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The Effect of Lovastatin on Proliferation of Cultured Rat Mesangial and Aortic Smooth Muscle Cells

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In order to investigate the anti-proliferative effect of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor, we evaluated the effects of lovastatin on DNA replication and the proliferation of rat mesangial and aortic smooth muscle cells, both of which were mesenchymal origin cells. Proliferations were determined by measuring [^{3}H]thymidine uptake, and counting the number of cells. Growth-arrested mesangial and aortic smooth muscle cells were exposed to platelet-derived growth factor (PDGF), endothelin (ET) and angiotensin II (Ang II) to stimulate mitogenesis. All agents exhibited dose-dependent stimulation of [^{3}H] thymidine uptake. PDGF was more potent than the others. Ang II increased [^{3}H] thymidine uptake without demonstrable mitogenic activity. Lovastatin inhibited PDGF (10 ng/ml in mesangial cell, 25 ng/ml in smooth muscle cell)-, ET (10^{-7}M)- and Ang II (10^{-7}M)-induced [^{3}H] thymidine uptake significantly in a dose-dependent manner in both cells. The increase of cell number in response to PDGF and ET treatment were also inhibited at 10 μM of lovastatin. The inhibitory effect of lovastatin was largely overcome in the presence of exogenous mevalonate at 200 μM, with 75.5% restoration from lovastatin-induced inhibition on PDGF-induced [^{3}H] thymidine uptake in mesangial cells (77.8% in aortic smooth muscle cells). However, the addition of cholesterol did not prevent inhibition by lovastatin. In conclusion, lovastatin had an inhibitory effect on mesangial and aortic smooth muscle cell proliferation, and mevalonate was essential for DNA replication in both types of cells. Lovastatin may reduce glomerular and atherosclerotic injury through an anti-proliferative effect on mesangial and vascular smooth muscle cells, in addition to lowering circulating lipids.

Key Words: Lovastatin, rat mesangial cell, aortic smooth muscle cell, proliferation

Many glomerular diseases are characterized by mesangial cell proliferation and accumulation of mesangial extracellular matrices. Indeed, these processes precede, and possibly cause, the development of glomerulosclerosis (Brenner 1985; Klahr *et al.* 1988). It has been reported previously in several experimental models of progressive glomerular disease that treatment with lovastatin, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor, reduced mesangial cellularity and extracellular matrix expansion. These effects of lovastatin were seen in obese Zucker rats, Dahl salt-sensitive rats, and rats with subtotal renal ablation (Kasiske *et al.* 1988a; Kasiske *et al.* 1988b; O'Donnell *et al.* 1992). Such beneficial effects of lovastatin were thought to be attributed to the lowering of circulating lipids (Keane *et al.* 1988).

HMG-CoA reductase is an intracellular en-

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zyme catalyzing the process of producing mevalonate from HMG-CoA. Products of the mevalonate pathway play a critical role in DNA replication and cell proliferation (Goldstein and Brown, 1990). Mevalonate metabolism yields a series of isoprenoid compounds which are incorporated into cholesterol, isopentenyl adenine, farnesylated proteins, and other end-products essential for cell growth (Quesney-Huneeus *et al.* 1979; Siperstein 1984). Inhibition of HMG-CoA reductase with agents such as compactin or lovastatin blocks the production of mevalonate and has been shown to inhibit DNA synthesis and proliferation in several cell types(Habenicht *et al.* 1980; Fairbanks *et al.* 1984). Therefore, in addition to the lipid-lowering effect of lovastatin, it is conceivable that lovastatin may also affect mesangial cell proliferation, directly.

In the pathogenesis of atherogenesis, vascular smooth muscle cell proliferation is emphasized as the key event in the development of the advanced lesions of atherosclerosis (Ross 1986). Vascular smooth muscle cell contains receptors to growth factors and vasoactive hormones, and respond to contract or proliferate to various stimuli, such as platelet-derived growth factor (PDGF), endothelin (ET), or angiotensin II (Ang II)(Thyberg *et al.* 1990). These cells are originated from mesenchymal cells, like mesangial cells, and also have similar intracellular signaling pathways to those of mesangial cells. Possibly, vascular smooth muscle cell growth may also be influenced by lovastatin. While lovastatin has been used frequently in treatment of hypercholesterolemia, the effect of lovastatin on smooth muscle proliferation is not well known.

This study was designed to investigate the possibility of the inhibitory effects of lovastatin on cultured rat mesangial and aortic smooth muscle cell proliferation. We conducted experiments evaluating the effects of lovastatin on DNA synthesis reflected by [^{3}H] thymidine uptake, and cell proliferation assessed by counting the number of cells, following stimulation by PDGF, ET, and Ang II, in both cells.

SUBJECTS AND METHODS

Reagents: RPMI-1640, insulin-transferrin-selenite media supplement, Eagle's minimum essential medium(EMEM), mevalonic acid lactone, LDL (low-density lipoprotein)-cholesterol, PDGF-BB, ET, Ang II were obtained from Sigma Chemical Co., St. Louis MO. Fetal calf serum, trypsin-EDTA and collagenase were purchased from Gibco Laboratories, Grand Island, NY. Lovastatin was generously provided by the Joongwoe Pharmaceutical Co. Seoul, Korea.

Mesangial cell culture

Primary cultures of rat mesangial cells were prepared according to the methods of Takeda *et al.*(1988). Briefly, the renal cortices from six to eight 200 to 250 g Sprague Dawley rats were collected, minced, and passed through successive stainless steel sieves of 200, 150, and 75 μm pore size. The glomeruli collected from the 75 μm sieve were plated in RPMI 1640 supplemented with 20% fetal calf serum (FCS), 5 μg/ml human transferrin, 100 U/ml penicillin, and 100 μg/ml streptomycin. The first outgrowths were epithelial cells, peaking at 5~6 days, and then dying off. These cells were replaced by mesangial cells which grew to confluency by 21~28 days. Cells were subcultured in RPMI-1640 media containing 20% FCS at 7- to 10-days intervals on confluence of the cells in 75 mm^2 culture flasks, and incubated in a humidified incubator at 5% CO_2, 37℃. Fibroblast growth was prevented by substituting L-valine in the RPMI medium for D-valine. For these experiments, mesangial cells between the twelfth and twenty fifth passage were used. Morphological examination confirmed a homogeneous population of stellate-shaped cells typical of mesangial cell. Immunoperoxidase stain showed anti-cytokeratin antibody (DAKO Japan Co., Kyoto, Japan) negative, and anti-desmin antibody (DAKO Japan Co., Kyoto, Japan) positive, suggestive of non-epithelial, mesenchymal origin cells.

Aortic smooth muscle cell culture

Rat aortic smooth muscle cells were isolated using a modified method described by Meyer-Lehnert *et al*(1988). The thoracic aortas from six to eight male Sprague-Dawley rats were cleaned in 4℃ phosphate buffered saline, and incubated at 37℃ for 30 minutes in 7.5 ml of Eagle's minimum essential medium (EMEM) containing 1.72 mg/ml collagenase, penicillin (100 U/ml) and streptomycin (100 mg/ml). After dissecting the adventitia, the aortas were incubated again in collagenase mixture for 30 minutes at 37℃.

The fragile aortas were cleaned again through further removal of adventitia, and minced with sterile razor blades. Small fragments of aorta were transferred to 50 ml tubes and incubated again at 37℃ in 7.5 ml of collagenase mixture for 1~1.5 hours with continuous shaking. After this incubation, the cell suspension was centrifuged for 5 minutes at 1,000 rpm, twice. The cell pellet was resuspended in fresh incubation medium containing 10% FCS. The cells were plated onto 35 mm^2 culture dishes and grown at 37℃ in a humidified atmosphere of 95% air and 5% CO_2. The cultures reached confluence after 7~10 days and were then used for experiments. Subcultured cells (10th~20th passages) were used in all experiments. Immunoperoxidase cell staining revealed anti-factor VIII antigen antibody (DAKO Japan Co., Kyoto, Japan) negative, and anti-α1-actin antibody (DAKO Japan Co., Kyoto, Japan) positive, being elliptical in shape and containing myofibril filaments.

DNA synthesis

Cellular incorporation of [^{3}H] thymidine (Du Pont Co., Wilmington, DE, USA) was used to assess DNA replication. Cells were seeded in 96 well culture plates at a density of 1×10^4 cells per well. At confluency, the cells were synchronized into a quiescent state by incubation in 0.5% (0.1% in smooth muscle cell) FCS RPMI-1640(EMEM in smooth muscle cell) containing insulin(5 μg/ml) and transferrin(5 μg/ml) for 48 hours (72 hours in smooth muscle cell), and cells were exposed to PDGF-BB, ET or Ang II at planned concentrations for 24 hours, respectively, to stimulate mitogenesis. After this, [^{3}H] thymidine (2 μCi/well)was added to all wells, and the total [^{3}H] thymidine uptake was measured over 6 hours. Cells were harvested using a cell harvester (Titertek Cell Harvester 550, Flow Laboratories, Irvine, Scotland, U.K.), collected on glass fiber filters, and placed in a 3 ml scintillation cocktail for determination of total radioactivity. The radioactivity of [^{3}H] thymidine uptake was measured in a β-counter(TL 5000S, Beckman Instruments Inc., Fullerton, CA, USA).

Cell proliferation

For determination of cell proliferation, cells were seeded in 24 well culture plates at a density of 2×10^4 cells per well. At 60% confluency, cells were synchronized to quiescence, and after 48 hours, the cells were each exposed to growth promoters, as described above. After stimulation for 5 days, cellular proliferations were assessed by counting viable cells using a hemocytometer under a microscope. Cell viability was checked by the exclusion of trypan blue (0.4%).

The effect of lovastatin on mesangial and aortic smooth muscle cell proliferation

Lovastatin salt(90 mg) was dissolved in 1.8 ml of warm ethanol, after which 0.9 ml of 0.6 N NaOH and 18 ml of water were added to form sodium salt (Kita *et al.* 1980). The lovastatin solution (4 mg/ml) was stored in multiple 0.5 ml aliquots at −20℃ until use. Lovastatin was added to each well at concentrations of 0.5, 1, 5, 10 μM with each growth promoter at concentrations having maximal stimulatory effect, together. After this, the anti-proliferative effects were evaluated through [^{3}H] thymidine incorporation and cell numbers, as described above.

Reversibility of the inhibitory effect of lovastatin on mesangial and aortic smooth muscle cell proliferation by mevalonate

Mevalonic acid lactone (32.5 mg)was solubilized in 2 ml absolute ethanol and added to

23 ml RPMI-1640(EMEM for smooth muscle cell), pH 7.40, to produce mevalonate salt. Mevalonate salt was diluted in a culture medium and added to cells in concentrations 50, 100 and 200 μM with lovastatin and each growth factor. For comparison with mevalonate, LDL-cholesterol (100 μM) was also tried.

Statistical analysis

Data are expressed as means±S.E. of at least three experiments carried out on different cultures in duplicate or triplicate. Statistical significance was assessed using one-way analysis of variance combined with Scheffe's test, and a probability level of $P<0.05$ was regarded as significant.

RESULTS

The effects of PDGF, ET, and Ang II on [^{3}H] thymidine uptake of mesangial cell

The treatment with PDGF at concentrations between 1 ng/ml and 50 ng/ml stimulated [^{3}H] thymidine uptake of quiescent mesangial cells in a dose-dependent manner(5 ng/ml: 1682±178 cpm, 50 ng/ml: 3641±244 vs 512±38, $p<0.05$). Exposure of mesangial cells to ET or Ang II at concentrations ranging from 10^{-10} to 10^{-6} M also increased [^{3}H] thymidine uptake, dose-dependently. The addition of ET at a concentration of 10^{-8} M stimulated [^{3}H] thymidine uptake significantly, compared to the control(10^{-8} M: 1350±250 cpm vs 650±50, $p<0.05$), and the treatment of Ang II at a concentration of 10^{-7} M also increased [^{3}H] thymidine uptake significantly (10^{-7} M : 1300±140 cpm vs 524±45, $p<0.05$).

The effect of Lovastatin on mesangial cell proliferation

The treatment with lovastatin at concentrations ranging from 0.5 μM to 10 μM caused a dose-dependent reduction of PDGF (10 ng/ml)-induced [^{3}H] thymidine uptake. At 0.5, 1, 5, 10 μM concentration of lovastatin, [^{3}H] thymidine uptake was 2354±334, 2210±358, 1528±171, 668 ±57 cpm, respectively, and the values at a greater than 5 μM concentration of lovastatin

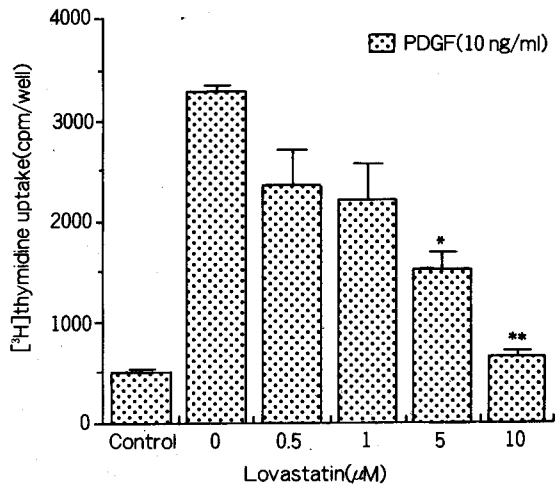

Fig. 1. *Effect of lovastatin on PDGF-induced [^{3}H] thymidine uptake in mesangial cells. Treatment with lovastatin inhibits [^{3}H] thymidine uptake following PDGF (10 ng/ml) stimulation, dose-dependently (n=5). *: $p<0.05$ vs PDGF alone, **: $p<0.05$ vs PDGF alone, and 0.5 μM lovastatin.*

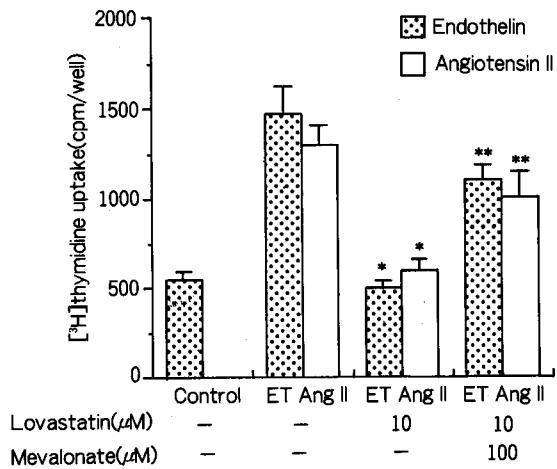

Fig. 2. *The inhibitory effect of lovastatin and its reversibility by mevalonate on ET- and Ang II-induced [^{3}H] thymidine uptake in mesangial cells. Treatment with lovastatin (10 μM) reduces [^{3}H] thymidine uptake in response to ET (10^{-7} M) and Ang II(10^{-7} M), markedly. The addition of mevalonate restored the reduction of [^{3}H] thymidine uptake significantly. *: $p<0.05$ vs ET or Ang II alone (n=3). **: $p<0.05$ vs treatment with lovastatin.*

were significantly lower than [^{3}H] thymidine uptake stimulated by PDGF, 3382±478 cpm($p<0.05$, Fig. 1). The addition of 10 μM lovastatin also markedly inhibited [^{3}H]thymidine uptake following ET (10^{-7} M) or Ang II(10^{-7} M) stimulation(Fig. 2).

Regarding the evaluation of cell proliferation, the exposure of quiescent cells to PDGF caused a marked increase of the number of cells. The treatment with lovastatin at a concentration from 1 μM to 10 μM significantly inhibited the increase of the number of cells following PDGF stimulation, and this was dose-dependent(Table 1). The proliferation induced by 10^{-7} M ET was also significantly inhibited by the addition of 10 μM lovastatin (Table 1). In Ang II-induced proliferation, the exposure of cells to 10^{-7} M Ang II showed approximately a three fold increase of [^{3}H] thymidine uptake but did not increase the number of cells, which indicated no detectable mitogenic activity(Table 1).

Reversibility of the inhibitory effect of lovastatin on [^{3}H]thymidine uptake in mesangial cells

Inhibitions of PDGF-, ET- and Ang II-induced [^{3}H] thymidine uptake by lovastatin were prevented by the presence of mevalonate. The addition of mevalonate at concentrations of 50, 100, and 200 μM restored [^{3}H] thymidine uptake from 668±57 cpm to 2174±150 (57.2%), 2409±369 (66.1%), and 2656±275 (75.5%), respectively ($p<0.05$, Fig. 3). Regarding inhibition of ET(10^{-7} M)- and Ang II(10^{-7} M)-induced [^{3}H] thymidine uptake by lovastatin, inhibition was also prevented largely by treatment with 100 μM mevalonate (Fig. 2). In contrast, the inhibitory effect of lovastatin on PDGF-induced [^{3}H] thymidine uptake was not changed by the addition of 100 μM LDL-cholesterol (Fig. 3).

Table 1. Effect of lovastatin on mesangial cell proliferation when stimulated by PDGF, endothelin, and angiotensin II

	Cell number (10^3/well)
Control	24.3±3.8
PDGF, 10 ng/ml	126.3±11.0*
PDGF, 10 ng/ml+Lovastatin, 0.5 μM	83.0±6.1
Lovastatin, 1 μM	45.0±5.5**
Lovastatin, 5 μM	27.7±3.7**
Lovastatin, 10 μM	21.7±3.2**
Endothelin, 10^{-7}	45.7±6.5*
Endothelin, 10^{-7}M+Lovastatin, 10 μM	20.2±5.1**
Ang II, 10^{-7}M	26.5±7.3
Ang II, 10^{-7}M+Lovastatin, 10 μM	22.2±4.8

Cell numbers are in single well of a 24-well culture plate (n=3 in triplicate).

*: $p<0.05$ versus control

**: $p<0.05$ versus treated with PDGF or endothelin alone.

The effects of PDGF, ET, and Ang II on [^{3}H] thymidine uptake of aortic smooth muscle cell

Exposure of quiescent aortic smooth muscle cells to PDGF at concentrations between 1 ng/ml and 50 ng/ml caused an increase of [^{3}H] thymidine uptake, dose-dependently, in a simi-

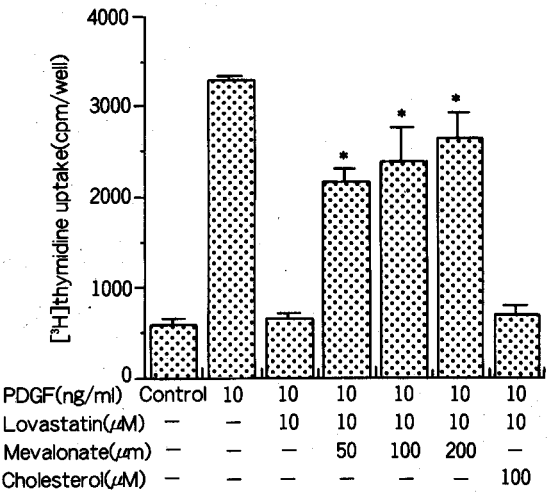

Fig. 3. *Reversibility of the inhibitory effect of lovastatin on PDGF-induced [^{3}H] thymidine uptake by mevalonate in mesangial cells. The addition of mevalonate at concentrations greater than 50 μM restores the reduction of [^{3}H] thymidine uptake by lovastatin (10 μM), significantly ($n=5$). *: $p<0.05$ vs lovastatin plus PDGF*

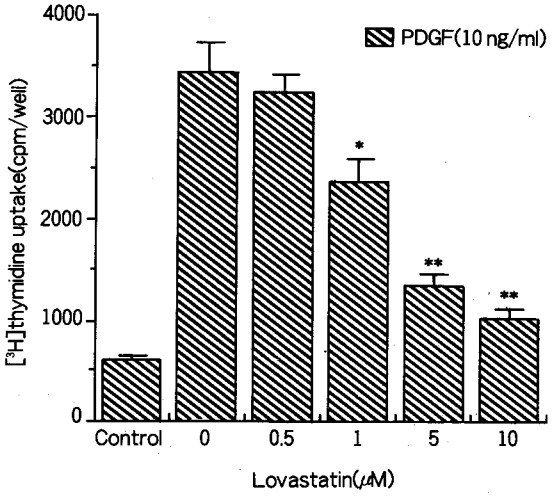

Fig. 4. *Effect of lovastatin on PDGF-induced [³H] thymidine uptake in aortic smooth muscle cells. Treatment with lovastatin inhibited [³H] thymidine uptake following PDGF (25 ng/ml) stimulation, dose-dependently (n=5). *: p<0.05, vs PDGF alone, **: p<0.05, vs PDGF alone, 0.5 μM lovastatin, and 1 μM lovastatin.*

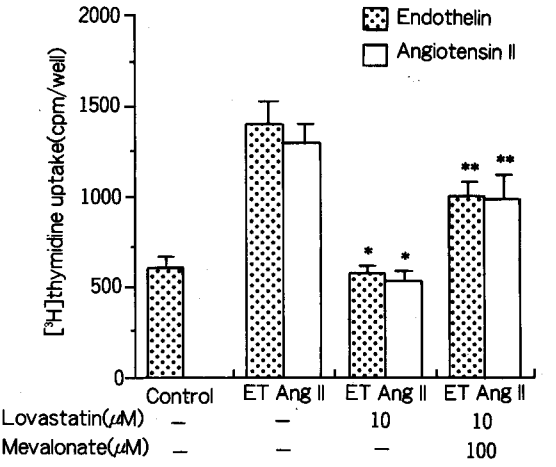

Fig. 5. *The inhibitory effect of lovastatin and its reversibility by mevalonate on ET- and Ang II-induced [³H] thymidine uptake in aortic smooth muscle cells. Treatment with lovastatin (10 μM) reduces [³H] thymidine uptake in response to ET(10^{-7} M) and Ang II (10^{-7} M), markedly. The addition of mevalonate (100 μM) restores the reduction of [³H] thymidine uptake significantly (n=3) *: p<0.05 vs ET or Ang II alone. **: p<0.05 vs treatment with lovastatin.*

lar manner to mesangial cells. At concentration of 10 ng/ml or greater, [³H] thymidine uptakes were significantly higher than those of the control (10 ng/ml: 2176±110 cpm, 50 ng/ml: 3444±275 vs 619±33, $p<0.05$). The treatment with ET or Ang II at concentrations ranging from 10^{-10} to 10^{-6} M also increased [³H] thymidine uptake dose-dependently. The addition of 10^{-9} M ET stimulated [³H] thymidine uptake significantly, compared to that of the control (1100±55 cpm vs 620±45, $p<0.05$), and treatment with 10^{-8} M Ang II also increased [³H] thymidine uptake significantly, compared to that of the control (1150±90 vs 580±50, $p<0.05$)

The effect of lovastatin on aortic smooth muscle cell proliferation

The treatment with lovastatin inhibited PDGF(25 ng/ml)-induced [³H] thymidine uptake, in a dose-dependent manner. At 0.5, 1, 5 and 10 μM of lovastatin, [³H] thymidine uptakes were 3245±172 cpm, 2378±220, 1353±

Table 2. Effect of lovastatin on aortic smooth muscle cell proliferation when stimulated by PDGF, endothelin, and angiotensin II

	Cell number (10^3/well)
Control	23.7±3.5
PDGF, 25 ng/ml	114.3±11.0*
PDGF, 25 ng/ml+Lovastatin, 0.5 μM	75.3±5.5
Lovastatin, 1 μM	39.0±4.1**
Lovastain, 5 μM	22.3±3.3**
Lovastatin, 10 μM	18.7±2.2**
Endothelin, 10^{-7}	40.6±7.5*
Endothelin, 10^{-7}M+Lovastatin, 10 μM	17.2±6.3**
Ang II, 10^{-7}M	27.5±5.2
Ang II, 10^{-7}M+Lovastatin, 10 μM	20.1±5.0

Cell numbers are in single well of a 24-well culture plate (n=3 in triplicate).

*: $p<0.05$ versus control

**: $p<0.05$ versus treated with PDGF or endothelin alone.

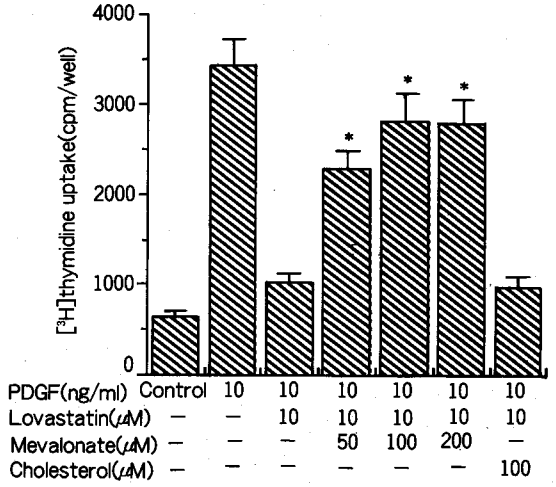

Fig. 6. *Reversibility of the inhibitory effect of lovastatin on PDGF-induced [^{3}H] thymidine uptake by mevalonate in aortic smooth muscle cells. The addition of mevalonate at concentration greater than 50 μM restores the reduction of [^{3}H] thymidine uptake by lovastatin (10 μM) significantly (n=5). *: p<0.05 vs lovastatin plus PDGF*

115, 1035±75, respectively, and the values at greater than 1 μM of lovastatin were significantly lower than when stimulated by PDGF, 3308±106 cpm ($P<0.05$, Fig. 4). ET(10^{-7} M)- and Ang II(10^{-7} M)-induced [^{3}H] thymidine uptakes were markedly inhibited by lovastatin at 10 μM($p<0.05$, Fig. 5).

Concerning the evaluation of cell proliferation, the exposure of quiescent cells to 25 ng/ml PDGF increased the number of cells significantly. The treatment with lovastatin at a concentration from 1 μM to 10 μM significantly inhibited PDGF-induced proliferation (Table 2). Proliferation induced by 10^{-7} M ET was also nearly abolished by the addition of 10 μM lovastatin (Table 2). In an Ang II-induced proliferation experiment, while the treatment with 10^{-7} M Ang-II stimulated [^{3}H] thymidine uptake significantly, the number of cells did not increase, indicating that Ang II induced [^{3}H] thymidine uptake with no mitogenic activity (Table 2).

Reversibility of the inhibitory effect of lovastatin on [^{3}H] thymidine uptake in aortic smooth muscle cells

Compared to the results in mesangial cells, the addition of mevalonate prevented the inhibitory effect of lovastatin on PDGF-, ET-, and Ang II-induced [^{3}H] thymidine uptake of aortic smooth muscle cells. The treatment with mevalonate at concentrations of 50, 100, and 200 μM restored [^{3}H] thymidine uptake from 1035±75 cpm on the treatment with 10 μM lovastatin and 25 ng/ml PDGF to 2299±199 cpm(55.6%), 2823±311(78.6%), 2803±265(77.8%), respectively ($p<0.05$, Fig. 6). Regarding the inhibition of ET (10^{-7} M)- and Ang II(10^{-7} M)-induced [^{3}H] thymidine uptake by lovastatin, inhibition was also prevented by treatment with 100 μM mevalonate (Fig. 5). In contrast, the addition of 100 μM LDL-cholesterol did not restore the inhibition of PDGF-induced [^{3}H] thymidine uptake by lovastatin (Fig. 6).

DISCUSSION

The mesangial cell proliferation is a important finding in proliferative glomerulonephritis, which is a major causative disease in chronic renal failure (Floege *et al.* 1991a; Abboud 1993). This proliferation is mediated by various mediators such as growth factors or cytokines, and many studies have been performed in order to prevent progression of glomerular injury by inhibition of mesangial proliferation (Floege *et al.* 1991b; Grandaliano *et al.* 1993). Recently, in remnant kidney models and obese Zucker rats, hypolipidemic therapy by HMG-CoA reductase inhibitor demonstrated retardation of the progress of glomerulosclerosis, and it was proposed that in addition to the lipid lowering effect, glomerular injury was reduced in part by inhibiting mesangial cell proliferation (Kasiske *et al.* 1988b; O'Donnell *et al.* 1993a).

Regarding the "response-to-injury" hypothesis, vascular smooth muscle cell proliferation seems to be central to the pathogenesis (Ross 1986). Several growth factors released

from platelets and endothelial cells following injury induce medial smooth muscle cell proliferation, forming atheroma (Walker *et al.* 1986; Grunwald *et al.* 1987). Hypolipidemic agents for reducing hypercholesterolemia were frequently used in treatment of atherosclerosis. Considering the fact that the vascular smooth muscle cell has similar characteristics to those of the mesangial cell, one of the HMG-CoA reductase inhibitors, lovastatin, may also influence proliferation of vascular smooth muscle cells.

Among the mediators of glomerular and vascular injury, PDGF is a 28- to 32-kD basic glycoprotein that contains two peptide chains that exist in disulfide linkage either as a heterodimer or a homodimer, resulting in three PDGF isoforms (PDGF-AA, -AB, and -BB)(Williams 1989). PDGF is also synthesized and released by glomerular endothelial cells, mesangial cells and smooth muscle cells, thereby providing a mechanism for autocrine mediated proliferation (Walker *et al.* 1986; Abboud *et al.* 1987). In our experiment, PDGF-BB was used for stimulating mesangial cell proliferation. Vasoactive substances, such as ET and Ang II were mainly released from endothelial cells, and acted in vascular lumens. Thus these substances easily contacted glomerular mesangial cells and vascular smooth muscle cells. ET, a novel endothelium-derived vasoconstrictive 21-amino acid polypeptide, had an effect on vascular smooth muscle cell proliferation. Ang II, a potent vasoconstrictor, has been reported to induce hypertrophy in vascular smooth muscle cells (Geisterfer *et al.* 1988; Hirata *et al.* 1989). We evaluated [^{3}H] thymidine uptake in response to these two hormones.

The present study has shown that both PDGF and ET increased [^{3}H] thymidine uptake and cellular proliferation in mesangial and aortic smooth muscle cells. In Ang II stimulation, while [^{3}H] thymidine uptake was significantly increased, the increase of cell number was not evident, suggesting that Ang II was a hypertrophic agent in both cells. Geisterfer *et al.* (1988) have also reported cellular hypertrophy following Ang II stimulation in rat aortic smooth muscle cells, which was associated with either arrest of cells in the G_2 phase of the cell cycle or development of tetraploidy.

The treatment of mesangial cells with lovastatin inhibited PDGF-induced [^{3}H] thymidine uptake in a dose-dependent manner. ET- and Ang II-induced [^{3}H] thymidine uptakes were also almost completely inhibited by the addition of lovastatin. In aortic smooth muscle cells, the treatment with lovastatin produced a dose-dependent inhibition of PDGF-induced [^{3}H] thymidine uptake, with a comparable dose in mesangial cells. There was no difference on the inhibitory effect of lovastatin on [^{3}H] thymidine uptake according to stimulatory agents among PDGF, ET and Ang II. This finding suggests that lovastatin had a direct anti-proliferative effect in mesangial cells and aortic smooth muscle cells.

There are two possibilities regarding mechanisms mediating the anti-proliferative effect of lovastatin. The first possibility is that it affected the hormonal stimulatory signaling pathways, and the other is that it altered mevalonate metabolism. McGuire *et al.* (1993) have shown that lovastatin inhibited DNA replication through affecting signaling pathways, early activation part in proliferative response in NIH-3T3 cells. The pretreatment of cells with 10 μM lovastatin inhibits the stimulation of phosphatidylinositol 3-kinase(PI-3-kinase), a putative second messenger-generating enzyme to be required for the mitogenic activity of PDGF (McGuire *et al.* 1993). PDGF-dependent association of PI-3-kinase p85 subunit with tyrosine-autophosphorylated PDGF receptor was also decreased significantly. This inhibition is one of potential mechanisms by which lovastatin inhibits cell growth, and the inhibitory effect of lovastatin on proliferation can be mediated by influencing early common signaling pathway of PDGF, ET and Ang II. But, further studies about early events in signal transduction pathway, such as PLC or protein kinase C activation, will be needed.

Alteration of mevalonate metabolism is proposed as one of the anti-proliferative mechanisms of lovastatin. Lovastatin inhibits HMG-CoA reductase, which mediates the conversion

from HMG-CoA to mevalonate, meaning the production of mevalonate should be reduced by lovastatin treatment. Mevalonate generates several mediators which play important roles in cell proliferation, isopentenyl pyrophosphate, geranyl- and farnesyl-pyrophosphate (Goldstein and Brown 1990; Grandaliano *et al.* 1993). We evaluated the effect of mevalonate on the inhibition of PDGF-, ET-, and Ang II-induced [^{3}H] thymidine uptake by lovastatin. The inhibitory effect of lovastatin was reversed by the addition of mevalonate but not LDL-cholesterol, suggesting the importance of the products of the mevalonate pathway in growth factor-induced DNA synthesis in both cells. Therefore, it seems that the alteration of mevalonate metabolism is a potential mechanism mediating the anti-proliferative effect of lovastatin, although the eventual mediator has not yet been defined.

Among mevalonate metabolites, one likely candidate mediating the anti-proliferative effect, is isopentenyl pyrophosphate, which is the precursor for isopentenyladenine. Previous studies have shown that isopentenyladenine can effectively reverse DNA synthesis after HMG-CoA reductase inhibition (O'Donnell *et al.* 1993b). This compound is reported to be essential for cells in late G_1 to carry out S phase DNA replication. O'Donnell *et al.* (1993b) have reported that the inhibition of fetal calf serum-stimulated DNA synthesis by lovastatin was prevented by the addition of isopentenyladenine. Geranyl- and farnesyl-isoprenylated proteins are other important candidates, which are formed from isopentenyl pyrophosphates. One of isoprenylated proteins is the $p21^{ras}$, the product of the ras oncogene, which covalently bond farnesol (Casey *et al.* 1989; Grandaliano *et al.* 1993). The $p21^{ras}$ attaches to the inner surface of the cell membrane and may serve as a signaling mechanism for growth factors. Therefore the inhibition of DNA replication by lovastatin seems to be caused in part by the alteration of mevalonate metabolism.

Vasoconstrictor and mitogenic peptide enhance lipoprotein receptor activity and have a synergistic effect on the mitogenic effect of LDL in human mesangial cells (Grone *et al.* 1992). Preexposure to ET-1 and PDGF for 16 or 15 hours with doubled uptake of LDL resulted in an elevation of c-*fos* and c-*jun* mRNA. In monkey arterial smooth muscle cells and Swiss 3T3 cells, the addition of PDGF to the quiescent cultures induced a marked increase in the biosynthesis of cholesterol within 3 to 6 hours, an increase in DNA synthesis within 16 to 20 hours, and subsequent cell division, suggesting the possible role of cholesterol in cell proliferation (Habenicht *et al.* 1980). We have evaluated the reversibility of the anti-proliferative effect of lovastatin by LDL-cholesterol, but the treatment with LDL-cholesterol did not prevent the inhibitory effect of lovastatin on [^{3}H] thymidine uptake. Grandaliano *et al.* (1993) report similar results of experiments using simvastatin and LDL-cholesterol in human mesangial cells. On the other hand, Habenicht *et al.* (1980) have reported that the inhibition of DNA synthesis by 2.5 μM compactin, a different inhibitor of HMG-CoA reductase, was prevented by adding cholesterol in monkey arterial smooth muscle cells. There seems to be a difference in reversibility of the HMG-CoA reductase inhibitor-induced inhibition of DNA synthesis by cholesterol, according to species. From this, we may be able to infer that the inhibition of DNA synthesis in lovastatin-treated mesangial cells appears not to be mediated by reducing the availability of cholesterol. It is likely that some other product of mevalonate metabolism plays a role in mediating the anti-proliferative effect of lovastatin.

In conclusion, lovastatin has an inhibitory effect on rat mesangial and aortic smooth muscle cell proliferation in response to growth factors and vasoactive substances, and the inhibitory effect is mediated in part by altering the mevalonate pathway, which seems to be essential to DNA replication of both cells. It appears that lovastatin may ameliorate glomerular or atherosclerotic pathology by a direct effect on mesangial and vascular smooth muscle cell proliferation, in addition to lowering circulating lipids.

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