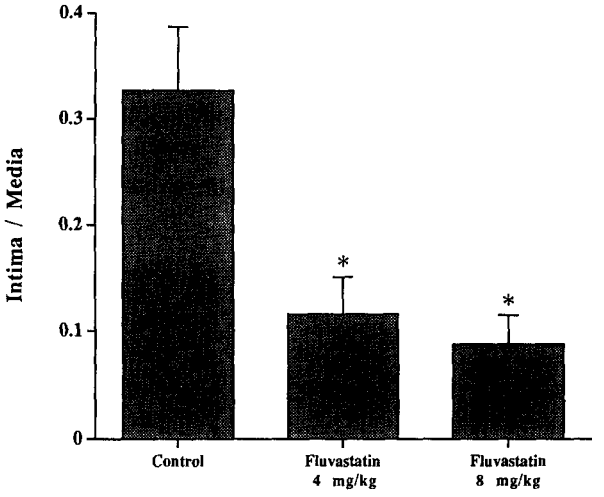

Fig. 3. Effects of fluvastatin on intimal thickening induced by balloon catheterization. Fluvastatin was given orally at doses of 4 and 8 mg/kg per day after the balloon catheterization for 2 weeks. The ratio of intima area to media area is given as the mean ± S.E.M. * $P < 0.05$, compared with the control (Tukey-Kramer multiple test).

Intimal thickening was attenuated markedly by treatment with fluvastatin (Fig. 3C,D). As shown in Fig. 3, the I/M ratio in the control group for catheterization was reduced significantly by oral administration with fluvastatin at doses of 4 and 8 mg/kg for 2 weeks. The reduction of neointimal formation by fluvastatin was dose-dependent. Simultaneous treatment with mevalonate reversed the inhibitory effect of 8 mg/kg fluvastatin on intimal hyperplasia (Fig. 4). The effects of fluvastatin on intimal thickening were compared with those of pravastatin at the same dose of 8 mg/kg. In contrast with fluvastatin, the I/M ratio was not significantly lowered by pravastatin (Fig. 4).

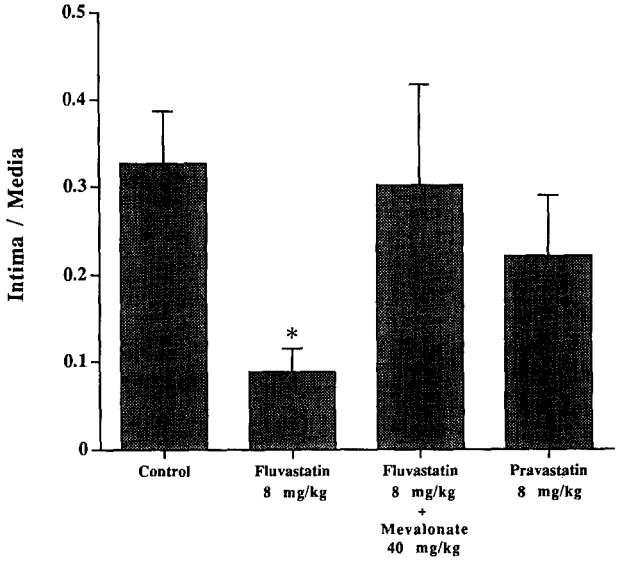

Fig. 4. Effects of fluvastatin, pravastatin and mevalonate on intimal thickening induced by balloon catheterization. Fluvastatin and pravastatin were given orally at dose of 8 mg/kg per day for 2 weeks after the balloon catheterization. Mevalonate was administered orally twice a day at a daily dose of 40 mg/kg in fluvastatin-treated rabbits. The ratio of intima area to media area as given as the mean ± S.E.M. * $P < 0.05$, compared with the control (Tukey-Kramer multiple test).

4. Discussion

Although there was no significant change in serum lipids, the femoral artery injured surgically by balloon catheter demonstrated morphological deterioration of the intima in rabbits. Intimal thickening was formed markedly after balloon catheterization. Excess proliferation of vascular smooth muscle cells which were identified in the intima was suppressed by chronic treatment with fluvastatin at 4 mg/kg.

It has been demonstrated that the mevalonate pathway plays a role in cell growth (Habenicht et al., 1980; Fairbanks et al., 1984; Doyle and Kandutsch, 1988; Maltese, 1990), and that the inhibitors, such as fluvastatin and simvastatin, inhibit the proliferation of vascular smooth muscle cells in vitro (Corsini et al., 1993). In the present study, combined treatment with mevalonate at 40 mg/kg reversed the anti-intimal thickening responses to 8 mg/kg fluvastatin. Thus, it is likely that the inhibitory effect of fluvastatin on intimal thickening is due to reduced production of mevalonate pathway metabolite(s) which are postulated to generate excessively after intimal injury. Therefore, intermediates as well as cholesterol may play a role in the over-proliferation of the smooth muscle cells. Potential intermediates for the intimal thickening, which HMG-CoA reductase inhibitors modulates, may be isoprenoid metabolites, like geraniol, farnesol and geranylgeraniol (Corsini et al., 1993). Although no inhibitor of the cholesterol biosynthesis pathway after squalene was examined in the present study, Rogler et al. (1995) demonstrated in cultured arterial myocytes that inhibition by fluvastatin of cell growth is reversed by mevalonate but not by squalene or cholesterol. Thus, specific role of isoprenoid metabolites in regulating cell proliferation is raised in vitro, but it remained to determine of whether or not squalene synthesis inhibitor may elicit an inhibitory effect on intimal thickening. It would be also of interest to examine effects of squalene synthesis inhibitor on proliferation of the smooth muscle cells in the presence and absence of mevalonate. Besides the mevalonate pathway, the ras oncogene has been proposed to play a central role in the over-proliferation of the smooth muscle cells following angioplasty, as transdominant negative H-ras mutants inhibit the proliferation (Indolfi et al., 1995). It remains to be determined whether or not fluvastatin suppresses the expression of the ras oncogene.

The 4 mg/kg dose of fluvastatin used in the present study is approximately one-fifth of those used elsewhere (Soma et al., 1993). It is also less than the dose required to elicit an inhibitory effect on tumor growth by mevilonin

(Maltese et al., 1985) which causes a lipid-lowering effect at similar doses to fluvastatin. It is, thus, postulated that the HMG-CoA reductase inhibitor acts more on excessive proliferation of smooth muscle cells, presumably of the synthetic phenotype (Chamley-Champbell and Champbell, 1981; Chamley-Champbell et al., 1981).

The proliferation of smooth muscle cells in vitro is inhibited by fluvastatin with the IC_{50} of 2.2 μM (Corsini et al., 1993). This is more than the expected plasma concentrations; 0.5 μM at the peak (Masuda et al., 1995). While the experimental conditions in vitro are not necessarily the same as in plasma, it is speculated that fluvastatin may reduce the production of growth factors or the expression of atherogenic factors, and that fluvastatin at higher doses suppresses the growth of smooth muscle cells in a direct manner. Our preliminary study (Niwa et al. unpublished data) showed an inhibitory effect on the expression of lymphocyte function associated antigen-1 (LFA-1), an adhesion molecule for an atherogenic factor in human monocytes.

In contrast with fluvastatin, pravastatin did not suppress intimal thickening following balloon catheterization. The effective doses to lower lipids in plasma to a critical extent are 12.5 mg/kg in Watanabe heritable hyperlipidemic (WHHL) rabbits for both fluvastatin and pravastatin (Tsujita et al., 1986; Shiomi et al., 1994). Thus, it is not likely that the difference in the anti-atherosclerotic effect is due to unbalanced doses of the two enzyme inhibitors in terms of lipid-lowering effectiveness. Pravastatin inhibits HMG-CoA reductase in the liver to a greater extent than in other organs, and it is permeable to the hepatic tissue membrane (Scott, 1989). If fluvastatin, with more lipophilicity than pravastatin, is more permeable to the smooth muscle membrane, it may contribute to the difference in anti-atherosclerotic effect. Alternatively, fluvastatin may attenuate the release/production of vascular smooth muscle growth factor(s) preferentially following intimal injury. There is the possibility that the greater potency of fluvastatin for inhibiting cholesterol synthesis in vitro in hepatic cell lines (Tsujita et al., 1986) may simply account for the difference.

The medial layer in the vessel for the muscle contribution was not affected by fluvastatin. This would be advantageous for the vascular action, if fluvastatin has the phenotype selectivity of action but no weakening of the vascular wall. On the one hand, fluvastatin is likely to act on the cell in particular in the growth cycle accelerated and/or in the intimal environment specifically, which remains to be determined.

In conclusion, fluvastatin at low doses insufficient to lower lipids in serum attenuated the progression of intimal thickening following balloon catheterization. It is conceivable that the excessive growth of vascular smooth muscle cells is suppressed via a decrease in mevalonate pathway metabolite(s) in the cell or indirectly through inhibition of atherogenic factor(s).

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