EXPERIMENTAL STUDIES

Effects of Hydroxymethylglutaryl Coenzyme A Reductase Inhibitor Simvastatin on Smooth Muscle Cell Proliferation In Vitro and Neointimal Formation In Vivo After Vascular Injury

Ciro Indolfi, MD, FACC, Angelo Cioppa, MD, Eugenio Stabile, MD, Emilio Di Lorenzo, MD, Giovanni Esposito, MD, Alfonso Pisani, MD, Antonio Leccia, MD, Luigi Cavuto, MD, Angela Maria Stingone, MD, Alaide Chieffo, MD, Claudia Capozzolo, MD, Massimo Chiariello, MD, FACC
Naples, Italy

OBJECTIVES
We sought to evaluate the effects of hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase inhibitors on vascular smooth muscle cell (VSMC) proliferation in vitro and neointimal formation in vivo after vascular injury.

BACKGROUND
Neointimal hyperplasia after vascular injury is responsible for restenosis after arterial stenting, whereas arterial remodeling and neointimal formation are the causes of restenosis after percutaneous transluminal coronary angioplasty.

METHODS
We assessed the effect of simvastatin on in vitro VSMC proliferation. To study the effects of simvastatin in vivo, balloon injury and stent deployment were performed in the common carotid artery of rats. Neointimal area was measured two weeks later in the balloon injury model and three weeks after stent deployment.

RESULTS
Simvastatin markedly inhibits VSMC proliferation in vitro. In vivo, simvastatin reduced, in a dose-dependent manner, the neointimal area and the neointima-media ratio after balloon injury from 0.266 ± 0.015 mm² to 0.080 ± 0.026 mm² and from 1.271 ± 0.074 to 0.436 ± 0.158 (p < 0.001 vs. control rats) at the highest dose. Simvastatin also significantly reduced the neointimal formation and the neointima-media ratio after stenting from 0.508 ± 0.035 mm² to 0.362 ± 0.047 mm² (p < 0.05 vs. control rats) and from 2.000 ± 0.136 to 1.374 ± 0.180 (p < 0.05 vs. control rats). The vessel thrombosis rate after stent deployment was 30% in the control group and 11.1% in the treated group (p = NS). Moreover, the systemic administration of simvastatin did not affect hepatic and renal functions, blood pressure or heart rate.

CONCLUSIONS
Simvastatin potently inhibits VSMC proliferation in vitro and reduces neointimal formation in a rat model of vascular injury. (J Am Coll Cardiol 2000;35:214–21) © 1999 by the American College of Cardiology

Several clinical studies have demonstrated the beneficial effects of hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase inhibitors in primary (1,2) and secondary prevention in reducing cardiovascular mortality (3–5). The antiatherosclerotic effect of these drugs has been linked to their hypolipidemic properties (6–9). However, clinical studies have demonstrated that the potency of these drugs in reducing cardiovascular events is independent of the basal cholesterol levels (5,10,11). In fact, the HMG-CoA reductase inhibitors, such as simvastatin, not only reduce the plasma cholesterol levels but also competitively inhibit intracellular synthesis of mevalonate, a precursor of nonsterol compounds, such as geranylgeranyl and farnesyl, involved in cell functions and proliferation (12–18).

The effect of simvastatin on the synthesis of farnesyl radicals is responsible for the intracellular inhibition of the ras-raf-MAPKK (mitogen-activated protein kinase kinase) protein’s signal transduction pathway activation (12). Ras proteins and their relatives play a critical role in the control of normal and transformed cell growth. Ras proteins are members of a family of glutamyl transpeptidases (GTPases), which includes proteins involved in protein synthesis and
growth inhibition experiments, 2 g/liter glucose and 1.0 mmol/liter sodium pyruvate. For proliferation in vitro. Cells were grown in monolayers at 37°C in a humidified atmosphere at 95% carbon dioxide in 10% fetal calf serum (FCS)–Dulbecco Modified Eagles (33,34).

Accordingly, the aims of the present study were to assess the effect of simvastatin on in vivo neointimal formation induced by vascular injury. The role of the HMG-CoA reductase inhibitor simvastatin on vascular smooth muscle cell (VSMC) proliferation in vitro was also assessed.

METHODS

Cell culture. Smooth muscle cells (A10, thoracic aorta of the rat) were used to study the effect of simvastatin on cell proliferation in vitro. Cells were grown in monolayers at 37°C in a humidified atmosphere at 95% carbon dioxide in 10% fetal calf serum (FCS)–Dulbecco Modified Eagles Medium (DMEM) with 4 mmol/liter L-glutamine, 4.5 g/liter glucose and 1.0 mmol/liter sodium pyruvate. For growth inhibition experiments, 2 × 10^4 cells were plated into a 35-mm plate and grown in 10% FCS–DMEM in the presence of simvastatin in lactone form (2 μmol/liter) that was brought into solution by 0.1 mol/liter NaOH to give the active form, and the pH was adjusted to 7.4 by adding 0.1 mol/liter hydrochloride (Merk–Sharp & Dhome Research, Woodbridge, New Jersey), 25-OH-cholesterol (10 μg/ml) or mevalonic acid (MVA) (100 μmol/liter) (Sigma Chimica, Milan, Italy), or in the absence of simvastatin (control). Cell numbers in both conditions were assessed every 48 h for six days.

Vascular injury induced by balloon dilation or stent deployment. The rats in this study were handled according to the animal welfare regulations of the University Federico II of Naples, and the protocol was approved by the Animal Use Committee of this institution. All animals received human care in accordance with the Animal Use Principles of the American Society of Physiology. All rats were maintained under identical conditions of temperature (21 ± 1°C), humidity (60 ± 5%) and light-dark cycle and had free access to normal rat chow.

Ballooning injury. Forty-five Wistar male rats weighing 350 to 400 g (at 14 weeks of age), purchased from Charles River (Calco, Italy), were included in the present study. The rats were anesthetized with an intramuscular injection of 100 mg/kg ketamine (Sigma Chimica, Milan, Italy) and 5 mg/kg xylazine (Sigma Chimica). Angioplasty of the carotid artery was performed using a balloon embolectomy catheter, as previously described and validated in our laboratory (18,35–37). In brief, the balloon catheter (2F Fogarty, Edwards Laboratories, Santa Ana, California) was introduced through the right external carotid artery into the aorta, and the balloon was inflated at 1.5 atm using a calibrated, commercially available inflation device (Indeflator Plus 20, Advanced Cardiovascular System, Inc., Temecula, California). The vessel was damaged by passing an inflated balloon through the lumen three times. Previous studies performed in our laboratory demonstrated that the time necessary to pass the inflated balloon catheter back and forth into the carotid artery three times is 18 s (36). Therefore, to keep constant the time of the injury (that might influence the VSMC proliferation) we maintained constant the time of balloon inflation to 18 s.

Stent deployment. Scimed NIR stents (Boston Scientific Corporation, Galway, Ireland) (7-cell, 4-mm length), etched in 316LVM stainless steel and made of a continuous uniform multicellular design, were implanted. The stents were manually crimped on a 1.5-mm PTCA balloon catheter (ACS RX Comet, Advanced Cardiovascular System, Inc.) inflated at 11 atm for 60 s to expand the stent and to optimize the struts’ apposition against the arterial wall. At the time of stent implantation, 100 U/kg of heparin was injected intraperitoneally. No other anticoagulant or antiplatelet therapy was given either before or after stent deployment.

The balloon catheter loaded with the stent was introduced using a dissecting microscope (Leica, GZ4) through
the right external carotid artery into the common carotid artery and then inflated. The effect of stent implantation on neointimal formation was assessed 21 days later.

**Drug dosage and administration.** The HMG-CoA reductase inhibitor simvastatin was randomly administered in three different protocols: Protocol 1—the drug was administered orally at the daily dose of 40 mg/kg (n = 8), 16 mg/kg (n = 7), 4 mg/kg (n = 7), 0.04 mg/kg (n = 9) 14 days before and after balloon injury. Eight animals were used as the control group: Protocol 2—simvastatin was administered at 40 mg/kg as in protocol 1, although at the time of balloon injury MVA (10 mmol/liter) was locally administered to the injured artery using pluronic gel (n = 5), with a technique previously described (21,37). Protocol 3—40 mg/kg simvastatin (n = 10) was administered daily 14 days before and 21 days after stent deployment in the rats, although in a control group of nine animals the stent was deployed without drug administration.

**Toxicity.** To study simvastatin toxicity, laboratory studies were performed at baseline and two weeks after daily drug administration (40 mg/kg) (n = 8).

**Morphology.** At the time of the final experiment (two weeks later), the animals were anesthetized with an intramuscular injection of 100 mg/kg ketamine and 5 mg/kg xylazine, and the carotid arteries were fixed by perfusion at 120 mm Hg with 100 ml of phosphate-buffered saline (PBS, pH 7.2), followed by 80 ml containing 4% paraformaldehyde through a large cannula placed in the left ventricle. The carotid arteries were removed and six cross sections were cut (each 6 μm thick) from the approximate mid-portion of the artery, with three of the sections stained with hematoxylin-eosin to demarcate cell types. The remaining three sections were stained with aldehyde fuchsin and counterstained with Van Gieson's solution to demarcate the internal elastic lamina. The sections were photographed under low power, blindly videodigitized and stored in the image analysis system (Mipron, Kontron Electronics, Eching, Germany) in a 512 × 512 matrix with an eight-bit gray scale and a 12-field view. The media, neointima and vessel wall were traced carefully, and the ratios between the neointima and media were calculated (36,37). The intraobserver variability was already documented (21,36).

In an additional group (n = 7) the effects of the anesthesia and the surgical procedure (without the balloon injury) on VSMC proliferation were also assessed.

**Pathologic evaluation.** Immediately after the rats’ death, the arteries were dehydrated and cleared, and then infiltrated and embedded in a combination of polymethylmethacrylate, n-butyl phthalate and benzoil peroxide, as previously described (38). A tungsten-carbide knife was used to obtain 6-μm sections from the middle of the stented arteries. Modified hematoxylin-eosin and elastin stains were used to stain the plastic-embedded stented segments. The neointimal area and the neointima-media ratio were calculated in a blinded manner as described earlier.

**Doppler evaluation.** To assess vessel patency, right and left Doppler probes were applied at the time of the final experiment.

**Statistical analysis.** All data are presented as the mean value ± SEM. Statistical analysis between the groups was performed by analysis of variance and the unpaired t test using a Systat program (39). Tukey’s test was applied to compare single mean values (40). A p value <0.05 was considered significant.

**RESULTS**

**Effect of simvastatin on VSMC proliferation in vitro.** To evaluate the effect of simvastatin on VSMC proliferation, we treated cultures of VSMCs with 2 μmol/liter simvastatin and analyzed cell growth at different times. Figure 1 shows that simvastatin markedly inhibited the proliferation of VSMCs. To demonstrate that the effect of simvastatin on VSMC proliferation was due to the inhibition of HMG-CoA reductase, we investigated the effects of this statin on VSMC proliferation in the presence of 100 μmol/liter MVA and 25-OH-cholesterol (2 μg/ml). The ability of MVA to restore VSMC proliferation indicates that the effect elicited by simvastatin was related to inhibition of the MVA pathway, whereas the lack of this effect in the presence of 25-OH-cholesterol may suggest that the antiproliferative effect of simvastatin is independent of cholesterol in the serum (Fig. 1).

**Effect of simvastatin on neointimal formation after balloon dilation in vivo.** In the sham-operated rats not subjected to vascular injury, no neointimal formation was detected and the endothelium was found intact 14 days after the operation in both carotid arteries of all rats. A repro-
ducible neointimal formation was found 14 days after balloon injury in the control group (neointima $0.266 \pm 0.015$, neointima-media ratio $1.271 \pm 0.074$). In animals treated with systemic administration of simvastatin, we observed a significant reduction of both the neointima and neointima-media ratio in a dose-dependent manner (Table 1).

Table 1. Arterial Morphologic Characteristics of Different Groups of Balloon-Injured Animals Included in the Study

<table>
<thead>
<tr>
<th>Group</th>
<th>Neointima (mm²)</th>
<th>% Change vs. Control Group</th>
<th>P Value vs. Control Group</th>
<th>Media (mm²)</th>
<th>P Value vs. Control Group</th>
<th>N/M Ratio</th>
<th>P Value vs. Control Group</th>
<th>% Change vs. Control Group</th>
<th>P Value vs. Control Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control rats (n = 8)</td>
<td>$0.266 \pm 0.015$</td>
<td>—</td>
<td>—</td>
<td>$0.208 \pm 0.035$</td>
<td>—</td>
<td>$1.271 \pm 0.074$</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Simvastatin 0.04 mg/kg (n = 9)</td>
<td>$0.219 \pm 0.020$</td>
<td>$-24%$ NS</td>
<td>$0.210 \pm 0.020$</td>
<td>NS</td>
<td>$1.045 \pm 0.033$</td>
<td>$-18%$ NS</td>
<td>$1.023 \pm 0.040$</td>
<td>$-20%$ NS</td>
<td>$1.045 \pm 0.033$</td>
</tr>
<tr>
<td>Simvastatin 4 mg/kg (n = 7)</td>
<td>$0.212 \pm 0.006$</td>
<td>$-26%$ NS</td>
<td>$0.208 \pm 0.018$</td>
<td>NS</td>
<td>$1.023 \pm 0.040$</td>
<td>$-20%$ NS</td>
<td>$1.023 \pm 0.040$</td>
<td>$-20%$ NS</td>
<td>$1.023 \pm 0.040$</td>
</tr>
<tr>
<td>Simvastatin 16 mg/kg (n = 7)</td>
<td>$0.176 \pm 0.009$</td>
<td>$-32%$ &lt; 0.05</td>
<td>$0.205 \pm 0.028$</td>
<td>NS</td>
<td>$0.866 \pm 0.050$</td>
<td>$-32%$ &lt; 0.05</td>
<td>$0.866 \pm 0.050$</td>
<td>$-32%$ &lt; 0.05</td>
<td>$0.866 \pm 0.050$</td>
</tr>
<tr>
<td>Simvastatin 40 mg/kg (n = 8)</td>
<td>$0.080 \pm 0.026$</td>
<td>$-69%$ &lt; 0.001</td>
<td>$0.170 \pm 0.064$</td>
<td>NS</td>
<td>$0.436 \pm 0.158$</td>
<td>$-66%$ &lt; 0.001</td>
<td>$0.436 \pm 0.158$</td>
<td>$-66%$ &lt; 0.001</td>
<td>$0.436 \pm 0.158$</td>
</tr>
<tr>
<td>Simvastatin 40 mg/kg + mevalonate 10 mmol/liter (n = 5)</td>
<td>$0.256 \pm 0.011$</td>
<td>$-1%$ NS</td>
<td>$0.207 \pm 0.040$</td>
<td>NS</td>
<td>$1.258 \pm 0.084$</td>
<td>$-2%$ NS</td>
<td>$1.258 \pm 0.084$</td>
<td>$-2%$ NS</td>
<td>$1.258 \pm 0.084$</td>
</tr>
</tbody>
</table>

Data are expressed as the mean value ± SEM.

N/M = neointima-media.

Figure 2. Representative histologic sections stained with hematoxylin-eosin of the common carotid arteries from (a) a control rat subjected to only balloon injury; (b) a rat treated with 0.04 mg/kg simvastatin; (c) a rat treated with 4 mg/kg simvastatin; (d) a rat treated with 16 mg/kg simvastatin; (e) a rat treated with 40 mg/kg simvastatin; and (f) a rat treated with 40 mg/kg simvastatin plus local administration of mevalonate (10 mmol/liter).

Figure 3. Neointima-media (N/M) ratio and neointimal cross-sectional area (neointima) of common carotid arteries from rats subjected to only balloon injury (solid bars), rats treated with 40 mg/kg per day of simvastatin (open bars) and rats treated with simvastatin at the highest dose and local administration of mevalonate (stripped bars). *p < 0.01 vs. control group and mevalonate group.

Interestingly, the local delivery of mevalonate in vivo prevented the beneficial effects of simvastatin on neointimal formation. In fact, the neointima-media ratio was $1.271 \pm 0.074$ in control rats, $0.436 \pm 0.158$ after simvastatin and $1.258 \pm 0.084$ after systemic simvastatin administration and local delivery of mevalonate (Figs. 3,4).
Effect of systemic simvastatin administration on neointimal formation after arterial stenting. As shown in Table 2 in control rats 21 days after arterial stenting, a significant neointimal formation and neointima-media ratio were observed (0.508 ± 0.035 mm² and 2.008 ± 0.136). The systemic administration of simvastatin reduced the neointimal formation and the neointima-media ratio (0.362 ± 0.047 mm² [p < 0.05 vs. control rats], 1.374 ± 0.180 [p < 0.05 vs. control rats]) (Figs. 5, 6). The rate of stent thrombosis was 30% in the control group and 11% in the simvastatin group (p = NS).

Effect of systemic simvastatin administration. No differences in arterial pressure and heart rate were found between the sham-operated rats and the experimental rats (data not shown). No hematopoietic, hepatic and renal function alterations were associated with simvastatin administration (Table 3). Histologic studies of kidney and liver sections confirmed these data (data not shown).

DISCUSSION

The major findings of the present study are that simvastatin reduces the VSMC proliferation in vitro and neointimal formation induced by vascular injury in vivo.

Mechanisms of restenosis. Treatment of symptomatic atherosclerotic heart disease has become increasingly focused on percutaneous catheter-based techniques. However, restenosis limits the benefit of PTCA occurring in 30% to 60% of patients (41–44). Recently the use of the stent has been introduced to treat vascular dissections and to achieve a larger lumen diameter to reduce restenosis (29,30,45). Unfortunately, the stent also markedly induced proliferation of VSMCs. In fact, neointimal formation is the only mechanism responsible for the restenosis after stent deployment (28) that occurs in ~20% of BENESTENT-like lesions (29,30) and in ~50% of long lesions and vein grafts (45–49). Previous studies using intravascular ultrasound demonstrated that stent deployment abolishes arterial remodeling but triggers and may actually increase the neoin-

Table 2. Arterial Morphologic Characteristics After Stent Deployment

<table>
<thead>
<tr>
<th>Neointima (mm²)</th>
<th>% Change vs. Control Group</th>
<th>p Value vs. Control Group</th>
<th>Media (mm²)</th>
<th>p Value vs. Control Group</th>
<th>N/M Ratio</th>
<th>% Change vs. Control Group</th>
<th>p Value vs. Control Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control rats (n = 6)</td>
<td>0.508 ± 0.035</td>
<td>—</td>
<td>—</td>
<td>0.250 ± 0.007</td>
<td>—</td>
<td>2.008 ± 0.136</td>
<td>—</td>
</tr>
<tr>
<td>Simvastatin 40 mg/kg (n = 9)</td>
<td>0.362 ± 0.04</td>
<td>−28%</td>
<td>0.05</td>
<td>0.264 ± 0.007</td>
<td>NS</td>
<td>1.374 ± 0.180</td>
<td>−32%</td>
</tr>
</tbody>
</table>

Data are expressed as the mean value ± SEM.

N/M = neointima-media.
timal proliferation responsible for neointimal formation (31–33). Therefore, increasing evidence suggests a central role of VSMC proliferation in restenosis after stent deployment (29) and a relative importance of VSMCs in restenosis after balloon angioplasty, especially in patients with diabetes (50–52), unstable angina or hypertension (53,54). A previous study from our laboratory demonstrated that the local delivery of a transdominant negative H-ras gene markedly prevented neointimal formation after balloon injury in rats (21). This finding was recently confirmed using adenovirus-mediated gene transfer (55). The inhibition of a protein downstream from ras, the MAPKK, also prevents neointimal formation after balloon injury (37). Other investigators have also shown that gene therapy approaches could be used in experimental animal models to reduce neointimal formation after balloon injury (56–58). Several studies using adenovirus gene transfer have shown efficacy in preventing neointimal proliferation after balloon injury (59–62). These data, demonstrating the ability of adenoviral vectors to deliver genes efficiently in VSMCs, could predict their use in the clinical setting. However, careful and objective trials in humans are needed to validate this hypothesis.

Inhibition of neointimal hyperplasia by statins. At the present time, inhibition of the ras pathway is pharmacologically feasible in the clinical setting using inhibitors of farnesyl radicals synthesis, such as simvastatin or other HMG-CoA reductase inhibitors (17,23–25,63). To demonstrate that the effect of simvastatin was independent of the cholesterol concentration and dependent on the inhibition of the mevalonate pathway, we performed additional in vitro and in vivo experiments. The cholesterol that was added in the in vitro VSMCs did not affect the inhibition of cell proliferation by simvastatin. In contrast, MVA completely abolished the effects of simvastatin on cell growth (Fig. 1). These data were also confirmed in vivo. Our method, the local administration of mevalonate in pluronic gel (21,36), allows the study of regional drug administration without systemic toxic effects and demonstrates, for the first time in vivo, that the effects of simvastatin on cell proliferation was dependent of the inhibition of mevalonate (Fig. 3). In the present study, we also assessed the dose response of simvastatin (Table 1, Fig. 2). The dose that potently reduced neointimal formation is high as compared with the dose used in the clinical setting. However, previous studies demonstrated a species differences in simvastatin metabolism; in fact, the DL50 of this drug in rats is 5 g/kg per day (Merk-Sharp & Dhome data base), and other animal studies also used the dose of 10 to 150 mg/kg per day (10,64).

In this study we used a highly standardized model of balloon injury introduced by Clowes et al. (65). It has been demonstrated that in this model the amount of neointimal hyperplasia is maximal, reaching the plateau at 14 days after the procedure (66). Therefore, we decided to evaluate the neointimal area 14 days after balloon injury (36). In contrast, we evaluated the effect of stent deployment 21 days after the procedure. We found, in this model, that the neointimal formation reaches the maximum at that time (unpublished data). We started simvastatin administration 14 days before the procedure to achieve a biologic effect. Shorter pretreatment did not influence neointimal proliferation after balloon injury and stent deployment.

The difference in the amount of neointima in balloon-injured arteries and stented arteries may be due to differences in the nature and extent of vessel injury in the two models. In fact, stent deployment with a semicompliant PTCA balloon inflated at high pressures induces a greater circumferential stretch to the vessel wall, which causes greater barotrauma as compared with balloon injury alone. In a previous study, a Silastic collar was applied on the external surface of the artery, and the effect of simvastatin and other HMG-CoA reductase inhibitors was assessed (10). Although the animal model used might be different in terms of the vascular effects induced by balloon catheter dilation and stent deployment, a reduction in the neointimal area in the simvastatin-treated group was documented (10). Our data also demonstrate that the systemic administration of simvastatin, at the highest dose used, is able to reduce neointimal formation by 66% after balloon injury. More interestingly, the dose of simvastatin used in the present study did not affect heart rate, blood pressure or liver and renal function (Table 3) or histology (data not shown). This

Table 3. Effect of Simvastatin on Hematologic Variables in Rats After Balloon Angioplasty

<table>
<thead>
<tr>
<th></th>
<th>Control Group</th>
<th>SIM Group (40 mg/kg)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BUN (mg/dl)</td>
<td>43.1 ± 3.5</td>
<td>35.16 ± 8.4</td>
<td>NS</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.56 ± 0.08</td>
<td>0.41 ± 0.05</td>
<td>NS</td>
</tr>
<tr>
<td>GOT (IU/liter)</td>
<td>122.5 ± 12.6</td>
<td>129 ± 11.5</td>
<td>NS</td>
</tr>
<tr>
<td>GPT (IU/liter)</td>
<td>39.16 ± 8.6</td>
<td>43.1 ± 6.7</td>
<td>NS</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>59.33 ± 8.23</td>
<td>49.5 ± 6.23</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>CK (IU/liter)</td>
<td>919 ± 24</td>
<td>926 ± 32</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are expressed as the mean value ± SEM.
BUN = blood urea nitrogen; CK = creatine kinase; GOT = glutamic oxaloacetic transaminase; GPT = glutamic pyruvic transaminase; SIM = simvastatin.
is an important finding; in fact, our study demonstrates that commercially available drugs such as simvastatin, systemically administered, can prevent a very localized phenome-
on such as neointimal hyperplasia after vascular injury. Systemic administration of a therapeutically active agent may represent a distinct advantage over local delivery systems. In fact, previous studies from our and other laboratories demonstrated the efficacy of using pluronic gel applied on the external surface of the artery or adenovirus-mediated gene therapy to prevent the in vivo proliferation of VSMCs. However, these techniques are not clinically fea-
sible (18,21,35,37,55,59–62). In addition, intravascular brachytherapy is an emerging therapeutic strategy to prevent restenosis after angioplasty and stenting. It is obvious that the unwanted biologic effects of radiation and the effort to organize the use of radiation in the catheterization labora-
tory may limit the extensive use of this new therapeutic option.

Study limitations. Extreme caution should be used in extrapolating the experimental data presented in this study, using VSMC culture or the rat angioplasty model, to the clinical setting. The role of other statins should also be studied. Therefore, further studies should be performed to evaluate the effects of HMG-CoA reductase inhibitors on stent restenosis in a model of larger animals and eventually in humans.

Conclusions. Simvastatin potently affects in vitro VSMC proliferation. More interestingly, the HMG-CoA reductase inhibitors prevented neointimal formation after vascular injury. This beneficial effect was abolished using local administration of mevalonate.

Acknowledgment
We thank Armando Coppola for his excellent technical assistance.

Reprint requests and correspondence: Dr. Ciro Indolfi, Labora-

tory of Interventional Cardiology, Division of Cardiology, Uni-

versity Federico II, Via Pansini, 5, 80131 Napoli, Italy. E-mail: Indolfi@unina.it.

REFERENCES
1. Lipid Research Clinics Program. The Lipid Research Clinics Coro-
8. Wang-Hung L, Chu-Pak L, Cheuk-Kit W. Beneficial effect of cholesterol-lowering therapy on coronary endothelium-dependent relax-
10. Soma MR, Donetti E, Parolini C, et al. HMG-CoA reductase inhibitors: in vivo effects on carotid intimal thickening in normocho-
11. Long-term Intervention with Pravastatin in Ischaemic Disease (LIP-
13. Maltese WA. Post translational modifications of proteins by isopre-

17. Guijarro C, Blanco-Colio LM, Ortego M, et al. 3-Hydroxy-3-


