Local statin therapy differentially interferes with smooth muscle and endothelial cell proliferation and reduces neointima on a drug-eluting stent platform

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Abstract

Objective: Therapeutic strategies to provide local inhibition of mitogen mediated proliferation and migration of human coronary artery smooth muscle cells (CASMC) by means of drug-eluting stents have been shown to enable effective limitation of neointimal hyperplasia. However, currently available drug-eluting stents utilize compounds that may also adversely affect endothelial regrowth, thus possibly precipitating subsequent cardiac events. Accordingly, identification of compounds that differentially inhibit smooth muscle and endothelial cell migration and proliferation could be of substantial clinical usefulness.

Methods and results: In addition to lipid lowering, statins are known to display auxiliary pleiotropic activities. The purpose of this study was to evaluate the effect of local administration of cerivastatin on proliferation, migration and cytotoxicity of CASMC as well as coronary artery endothelial cells (CAEC) and to evaluate the effect of cerivastatin-coated stents on the inhibition of neointima formation as well as endothelial regrowth within the stented vessel. Cerivastatin displayed a differential effect on CASMC as compared to CAEC with regard to proliferation and migration; both were more profoundly inhibited in CASMC. Appreciable cytotoxicity and pro-apoptotic effects were low in both cell lines at therapeutic concentrations. Cerivastatin-elution led to significant inhibition of neointima formation in the rat carotid stent model, endothelial coverage of in-stent vascular tissue was similar with control and cerivastatin-eluting stents.

Conclusions: As proof of principle, our study provides evidence that local application of a HMG-CoA reductase inhibitor on a drug-eluting stent platform can efficiently limit neointima formation. Consequently, these compounds warrant further clinical evaluation to confirm this finding. Our data further suggest that the anti-restenotic effect of local statin administration might be associated with a more protective interaction with the endothelium than that observed with compounds currently employed on drug-eluting stents.

Keywords: Drug-eluting stents; Neointimal hyperplasia; Statin; Endothelium; Cell cycle

1. Introduction

Percutaneous coronary interventions (PCI) to treat coronary artery disease are performed world wide in steadily increasing numbers [1]. Since coronary stents lead to improved acute and chronic outcomes compared to conven-
tional balloon angioplasty [2], they are now used in more than 80% of PCI. Injury induced migration and proliferation of coronary artery smooth muscle cells (CASMC) is the major pathophysiological event that leads to neointima formation [3], anatomically correlating with in-stent restenosis. Numerous studies with 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors or statins have shown efficacy for primary [4] and secondary prevention [5] of coronary artery disease (CAD). The lipid profile of an individual patient may play an important role in the formation of an atherosclerotic plaque since the uptake of modified LDL contributes to the lipid core of the plaque [6]. As serum cholesterol levels are associated with the development of CAD and acute coronary events [7], it has been assumed previously that the beneficial effect of statin therapy in CAD is exclusively due to lowering effects on total and LDL cholesterol as well as triglycerides together with an increase in HDL cholesterol. However, there is compelling evidence that statins may also exhibit non cholesterol dependent properties, known as pleiotropic effects [8].

Pleiotropic properties of statins comprise effects on smooth muscle cell proliferation as well as on endothelial function, vascular inflammation and platelet activation. Systemic statin therapy is not effective for the limitation of human coronary artery restenosis [9]. The rate of in-stent restenosis is, however, substantially reduced by drug-eluting stents coated with either sirolimus or paclitaxel [10,11]. These compounds also cause significant inhibition of endothelial cell proliferation [12]. This is a major concern since impairment of endothelial restoration and function may lead to late thrombotic events [13]. In fact, there is evidence that sirolimus-eluting stents may elicit late stent thrombosis in some cases [14], a phenomenon which is virtually unknown for bare-metal stents. Therefore, a stent-based compound which would provide effective inhibition of smooth muscle cell proliferation without significant interference of post-procedural endothelial proliferation is regarded as the main target for evolutionary concepts toward the development of next generation drug-eluting stents [13,15]. Since statins display anti-proliferative properties but are known to improve endothelial function, the purpose of the present study was to evaluate the effect of cerivastatin on CASMC and CAEC proliferation and migration as well as to determine the impact of cerivastatin-coated stents on the inhibition of neointima formation and endothelial regrowth in an experimental animal model of vascular injury and stenting.

2. Methods

2.1. Materials

Cerivastatin, a 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor with a molecular weight of 481.5 g/mol was provided by Bayer, Leverkusen, Germany.

2.2. Cell culture

Human coronary artery smooth muscle cells (CASMC; #CC-2583, Cambrex, East Rutherford, USA) as well as human coronary artery endothelial cells (CAEC; #CC-2585) were obtained at passage 3 and used in passages not higher than 10. CASMC were cultured in smooth muscle cell basal medium (SmBM®, #CC-3181) supplemented with SmGM-kit (#CC-4149) including 5% FBS. CAEC were maintained in endothelial cell basal medium (EBM-2, #CC-3156) supplemented with EGM-2-kit (#CC-4176), including 2% FBS, both from Cambrex. Treatment with cerivastatin was performed in fully supplemented media for the indicated time and concentration ranges.

2.3. Cell cycle and proliferation assessment (BrdU ELISA, FACS analysis, pRb ELISA, kinase assay)

For cell counting, cells were seeded in 35 mm plates the day before incubation with various concentrations of cerivastatin (triplicates). After 48 h, cells on three randomly assigned 40 x power fields per well were counted manually. Assessment of cellular proliferation via measurement of DNA synthesis was analyzed by a colorimetric bromodeoxyuridine (BrdU) ELISA kit (Roche, Mannheim, Germany, #1647229) as previously described [16]. Growing cells were pre-incubated with cerivastatin for 24 h followed by exposition to a 15 h 10 μM BrdU pulse. Assessment of cell cycle distribution was carried out by flow cytometry using propidium iodine for DNA labeling as described [16]. For this particular experiment, CASMC and CAEC medium was additionally supplemented with 10 ng/ml PDGF or 1 μl/ml VEGF, respectively. Software based cell cycle analysis was performed using ModFit 2.0 software (Verity, Topsham, USA). The phosphorylation state of retinoblastoma protein (Rb) was analyzed by a pRb ELISA (BioSource, Camarillo, USA, #KH-O0021) according to the provided protocol by the manufacturer after 24 h cerivastatin treatment. This assay recognizes the phosphorylation of threonine 821, which is catalyzed by cyclin E/CDK2 and cyclin A/CDK2. Additionally, Western blotting was performed by use of an Rb antibody (Becton Dickinson, Heidelberg, Germany #554141) which recognizes different phosphorylation forms of Rb. For kinase assay, CAEC and CASMC were lysed in lysis buffer (0.2% NP-40, 20 mM Tris (pH 7.4), 150 mM NaCl, 1 mM EDTA, 1.5 mM MgCl₂, 1 mM Na-ortho-vanadate), sonified and supernatants normalized for equal amounts of protein. Cyclin A/CDK2 complexes were immunoprecipitated using a polyclonal cyclin A antibody as described previously [17]. The radioactive reactions were performed at 30 °C for 45 min with 10 mCi γ-32P-ATP and 20 μM cold ATP. Cyclin A/CDK2 activity was quantified by phosphorimaging.
2.4. Migration assay

Influence of cerivastatin on cell migration was measured in a Boyden chamber system. The QCM™-FN quantitative cell migration assay (#ECM500) from Chemicon (Temecula, USA) was applied according to the protocol of the manufacturer. This assay allows measurement of cell migration (haptotaxis) towards a fibronectin gradient. As controls, BSA coated chambers were used. After 18 h, cells on the bottom side of the membrane were fixed, stained with crystal violet and manually counted on an inverted microscope.

2.5. Western blotting

Western blotting was performed as previously described [16]. Membranes were probed with antibodies directed against PCNA (Santa Cruz, Santa Cruz, USA #sc-56), p21cip1 (BD, #610233), p27kip1 (BD, #610241), cyclin A1/A2 (Santa Cruz, #sc-751) and cyclin D1 (Santa Cruz, #sc-8396), respectively. HRP-conjugated secondary antibodies were obtained from Santa Cruz. Quantitative assessment was carried out using a GS-800 imaging densitometer (BioRad, Hercules, USA, #GS-800) and quantitated by Quantity One 4.1 software (BioRad).

2.6. Assessment of cytotoxicity and apoptosis

Cytotoxic cell injury leads to LDH release from the cytosol of damaged cells into the supernatant. LDH activity in cell-free supernatants was measured 48 h after exposure to cerivastatin using a colorimetric assay (Roche, Mannheim, Germany, #1644793) as previously described [16]. Maximum LDH release was determined by 1% Triton X-100 (Sigma) treatment. Cellular apoptosis was quantitated by a single stranded DNA apoptosis ELISA kit obtained from Chemicon (Temecula, USA, #APT225) according to the supplied protocol. As a control, apoptosis was induced in both cell lines by treatment with 100 mM FeSO4 and 100 mM H2O2 in HBSS for 2 h at 37 °C.

2.7. Stent coating and HPLC-based analysis of release kinetics

BiodivYsio® (Biocompatibles, Galway, Ireland) Matrix™ LO DD stents (10 mm length, 2.0 mm diameter) were dip coated in 5 mg/ml cerivastatin solution for 10 min and air dried for 10 min. Control stents were identically pre-treated but were not coated with cerivastatin. For determination of release kinetics, the stent was deployed and submersed in 2 ml medium. Every hour, medium was changed and subjected to HPLC analysis to determine eluted cerivastatin concentrations.

2.8. Experimental model of rat carotid stent placement and histomorphometry

To determine the effect of cerivastatin on neointimal hyperplasia, the rat carotid stent model was utilized as previously described [16,18]. In brief, the left common carotid artery was exposed and injured by triple withdrawal of an inflated 2 French Fogarty catheter. Thereafter, the pre-mounted 2.0/10 mm long drug-eluting stent system was inserted through the identical access site via the external carotid artery (ECA); placed into the common carotid artery and deployed at 10 atm (n = 14 for cerivastatin-coated, n = 17 for control stents), yielding a balloon-to-vessel ratio of 1.5:1. ECA was ligated after stenting. During surgery, animals received 80 IE of heparin. 14 days after surgery, animals were examined by duplex sonography [18] to determine stent patency and subsequently sacrificed. The common carotid artery was embedded in methylmethacrylate [16]. For histomorphometric analysis and assessment of stent strut coverage and reendothelialization, sections were cut with a Leitz saw microtome. Stent segments were Paragon stained according to an established protocol [19] and analyzed at a thickness of 10 µm (3 segments for each stent). Stent endothelialization was defined as the extent of the circumference of the arterial lumen covered by endothelium: 1 = 25%; 2 = 25 to 75% and 3 = 75 to 100% coverage according to a published score [20] and was carried out on Paragon stained tissue. Histomorphometric analysis was carried out as described [16]. For immunohistochemistry, tissue samples from methanol fixed stent explants were removed by microdissection, infiltrated overnight with a 5% sucrose solution in PBS and cryosectioned at 12 µm. Sections were labeled with a monoclonal antibody directed against factor VIII (von Willebrand factor, Serotec, Düsseldorf, Germany, # MCA127). Non-specific binding of antibodies was controlled for by omitting the primary antibody. Antibody binding was detected with a Vectastain ABC Elite avidin/biotin peroxidase kit (Vector Labs, Burlingame, USA).

2.9. Statistical analysis

Results are expressed as mean±standard deviation. The significance of variability among the means of the experimental groups was determined by one or two way analysis of variance (ANOVA), using SPSS for Windows V10.0 software. Differences among experimental groups were regarded to be statistically significant when P<0.05.

3. Results

3.1. Differential inhibition of smooth muscle and endothelial cell proliferation by cerivastatin

Initiation and maintenance of CASMC proliferation is a critical event in the pathogenesis of neointima formation and thus in-stent restenosis. However, simultaneous inhibition of endothelial cell growth is regarded as detrimental since retarded reendothelialization increases the chance for stent thrombosis. Human CASMC proliferation was inhibited by
cerivastatin at concentrations as low as 5 nM, the same concentration had no significant effect on CAEC proliferation (Fig. 1A and B). Treatment with cerivastatin at dosages of 50 nM or higher led to complete growth arrest in CASMC but not CAEC. Remarkably, BrdU incorporation was constantly maintained at significantly higher levels in CAEC with increasing relative differences at higher cerivastatin concentrations (Fig. 1B). Accordingly, FACS based cell cycle assessment revealed a higher percentage of cycling CAEC when treated with 50 nM cerivastatin compared to CASMC (Fig. 1C). Consequently, these results reveal a minor responsiveness of coronary artery endothelial cells to the anti-proliferative effect of cerivastatin compared to smooth muscle cells of coronary origin, suggesting a therapeutic gap which might be advantageous in the vascular interventional setting. To ensure that this is a class effect for statins, we repeated the experiments with fluvastatin, yielding similar results on differential inhibition of CASMC versus CAEC proliferation, however, at approximately 10-fold higher concentrations compared to cerivastatin (data not shown).

Rb function, including the ability to interact with E2F, is regulated by phosphorylation. Therefore, phosphorylation of the retinoblastoma protein (Rb) is a critical event in cellular proliferation and takes place in G1/S transition [21]. Whereas Thr821 phosphorylation of Rb was abrogated with increasing cerivastatin dosages in CASMC, this was apparently not the case in CAEC (Fig. 2). This is conclusive with the previous findings that proliferation and S-phase progression are less attenuated by cerivastatin in CAEC than CASMC.

3.2. Cerivastatin significantly inhibits migration of human coronary artery smooth muscle but does not negatively interfere with endothelial cell migration

Another key mechanism of neointima formation besides proliferation of vascular smooth muscle cells is mitogen mediated migration of these particular cells into the neo-
intima. Therefore, the inhibitory effect of cerivastatin on mitogen induced cellular migration was analyzed using a Boyden chamber assay. Cerivastatin led to dose dependent inhibition of CASMC migration towards a fibronectin gradient after 18 h of migration time (Fig. 3). The migratory activities at different concentrations were quantified by cell count and showed significant reduction compared to untreated controls. Migration of endothelial cells from non-injured endothelial layers is a critical event in the process of reendothelialization. Therefore, negative interference of this physiological response to injury should be avoided. In contrast to CASMC, cerivastatin had no detectable effect on migration of CAEC at concentrations of 50 and 100 nM in vitro (Fig. 3), suggesting a protective effect on CAEC migration and an inhibitory effect on CASMC migration at the investigated concentrations. Similar results were obtained by means of a vitronectin-based Boyden chamber system, thus confirming the protective statin effect on CAEC migration (data not shown).

3.3. Cerivastatin differentially interferes with kinase activity and proteome expression of distinct cell cycle regulating genes in smooth muscle compared to endothelial cells

Cyclins and cyclin-dependent kinases are essential for cell cycle progression and can be inhibited by cyclin-dependent kinase inhibitors (CKI). CKI of the cip/kip family such as p21cip1 and p27kip1 have been shown to be activated in vascular proliferative disease and are negative regulators of neointima formation [22,23]. Consequently, overexpression of p21cip1 or p27kip1 leads to attenuation of neointima formation following vascular injury [24,25]. Cerivastatin treatment of mitogen stimulated CASMC led to rapid increase of both p21cip1 and p27kip1 protein levels, this effect was less pronounced in CAEC (Fig. 4B). Similarly, downregulation of cyclin A1/A2 and cyclin D1 expression was detected to a higher extent in CASMC compared to CAEC. PCNA (proliferating cell nuclear antigen) is a member of the so called DNA sliding clamp family and has an essential function on providing replicative polymerases required to duplicate the entire genome [26]. Therefore, PCNA is mainly expressed in G1/S transition and S-phase. PCNA protein levels were significantly decreased in CASMC treated with cerivastatin (Fig. 4A). In contrast, PCNA expression remained less downregulated in CAEC, indicating maintained G1/S phase progression in endothelial cells. Cell cycle kinase activity reflects the integral of signaling events interfering with cell cycle regulation. Consequently, cerivastatin inhibited cyclin A/CDK2 kinase activity, essential for G1/S and S phase progression, more efficiently in smooth muscle compared to endothelial cells (Fig. 4C).

3.4. Cerivastatin does not induce appreciable cytotoxicity or apoptotic cell death in both CASMC and CAEC

The biological safety profile is crucial for drug-eluting intravascular stents since cell injury may lead to delayed vascular healing and possibly to stent thrombosis and increased rates of myocardial infarction [27]. Therefore, we examined whether cerivastatin imposes cytotoxic and/or pro-apoptotic effects in both coronary artery smooth
muscle and endothelial cells. Assessment of cytotoxic effects was performed by measurement of LDH release in both cell lines. Concentrations of up to 500 nM cerivastatin were not sufficient to induce a marked increase in cytotoxicity (Fig. 5A and B), however, a small increase was detectable. Corresponding results were observed for quantification of apoptosis rates in cerivastatin treated CASMC and CAEC showing a slight increased apoptosis rate at higher concentrations for CASMC but not CAEC (Fig. 5C and D). These results suggest that concentrations notably affecting CASMC proliferation have no pronounced effect on CASMC and CAEC viability. This implicates a broad therapeutic range for local cerivastatin treatment which is an important parameter for the drug’s safety profile regarding local drug transfer into the vessel wall.

3.5. Cerivastatin coated stents do not negatively interfere with reendothelialization and significantly inhibit neointima formation in vivo

To determine the efficacy of local cerivastatin release for the limitation of neointimal hyperplasia, BiodivYsio® Matrix LO stents were coated with 5 mg/ml cerivastatin. To determine in vitro release kinetics, released cerivastatin dosages were determined by HPLC analysis. Cerivastatin release was detectable for up to 3 h (Fig. 6A). The total loading dose of cerivastatin on the stent was 11.1 μg per 10
mm stent. In the rat carotid stent model, 14 days after deployment of coated and identical but non-cerivastatin-coated stents, duplex sonography showed a similar patency rate in both stent groups (n = 3/14 cerivastatin vs. 4/17 for control stents, P = N.S.) indicating no increase in the rate of stent thrombosis. Despite relatively short release kinetics, histomorphometric analysis of neointimal area revealed a significant reduction of in-stent stenosis by cerivastatin-eluting stents (Fig. 6B and C).

3.6. Vascular reendothelialization is not altered by cerivastatin-eluting stents compared to non-coated stents

Statins are known to be capable of positively influencing endothelial function. The process of lesion endothelialization following vascular intervention is important to assure an appropriate clinical outcome. Therefore, we assessed stent endothelial coverage of strut-associated tissue and magnitude of endothelialization at the end of the observation period at 14 days. As depicted in Fig. 6D, reendothelialization was not different in animals receiving cerivastatin-coated and non-coated stents. Concordantly, immunohistochemical assessment of stented segments with an endothelial cell marker (von Willebrand factor) (n = 3 stents each group) revealed no appreciable difference between animals receiving cerivastatin-coated or non-coated stents (Fig. 6C e–f). These data suggest that cerivastatin may not negatively interfere with the in-stent endothelialization process.

4. Discussion

Cholesterol-independent effects of statins are widely recognized [28] and account for clinical benefits of patients receiving oral therapy with HMG-CoA reductase inhibitors [29]. In addition to their effect on lowering lipid levels, statins have been shown to improve endothelial function by transcriptionally activating vascular NO production [30] via induction of endothelial NO synthase (eNOS) [31]. Moreover, statins inhibit mitogen induced smooth muscle cell proliferation [32], e.g. by inhibiting Rho-GTPase mediated downregulation of p27cip1 [33]. HMG-CoA reductase inhibitors are also known to stabilize atherosclerotic plaques by inhibition of oxidized LDL mediated macrophage proliferation as well as inhibition of migration and reduction of the accumulation of cholesteryl esters in macrophages exposed to oxidized LDL [34]. They also display anti-inflammatory properties which include attenuation of P-selectin expression in vivo [35]. Interestingly, statins have
also been shown to inhibit platelet function [36]. This entire spectrum of actions would be the potential result of local drug delivery to the vascular wall.

Systemic treatment with simvastatin, albeit in much higher doses than used in humans, reduces neointimal thickening in LDL receptor-deficient mice [37]. In this context, short-term treatment with a farnesyl transferase inhibitor, which catalyzes Rho farnesylation and, therefore, renders inhibition of smooth muscle cell proliferation similar to that of statins, demonstrated efficacy in prevention of neointima formation in a porcine angioplasty model [38]. However, oral statin therapy does not appear to inhibit in-stent restenosis in humans [9], presumably due to insufficient local drug concentrations.

Cerivastatin has been shown to be the most effective statin for direct inhibition of vascular smooth muscle cell proliferation [32]. This is important for the application on a drug-eluting stent platform since drug loading capacity is limited. In this study, we found that cerivastatin inhibits mitogen mediated coronary artery smooth muscle cell proliferation even at low dosages.

Notably, there is a clear differential inhibitory effect on smooth muscle and endothelial cell proliferation. In the injured artery, this effect might be beneficial for the process of reendothelialization. Further, at dosages with a prominent anti-proliferative effect on CASMC, cerivastatin inhibited coronary artery smooth muscle cell migration in the Boyden chamber with, apparently, no appreciable effect on CAEC migration. Migratory cell activity in this model corresponds to invasive cellular capacity in vivo [39]. Interference with CASMC migration might be explained by up-regulation of p27kip1. This cyclin-dependent kinase inhibitor exhibits anti-migratory properties in CASMC [17,40]. The attenuated anti-proliferative effect of cerivastatin on coronary artery endothelial cells could be due to increased eNOS levels through a PI3K/Akt dependent signaling pathway. eNOS is known to promote endothelial function and proliferation [41]. Differential inhibition of CASMC versus CAEC.
proliferation can be considered a class effect of statins since we found similar effects of fluvastatin on CASMC and CAEC proliferation (data not shown), however, these effects were only found at 10-fold higher drug concentrations compared to cerivastatin.

Drug-eluting stents coated with either sirolimus or paclitaxel have been shown to inhibit in-stent restenosis [10,11]. Both compounds elicit profound cell-cycle inhibitory properties. For example, sirolimus, upon binding to its intracellular receptor FKBP12, increases levels of the CDK inhibitor p27kip1, thus leading to cell cycle arrest [42]. Cell-cycle inhibitory properties should be regarded as mandatory for a compound which is considered for application on drug-eluting stents. Similar to rapamycin, we have shown that cerivastatin inhibits the CASMC cell cycle in G1 and leads to upregulation of p27kip1 but also p21cip1 as well as to downregulation of cyclin A1/A2 and D1 protein levels. In consequence, kinase activity of cyclin A/CDK2 kinase was found to be extensively downregulated. We further show that these effects occur to a considerably lower extend in CAEC, confirming the differential statin effect on endothelial cell proliferation.

An important issue for local vascular drug delivery is the safety and therapeutic range of the drug. Cytotoxicity may lead to an increase in the incidence of life-threatening stent thrombosis [27] as well as accelerated neointimal growth as evident in human trials [43]. Our data suggest a safe drug profile for local delivery of cerivastatin. Duplex sonography and histology revealed no accelerated stent thrombosis rate in cerivastatin-eluting stents as compared to non-coated stents in this model. In vitro release kinetics of cerivastatin demonstrated rapid elution with a total release of the drug within the first 3 h. This rapid release profile is not unusual for this particular drug-eluting stent platform and has been also shown for other compounds [16,44]. Coated with 17β estradiol, the identical stent platform decreased in-stent restenosis in the human EASTER trial [45]. Similar to a published animal study with a farnesyl transferase inhibitor [38], short-term but stent-based cerivastatin release proved sufficient to yield significant inhibition of neointima formation in vivo. This demonstrates proof of principle; however, protracted cerivastatin release by an alternative drug-eluting stent platform might be preferential and could lead to an even better outcome.

4.1. Limitation of the study

We examined the therapeutic effect of cerivastatin-eluting stents in the rat carotid stent model. In a recently published consensus manuscript it was stated that “the ideal animal model for drug-eluting stent evaluation is uncertain” and “it is unclear that any single animal species is any more predictive of human response and for specific indications” [46]. Thus, as is the case for any animal model, extrapolation of the data to the human clinical situation remains uncertain [47].

In conclusion, this is the first report demonstrating successful local, stent-based statin treatment to inhibit in-stent neointima formation. Statins display a favorable effect on endothelial cell proliferation and migration as well as a profound inhibitory effect on coronary artery smooth muscle proliferation. The endothelium is now considered of substantial importance for the development of next generation of drug-eluting stents [15] and the “next target in restenosis prevention”[13]. Recent clinical reports [48] support the assumption that (late) stent thrombosis might be increased in subsets of patients receiving currently available drug-eluting stents. Hence, identification of novel compounds with differential inhibitory effect on smooth muscle cell versus endothelial cell proliferation and migration is warranted. Therefore, together with the well recognized pleiotropic effects of statins, it is tempting to speculate that HMG-CoA reductase inhibitors might be suitable for the utilization on drug-eluting stents in humans.

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