Simvastatin reduces human atrial myofibroblast proliferation independently of cholesterol lowering via inhibition of RhoA

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Abstract

Objective: Adverse atrial and ventricular myocardial remodeling is characterized by fibrosis, myocyte death or hypertrophy and fibroblast proliferation. HMG-CoA reductase inhibitors (statins) are widely prescribed cholesterol-lowering drugs that also appear to have beneficial effects on myocardial remodeling. Although statins are known to reduce myocyte hypertrophy, their effect on cardiac fibroblast proliferation is unknown. The purpose of this study was to investigate the effects of simvastatin on human atrial myofibroblast proliferation. Methods: Cardiac myofibroblasts were cultured from biopsies of human right atrial appendage. Proliferation was quantified by cell counting and cell cycle progression determined by immunoblotting for Cyclin A. The expression, activation and intracellular localization of RhoA were investigated using immunoblotting and immunocytochemistry. Results: Simvastatin (0.1 – 1.0 $\mu$mol/l) inhibited serum-induced myofibroblast proliferation in a concentration-dependent manner at a point upstream of Cyclin A expression. These effects were reversed by mevalonate or geranylgeranyl pyrophosphate (GGPP), but not squalene or farnesyl pyrophosphate (FPP), indicating a mechanism involving inhibition of Rho-family GTPases and independent of cholesterol synthesis. The effects of simvastatin were mimicked by inhibiting Rho geranylgeranylation or Rho-kinase activation. Furthermore, we demonstrated that simvastatin inhibited RhoA function by preventing its association with the plasma membrane and hence, its interaction with downstream effectors required for cell proliferation. Conclusions: Simvastatin reduced proliferation of cultured human atrial myofibroblasts independently of cholesterol synthesis via a mechanism involving inhibition of RhoA geranylgeranylation. Statins may therefore have an important role in preventing adverse myocardial remodeling associated with cardiac myofibroblast proliferation.

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1. Introduction

Cardiac fibroblasts account for up to two-thirds of the total cells in the normal heart and are responsible for maintaining its structural integrity through controlled proliferation and extracellular matrix turnover. However, fibroblasts are capable of changing from a quiescent phenotype in the normal heart to a proliferative and invasive myofibroblast phenotype, for example, after myocardial infarction [1]. Adverse myocardial remodeling is characterized by fibrosis, cardiac myocyte death, hypertrophy of surviving myocytes and proliferation of cardiac fibroblasts [2–4].

Although these events serve initially as an important adaptive response, ultimately, they contribute to adverse remodeling and depressed contractile function. Similar adverse changes may be of significance in the impairment of cardiac function seen with aging and hypertension [5,6].

In contrast to studies of the ventricle, remodeling of the atrium has been largely neglected, yet it may have important clinical implications in one of the commonest arrhythmias in man, that of atrial fibrillation (AF) [7]. Structural changes in the atria may play an important role in perpetuating AF [8] and indeed in its origin [9,10]. Controlled regulation of cardiac myofibroblast proliferation is therefore essential to the maintenance of both atrial and ventricular architecture.

Statins are now widely prescribed to patients with ischemic heart disease, and it is increasingly clear that they have favorable effects unrelated to lipid lowering [11]. For
example, pravastatin has been claimed to reduce left-ventricular (LV) mass in hypertensive patients [12] and in the 4S study patients receiving long-term simvastatin treatment had a reduced incidence of heart failure [13]. Statins have also recently been shown to prevent stroke [14], and the mechanism is likely to be multifactorial. Finally, in a very recent study, statins were observed to reduce the incidence of AF in patients with coronary artery disease [15].

The cellular effects of statins arise from inhibition of HMG-CoA reductase, a key enzyme in the cholesterol-synthetic pathway, resulting in deprivation of intracellular mevalonate. In addition to its central role in cholesterol synthesis via squalene, mevalonate is also a precursor of several isoprenoid derivatives, including farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP). Both FPP and GGPP are required for posttranslational prenylation of a number of proteins, including small GTPases of the Ras and Rho families [16]. FPP is required for Ras farnesylation and GGPP for Rho geranylgeranylation. Both Ras and Rho are necessary for a diversity of cellular events, and prenylation is essential for their membrane localization and function.

Animal studies have demonstrated that statins have beneficial effects on adverse ventricular myocardial remodeling [17–21]. Moreover, these studies have all utilized normcholesterolemic animals with no induction of atherogenesis; therefore, the reported effects were independent of correction of hyperlipidemia. Although in vivo models are useful for analyzing intact cardiac tissue, they do not permit the examination of individual cell types within the myocardium. When studies have been performed on specific cell types, the focus has been on cardiac myocytes, largely animal and predominantly neonatal [22–24].

In spite of the pivotal role of myofibroblasts in the myocardial remodeling process, the effect of statins on cardiac fibroblast function in man is unknown. The present study was designed to investigate whether simvastatin, a commonly prescribed statin, can inhibit human atrial myofibroblasts proliferation, and if so, to identify the intracellular mechanisms involved.

2. Methods

2.1. Reagents

All cell culture reagents were purchased from Invitrogen (Paisley, UK), with the exception of fetal calf serum (FCS) that was from LabTech International (Ringmer, East Sussex, UK). Cyclin A antibody and agarose-conjugated rhotekin-RBD were from Upstate Biotechnology (Milton Keynes, UK). Phospho-specific p44/p42-mitogen-activated protein kinase (MAPK) and p44/p42-MAPK expression antibodies were obtained from Cell Signaling Technology (Hitchin, Herts., UK), and the RhoA and epidermal growth factor (EGF) receptor antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Horseradish peroxidase-conjugated secondary antibodies were purchased from Amersham Life Science (Amerham, Bucks., UK). Vimentin antibody and FITC- and Cy3-conjugated secondary antibodies were from Jackson ImmunoResearch Laboratories (West Grove, PA, USA). Simvastatin was a gift from Merck, Sharp and Dohme (Hoddesdon, Herts., UK). α-Smooth muscle actin (α-SMA) monoclonal antibody, mevalonate, squalene and platelet-derived growth factor-BB (PDGF) were obtained from Sigma (Poole, Dorset, UK). GGPP and FPP were from Alexis Biochemicals (Nottingham, UK), and manumycin A, GGTI-286 and Y27632 were from Calbiochem (Nottingham, UK).

2.2. Human atrial myofibroblast culture

Biopsies of right atrial appendage (approximately 100 mg) were obtained from patients without LV dysfunction (ejection fraction normal by cardiac ultrasound and/or LV angiography) undergoing elective coronary artery bypass surgery. Local ethical committee approval and informed patient consent were obtained. Primary cultures of cardiac myofibroblasts were harvested, characterized by immunofluorescence microscopy and cultured as we have described previously [25]. Experiments were performed on cells from passages 2–5 from more than 20 different patients. The investigations conformed to the principles outlined in the Declaration of Helsinki, 1997.

2.3. Proliferation assays

Cardiac myofibroblasts were trypsinized and plated into 24-well tissue culture plates at a density of 2 × 10⁴ cells/well in growth medium comprising Dulbecco’s Modified Eagle’s Medium supplemented with 10% FCS. After incubation overnight, cells were rendered quiescent in serum-free medium (SFM) for 72 h before addition of fresh growth medium containing the appropriate supplements. Medium and drugs were replaced every 2 days. Cell number was determined in triplicate using a hemocytometer and comparisons made by measuring areas under growth curves.

2.4. Immunoblotting

Sub-confluent myofibroblasts were incubated in SFM for 72 h before exposure to growth medium (10% FCS) in the presence of appropriate supplements for up to 24 h at 37 °C. Whole cell homogenates were prepared, protein-standardized and immunoblotted as described previously [26]. Antibodies for Cyclin A, vimentin, phospho-p44/p42-MAPK, p44/p42-MAPK expression, RhoA and EGF receptor were diluted 1:350, 1:2000; 1:1000, 1:500, 1:200 and 1:1000, respectively. Densitometric analysis was performed using a
2.5. RhoA activation assay

RhoA activation was determined using an affinity precipitation assay incorporating the Rho-binding domain (RBD) of rhotekin, which binds only the active GTP-bound form of Rho [27]. Sub-confluent cardiac myofibroblasts were incubated in SFM for 48 h before addition of appropriate supplements in SFM for a further 24 h. Cells were then stimulated with 10% FCS for 20 min at 37 °C before addition of lysis buffer (25 mmol/l HEPES, pH 7.5, 150 mmol/l NaCl, 1% Igepal CA-630, 10 mmol/l MgCl₂, 1 mmol/l EDTA, 10% glycerol, 1 μg/ml aprotinin, 10 μg/ml leupeptin and 1 mmol/l Na₃VO₄) at 4 °C. Whole cell lysates were incubated with agarose-conjugated rhotekin-RBD for 45 min at 4 °C then washed three times with lysis buffer. Agarose beads were boiled in SDS-PAGE sample buffer to release active Rho.
and samples resolved on a 12.5% polyacrylamide gel followed by immunoblotting with RhoA antibody.

### 2.6. RhoA immunofluorescence confocal microscopy

Cardiac myofibroblasts were grown to ~50% confluence on Lab-Tek chamber slides. After serum deprivation for 48 h, cells were exposed to appropriate supplements in SFM for a further 24 h. Myofibroblasts were subsequently exposed to 10% FCS for 40 min, then immediately fixed in 4% paraformaldehyde for 30 min and permeabilized with 1% Triton X-100. Slides were incubated with RhoA antibody (1:100) at 4°C overnight, followed by Cy3-conjugated anti-mouse antibody (1:200) at room temperature for 4 h in the dark. Image analysis was performed using a Zeiss LSM-510 confocal microscope.

### 2.7. Preparation of membrane fractions

After serum deprivation for 48 h, cardiac myofibroblasts were exposed to simvastatin with or without GGPP for 24 h before addition of 10% FCS for 40 min. Membrane fractions were prepared using the ProteoExtract subcellular proteome extraction kit (Calbiochem) according to the manufacturer’s instructions. Equal volumes of membrane samples were resolved by SDS-PAGE and immunoblotted for RhoA as described above. Equal loading of samples was confirmed by immunoblotting for the EGF receptor. Membrane fractions were also analyzed for RhoA activation using the rhotekin affinity precipitation assay (Section 2.5).

### 2.8. Statistical analysis

All results are expressed as mean ± S.E.M. Analysis of variance (ANOVA) and paired t-tests were performed for statistical analysis as appropriate. P<0.05 was considered to be statistically significant.

### 3. Results

#### 3.1. Simvastatin inhibits human atrial myofibroblast proliferation

Immunofluorescent characterization of cells cultured from human atrial appendage revealed coexpression of vimentin and α-SMA (Fig. 1), indicative of a myofibroblast phenotype comparable with the activated myofibroblasts that play a pivotal role in the myocardial remodeling process [28]. Homogeneity of cell populations was maintained throughout passages 2–5 (data not shown).

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**Fig. 3. Effect of passage number and serum starvation period on the antiproliferative effect of simvastatin.** Left panel: The inhibitory effect of 1 μmol/l simvastatin on 10% FCS-induced proliferation was compared in cells of passage numbers 1–5 from different patients. Right panel: The inhibitory effect of 1 μmol/l simvastatin on 10% FCS-induced proliferation was compared in P3 cells from two different patients that were serum starved for either 72 or 24 h. All data are expressed as % control cell number after 5 days.

**Fig. 4. Effect of mevalonate and squalene.** (A) Cells were treated with 10% FCS alone (control, C) or supplemented with 1 μmol/l simvastatin (SIM) alone or in combination with 100 μmol/l mevalonate (MEV) or 10 μmol/l squalene (SQ). Data are expressed as % control cell number after 5 days. **P<0.01 vs. control (n=3).** (B) Cells were treated for 24 h exactly as described above before measuring Cyclin A expression. Associated densitometry data are expressed as % control. ***P<0.001 vs. control (n=3–4). Equal loading of samples was confirmed by immunoblotting with vimentin antibody.
Simvastatin significantly inhibited 10% FCS-induced myofibroblast proliferation in a concentration-dependent manner (P < 0.0001, ANOVA) (Fig. 2A). Proliferation was reduced by 34% (P < 0.01, Student’s t-test), 47% (P < 0.01) and 68% (P < 0.001) with 0.1, 0.5 and 1 μmol/l simvastatin, respectively. Simvastatin had no effect on cell viability as determined by Trypan Blue exclusion and counting detached cells in the medium (data not shown).

Statins have been shown in other cell types to arrest growth in the G1 phase of the cell cycle [29–31]. We therefore used immunoblotting to monitor the expression of Cyclin A, a protein specifically expressed during S-phase. Cyclin A expression was undetectable in quiescent cardiac myofibroblasts, but increased in a time-dependent manner commencing 16–18 h after stimulation with 10% FCS, with marked expression apparent after 22–24 h (Fig. 2B). Simvastatin (0.1–1 μmol/l) inhibited Cyclin A expression in a concentration-dependent manner (Fig. 2C), confirming that proliferation was inhibited at a point upstream of Cyclin A expression.

To investigate any effect of passage number on the anti-proliferative effect of simvastatin, we performed experiments on P1–P5 cells and compared the degree of inhibition induced by 1 μmol/l simvastatin. As shown in Fig. 3 (left panel), the anti-proliferative effect of simvastatin was comparable between cells of different passage number. We also investigated whether the duration of serum starvation influenced the anti-proliferative effect of simvastatin. As shown in Fig. 3 (right panel), simvastatin had similar effects on cells that were serum starved for 24 or 72 h. All subsequent proliferation assays were therefore performed on P2–P5 cells that were serum-starved for 72 h.

### 3.2. Reversal of the anti-proliferative effect of simvastatin by mevalonate but not squalene

Inhibition of HMG-CoA reductase by simvastatin reduces intracellular levels of mevalonate. As expected, co-incubation with mevalonate prevented the inhibitory effects of simvastatin on myofibroblast proliferation (Fig. 4A) and Cyclin A expression (Fig. 4B). To determine whether the anti-proliferative effect of simvastatin was due...
to inhibition of cholesterol synthesis, we examined the ability of squalene, an essential intermediate in the synthesis of cholesterol from mevalonate, to reverse its effects. Supplementation with squalene did not overcome the inhibitory effect of simvastatin on cell proliferation (Fig. 4A) or Cyclin A expression (Fig. 4B), indicating a mechanism independent of effects on cholesterol synthesis.

3.3. Reversal of the anti-proliferative effect of simvastatin by GGPP but not FPP

We next investigated whether co-incubation with isoprenoid metabolites of mevalonate could reverse the anti-proliferative effects of simvastatin. Repeated exposure of myofibroblasts to 10 μmol/l GGPP resulted in a tendency for cells to become detached (data not shown). Cells were therefore counted after a 3-day treatment interval and the effects of GGPP compared between groups treated with FCS alone and those receiving FCS plus simvastatin (Fig. 5A). For consistency, the effects of FPP were determined in the same way. GGPP supplementation prevented the inhibitory effects of simvastatin on both proliferation (Fig. 5A) and Cyclin A expression (Fig. 5B), indicating a mechanism involving inhibition of geranylgeranylation. In contrast, FPP had no effect, thus eliminating an anti-proliferative mechanism related to inhibition of farnesylation and subsequent Ras activation. This was further strengthened by our observation that
Simvastatin had no effect on PDGF-induced p44/p42-MAPK activation, a Ras-dependent process that was inhibited by manumycin A, a farnesyl transferase inhibitor (Fig. 6).

3.4. GGTase-I and Rho kinase inhibition mimic the anti-proliferative effects of simvastatin

To determine whether geranylgeranylation was essential for FCS-induced proliferation, we investigated the effects of GGTI-286, a specific inhibitor of geranylgeranyl transferase-I (GGTase-I), the enzyme that regulates geranylgeranylation of Rho-family GTPases. GGTI-286 significantly inhibited FCS-induced myofibroblast proliferation (Fig. 7A) and Cyclin A expression (Fig. 7B).

Previous studies have shown that a number of the downstream effects of Rho activation are mediated via activation of Rho-kinase (ROCK). ROCK is activated by RhoA, but not Rac or cdc42 [16]. We therefore determined the effects of the ROCK inhibitor Y27632 on myofibroblast proliferation. Y27632 significantly inhibited FCS-induced proliferation (Fig. 7A) and Cyclin A expression (Fig. 7B).

3.5. Effect of simvastatin on RhoA expression, activation and localization

The data presented thus far suggested that inhibition of Rho geranylgeranylation was the most likely mechanism to explain the anti-proliferative effects of simvastatin on human cardiac myofibroblasts. We therefore investigated the effects of simvastatin on RhoA expression, activation and localization.

A 24-h pretreatment with simvastatin resulted in a fourfold increase in cellular RhoA expression, an effect mimicked by GGTI-286 (Fig. 8A). The stimulatory effect

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Fig. 9. RhoA localization using immunofluorescence microscopy. RhoA was localized in both the cytosol and membrane in quiescent cells in SFM (A); however, exposure to FCS for 40 min resulted in a marked redistribution to the plasma membrane (arrowheads) (B). Simvastatin (SIM; 1 μmol/l) prevented FCS-induced RhoA translocation (C), an effect reversed by 10 μmol/l GGPP (D), but not 10 μmol/l FPP (E). GGTI-286 (GGTI; 10 μmol/l) also prevented FCS-induced RhoA translocation (F). Note also the increased intensity of fluorescence following treatment with simvastatin (C and E) or GGTI-286 (F). Scale bar = 25 μm. Figure is representative of three separate experiments.
of simvastatin on RhoA expression was reversed by GGPP but not FPP (data not shown).

Minimal amounts of active (i.e., GTP-bound) RhoA were observed in serum-starved cells; however, RhoA activation increased significantly following 20 min exposure to 10% FCS (Fig. 8B). Interestingly, neither simvastatin nor GGTI-286 inhibited FCS-induced activation of total cellular RhoA; on the contrary, a trend towards increased activation was observed (Fig. 8B).

Upon activation, RhoA translocates from the cytosol to the plasma membrane, a phenomenon that requires geranylgeranylation of Rho [16]. Using immunofluorescence confocal microscopy, we investigated the effects of simvastatin on the intracellular localization of RhoA. RhoA was located in both the cytosol and membrane of serum-starved cells (Fig. 9A). Stimulation with FCS (40 min) resulted in increased association of RhoA at the plasma membrane (Fig. 9B). Simvastatin pretreatment (24 h) prevented this translocation (Fig. 9C), an effect restored by GGPP (Fig. 9D) but not FPP (Fig. 9E). Moreover, GGTI-286 mimicked the effects of simvastatin by preventing translocation of RhoA (Fig. 9F). In agreement with our immunoblotting data (Fig. 8A), we also observed increased cytosolic expression of RhoA in cells treated with simvastatin (Fig. 9C) or GGTI-286 (Fig. 9F). This simvastatin-induced increase in RhoA expression was prevented by supplementation with GGPP (Fig. 9D), but not FPP (Fig. 9E).

To quantify changes in membrane localization, we used immunoblotting to determine the levels of membrane-associated RhoA (Fig. 10A). Simvastatin treatment almost completely abolished association of RhoA with the membrane, an effect fully reversed by co-incubation with GGPP. Having already determined that simvastatin did not reduce total cellular RhoA activation (Fig. 8B), we proceeded to study the effect of simvastatin on membrane-associated RhoA activation. As shown in Fig. 10B, FCS-induced activation of membrane-associated RhoA was reduced by 90% following treatment with simvastatin.

4. Discussion

The weight of evidence from animal studies [17–21] suggests that the beneficial effects of statins on myocardial remodeling occur independently of the cholesterol-lowering properties of this drug class. These observations in animals are further supported by in vitro evidence that statins prevent hypertrophy of cultured rat cardiac myocytes [22–24]. Although previous studies have demonstrated that statins inhibit proliferation in a variety of cell types, including vascular smooth muscle cells [31–33], their effects on cardiac fibroblast/myofibroblast proliferation are unreported. Since excessive cardiac myofibroblast proliferation is a well-recognized feature of both atrial and ventricular remodeling, we investigated whether simvastatin could attenuate proliferation of human atrial fibroblasts in vitro. The cells used in the present study were myofibroblasts that are commonly observed in primary cultures of fibroblasts due to phenotypic drift [34]. Herein, we demonstrate that therapeutic concentrations of simvastatin significantly reduce myofibroblast proliferation and furthermore provide the mechanism (inhibition of the RhoA/ROCK pathway) through which this is achieved.

Previous studies in other cell types have shown that statins can inhibit cell cycle progression in the late G1 phase by regulating cyclins and/or cyclin-dependent kinase (CDK) inhibitors, although the exact mechanisms vary between cell types [29,30]. These anti-proliferative effects can be reversed by GGPP [23,29,32], indicating a mechanism involving inhibition of geranylgeranylation. Rho-family GTPases can induce cell cycle progression via upregulation of G1 phase cyclins and degradation of CDK inhibitors [35]. The resultant activation of CDKs induces phosphorylation of retinoblastoma (Rb) protein, ultimately...
leading to increased transcription of S-phase cell cycle genes (e.g., Cyclin A). Our data support these observations by clearly demonstrating that simvastatin inhibits cell cycle progression at a point upstream of Cyclin A expression.

Our studies indicate that the principal effect of simvastatin on RhoA function is to prevent its membrane association rather than inhibiting its activation per se. Simvastatin and GGTI-286, an inhibitor of Rho-family geranylgeranlylation, increased cellular RhoA expression by three- to fourfold, probably due to a negative feedback mechanism similar to that previously described in endothelial cells [36]. Simvastatin did not reduce total cellular RhoA activation, but rather, we observed a trend towards increased activation. The lack of an inhibitory effect of simvastatin on RhoA activation is not unexpected given that activation of Rho (i.e., binding of GTP) precedes membrane translocation and does not require Rho to be prenylated [37]. Immunofluorescence microscopy revealed that RhoA translocated from the cytosol to the plasma membrane following stimulation with FCS, as previously reported in other cell types [38]. Simvastatin treatment markedly reduced the membrane association of RhoA due to inhibition of geranylgeranlylation with a resultant loss of membrane-associated active RhoA, in agreement with previous reports in endothelial cells [36,39,40]. Simvastatin therefore prevents membrane association of active RhoA and disrupts its targeting to the downstream effectors necessary for cell proliferation (including membrane-associated ROCK). This proposed mechanism for the anti-proliferative effect of simvastatin on human atrial myofibroblasts is summarized in Fig. 11.

Two recent studies reported a role for Rho and ROCK in the pathogenesis of cardiac dysfunction and demonstrated that myocardial RhoA expression was elevated in rat [41] and canine [42] models of heart failure. Furthermore, inhibition of ROCK reduced LV remodeling [41]. Modulation of Rho/ROCK function may therefore represent a useful strategy for the control of myocardial remodeling, a scenario in which statins may offer therapeutic potential.

A major strength of our study was the use of adult human cardiac myofibroblasts, as opposed to animal (often neonatal) cells that are more commonly employed for studying fibroblast function. Primary cultures of animal cardiac fibroblasts have been shown to exhibit important differences in their biological properties compared with human cardiac fibroblasts. For example, young adult human cardiac fibroblasts exhibit significant differences in their proliferative responses compared with equivalent rabbit cardiac fibroblasts [43]. Furthermore, significant differences in biological responses are observed between neonatal and adult cardiac fibroblasts from the same animal species [44]. While studies using animal cells provide useful data, it cannot be assumed, however, that human cardiac fibroblasts will behave similarly. Characterization of the cells used in the present study showed them to be myofibroblasts, the act-

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**Fig. 11.** Schematic representation of the proposed mechanism underlying the anti-proliferative effect of simvastatin on human atrial myofibroblasts. In the absence of statin, HMG-CoA reductase catalyses the conversion of HMG-CoA to mevalonate which is a precursor for GGPP. FCS treatment of myofibroblasts leads to activation (GTP/GDP exchange) and membrane translocation of RhoA. At the plasma membrane, GGTase-I catalyses the geranylgeranlylation of active RhoA, thus anchoring it at the membrane. RhoA then activates membrane-bound ROCK which is required for induction of Cyclin A expression and cell proliferation. Inhibition of HMG-CoA reductase by simvastatin reduces the availability of GGPP, which in turn decreases membrane attachment of active RhoA. This leads to reduced ROCK activation and Cyclin A expression, thereby inhibiting cell proliferation. GGTI-286, a GGTase-I inhibitor, or Y27632, a ROCK inhibitor, can mimic the anti-proliferative effects of simvastatin.
vated fibroblasts that play a role in myocardial remodeling. Whether atrial and ventricular myofibroblasts respond differently to statins is an intriguing issue worthy of further study. Our observations seem likely to apply to statins as a class of drugs, but an important factor in our study was the use of simvastatin at concentrations that may be achieved therapeutically (Merck, Sharpe and Dohme, unpublished data, 1999).

In conclusion, we report here for the first time that simvastatin inhibits proliferation of human atrial myofibroblasts via a mechanism involving inhibition of the RhoA/ROCK pathway and subsequent cell cycle arrest, important effects independent of cholesterol lowering. These findings highlight a potential mechanism by which HMG-CoA reductase inhibitors may reduce adverse remodeling of the heart in man, with important implications for the potential future use of statins.

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