Atorvastatin attenuates angiotensin II-induced inflammatory actions in the liver

Montserrat Moreno, 1* Leandra N. Ramalho, 1* Pau Sancho-Brú, 1 Marta Ruiz-Ortega, 2 Fernando Ramalho, 1 Juan G. Abraldes, 1 Jordi Colmenero, 1 Marlene Domínguez, 1 Jesús Egido, 2 Vicente Arroyo, 1 Pere Ginès, 1 and Ramón Bataller 1

1Liver Unit, Institut de Malalties Digestives i Metabòliques, Hospital Clínic, Institut d’Investigacions Biomèdiques August Pi i Sunyer, Centro de Investigación Biomédica Esther Koplowitz, Barcelona, Catalonia, Spain; and 2Vascular and Renal Research Laboratory, Fundación Jiménez Díaz, Universidad Autònoma, Madrid, Spain.

Submitted 6 October 2007; accepted in final form 21 November 2008

Atorvastatin attenuates angiotensin II-induced pathogenic effects in the liver. Male Wistar rats were infused with saline or angiotensin II (100 ng·kg⁻¹·min⁻¹) for 4 wk through a subcutaneous osmotic pump. Rats received either vehicle or atorvastatin (5 mg·kg⁻¹·day⁻¹) by gavage. Angiotensin II infusion resulted in infiltration of inflammatory cells (CD43 immunostaining), oxidative stress (4-hydroxynonenal), hepatic stellate cells (HSC) activation (smooth muscle α-actin), increased intercellular adhesion molecule (ICAM-1), and interleukin-6 hepatic gene expression (quantitative PCR). These effects were markedly blunted in rats receiving atorvastatin. The beneficial effects of atorvastatin were confirmed in an additional model of acute liver injury (carbon tetrachloride administration). We next explored whether the beneficial effects of atorvastatin on angiotensin II-induced actions are also reproduced at the cellular level. We studied HSC, a cell type with inflammatory and fibrogenic properties. Angiotensin II (10⁻⁷M) stimulated cell proliferation, proinflammatory actions (NF-κB activation, ICAM-1 expression, interleukin-8 secretion) as well as expression of procollagen-α1(1) and TGF-β1. All of these effects were reduced in the presence of atorvastatin (10⁻⁷M). These results indicate that atorvastatin attenuates the pathogenic events induced by angiotensin II in the liver both in vivo and in vitro. Therefore, statins could have beneficial effects in conditions characterized by hepatic inflammation.

chronic inflammation of the hepatic parenchyma eventually leads to fibrosis. Liver fibrosis is the excessive accumulation of extracellular matrix proteins, including collagen, which occurs in most types of chronic liver diseases. Advanced liver fibrosis results in cirrhosis, liver failure, and portal hypertension and often requires liver transplantation (2). Drugs capable of attenuating inflammation and/or fibrosis progression in patients with chronic liver diseases are currently under investigation.

Experimental and clinical data strongly indicate that the renin-angiotensin system may play a major role in liver fibrosis by promoting inflammation and collagen synthesis (2, 7). Angiotensin II (ANG II), the main effector of this system, exerts an array of inflammatory and fibrogenic actions in hepatic stellate cells (HSC), the major fibrogenic cell type in the injured liver (5). Moreover, we previously demonstrated that ANG II infusion into normal rats induces HSC activation and proinflammatory events in the liver (3, 4). Most importantly, pharmacological inhibition of the renin-angiotensin system attenuates liver fibrosis in rodents (13, 18, 22, 31, 33, 35, 42, 46, 47, 49).

A large body of evidence indicates that 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, so-called statins, have beneficial properties in atherosclerosis (38, 43). Moreover, statins modulate the deleterious effects of the renin-angiotensin system in several organs (34). Atorvastatin, a widely used statin, reduces NF-κB activation and chemokine expression induced by ANG II in vascular smooth muscle cells (32), as well as reduces free radical production (44). Statins also exert anti-inflammatory and antifibrogenic activity in the kidney in vitro and in vivo (10, 29). These effects are due to a decrease in serum lipid levels and to its lipid-independent, pleiotropic effects.

Recent reports suggest that atorvastatin may have beneficial effects in patients with nonalcoholic steatohepatitis associated with the metabolic syndrome, suggesting a potential usefulness for this drug in the treatment of chronic liver diseases (20). In addition, lovastatin and simvastatin inhibit cell growth of cultured HSC (36). The combinatory use of pitavastatin and candesartan, an ANG II receptor, type 1 (AT1) blocker, inhibits liver fibrogenesis in carbon tetrachloride (CCL4)-treated rats (28). Nevertheless, simvastatin, used without an ANG II type 1-receptor blocker, does not seem to affect liver fibrogenesis in vivo (30).

To provide novel insights on the potential effects of statins on liver inflammation, the current study investigates whether atorvastatin modulates the pathogenic effects of ANG II on the liver both in vitro and in vivo as well as its effect on a model of acute liver injury (CCL4-induced liver damage). Here, we provide evidence that atorvastatin markedly reduces the deleterious effects induced by ANG II and CCL4. These results reinforce the hypothesis that statins may have beneficial effects in patients with chronic liver diseases.

* M. Moreno and L. N. Ramalho contributed equally to this work.

Address for reprint requests and other correspondence: R. Bataller, Liver Unit, Hospital Clinic, Villarroel 170, 08036-Barcelona, Catalonia, Spain (e-mail: bataller@clinic.ub.es).

http://www.ajpgi.org 0193-1857/09 $8.00 Copyright © 2009 the American Physiological Society

G147
Experimental Procedures

Experimental protocol. For the ANG II experimental model, male 200 g Wistar rats were infused with either saline or ANG II (Sigma-Aldrich, St. Louis, MO) at a dose of 100 ng·kg⁻¹·min⁻¹, dissolved in saline through an osmotic minipump (Alza, Palo Alto, CA) for 4 wk as described previously (4). Minipumps were placed subcutaneously and replaced after 2 wk. Rats were treated daily with either vehicle or atorvastatin (Pfizer, Madrid, Spain) (at 5 mg·kg⁻¹·day⁻¹) by gavage. Before death, systolic arterial pressure was measured by a tail-cuff plethysmograph (Narco Bio-Systems, Houston, TX), as previously described (11). Ten rats were included in each group. At the end of the infusion period, rats were weighed and killed and liver and blood samples were harvested. For the CCl₄ experimental model, rats were administered vehicle, atorvastatin (at 5 mg·kg⁻¹·day⁻¹), losartan (Pfizer, Madrid, Spain) (at 10 mg·kg⁻¹·day⁻¹), or atorvastatin plus losartan by gavage at day 0 and at day 1. At day 1 rats were also administered vehicle (olive oil) or 30% CCl₄ (Sigma, Madrid, Spain) (at 1 ml/kg) by a single intraperitoneal injection. Twenty-four hours later, rats were killed and liver and blood samples were harvested. Eight rats were included in each group. Animal protocols were reviewed and approved by the local committee according to the guidelines for ethical care of experimental animals of the European Community.

Biochemical analysis. Serum alanine aminotransferase (ALT) was measured with an automatic biochemical analyzer.

Histological analysis. Livers were fixed in 10% phosphate-buffered formalin for 24 h at room temperature and then embedded in paraffin. Liver inflammation and fibrosis were assessed in 5-μm sections, which were stained with hematoxylin and eosin and Sirius red, respectively. Samples were blindly scored by an expert pathologist (L. N. Ramalho). For the ANG II infusion model, the scoring system used was: inflammation (0 = absence, 1 = mild, 2 = moderate, 3 = severe) and fibrosis (0 = absence, 1 = portal fibrosis, 2 = portal fibrosis and few septa, 3 = evident septal fibrosis without cirrhosis, 4 = cirrhosis) (9). For the CCl₄ model, hepatic necroinflammation was estimated by quantifying the presence of necrosis, hepatocytes ballooning, and/or swelling, inflammatory cell infiltration, and lipid droplets. The degree of necroinflammatory changes was assessed as the percentage of hepatic parenchyma with any of the above-described changes: 0 = lower than 20%; 1 = 20–40%; 2 = 40–60%; 3 = 60–75%; 4 = >75%. For immunohistochemical analysis, sections were deparaffinized, rehydrated, and stained by using the Dako Envision system (Dako, Carpinteria, CA). Sections were incubated with anti-CD43 (1:1,000; Serotec, Raleigh, NC), anti-(E)-4-hydroxy-3-(4-hydroxyphenyl)acrylamide (H-4-HT) (1:500, Chemicon, Temecula, CA) for 30 min at room temperature. As negative controls, all specimens were incubated with an isotype-matched antibody. Morphometric assessments were performed using an optic microscope (Eclipse E600; Nikon, Kanagawa, Japan) connected to a high-resolution camera (model CC12; Soft-Imaging System, Münster, Germany) as described previously (12).

Analysis of gene expression. RNA was isolated from either frozen liver samples and cultured cells using RNeasy mini kit (Hilden, Germany) and Trizol (Life Technologies, Rockville, MD), respectively. Retroscription was performed to obtain cDNA. Quantitative PCR was performed with predesigned TaqMan Gene Expression Assay probes and primer pairs for collagen-α₁, transforming growth factor-β₁ (TGF-β₁), intercellular adhesion molecule-1 (ICAM-1), interleukin-6 (IL-6), Rac1, AT1, and ribosome subunit 18S, as described previously (40). Information on these assays is available at: http://www.appliedbiosystems.com. TaqMan reactions were carried out in duplicate on an ABI PRISM 7900 Machine (Applied Biosystems, Foster City, CA). Results were normalized to 18S expression. Results are expressed as fold respect to saline.

Isolation and culture of primary human HSC. HSC were isolated from fragments of normal human livers obtained from resections of liver metastasis, as described previously (5). Briefly, liver tissues were digested by two enzymatic solutions. First, digestion was performed in Gey’s balanced salt solution containing 0.33% pronase, 0.035% collagenase, and 0.001% DNase for 30 min at 37°C (all from Roche Diagnostics, Mannheim, Germany). Second, digestion was performed in Gey’s balanced salt solution containing 0.06% pronase, 0.035% collagenase, and 0.001% DNase for 30 min at 37°C. The resulting cell pellet was centrifuged over a gradient of 10% Nycodenz (Sigma-Aldrich). Average yield per isolation was 5×10⁶ cells/g liver. A subset of immunocytochemistry studies was performed in HSC freshly isolated from normal human livers (quiescent phenotype). In all cell cultures, no staining was found for CD45, factor VIII-related antigens, and CAM 5.2 (Dako), indicating the absence of mononuclear, macrophagic, endothelial, and epithelial cells. HSC were studied after the second serial passage (culture-activated phenotype). Cells were cultured in standard conditions in DMEM (BioWhittaker, Verviers, Belgium) containing 15% fetal bovine serum, glutamine, sodium pyruvate, nonessential amino acids and insulin. Cells were serum starved for at least 12 h before the experiments. The protocol was approved by the Ethical Committee of the Hospital Clinic of Barcelona.

Immunocytochemistry studies. HSC were stimulated for 12 h with agonists in the presence or absence of atorvastatin (10⁻⁷ M). Cells were then fixed in methanol at −20°C for 10 min, blocked in PBS containing 0.1% BSA for 30 min, and incubated with anti-p65 for 1 h (Santa Cruz Biotechnology, Santa Cruz, CA). Cells were incubated with fluorescein-labeled secondary antibody for 1 h. An isotype-matched antibody was used as a negative control. The p65 nuclear translocation was estimated as the media of the index of nuclear/cytoplasmatic staining in 10 fields at ×400 magnification.

Cell proliferation assay. DNA synthesis was estimated by [methyl-³H]-labeled thymidine (Amersham Biosciences, Buckinghamshire, UK) incorporation, as described in detail previously (6). Cells were serum starved for 24 h, stimulated for 18 h with agonists in the presence or absence of atorvastatin (10⁻⁷ M), and then pulsed for 6 h with 1 μCi/ml [methyl-³H]-labeled thymidine. Results are expressed as fold stimulation compared with cells incubated with buffer.

IL-8 and TGF-β₁ secretion. HSC were cultured in six-well plates at a density of 4×10⁵ cells/well for 24 h. Medium was removed, and cells were incubated in serum-free medium for 24 h in the presence of agonists. Supernatants were collected, and a sandwich ELISA for human IL-8 (BLK Diagnostics, Barcelona, Spain) (39), or TGF-β₁ (R&D Systems, Minneapolis, MN) was performed.
Western blot analysis. Whole cell extracts were obtained in lysis buffer containing protease and phosphatase inhibitors. Fifty micrograms were loaded onto 12% SDS acrylamide gels, electrophoresis was carried out, and proteins were blotted onto nitrocellulose membranes. Membranes were blocked for 2 h with nonfat milk and incubated with antibodies against AT1 (1:200; Santa Cruz Biotechnology), or phospho-extracellular-regulated kinase (1:1,000; Cell Signaling, Beverly, MA) overnight at 4°C. After extensive washing, membranes were incubated with horseradish peroxidase-conjugated secondary antibody. Proteins were
detected by enhanced chemiluminescence (Amerham, Buckinghamshire, UK).

**Rac1 pull-down assay.** Rac1 activity was determined by a pull-down assay kit (Assay Designs, Ann Arbor, MI). Briefly, after incubation with agonists, cell extracts were obtained in lysis buffer containing protease inhibitors. Part of the lysates was used to analyze total Rac1, and the rest was incubated with GST-human Pak1-PBD to pull down active Rac1 in the presence of a glutathione disc at 4°C for 1 h. After incubation, the mixture was centrifuged at 7200 g for 30 s to remove unbound proteins. The resins were rinsed with washing buffer, and the samples were eluted by adding 50 μl of SDS sample buffer. Half of the sample volume was loaded onto 12% SDS acrylamide gel, electrophoresis was carried out, and proteins were transferred onto a nitrocellulose membrane. Active Rac1 was detected by using a specific mouse monoclonal anti-Rac1 antibody diluted 1:1,000. Goat anti-mouse antibody conjugated with horseradish peroxidase (1:3,000; Cell Signaling, Beverly, MA) was used as the secondary antibody. Proteins were detected by enhanced chemiluminescence (Amerham, Buckinghamshire, UK).

**Data analysis.** Data presented herein are expressed as means ± SE. Histology data, liver serum enzymes, and gene expression are means of at least eight animals per group. In vitro assays are representative of five independent experiments. Statistical analysis was performed by Student’s t-test for pairwise comparisons and analysis of variance with a post hoc test of Tukey for multiple comparisons. The Kruskall-Wallis test with a post hoc Dunn’s test was used for multiple nonparametric analyses.

**RESULTS**

**Atorvastatin reduces ANG II-induced inflammation and oxidative stress in the liver.** ANG II infusion, either with saline or atorvastatin, was well tolerated in all rats. No rats showed noticeable side effects. ANG II infusion induced a marked increase in arterial pressure (157 ± 5 and 118 ± 7 mmHg in rats receiving ANG II and saline, respectively, P < 0.01). Concomitant administration by atorvastatin did not decrease ANG II-induced arterial hypertension (147 ± 18 mmHg, P = not significant vs. ANG II plus saline). ANG II induced a slight increase in ALT serum levels (42 ± 5.5 U/l vs. 28 ± 4.6 U/l in ANG II and saline-treated rats, respectively; P < 0.05). Histological examination of ANG II-infused livers showed preserved hepatic parenchyma with no apparent hepatocyte damage. Infiltration of mononuclear cells and thickening of the limiting membrane were observed in most portal tracts (Fig. 1A). The median inflammatory score in ANG II-treated rats was 2, while no inflammation was seen in saline-infused rats (Fig. 1B). To further demonstrate that ANG II infusion results in hepatic inflammation, infiltrating inflammatory cells were stained with CD43, a pan-leukocyte antibody. CD43 is typically expressed by infiltrating mononuclear cells and lymphocytes. Quantification of CD43-positive cells showed that ANG II infusion increased the amount of inflammatory cells infiltrating the hepatic parenchyma (Fig. 1, C and D). Concomitant treatment with atorvastatin, but not saline, resulted in reduced inflammatory changes, both the inflammatory degree (median inflammatory scores 1 and 2, respectively, P < 0.05) and the amount of CD43 positive infiltrating cells. As previously shown, ANG II infusion into normal rats did not cause parenchymal fibrosis (4). The degree of liver fibrosis and the amount of Sirius red staining as assessed using a computer-based morphometric method did not differ from rats receiving saline and ANG II (data not shown). HSC activation, as indicated by α-SMA immunostaining, was slightly increased in ANG II-treated rats. Atorvastatin treatment blunted this effect (Fig. 2, A and B).

**Atorvastatin modulates ANG II-induced expression of inflammatory and fibrogenic genes in the liver.** We previously demonstrated that ANG II stimulates the expression of genes involved in hepatic inflammation and fibrogenesis both in vivo and in vitro (3, 4, 8). We next studied whether atorvastatin modulates these effects. We assessed key genes involved in hepatic fibrogenesis [procollagen-α1(1) and TGF-β1], inflammation (ICAM-1, and IL-6), a chief component of the nonphagocytic NADPH oxidase (Rac1), and a key component of the renin-angiotensin system (AT1). ANG II infusion induced an upregulation of genes involved in fibrogenesis, inflammation, and Rac1, as described in Fig. 3. Atorvastatin treatment significantly reduced the expression of TGF-β1, IL-6, and Rac1. AT1 expression was not modified by any treatment. These results suggest that infusion of ANG II to normal rats stimulates inflammatory and fibrogenic gene expression, which is largely attenuated in the presence of atorvastatin.

**Atorvastatin reduces CCl4-induced acute liver damage.** We next investigated whether the protective effects induced by atorvastatin are reproduced in a different experimental model. For this purpose, rats were exposed to a single intraperitoneal injection of CCl4 in the presence or absence of atorvastatin. As expected, CCl4 administration caused profound hepatic histological changes, including inflammatory infiltrate, necrosis, hepatocytes ballooning, and steatosis. Importantly, atorvastatin administration resulted in a reduction of the necroinflammatory score (Fig. 1, E and F) and ALT serum levels (Fig. 1G). This result reinforces our hypothesis that atorvastatin exerts protective effects against liver injury. To explore whether endogenous ANG II participates in the pathogenesis of acute liver injury, a group of rats were exposed to losartan before CCl4 administration. We found that losartan also reduced the extent of liver damage induced by CCl4 (Fig. 1, E and F). Interestingly, concomitant treatment with atorvastatin and losartan did not induce any synergistic effect.

**Atorvastatin reduces the proinflammatory effects of ANG II in HSC.** We finally investigated whether the beneficial effects of atorvastatin in rats are reproduced at the in vitro level. For this purpose, we studied cultured HSC, a key fibrogenic cell type in the injured liver that also displays powerful inflammatory properties. We first studied HSC proliferation by measur-
ing [3H]-labeled thymidine incorporation. ANG II stimulated HSC growth, as shown in Fig. 4A. The effect induced by ANG II was significantly attenuated in the presence of atorvastatin. This result corroborates the antiproliferative effects of statins in HSC (27, 36). We next explored whether atorvastatin attenuates the inflammatory effects induced by ANG II in HSC. We studied the activation of the transcription factor NF-κB, a signaling pathway that participates in the inflammatory actions in HSC (24). ANG II and TNF-α, a powerful inflammatory cytokine, stimulated NF-κB activation, as indicated

Fig. 2. Effect of Ator on ANG II-induced effects in fibrosis and oxidative stress. A: smooth muscle α-actin (α-SMA) immunostaining of liver specimens from rats treated with saline and ANG II with or without Ator. ANG II infusion resulted in a mild increase in α-SMA immunostaining (arrows) that was reduced by Ator. Original magnification, ×400. B: quantification of the amount of α-SMA positive cells (*P < 0.05 vs. saline; *P < 0.05 vs. ANG II). C: ANG II infusion induced oxidative stress in the liver as assessed by increased detection of 4-hydroxynonenal (4-HNE) protein adducts in pericentral areas (arrow). Concomitant treatment with Ator reduced signs of oxidative stress (4-HNE immunohistochemistry; original magnification, ×200); D: morphometric quantification of % area stained with 4-HNE in all groups (*P < 0.05 vs. saline; *P < 0.05 vs. ANG II).
by increased nuclear translocation of the subunit p65 (Fig. 4, B and C). NF-κB activation by ANG II and TNF-α was markedly reduced by cell preincubation with atorvastatin. We next explored the secretion of IL-8, an inflammatory chemokine, involved in liver fibrogenesis (12). As we previously reported, ANG II stimulated the secretion of IL-8 by cultured HSC (8). Preincubation with atorvastatin markedly reduced IL-8 secretion to basal levels (Fig. 4D). Similarly, ANG II stimulated the expression of ICAM-1, a membrane protein involved in the interaction between HSC and lymphocytes. Again, this effect was blunted in the presence of atorvastatin (Fig. 5A). We next investigated whether atorvastatin regulates the expression of genes involved in liver fibrogenesis, such as procollagen-α(I) and TGF-β1. As previously described, ANG II induced an upregulation of both genes. This effect was attenuated in the presence of atorvastatin (Fig. 5B). Moreover, ANG II induced TGF-β1 release by HSC. As shown in Fig. 4E, this effect was attenuated by atorvastatin. However, atorvastatin did not modify α-SMA expression, a marker of HSC activation, (Fig. 4F). Next, we assessed whether atorvastatin modifies ANG II receptor activation by analyzing ERK phosphorylation, an important intracellular pathway stimulated by ANG II. Atorvastatin did not modify ERK phosphorylation (Fig. 4G). Overall, these results indicate that atorvastatin attenuates the inflammatory effects of ANG II in HSC. We then analyzed whether atorvastatin treatment reduces Rac1 activity. Atorvastatin did not modify Rac1 activation in cells treated with ANG II (Fig. 4H). Finally, we studied whether atorvastatin modulates AT1 expression. We found that atorvastatin did not modify AT1 expression in cultured HSC (Fig. 4I).

**DISCUSSION**

The present study investigates the effects of a statin (atorvastatin) on the inflammatory actions of ANG II in the liver. We provide evidence that atorvastatin attenuates the pathogenic events induced by ANG II in the liver, including oxidative stress, inflammatory events, and expression of profibrogenic genes. These results confirm previous observations that statins attenuate the atherogenic effects of ANG II (17). Moreover, our results indicate that atorvastatin exerts protective effects in a model of acute liver injury. Losartan (an AT1 antagonist) treatment also reduced the extent of liver damage, suggesting that endogenous ANG II plays a role in the pathogenesis of hepatic inflammation. Because ANG II is believed to play a role in liver inflammation both in rodents and in humans, the beneficial effects of atorvastatin suggest that this family of drugs could exert beneficial effects in the liver. Further studies should evaluate this hypothesis.
To test the effects of atorvastatin in the liver, we have used a well-characterized model of continuous infusion of ANG II into rats (4). This model was chosen since ANG II is a powerful proinflammatory substance that plays a role in the pathogenesis of liver inflammation. Moreover, drugs inhibiting ANG II generation and/or binding to its receptors (such as losartan) are considered the most promising approach to treat liver fibrosis in humans (7). The model of continuous infusion of ANG II has been widely used in other organs, such as the kidney and the heart (14, 37). Also, it was previously demonstrated that this model is associated with hepatic inflammation, oxidative stress, and activation of profibrogenic mediators, such as TGF-β1 (3, 16). To determine whether the effect of atorvastatin was specific for the ANG II infusion model of inflammation, we confirmed the beneficial effects of atorvastatin in a well-characterized model of liver injury (CCl4 administration). Besides, we tested the effects of atorvastatin in cultured HSC. This cell type plays a pivotal role in the hepatic wound healing response to injury (5). Moreover, HSC are an active source of free radicals during liver fibrogenesis and amplify the inflammatory response to injury (4, 8). Finally, there is strong evidence that ANG II is a powerful inflammatory and fibrogenic agonist for these cells (5, 19). Atorvastatin attenuated most of the pathogenic effects of ANG II in these cells. Further studies should investigate whether atorvastatin or other statins blunt the effects of ANG II on other nonparenchymal cell types such as Kupffer cells or sinusoidal endothelial cells.
Our results strongly suggest that statins exert anti-inflammatory effects in the liver. This effect was demonstrated in vivo and in cultured HSC. The mechanisms involved in this effect are largely unknown. Statins reduced the expression of proinflammatory cytokines, which promote recruitment of inflammatory cells (26). Moreover, atorvastatin reduced oxidative stress in the liver, which is an important event leading to hepatic inflammation (8). Finally, we recently demonstrated that statins decrease endothelial dysfunction in rats with experimental cirrhosis, which is a pathogenic event linked to local inflammation and fibrogenesis (1). Besides this effect, we showed that atorvastatin attenuates the expression of procollagen-α1(I) and reduced the accumulation of activated HSC. Further studies should investigate whether statins attenuate liver fibrogenesis.

A relevant result of this study is that atorvastatin reduces the prooxidant effects of ANG II in the liver. There is extensive evidence demonstrating that ANG II is a powerful prooxidant agent on the liver (4, 8). ANG II stimulates NADPH oxidase-derived reactive oxygen species generation in cultured HSC (8), and ANG II infusion induces hepatic oxidative stress in vivo (4). Importantly, mice lacking AT1 receptors do not develop oxidative stress following chronic liver injury, suggesting that local ANG II plays a key role in reactive oxygen species generation in chronically damaged livers (48). The antioxidant effects of statins have been previously suggested in different organs (25, 41). Inhibition of the small GTP-binding proteins, including Rac1, plays an important role in mediating the antioxidant effects of statins (25, 41). Membrane translocation of Rac1, which is required for the activation of NAD(P)H oxidase, is inhibited by atorvastatin in other organs (45). However, in our study, atorvastatin does not modulate ANG II-induced Rac1 activation in HSC. Further studies should investigate the molecular mechanisms involved in the antioxidant effect of atorvastatin.

At the cellular level, we explored the effects of atorvastatin on ANG II-induced biological effects in human primary HSC. Activated HSC proliferate and accumulate at the areas of active inflammation. This cell type plays a major role in the hepatic wound healing response to injury by promoting inflammation and fibrosis (2). ANG II is a powerful agonist for these cells, inducing cell growth and inflammatory and profibrogenic effects (5). We confirmed previous data that statins reduce proliferation of HSC (27, 36). Moreover, we provide evidence that atorvastatin reduces the inflammatory actions (IL-8 secretion and ICAM-1 expression) stimulated by ANG II (17, 23). This effect was associated with a reduction in ANG II-induced NF-κB activation (32). This biological effect of atorvastatin has been reported in hepatocytes (21). Importantly, we demonstrate that atorvastatin blunted the effect of ANG II on fibrogenic gene expression, including procollagen-α1(I) and TGF-β1. Moreover, atorvastatin reduced the effects of ANG II in TGF-β1 cell release in HSC. These results are relevant, since HSC are the major source of collagen in the injured liver and play a pivotal role in liver fibrogenesis (2). Further studies should evaluate whether statins attenuate fibrosis in experimental models of chronic liver injury.

Taken together, our results demonstrated that ANG II exerts inflammatory properties in the liver, both in vivo and in vitro. Administration of atorvastatin reduced mainly the inflammatory effects of ANG II in vivo, as well as inflammatory and profibrogenic events in vitro. These results suggest that statins, besides their lipid-lowering properties, may exert beneficial effects in patients with chronic liver injury.

ACKNOWLEDGMENTS

We thank Cristina Millan and Elena Juez for excellent technical support.

GRANTS

This work is supported by grants from the Ministerio de Ciencia y Tecnología, Dirección General de Investigación (SAF 2005-06245 and SAF 2005-03378) and the Instituto de Salud Carlos III (FIS 05150567). Leandra N. Ramalho had a grant from the Coordenación de Aperfeiçoamento de Pessoal de Nível Superior, from the Brazil Government. Montserrat Moreno and Marlene Dominguez each had a grant from the Institut d’Investigacions Biomèdiques...
REFERENCES


duced vascular fibrosis: role of RhoA/ROCK and MAPK pathways. 


