Simvastatin causes endothelial cell apoptosis and attenuates severe pulmonary hypertension

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Simvastatin causes endothelial cell apoptosis and attenuates severe pulmonary hypertension. Am J Physiol Lung Cell Mol Physiol 291: L668–L676, 2006. First published May 12, 2006; doi:10.1152/ajplung.00491.2005.—Severe pulmonary hypertension (SPH) is characterized by precapillary arteriolar lumen obliteration, dramatic right ventricular hypertrophy, and pericardial effusion. Our recently published rat model of SPH recapitulates major components of the human disease. We used this model to develop new treatment strategies for SPH. SPH in rats was induced using VEGF receptor blockade in combination with chronic hypoxia. A large variety of drugs used in this study, including anticancer drugs (cyclophosphamide and paclitaxel), the angiotensin-converting enzyme inhibitor lisinopril, the antiangiogenic agent thalidomide, and the peroxisome proliferator-activated receptor (PPAR)-γ agonist pioglitazone, failed to decrease mean pulmonary artery pressure (PAP) or right ventricular hypertrophy. In contrast, treatment of rats with established SPH with simvastatin markedly reduced mean PAP and right ventricular hypertrophy. Some of these results have been previously published in abstract form (46).

Simvastatin partially restored caveolin-1, caveolin-2, and phospho-caveolin expression in vessel walls. In rat primary pulmonary microvascular endothelial cells, simvastatin induced caspase 3 activation and Rac 1 expression while suppressing RhoA and ERK phosphorylation. We conclude that simvastatin is effective in inducing apoptosis in hyperproliferative pulmonary vascular lesions and could be considered as a potential drug for treatment of human SPH.

HUMAN SEVERE PULMONARY HYPERTENSION (SPH), including pulmonary hypertension secondary to congenital cardiac abnormalities, collagen vascular disorders, human immunodeficiency virus and human herpes virus-8 infection, anorexigen drug intake, and so-called idiopathic pulmonary hypertension, are unfortunately diagnosed when the pulmonary vascular bed already has been extensively remodeled and is not usually responsive to vasodilators (42). Although continuous infusion of prostacyclin has prolonged the survival of many patients with SPH and improved their ability to exercise and function (15, 39, 44), prostacyclin does not cure the disease. Vascular lesions are frequently localized at sites distal to arteriolar bifurcations (9) and contain phenotypically altered endothelial and smooth muscle cells (25, 52, 53) that have lost the expression of p27, caveolin-1, and -2 protein and of prostacyclin synthase and peroxisome proliferator-activated receptor (PPAR)-γ genes and proteins (1, 51). These endothelial cells also do not obey the “law of the monolayer” and instead form cell clusters or tumorlets (52, 53).

Our rat model of SPH (48, 50) resembles the human disease because it demonstrates endothelial cell proliferation and the occlusion of small precapillary arterioles. In this model, chronic VEGF receptor blockade in combination with chronic hypoxia causes the initial apoptosis of lung endothelial cells, which is followed by the selection of phenotypically altered apoptosis-resistant endothelial cells. Endothelial cell proliferation causes pulmonary artery pressures (PAP) similar to those encountered in human SPH (48).

Here, we used this model of SPH to screen various compounds to assess their effect on established SPH and on lumen-obliterating pulmonary vascular lesions. These experiments may provide guidance for treatment of human pulmonary hypertension. We found that treatment of SPH rats with the 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase inhibitor simvastatin caused apoptosis of lumen-obliterating cells, associated with a significant reduction of PAP and of right ventricular (RV) hypertrophy. Some of these results have been previously published in abstract form (46).

MATERIALS AND METHODS

Materials. First published 21 November 2005; accepted in final form 5 May 2006

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of three animals in the group died before the end of the treatment period of 3 wk.  

Simvastatin HMG-CoA reductase inhibitor 10 mg/kg by gavage 3 3 63  
Lisinopril ACE inhibitor 20 mg/kg by gavage 3 3 70  
Taxol (Paclitaxel) Anticancer drug 10 mg/kg ip 3 3 66  
B9430 Bradykinin antagonist 5 mg/kg sc 3 3 63  

Untreated 33 3 68  

were returned to Denver altitude (DA; 1,600 m) and treated with 

hypoxia (simulated altitude of 5,000 m in a hypobaric chamber) for 3 wk (48). After 3 wk of hypoxic high-altitude exposure, the animals were returned to Denver altitude (DA; 1,600 m) and treated with different drugs as described in the RESULTS.

Assessment of pulmonary hypertension and lung morphology. At the end of the treatment period, rats were weighed and anesthetized with 1 M ketamine hydrochloride (60 mg/kg) and xylazine (8 mg/kg) administered intramuscularly. PAP, RV hypertrophy, and lung morphology were measured as previously described (48).

IHC. IHC was performed as previously described (49). Controls included the omission of primary antibody and its replacement by rabbit nonimmune serum. For the mouse kit, a biotinylated anti mouse IgG (rat adsorbed, BA-2001, Vector Laboratories) at 10 mg/ml was employed to avoid background staining.

Cell proliferation assay in lung tissue. Bromodeoxyuridine (BrdU; Sigma) was administered via tail vein injection (40 mg/kg). Animals were killed at 2 h (baseline control) and 24 h after the injection. BrdU in paraffin-embedded lung tissue was detected immuno-  

Assessment of apoptosis. IHC for active caspase-3 was performed on paraffin-embedded tissue sections. Vessels (50–100 μm) were assessed for caspase-3-positive cells. Twenty-five totally occluded, partially occluded, and nonoccluded vessels per slide (1 slide/animal, n = 4) were counted. Data were expressed as percentages of caspase-3-positive vessels per 25 vessels.

Cell culture assays. Rat pulmonary microvasculature endothelial cells (RPMECs) and rat pulmonary artery smooth muscle cells (RPASMCs) were plated in 200 μl of 10% FBS-DMEM (CellGro, Mediatech) in 96-well culture plates (5 × 10^3 cells/well), grown overnight, synchronized in 0.5% heat-inactivated FBS-DMEM for 6 h, and treated for 18 h with different compounds as indicated in the results.

Cell proliferation was assessed using the CyQuant Cell Proliferation Assay Kit (Molecular Probes) as previously described (14).

Cell death was measured using Vybrant Apoptosis Assay Kit no. 3 (Molecular Probes), followed by flow cytometric analysis using a BD FACScalibur Flow Cytometer.

WB studies. Cells were lysed in HB buffer [20 mM HEPES (pH 7.55), 1.5 mM MgCl2, 150 mM NaCl, 10% glycerol, 1% Triton X-100, 2 mM EDTA, 2 mM Na3VO4, 50 mM NaF, 2 μg/ml aprotinin, 5 μg/ml leupeptin, and 1 mM PMSF] for 15 min at room temperature and centrifuged at 10,000 g for 10 min. The protein concentration was determined using Bradford reagent (Sigma). Proteins (25 μg) were subjected to electrophoresis on 4–12% gradient NuPAGE Bis-Tris gels (Invitrogen), transferred to a PolyScreen polynylidine difluoro-ride transfer membrane (NEN Life Science Products), and visualized using the Renaissance WB Chemiluminescence Reagent (NEN Life Science Products).

Statistical analysis. Statistical significance was determined using Student’s unpaired t-test (P < 0.05). Values are expressed as means ± SE.

RESULTS

Development of SPH in rats. Sprague-Dawley rats treated with a single subcutaneous injection of US5416 (20 mg/kg) and exposed to chronic hypoxia (simulated altitude of 5,000 m) for 3 wk developed SPH (Fig. 1A and Table 1) as reported previously (48). The mean PAP of US5416-treated animals (n = 24) was 47 ± 1.8 versus 32.3 ± 2.1 mmHg in vehicle-only treated hypoxic rats (n = 12). Of note, this treatment induced irreversible SPH. After rats were reexposed to normoxia (DA, 1,600 m) for an additional 3–4 wk, the mean PAP increased further to reach 68 ± 2.8 mmHg, whereas in control animals exposed to chronic hypoxia alone (plus vehicle), PAP decreased to normal 20 ± 2.8 mmHg levels. Eventually, the animals died, presumably from right heart failure. In rats treated with vehicle and exposed to hypoxia and subsequently for 3–4 wk at DA, mean PAP returned to 18.4 ± 0.5 mmHg. SPH was accompanied by pronounced RV hypertrophy (Table 1). The ratio of RV mass to left ventricle (LV) and septum (S) mass (RV/LV + S) of the US5416-treated chronically hypoxic animals (3 wk) was 0.53 ± 0.05 versus 0.28 ± 0.03 in vehicle-treated controls. When animals were reexposed to DA for an additional 3 or 4 wk, the RV hypertrophy ratio continued to increase further to 0.73 ± 0.03 in the US5416-treated animals, whereas the ratio in the hypoxia exposure-only animals returned to normal.

Histology of lungs of rats with SPH. Lungs of US5416-treated animals after 3 wk of exposure to hypoxia followed by 3 wk at DA showed vasoobliterative lesions characterized by luminal occlusion of medium-sized and precapillary arteries (Fig. 1). As demonstrated previously (48), the majority of these lesions showed lumen obliteration by endothelial cells, as

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mechanism of Action</th>
<th>Dosea</th>
<th>n</th>
<th>T, wk</th>
<th>PAP, mmHg</th>
<th>RV/LV + S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td></td>
<td>33</td>
<td>3</td>
<td>3</td>
<td>68±2.8</td>
<td>0.73±0.03</td>
</tr>
<tr>
<td>B9430</td>
<td>Bradykinin antagonist</td>
<td>5 mg/kg sc</td>
<td>3</td>
<td>3</td>
<td>63±2.0</td>
<td>0.63±0.02</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>Anticancer drug</td>
<td>20 mg/kg ip</td>
<td>3</td>
<td>3</td>
<td>66±2.6</td>
<td>0.76±0.04</td>
</tr>
<tr>
<td>Thalidomide</td>
<td>Angiogenesis inhibitor</td>
<td>10 mg/kg</td>
<td>3</td>
<td>3</td>
<td>65±3.7</td>
<td>0.73±0.05</td>
</tr>
<tr>
<td>Taxol</td>
<td>Anticancer drug</td>
<td>10 mg/kg</td>
<td>3</td>
<td>3</td>
<td>66±4.6</td>
<td>0.61±0.04</td>
</tr>
<tr>
<td>VEGF</td>
<td>Angiogenic growth factor</td>
<td>1 μg/kg by tail vein</td>
<td>3</td>
<td>3</td>
<td>83±3</td>
<td>0.62</td>
</tr>
<tr>
<td>Ibesartan</td>
<td>Angiotensin II receptor blocker</td>
<td>15 mg/kg by gavage</td>
<td>3</td>
<td>3</td>
<td>63±3.3</td>
<td>0.83±0.04</td>
</tr>
<tr>
<td>PG12</td>
<td>PPAR-γ agonist</td>
<td>200 μg/kg ip</td>
<td>3</td>
<td>3</td>
<td>64±3.3</td>
<td>0.66±0.02</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>Ca2+ channel blocker</td>
<td>10 mg/kg ip</td>
<td>3</td>
<td>3</td>
<td>63±3</td>
<td>0.61±0.01</td>
</tr>
<tr>
<td>Lisinopril</td>
<td>ACE inhibitor</td>
<td>20 mg/kg by gavage</td>
<td>3</td>
<td>3</td>
<td>70±3.9</td>
<td>0.63±0.02</td>
</tr>
<tr>
<td>Simvastatin</td>
<td>HMG-CoA reductase inhibitor</td>
<td>10 mg/kg by gavage</td>
<td>3</td>
<td>3</td>
<td>49±3.2</td>
<td>0.56±0.04</td>
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</tbody>
</table>

Values are means ± SE; n, no. of animals. Drugs were administered 5 times/wk. Untreated animals were animals with severe pulmonary hypertension (SPH) that did not receive drugs. T, duration of treatment with drug; PAP, pulmonary arterial pressure; RV/LV + S, ratio of the right ventricular mass to left ventricular plus septal mass; PPAR, peroxisome proliferator-activated receptor; ACE, angiotensin-converting enzyme; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA. *Two of three animals in the group died before the end of the treatment period of 3 wk.

AJP-Lung Cell Mol Physiol • VOL 291 • OCTOBER 2006 • www.ajplung.org
demonstrated by factor VIII staining (Fig. 1A). Cells in the lesions showed a higher proliferation rate as measured by BrdU incorporation within a 24-h period (Fig. 1, D and E) compared with the control lung, where we only occasionally found proliferating cells (Fig. 1, B and C). A quantitative analysis performed on 10 consecutive small pulmonary arteries (1 slide/animal, 5 animals/group) showed that, within the 24-h period, there were 5.5 ± 0.62 BrdU-positive cells/vessel in SU5416-treated rat lungs compared with 0.6 ± 0.16 BrdU-positive cells/vessel in the vehicle-treated control group.

**Treatment of animals with established SPH with different therapeutical agents.** All drug treatments were started after the animals had developed SPH due to the combined exposure to SU5416 and hypoxia and had been returned to DA. The criteria for a treatment response were a decrease in mean PAP and RV/LV + S and a decrease in the number of obliterative vascular lesions. The drugs used in this study are shown in Table 1. It was not the purpose of these studies to conduct drug dose ranging experiments. The dose of the drugs used was chosen based on previously published reports where drug effects had been obtained in adult rats. Only simvastatin decreased mean PAP and RV hypertrophy (Table 1).

Rats receiving simvastatin (10 mg/kg) by daily gavage for 3 wk showed a significant decrease in mean PAP (49 ± 3.2 vs. 68 ± 2.8 mmHg of untreated animals) and RV hypertrophy (0.56 ± 0.04 vs. 0.73 ± 0.03 of untreated rats). There was no difference in the hematocrit, and a slight decrease (about 10%) in body weight between untreated and simvastatin-treated animals. To determine whether the beneficial effect of simvastatin was mediated by NO, animals were treated simultaneously with simvastatin and the eNOS inhibitor N-nitro-L-arginine methyl ester (L-NAME; 10 mg/kg, daily by gavage) or with L-NAME alone. Fifty percent of the rats in both groups treated with L-NAME died within the first week, and the remaining 50% died within the second week of treatment. Thus, in this situation, simvastatin did not prolong survival, suggesting that it produced no effect in the presence of pharmacological eNOS blockade.

In the normal rat lung, blood vessels were negative for caspase-3 staining (Fig. 2A), and there was expression of caveolin-1, caveolin-2, and activated (phosphorylated) caveolin (Fig. 2, D, G, and J, respectively). Simvastatin induced apoptosis of cells in lumen-obliterating lesions (as shown by IHC of activated caspase-3; Fig. 2C) and partially restored...
caveolin-1, caveolin-2, and phospho-caveolin expression in precapillary blood vessels (Fig. 2, F, I, and L) rats. There were no caspase (Casp)-3 positive cells in the normal vasculature (A) and in pulmonary vascular lesions of SPH rats (B). D, G, and J: caveolin (Cav)-1 (D), Cav-2 (G), and phospho-Cav (pCav; J) expression in the normal vasculature. There was an absence of Cav-1 (H) and pCav (K) and decreased Cav-2 (H) expression in SPH rat lung vascular lesions. Treatment with SIM induced Casp-3 activation of endothelial cells in pulmonary lesions (C), partially restored the expression of Cav-1 (I), and restored the expression of Cav-2 (J) and activation of Cav (L). Original magnification: ×400.

**Simvastatin treatment causes activation of caspase-3 and significantly decreases the number of pulmonary vascular lesions.** Treatment with simvastatin induced apoptosis of endothelial cells in obliterated vessels (Fig. 3A). After 7 days of simvastatin treatment, 20% of totally occluded, 30% of partially occluded, and 5% of nonoccluded vessels stained positive for activated caspase-3, whereas in the untreated rat lung, there were very few caspase-3-positive cells. WBs of whole lung extracts showed more than threefold upregulation of activated caspase-3 at days 4, 7, and 14 of simvastatin treatment (Fig. 3B). Activated caspase-3 levels remained high for up to 2 wk of treatment. In contrast, in untreated lung tissue extracts, caspase-3 activity was not significantly elevated. IHC staining for the endothelial cell marker factor VIII of untreated and simvastatin-treated (Fig. 3C) rat lungs with SPH indicated a 30% decrease in the number of obliterated lung vessels (Fig. 3D; P < 0.0001).

**Effect of simvastatin on signaling pathways in RPMVECs and RPASMCs.** It is now becoming clear that statins can trigger apoptosis using caspase-dependent and caspase-independent Rho kinase-dependent pathways (20). WBs of cells showed that treatment with simvastatin resulted in a significant induction of caspase-3 activation as well as Rac 1 levels and decreased expression of RhoA in RPMVECs (Fig. 4, A and C). Simvastatin also decreased Akt and ERK activation but did not affect Bcl-2 and BclXL expression in RPMVECs (Fig. 4E). SU5416 alone had no effect on caspase-3 activation. The NOS inhibitor L-NAME somewhat augmented the effect of SU5416, whereas simvastatin significantly upregulated activated caspase-3 (Fig. 4A). Treatment with mevalonate (downstream metabolite) abolished the simvastatin effect and restored the survival signaling pathways in endothelial cells, suggesting the involvement of farnesyl protein phosphatase- or geranylgeranyl pyrophosphate-mediated effects (Fig. 4, A and E). There was no
difference in the protein expression pattern of untreated (Fig. 4, E and F) and mevalonate-alone (data not shown)-treated cells. Interestingly, in RPASMCs, the identical dose of simvastatin had very little effect on caspase-3 activation, was not affected by l-NAME (Fig. 4, B and D), had no effect on proapoptotic pathways (Fig. 4F), and seemed to work through a RhoA-independent mechanism. Simvastatin had no effect on total eNOS protein expression in both cell types (Fig. 4, E and F).

As shown in Fig. 5A, simvastatin at 10 μM almost completely inhibited RPMVEC proliferation. Flow cytometric analysis of simvastatin-treated RPMVECs clearly showed the induction of apoptosis. Almost 40% of cells treated with 2 μM

<table>
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<tr>
<th>% of caspase-3 positive vessels/25 vessels</th>
<th>totally occluded</th>
<th>partially occluded</th>
<th>non-occluded</th>
</tr>
</thead>
<tbody>
<tr>
<td>untreated</td>
<td>2.16 ± 0.43</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>SIM-treated</td>
<td>19.66 ± 1.85</td>
<td>29.7 ± 2.88</td>
<td>5 ± 0.83</td>
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</table>

Fig. 3. Treatment with SIM induced the activation of Casp-3 and decreased the number of obliterated pulmonary blood vessels in SPH rats. A: Casp-3 staining of untreated and SIM-treated rat lungs. Arrows indicate Casp-3-positive cells in totally occluded (1), partially occluded (2), and nonoccluded (3) precapillary (50–100 μm) vessels (insets). Quantitative analysis was performed by counting Casp-3-positive cells in 25 totally occluded, partially occluded, or nonoccluded vessels. Data were obtained from 4 lung sections from 4 different animals (1 section/animal). B: Western blot analyses of activated Casp-3 in whole lung extracts at days 1, 4, 7, 14 and 28 in SIM-treated rats and days 4 and 28 in untreated rats (*P < 0.005). Data are from 3 independent experiments. C: FVIII staining of untreated and SIM-treated rats with SPH. Red arrows indicate obliterated blood vessels. Original magnification: ×200. D: quantitative analysis of FVIII staining area based on 10 images/slide from 5 different animals. There was a significant decrease in the number of obliterated lung vessels in SIM-treated rats compared with untreated SPH rats (*P < 0.001).
Simvastatin were apoptotic (annexin V positive) at 18 h (Fig. 5B).

**DISCUSSION**

These experiments show that lung vascular structural alterations, once established, are very difficult to change (Table 1). In this regard, our model resembles human SPH, unlike the commonly used models of chronic hypoxia-induced or monocrotaline-induced pulmonary hypertension, which can be effectively treated with a number of different compounds (18, 30, 31, 34, 35, 37, 54). Because the severity of pulmonary hypertension and the extent of pulmonary vascular obliteration in this model is substantial, it provides a formidable therapeutic challenge, which may be based on the unique vascular lesions comprised of phenotypically altered endothelial cells (48). This conclusion is based on several observations: 1) the lumen-obliterating endothelial cells are apoptosis resistant and arise from cells that survive the initial endothelial cell apoptosis (48, 54) caused by VEGF receptor kinase inhibition (13) and 2) they are characterized by loss of 2 tumor suppressor proteins, caveolin, and PPAR-γ (1, 9). Vascular-obliterating lesions in human SPH are devoid of apoptotic cells and overexpress nitrotyrosine, and their cells have lost the expression of several tumor suppressor genes (1, 4, 9); thus our model of SPH

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**Fig. 4.** Effect of SIM in rat primary pulmonary microvascular endothelial cells (RPMVECs) and rat pulmonary artery smooth muscle cells (RPASMCs). RPMVECs (A, C, and E) and RPASMCs (B, D, and F) were treated with SIM in the absence or presence of SU, N-nitro-L-arginine methyl ester (L-NAME), or mevalonate (Mev) for 18 h. Immunoblotting was performed with the indicated antibodies. Immunoblots are representative of 3 independent experiments. The quantitative analysis (C and D) represents ratios of Casp-3 to β-actin and Rac to β-actin.
Recapitulates some, but not all, major components of the human disease, including dramatic RV hypertrophy, pericardial effusion, and death. Because of the angioproliferative nature of the lesions encountered in our model, treatment trials with antiangiogenic drugs and compounds appeared to be a logical choice, and drugs that are generally not successful in the treatment of lesions encountered in our model, treatment trials with antian-

Simvastatin treatment also, to some degree, normalized the vascular cell phenotype by partially restoring caveolin-1, caveolin-2, and phospho-caveolin expression (Fig. 2, D, F, and H). Recent data from Lisanti’s laboratory have demonstrated the critical role of caveolin-1 in endothelial cell proliferation and maturation (26, 27); loss of caveolin-1 gene expression caused a dramatic reduction in life span (40).

Simvastatin treatment of phenotypically normal RPMVECs caused a 10-fold induction of caspase-3 activity, increased the expression of Rac 1, and caused a 2-fold suppression of RhoA. Sander et al. (43) demonstrated that in NIH3T3 fibroblasts, Rac downregulates Rho activity directly at the GTPase level and that the balance of Rac and Rho activities determines the cellular phenotype. In this context, we consider that simvastatin in our experiments modulated cell proliferation and apoptosis of the vessel-obliterating lesion cells via inhibition of the Rho kinase pathway, given the known effects of Ras GTPase on cell proliferation and apoptosis (19, 28). In RPMVECs, simvastatin also decreased Akt and ERK phosphorylation. The ERK and p38 pathways may regulate endothelial cell-adaptive responses via Nrf2 translocation and activation of antioxidant response elements (5). Antiproliferative effects of the statins have been demonstrated in a large number of ex vivo experiments, and the effects of statins on eNOS expression and activity have been shown by many investigators (6, 11). The ability of HMG-CoA reductase inhibitors to induce apoptosis was demonstrated in rat pulmonary vein endothelial cells (17) and human hepatocytes (22). Recently, Lee et al. (24) have demonstrated that simvastatin inhibits cigarette smoking-induced emphysema and pulmonary hypertension in rat lungs. Here, we show for the first time that simvastatin induces caspase-3 activation and apoptosis in vivo (Fig. 3) as well as in RPMVECs in vitro (Fig. 5). This may be mediated by caspase-dependent and, as recently shown by Kim et al. (20), caspase-independent, RhoA-dependent mechanisms. Dimmel et al. (11) showed that three structurally different statins increased the number of differentiated adherent endothelial cells in vitro. Antiproliferative effects of simvastatin on pulmonary artery smooth muscle cells have also been demonstrated (16, 23). Normally, smooth muscle cells are resistant to Fas or cytokine-induced apoptosis, and simvastatin alone does not lead to activation of the caspase cascade in these cells. Recently, Knapp and coworkers (21) have demonstrated that pharmacological doses of some lipophilic statins, including simvastatin, can sensitize smooth muscle cells to Fas ligand- and cytokine-induced cell death.

Although the vascular protective effects of statins are well documented in cardiovascular diseases (29, 45), here we show for the first time that simvastatin promotes RPMVEC apoptosis in vitro and in vivo. In the monocrotaline/pneumonectomy rat model of SPH, which is characterized by vascular remodeling due to smooth muscle cell growth and/or hyperplasia, Kao and coworkers (34, 36) showed that simvastatin attenuates smooth muscle neointimal thickening and rescues rats from fatal pulmonary hypertension. Our data with cultured pulmonary endothelial cells indicate that simvastatin-induced endothelial cell apoptosis is associated with decreased levels of Akt and ERK

Fig. 5. SIM inhibits proliferation and induces apoptosis of endothelial cells in vitro. A: RPMVECs were incubated with different concentrations of SIM for 18 h. Cell proliferation was assessed using the CyQuant Cell Proliferation Assay Kit. SIM at 1 µM concentration inhibited proliferation of RPMVECs. B: quantitative flow cytometric analysis of RPMVECs treated with 2 µM SIM for 18 h using Vybrant Apoptosis Assay Kit. SIM at 1 µM concentration inhibited proliferation of RPMVECs. The presence of annexin V demonstrated a similar degree of reduction of pulmonary hypertension. Our data with cultured pulmonary endothelial cells indicate that simvastatin-induced endothelial cell apoptosis is associated with decreased levels of Akt and ERK

L674  A SIMVASTATIN AND PULMONARY HYPERTENSION

AJP-Lung Cell Mol Physiol • VOL 291 • OCTOBER 2006 • www.ajplung.org
phosphorylation. We suggest that the improvement of SPH by simvastatin in our rat model is caused by an in vivo induction of apoptosis in phenotypically abnormal pulmonary endothelial cells, partial restoration of the normal vascular architecture, and an associated reduction of PAP and RV pressure. However, it is clear that simvastatin treatment did not restore normal PAP—and that the mechanisms underlying apoptosis of the phenotypically altered endothelial cells in vivo may differ from those governing apoptosis induction in normal endothelial cells in vitro.

Given our previous results, which showed that initial inhibition of apoptosis by a broad-spectrum caspase inhibitor prevented the development of the VEGF receptor blocker/chronic hypoxia-induced SPH (48), it is remarkable that simvastatin induced apoptosis of obliterating phenotypically altered endothelial cells, leading to a significant improvement of this aggressively proliferative form of SPH. We assume that complex interactions take place in our animal model between endothelial cells and vascular smooth muscle cells, leading to obliteration of precapillary arterioles. The precise role of vascular smooth muscle cells in this process is unknown, and it is not clear to what extent simvastatin affected smooth muscle behavior in our model of SPH. Our data in vitro indicate that low doses of simvastatin (2 and 5 μM) did not induce proapoptotic signals in RPASMCs. Recently, Blanco-Colio and colleagues (3) reported that a high dose of simvastatin (100 μM) induced apoptosis in rat thoracic aorta smooth muscle cells. Whether statins could be used clinically to treat SPH in humans is uncertain, but our data should encourage the development of molecular strategies designed to induce apoptosis of phenotypically altered pulmonary vascular cells. Our study shows that precapillary pulmonary arteries that have been completely occluded by phenotypically altered endothelial cells can be reopened by treatment with simvastatin.

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