Differential effect of atorvastatin and tacrolimus on proliferation of vascular smooth muscle and endothelial cells

Arturo Giordano,1 Simona Romano,2 Mario Monaco,3 Antonio Sorrentino,2 Nicola Corcione,1 Anna Laura Di Pace,2 Paolo Ferraro,1 Giovanna Nappo,2 Michele Polimeno,1 and Maria Fiammetta Romano2

1Invasive Cardiology Unit and 3Cardiovascular Surgery Department, Pineta Grande Hospital, Castelvolturno; 2Department of Biochemistry and Medical Biotechnologies, University of Naples Federico II, Naples, Italy

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Giordano A, Romano S, Monaco M, Sorrentino A, Corcione N, Di Pace AL, Ferraro P, Nappo G, Polimeno M, Romano MF. Differential effect of atorvastatin and tacrolimus on proliferation of vascular smooth muscle and endothelial cells. Am J Physiol Heart Circ Physiol 302: H135–H142, 2012. First published November 4, 2011; doi:10.1152/ajpheart.00490.2011.—Although considered promising for use in drug-eluting stents (DES), tacrolimus failed clinically. Tacrolimus inhibits growth factor production but can also act as a growth factor on vascular smooth muscle cells (VSMC). This unexpected proliferative stimulus could reverse the beneficial effects of the drug on restenosis. We hypothesized that tacrolimus’ association with statins, which lower cholesterol and impair cell proliferation, could restore tacrolimus’ beneficial effect by abrogating the aberrant proliferative stimulus. Additionally, since maintenance of endothelial function represents a challenge for new-generation DES, we investigated the combined effect of tacrolimus and atorvastatin on endothelial cells. Human VSMC and umbilical vein endothelial cells (HUVEC) were incubated with 100 nM tacrolimus and increasing doses of atorvastatin (0–3.0 μM). Atrorvastatin plus tacrolimus dose-dependently inhibited VSMC proliferation. The percentage of cells incorporating 5-bromo-2’-deoxyuridine (BrdU) in their DNA was 49 ± 14% under basal conditions, 62 ± 15% (P = 0.01) with tacrolimus, 40 ± 22% with 3 μM atorvastatin, and 30 ± 7% (P < 0.05) with 3 μM atorvastatin plus tacrolimus. Atorvastatin downregulated β-catenin, Erk1 and Erk2, and cyclin B in tacrolimus-stimulated VSMC. In contrast, atorvastatin plus tacrolimus did not affect proliferation of endothelial cells. The percentage of HUVEC incorporating BrdU in their DNA was 47 ± 8% under basal conditions, 58 ± 6% (P = 0.01) with tacrolimus, 45 ± 4% with 3 μM atorvastatin, and 49 ± 1% with 3 μM atorvastatin plus tacrolimus. Both agents stimulated endoglin production by HUVEC. Taken together, these results suggest that, when combined with tacrolimus, atorvastatin acts in concert with tacrolimus in HUVEC to stimulate production of endoglin, a factor that has an important role in endothelial repair. Our study supports the conclusion that prevention of postcoronary in-stent restenosis and late thrombosis may benefit of concomitant association of tacrolimus and high doses of atorvastatin.

signal transduction; cyclins; endoglin

TACROLIMUS, OR FK506, IS A MACROCYCLIC AGENT WITH POTENT IMMUNOSUPPRESSANT ACTIVITY THAT WAS APPROVED BY THE Food and Drug Administration for use in organ transplantation in 1994 (12). A decade later, its use has been extended to the treatment of postangioplasty coronary restenosis, where it is loaded into drug-eluting stents (DES) (20). Tacrolimus is a potent inhibitor of lymphocyte activation (1). For this reason, it appeared to be a good candidate for counteracting the migration and proliferation of smooth muscle cells that give rise to the neointima, which in turn obstructs the vessel and provokes in-stent restenosis (6). Classically, tacrolimus binds to the FK506-binding protein 12, and the composite surface formed within this complex interacts with calcineurin (CaN) (27) to block CaN’s phosphatase activity. This results in deactivation of a transcriptional apparatus driven by nuclear factor of activated T cells (NFAT) (4). Such transcription factors activate the expression of genes encoding cytokines in infiltrating mononuclear cells in angioplasty-injured vessels (6). This in turn promotes the migration and proliferation of vascular smooth muscle cells (VSMCs) from the tunica media through the disruption of the endothelial barrier and generates a new layer of the vessel wall termed neointima (6).

A recent study in a porcine coronary artery model highlights the active role of the CaN/NFAT pathway in VSMC proliferation (11). Recently, our group found that tacrolimus can stimulate VSMC proliferation and collagen type I production through activation of transforming growth factor (TGF)-β signaling (9). β-Catenin and the extracellular signal-regulated kinase, Erk, were found to be important effectors of tacrolimus-induced proliferation (9). Taken together, these findings support the conclusion that interplay of pro- and anti-proliferative signals regulates cell cycle progression in VSMC stimulated by tacrolimus. Nonetheless, an overall proliferative effect seems to prevail, as supported by positive vascular remodeling after tacrolimus-eluting stent (TES) implantation associated with clinical failure (25, 29). Less is known regarding the mechanisms regulating proliferation of endothelial cells stimulated with tacrolimus. Nevertheless, tacrolimus has been suggested to provide a more favorable environment for reducing thromboses of DES compared with sirolimus (28).

Statins are drugs that lower cholesterol because of inhibition of the enzyme 3-hydroxy-3-methyl-glutaryl-(HMG)-CoA reductase in the cholesterol biosynthesis pathway (7). Statin use in the prevention (32) and treatment (26) of cardiovascular diseases is increasing. This class of drugs has additional beneficial anti-inflammatory, anti-proliferative, and proapoprotic properties that may reduce cardiac risk besides its primary cholesterol-reducing property (13). The anti-proliferative effects of statins are ascribed to mechanisms either dependent (13, 24) or independent (23) of HMG-CoA reductase inhibition. The metabolic products of this pathway can supply prenyl groups for the prenylation of several substrates, particularly G proteins (24). These proteins are involved in receptor-coupled

Address for reprint requests and other correspondence: M. Fiammetta Romano, Dept. of Biochemistry and Medical Biotechnologies, Federico II Univ., via Pansini, 5, 80131, Naples, Italy (e-mail: mariafiammetta.romano@unina.it).

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signal transduction (24). Their prenylation allows membrane anchoring, which is an important switch for growth and differentiation signals (18), including the TGF-β signal and its downstream effector Erk (18). These factors were previously found to be involved in tacrolimus-induced VSMC proliferation (9).

The aim of the present work was to investigate whether atorvastatin abrogated the TGF-β-mediated growth of VSMC, which could rescue the beneficial effects of TES on restenosis prevention (8, 9). At the same time, we investigated whether the association between tacrolimus and atorvastatin affected the regenerative capacity of the endothelium, which is essential for correct vascular repair after stent implantation.

**MATERIALS AND METHODS**

**Cell culture and reagents.** VSMCs and human umbilical vein endothelial cells (HUVEC) were purchased from Cambrex Bio Science (Cambrex Profarmaco, Milan, Italy). VSMC were human aortic smooth muscle cells that were cultured in Clonetics SmGM-3 Bullet-Kit medium [containing insulin, recombinant human fibroblast growth factor-β, and recombinant human epidermal growth factor (EGF)] according to the manufacturer’s instructions. HUVEC were cultured in Clonetics MEGM BulletKit medium, containing hydrocortisone, recombinant human EGF, and bovine brain extract. Cells were starved of supplement growth factors for 48 h, before stimulation with reagents. The experiments were performed when cells were at the 6–10 passage. Tacrolimus (Sigma Aldrich, St. Louis, MO) was used at the dose of 100 nM (9), and atorvastatin (kindly provided by Pfizer Italia) was used at the doses of 0.1, 1, and 3 μM. The choice of atorvastatin doses was in accordance with the plasma concentrations of the drug measured in subjects receiving atorvastatin (14). One micromolar atorvastatin was the highest plasma concentration of the drug measured after a single oral dose of 20 mg.
**Cell lysates and Western blot assay.** Whole cell lysates were prepared by homogenization in modified RIPA buffer. The lysate was cleared by centrifugation at 14,000 rpm for 20 min. The lysate was run on SDS polyacrylamide gel electrophoresis, transferred onto a membrane filter (Cellulosenitrate; Schleider and Schuell, Keene, NH), and incubated with the primary antibody.

The antibodies against β-catenin (mouse monoclonal; Cell Signaling Technology, Danvers, MA); phosphorylated Erk1/2 (mouse monoclonal; Santa Cruz Biotechnology, Santa Cruz, CA); Erk1/2 (rabbit polyclonal; Cell Signaling Technology), and anti-cyclin B1 (rabbit polyclonal; Santa Cruz Biotechnology) were all used diluted 1:200. Anti-glyceraldehyde-3-phosphate dehydrogenase (rabbit monoclonal; Cell Signaling) was used 1:1,000. After a second incubation with peroxidase-conjugated anti-rabbit IgG or anti-mouse IgG (Santa Cruz Biotechnology), the blots were developed with the enhanced chemiluminescence system (Supersignal West Pico, Celbio; Pierce, Rockford, IL).

**Immunofluorescence and proliferative assays.** Measurement of endoglin expression served to evaluate the involvement of this factor in endothelial cell proliferation. Endoglin expression was measured in flow cytometry (FACScan; Becton-Dickinson, San Diego, CA) by using the mouse monoclonal antibody CD105-phycoerythrin (PE) conjugated (eBioscience, San Diego, CA). Briefly, HUVEC and VSMC were unstimulated or stimulated with 100 nM tacrolimus, in the absence or the presence of 3.0 μM atorvastatin. After 48 h, cells were harvested, centrifuged for 5 min at 400 g, and incubated with the monoclonal antibody against CD105-PE. After incubation at 4°C for 30 min in the dark, cells were analyzed in flow cytometry. DNA synthesis was measured with 5-bromo-2′-deoxyuridine (BrdU) labeling and a detection kit (Detection Kit II; Roche Diagnostics, Indianapolis, IN) using the manufacturer’s instructions. Briefly, cells were plated on 24-well plates in the absence or presence of different doses of tacrolimus or atorvastatin. After incubation for 3 days, 10 μM BrdU was added to the cultures and, after a further 4 h, cells were collected, fixed with ethanol, and incubated with anti-BrdU monoclonal antibody. The percent of BrdU incorporation was measured in flow cytometry. Cell proliferation was measured using a Cell Counting Chamber and Trypan blue. Cells were seeded on a 96-microwell plate (2.0 × 10^3/well). After a 48-h incubation with 100 nM tacrolimus and/or 3.0 μM atorvastatin, cells were mixed with Trypan blue (0.8 mM in PBS) to mark dead cells, loaded onto a counting chamber covered with a cover slide, and then counted under a microscope.

**Cell transfection and short-interfering RNA.** Twenty-four hours before transfection of a short-interfering (si) RNA for silencing the endoglin gene (Qiagen, Valencia, CA) or of a scrambled duplex as control, HUVEC were incubated in medium without antibiotics at the concentration of 2.5 × 10^5/ml, to obtain 30–50% confluence at the time of transfection. The siRNA or the scrambled oligo was trans-

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**Fig. 2.** Atorvastatin reduces levels of β-catenin and phosphorylated (p) extracellular signal-regulated kinase (Erk) in VSMC stimulated with tacrolimus. A: Western blot assay of β-catenin and pErk1/2 levels in VSMC, unstimulated or stimulated with 100 nM tacrolimus, in the absence or the presence of 3.0 μM atorvastatin. Total Erk and actin served as a loading control. Results are representative of 3 independent experiments. B: graphic representation of mean values of β-catenin and pErk1/2 expression levels. Western blot bands were quantified by densitometry using ImageJ 1.42q for Macintosh and expressed as integrated optical density (OD) normalized to actin and Erk, respectively. Bars represent SDs.
ected at the final concentration of 50 nM using Metafectene (Biontex, Munich, Germany) according to the manufacturer’s recommendations, and, 72 h later, cells were harvested to prepare cell lysates. Real-time PCR validated the effect of siRNA on endoglin expression.

**Primary endothelial samples.** Arterial specimens were obtained by 20 patients in need of vascular surgery for significant carotid stenosis or for infrainguinal obstructive arterial disease at the Cardiovascular Department of Pineta Grande Hospital (see Table 1 for clinical details). Small arterial pieces were collected by the arterial branch of the external carotid or common femoral artery, which were sacrificed for surgical reasons (site of anastomosis or arterial mobilization to gain wider access). At the time of surgery, 10 out of the 20 patients were taking atorvastatin (80 mg/day) as single therapy, by at least 1 wk, and 10 patients were not taking statins. All patients underwent an index surgical procedure with the same anesthetic protocol. All patients provided written informed consent; the study conforms with the principles outlined in the Declaration of Helsinki and was approved by local ethics review board (Ethics Committee of the Azienda Sanitaria Locale Caserta). Endothelium was gently removed by abrasion of the internal surface of the vessel with a scalpel. For cell isolation, the tissue was washed three times with 20 ml RPMI 1640 medium (Lonza) supplemented with 10% heat-inactivated FC (Lonza) and subjected to mechanical (with scissors and homogenization into an ice-cold Dounce tissue homogenizer) and enzymatic (in 2% type II collagenase in PBS, for up to 120 min at room temperature) dissociation, followed by filtration through a nylon mesh, vortexing, and repeated aspiration through small-gauge needles. Isolated cells were then subjected to flow cytometry. Endothelial RNA was prepared by homogenized tissue using the High Pure FFPE RNA Micro kit (Roche, Mannheim, Germany), following the manufacturer’s instruction. Total RNA served for cDNA synthesis and a measure of endoglin transcript by real-time PCR (see **Real-time PCR**).

**Real-time PCR.** Total RNA was isolated by Trizol (Invitrogen, Carlsbad, CA) from cells harvested, according to the instructions of the manufacturer. One microgram of RNA was used for cDNA synthesis with Moloney murine leukemia virus reverse transcriptase (Invitrogen). Real-time PCR using the iQSYBR Green Supermix (Bio-Rad) and specific real-time-validated QuantiTect primers for endoglin (QT00013335: NM_000118 600 e 750 bp; NM_001114753 600 e 750 bp and cyclin D3 (QT00096796: NM_0001136017 500 e 700 bp; NM_0011760 500 e 700 bp) (Qiagen, Germantown, PA), and specific primers for β-actin (forward 5'-CGA CAG GAT GCA GAA GGA GA-3' , reverse 5'-CGTCAT ACT CCT GCT TGC TTG CTG-3') served to quantify gene expression.

**Statistical analysis.** The results reported are the means and the standard deviation of independent experiments. The statistical significance of differences between means was estimated using Student's t-test. Values of $P \leq 0.05$ were considered statistically significant.

**Fig. 3.** Effect of atorvastatin on tacrolimus-induced human umbilical vein endothelial cell (HUVEC) proliferation. A: graphic representation (left) and table (right) of mean BrdU incorporation values in HUVEC, unstimulated or stimulated with 100 nM tacrolimus, in the absence or the presence of 0.1, 1.0, and 3.0 μM atorvastatin. Cells were harvested after a 3-day culture. B: graphic representation of cell counts ($\times 10^4$) in HUVEC cultures, unstimulated or stimulated with 100 nM tacrolimus, in the absence or the presence of 3.0 μM atorvastatin. Cells were harvested after a 48-h incubation. C: Western blot assay of cyclin B levels in VSMC, unstimulated or stimulated with 100 nM tacrolimus, in the absence or the presence of 0.1, 1.0, and 3.0 μM atorvastatin. Cells were harvested after an 8-h culture. G3PDH served as a loading control. Results are representative of 3 independent experiments.
RESULTS

**Atorvastatin inhibits tacrolimus-induced proliferation of VSMC.** To investigate the effect of tacrolimus and atorvastatin on VSMC growth, cells were incubated in media containing 100 nM tacrolimus and in the absence or the presence of 0.1, 1.0, or 3.0 μM atorvastatin. One micromolar corresponds to the highest plasma concentration measured in subjects receiving a single oral dose of 20 mg atorvastatin (14). Proliferation was investigated by measuring BrdU incorporation in cellular DNA and cell counts. Mean values of BrdU incorporation in VSMC, obtained from three different experiments, are represented in Fig. 1A. The percentage of cells incorporating BrdU into their DNA was 49 ± 14% in the absence of stimulant, 62 ± 15% (P = 0.01) in cultures containing 100 nM tacrolimus, 40 ± 22% with 3 μM atorvastatin, and 30 ± 7% (P = 0.04) with 3 μM atorvastatin plus tacrolimus. Cell counts after a 48-h incubation with 100 nM tacrolimus and/or 3.0 μM atorvastatin confirmed the proliferative effect of tacrolimus (P = 0.02). Atorvastatin alone produced a significant decrease in cell counts (P = 0.04) compared with unstimulated cells. Adding atorvastatin to tacrolimus produced a significant decrease in cell counts with respect to either unstimulated (P = 0.0001) and atorvastatin-stimulated (P = 0.006) or tacrolimus-stimulated (P < 0.0001) cells (Fig. 1B). A semiquantitative measure of cyclin B levels by Western blot assay of cellular lysates showed very low levels of this mitotic protein in VSMC cultured with 3 μM atorvastatin (Fig. 1C).

**Atorvastatin downmodulates β-catenin and phospho-Erk in tacrolimus-stimulated VSMC.** Tacrolimus-induced proliferation of VSMC is accompanied by an increase in levels of β-catenin and Erk (9). We investigated the effect of atorvastatin on this molecular pattern. A representative result of three independent experiments is shown in Fig. 2A. Western blot assay of whole cell lysates prepared from VSMC after 1 and 4 h of incubation showed that atorvastatin counteracted the increase in β-catenin and phospho-Erk levels stimulated by tacrolimus. Figure 2B is a graphic representation of β-catenin, phospho-Erk1, and phospho-Erk2 expression levels, quantitated by densitometry and expressed as integrated optical density normalized to actin and overall Erk levels, respectively. Levels of β-catenin were significantly increased after 1 h (P = 0.001) and 4 h (P = 0.05) in tacrolimus cultures compared with β-catenin levels in untreated cells. Addition of atorvastatin to tacrolimus significantly counteracted these increases (P < 0.001) after either 1 or 4 h. Interestingly, levels of phospho-Erk changed in accordance with β-catenin levels.

**Atorvastatin does not inhibit tacrolimus-induced proliferation of HUVEC.** To investigate the effect of tacrolimus and atorvastatin on HUVEC growth, cells were incubated with 100 nM tacrolimus and in the absence or presence of 0.1, 1.0, or 3.0 μM atorvastatin. Mean values of BrdU incorporation, obtained from three different experiments, are represented in Fig. 3A. The percentage of HUVEC incorporating BrdU into their DNA was 47 ± 8% under basal conditions, 58 ± 6% (P = 0.01) in 100 nM tacrolimus cultures, 45 ± 4% with 3 μM atorvastatin, and 49 ± 1% with 3 μM atorvastatin plus tacrolimus. Cell counts after a 48-h incubation with 100 nM tacrolimus and/or 3.0 μM atorvastatin confirmed the proliferative effect of tacrolimus (P = 0.04). Atorvastatin alone did not significantly change cell counts compared with unstimulated cells. Addition of atorvastatin to tacrolimus did not affect cell counts compared with unstimulated (P = 0.26) or tacrolimus-stimulated (P < 0.26) cells (Fig. 1B). Cell number increased in tacrolimus- plus atorvastatin-treated cultures compared with atorvastatin-treated cultures (P = 0.02) (Fig. 3B). Cyclin B levels were not decreased after addition of atorvastatin to tacrolimus-cultured HUVEC (Fig. 3C).

**Tacrolimus and atorvastatin stimulate endoglin production in HUVEC.** CD105, or endoglin, is a component of the TGF-β receptor-signaling pathway with proangiogenic properties (10, 16). Because endoglin promotes proliferation and is predominantly expressed by endothelial cells (16), we hypothesized a

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role for this factor in the lack of an effect by atorvastatin on tacrolimus-induced proliferation. To verify this hypothesis, cells were incubated in the absence or presence of tacrolimus (100 nM) and with or without 3 μM atorvastatin. After 48 h, the cells were harvested, and CD105 expression was measured by flow cytometry. A result representative of three different experiments is shown in Fig. 4A. CD105 was expressed in HUVEC but not VSMC. Both atorvastatin and tacrolimus enhanced the expression of CD105 in HUVEC but not VSMC. The enhancing effect in HUVECs appeared to be cooperative between the two drugs. In Fig. 4B, the kinetics of CD105 expression in HUVEC is shown. Results of the quantitative measure of CD105 mRNA levels by real-time PCR were consistent with data from protein levels. In fact, an increase in the expression of CD105 mRNA was observed in atorvastatin-treated cultures and, most remarkably, in atorvastatin plus tacrolimus cultures (Fig. 5A). The endoglin transcript was clearly reduced when a specific siRNA (Fig. 5A) was applied, and apparently also endoglin regulated cyclin D levels, as suggested by the reduction of these levels in HUVECs depleted of endoglin (Fig. 5B). Indeed, tacrolimus increased the number of cyclin D transcripts by 5.7- and 2.5-fold in the absence or the presence of endoglin siRNA, respectively. The cyclin D mRNA increase in atorvastatin-treated samples was negligible. The combination of atorvastatin and tacrolimus produced a 4.2-fold increase in cyclin D transcripts but a 2.2-fold increase when endoglin was downmodulated. Endoglin expression in endothelium specimens appeared increased in statin recipients compared with specimens from subjects who were not taking statins (see Table 1 for clinical characteristics of subjects). Figure 6A shows flow cytometric analysis of CD105 expression in endothelial cells separated from these specimens, and Fig. 6B shows the results of endoglin mRNA transcript measurements (Fig. 6B).

**DISCUSSION**

DES are used to prevent coronary postangioplasty restenosis. It is well known that migration and proliferation of smooth muscle cells, stimulated by cytokines released by infiltrating mononuclear cells in injured vessels, are the prime mechanism whereby restenosis occurs (5). Tacrolimus appeared to be a good candidate to counteract this phenomenon because it is a potent inhibitor of lymphocyte activation (1). In addition, CaN is a regulator of VSMC proliferation (11). Contrary to sirolimus, tacrolimus does not affect re-endothelialization (3, 28), which is critical for preventing late thrombosis. For all these reasons, tacrolimus appeared to be a good candidate for use in DES, although it is less potent than sirolimus as an antiproliferative agent (19). Contrary to expectations, the outcome of patients receiving TES was not favorable (25, 29). In line with clinical results, an in vitro study from our group (9) showed that tacrolimus activated TGF-β signaling in VSMC and induced proliferation mediated by the vascular remodeling factor β-catenin (34). In the present study, we show that atorvastatin downregulates β-catenin, its downstream effector kinases Erk1 and -2 and the cell cycle regulator cyclin B and counteracts proliferation of VSMC stimulated by tacrolimus. These results are in line with data obtained by analyzing the RNA expression profile of vascular cells treated with statins, showing that statins potently suppress cyclin B mRNA levels in VSMC (21). Moreover, BrdU incorporation into cellular DNA showed that atorvastatin inhibited tacrolimus-induced proliferation in a dose-dependent manner. Apparently, atorvastatin could suppress tacrolimus’ effect on BrdU incorporation at the low dose of 0.1 μM. Nevertheless, a higher (3 μM) concentration was needed to obtain a striking reduction in DNA synthesis below basal levels. Cell counts confirmed that the addition of 3 μM atorvastatin to tacrolimus remarkably impaired cell proliferation. Overall, proliferation data suggest that the association between atorvastatin and tacrolimus has a better inhibitory efficacy on VSMC growth compared with atorvastatin alone. The cooperation between atorvastatin and tacrolimus may be explained by atorvastatin-mediated blockage of the proliferative circuit engaged by the TGF-β signal, making it less latent or even amplifying the effect of CaN inhibition (11) by tacrolimus. Also, in accordance with results from an RNA profile study (21), HUVEC proliferation and
cyclin B expression levels were not reduced by atorvastatin. These findings suggest that tacrolimus activates a proliferative signal in HUVECs that atorvastatin was unable to counteract. Both tacrolimus and atorvastatin increased expression of endoglin in HUVEC. These results are in line with recent studies showing that tacrolimus increases endoglin expression in endothelial cell cultures (2) and that atorvastatin increased the expression of endoglin in the aortas of apolipoprotein E/low density lipoprotein receptor double-knockout mice (22). Moreover, we found that the enhanced levels of cyclin D in tacrolimus-treated cultures were, at least in part, dependent on endoglin induction. A slight increase in cyclin D transcripts was observed in atorvastatin-treated samples, despite that atorvastatin induced high levels of endoglin. This may be ascribed to an opposing effect of the cell-cycle progression inhibitory protein p27Kip1, which is also upregulated by statins (15).

Endoglin, or CD105, has an important role in angiogenesis (30) and vascular repair (33). It is an accessory receptor of the TGF-β family of proteins, which includes TGF-β, bone morphogenetic proteins (BMPs), and activins (30). Endoglin can modulate TGF-β signaling by regulating the access of ligands to signaling receptors. Endoglin knockout mice die in uterus because of defects in vasculogenesis, and mutations in endoglin result in the human vascular disease hereditary hemorrhagic telangiectasia (31). In several systems, endoglin expression promotes the BMP signaling pathway (5), a signal active during embryonic development that is reactivated during adult vasculogenesis. Results of endoglin mRNA measurements in primary endothelial samples from surgical patients appear to be in line with data obtained with HUVECs. Indeed, endoglin mRNA levels in the endothelium from statin recipients were higher than those obtained from subjects who were not statin recipients. Taken together, these findings support the conclusion that, in endothelium, pro-angiogenic signals driven by endoglin may neutralize anti-proliferative inputs found to play a role in atorvastatin-mediated decreased VSMC proliferation.

In conclusion, our study suggests that, when combined with tacrolimus, atorvastatin exerts a striking, dose-dependent, anti-proliferative effect on VSMC while improving endothelial renewal. Although tacrolimus is a potential anti-proliferative agent because of its ability to inhibit CaN (11), this drug also has a compensatory effect on the proliferation of VSMC by activating the TGF-β. Our findings suggest that statins counteract this compensatory loop and apparently reinforce the CaN inhibitory effects of tacrolimus. The combination of high-dose atorvastatin and tacrolimus results in significant impairment of VSMC growth. In contrast, in endothelial cells, atorvastatin and tacrolimus exert a cooperative stimulatory effect on endoglin production, which reasonably maintains the regenerative potential of this cell. Our findings support the conclusion.
that the concomitant association between tacrolimus and high-dose atorvastatin could be beneficial in preventing postcoronary in-stent restenosis and late thrombosis. A pharmacokinetic study of atorvastatin and its metabolites, performed in healthy volunteers undergoing concomitant exposure to tacrolimus, demonstrated the safety of this pharmacological association (17). Future directions in DES designs point toward new drugs and platforms that preserve endothelial function (8). The association between HMG-CoA reductase inhibitors and tacrolimus represents a promising approach in this direction, which can counteract restenosis, promote physiological healing, and treat local atherosclerotic disease.

GRANTS

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DISCLOSURES

None declared.

REFERENCES


