Simvastatin attenuates renal inflammation, tubular transdifferentiation and interstitial fibrosis in rats with unilateral ureteral obstruction

José Mauro Vieira Jr, Eduardo Mantovani, Leonardo Tavares Rodrigues, Humberto Dellé, Irene Lourdes Noronha, Clarice Kazue Fujihara and Roberto Zatt

Internal Medicine, Renal Division, University of São Paulo, São Paulo, Brazil

Abstract

Background. The pleiotropic actions of statins have been largely explored. These drugs have been tested in several models of progressive renal disease, most of them accompanied by hypertension. We sought to investigate more closely the effects of simvastatin on renal interstitial fibrosis due to unilateral ureteral obstruction (UUO).

Methods. Munich-Wistar rats were submitted to UUO and studied after 14 days. Animals were divided into two groups: vehicle (VH) or simvastatin (SIMV) 2 mg/kg b.i.d. by gavage. At sacrifice kidneys were harvested for morphology, mRNA and protein analysis. RT–PCR was done to assess expression of collagen I and III, fibronectin, MCP-1, TGF-β1 and bFGF. Protein expression was assessed by western blot (TGF-β) and immunostaining (macrophage, lymphocyte, PCNA, vimentin and α-smooth muscle actin). Contralateral kidneys (CL) were used as controls.

Results. SIMV-treated animals had less severe renal inflammation. MCP-1 was markedly expressed in obstructed kidneys and diminished with SIMV (48.9 ± 2.5 vs 64.3 ± 3.1 OD in VH, \textit{P} < 0.01). Interstitial fibrosis (IF) was significantly attenuated with SIMV (8.2 ± 1.3 vs 13.2 ± 0.6%, \textit{P} < 0.01 SIMV vs VH), which was confirmed by a decrease in collagen I and fibronectin renal expression. Vimentin, a marker of dedifferentiation, was expressed in tubular cells of VH and decreased with SIMV treatment. α-SMA, a marker of myofibroblast-type cells, was increased in renal interstitium of VH rats and SIMV significantly reduced its expression. PCNA was increased in the UUO kidneys, but SIMV did not decrease tubular or interstitial proliferating cells. TGF-β1, which was highly induced in the obstructed kidneys, decreased at the post-transcriptional level with SIMV treatment (5.35 ± 0.75 vs 13.10 ± 2.9 OD in VH, \textit{P} < 0.05). bFGF mRNA was also overexpressed in the obstructed kidneys, although SIMV treatment did not significantly decrease its expression.

Conclusions. SIMV had an evident protective effect on renal interstitial inflammation and fibrosis. It is conceivable that by attenuating inflammation, SIMV prevented tubular activation and transdifferentiation, two processes largely involved in the renal fibrosis of the UUO model.

Keywords: inflammation; interstitial fibrosis; MCP-1; simvastatin; transdifferentiation; unilateral ureteral obstruction

Introduction

HMG-CoA reductase inhibitors, a class of drugs known as statins and initially described as lipid-lowering drugs, have anti-inflammatory, antiproliferative and immunomodulatory actions \textit{in vitro} and \textit{in vivo} [1,2]. Their effects include the blockade of the transcription factor NFκB signalling pathway and therefore statins prevent the expression of downstream inflammatory mediators [3]. Besides their actions on atherosclerosis and cardiovascular diseases [4], several beneficial effects of statins have been described in experimental models of renal disease, most of them accompanied by hypertension and vascular injury [5,6]. Recently a statin was used successfully to decrease renal interstitial fibrosis in a model related to cyclosporine nephrotoxicity [7]. However, it is well known that this model encompasses arteriolar injury and renal ischemia as well [7,8].

In this study we sought to determine the effects of simvastatin in a model of pure tubulointerstitial renal injury, the one related to unilateral ureteral obstruction (UUO). In order to explain the mechanisms of action of simvastatin (SIMV) we studied the expression of monocyte chemotactrant protein-1 (MCP-1), one of
the most important mediators of the inflammatory process within the kidney, and the resulting renal infiltration by inflammatory cells.

Furthermore, it has recently been demonstrated that progressive renal disease is accompanied by epithelial-myoﬁbroblast transdifferentiation (EMT), a pathophysiological mechanism that helps to explain renal ﬁbrosis [9–11]. It appears that in several models of renal disease, tubular cells are activated and undergo dedifferentiation, followed by migration and acquisition of a phenotype of myoﬁbroblast. EMT could account for the large number of myoﬁbroblasts in renal progressive diseases, particularly in the UUO model [11]. We investigated the effects of SIMV on surrogate markers of EMT, and the impact of this treatment on the expression of proﬁbrogenic cytokines TGF-β and bFGF, two possible mediators of EMT and the resulting renal ﬁbrosis.

Subjects and methods

Male Munich-Wistar rats weighing ~250 g, obtained from a local colony at the University of São Paulo, were used in this study. Rats were maintained with free access to regular food and water, at 22 ± 1 °C under a 12/12 h light/dark cycle. All experimental procedures were conducted according to our institutional guidelines.

Experimental design

Rats were randomly assigned to receive either vehicle (VH, n = 7), or SIMV (n = 7), for 2 days prior to surgical procedure. Then the UUO was performed, after anaesthesia with 50 mg/kg i.p. sodium pentobarbital injection, through a ventral laparotomy. The left ureter and kidneys were exposed and the ureter was then ligated next to the uretero-pelvic junction. After abdominal closure rats were allowed to recover and returned to cages to follow VH or SIMV treatment for 14 days after obstruction. The right contralateral kidneys of rats from both groups were harvested to recover and return to cages to follow VH or SIMV treatment for 14 days after obstruction. The right contralateral kidneys of rats from both groups were harvested to recover and return to cages to follow VH or SIMV treatment for 14 days after obstruction. The right contralateral kidneys of rats from both groups were harvested to recover and return to cages to follow VH or SIMV treatment for 14 days after obstruction.

Morphology

At the end of the study (day 14), rats were anaesthetized with 50 mg/kg i.p. sodium pentobarbital injection. The abdominal wall was sectioned and the left kidney was then prepared for analysis by either of two methods. (i) For parafﬁn embedding, half of the left kidney, obtained through a midcoronal section, was ﬁxed with Dubosq–Brazil solution for 30 min, followed by a post ﬁxation in buffered 10% formaldehyde solution. The material was then embedded in parafﬁn for assessment of renal cortical interstitial injury and for immunohistochemical studies. (ii) The other half of left kidneys were snap-frozen in liquid nitrogen, and stored at −80 °C for further mRNA and protein analysis (see below).

Parafﬁn-embedded renal tissue was dewaxed using standard sequential techniques, and 2–3 μm-thick sections were stained with Masson’s trichrome technique. The fraction of renal cortex occupied by interstitial tissue (%INT) was estimated in Masson-stained sections by a point-counting technique as described previously [13,14]. Briefly, the percentage of the renal cortical area occupied by interstitial tissue was measured blindly by a single observer. Before sacrifice, blood was drawn to measure cholesterol serum levels.

Immunohistochemistry

The inflammatory inﬁltrate was analysed by assessing the number of macrophages and lymphocytes in the cortex of the renal tissue. In addition, the expression of vimentin, a marker of dedifferentiated mesenchymal cells, α-SMA, a protein expressed in ﬁbroblast-like cells, and proliferating cell nuclear antigen (PCNA) were also investigated. The inﬂammatory cells and the expression of vimentin, PCNA and α-SMA were identiﬁed by immunohistochemistry using standard techniques as described previously [14,15]. Briefly, 4 μm-thick sections obtained from parafﬁn-embedded tissue were dewaxed and mounted using conventional techniques. Sections were then subjected to microwave irradiation in citrate buffer to enhance antigen retrieval and preincubated with 5% normal rabbit or horse serum (Vector Labs, Burlington, MA, USA) in Tris-buffered saline (TBS). The renal tissue was then incubated with 1:200 monoclonal anti-ED1 antibody (Serotec, Oxford, UK) for macrophage detection, 1:100 anti-CD3 (Harlan Seralab, Oxford, UK) for lymphocyte, 1:200 anti-vimentin (Santa Cruz Biotechnology, CA, USA), 1:800 anti-CD3 (Harlan Seralab, Oxford, UK) for lymphocyte, 1:200 anti-vimentin (Santa Cruz Biotechnology, CA, USA), 1:800 anti-α-SMA (Dako, Copenhagen, Denmark), and 1:100 anti-rat PCNA (Dako). The incubation of primary antibodies was carried out overnight at 4 °C in a humidified chamber. Sections were then incubated with appropriate species-speciﬁc secondary antibodies for 45 min at room temperature. To complete the detection of antigens, we used the complex of alkaline phosphatase anti-alkaline phosphatase method (Dako) for ED1 or the avidin-biotinylated horseradish peroxidase technique (Dako) for CD3, vimentin, PCNA and α-SMA [14,15]. Negative control experiments were routinely performed by (i) omitting the incubation with the primary antibody, and (ii) replacing the primary antibody with unspeciﬁc rat IgG.

The quantiﬁcation of ED1, CD3 positive cells and PCNA was carried out in a blinded fashion under 200× magniﬁcation and expressed as cells/0.5 mm². Vimentin expression was quantiﬁed by counting positive tubules (at least one positive cell/tubule proﬁle) per 200× magniﬁcation ﬁeld. α-SMA expression in the interstitium was assessed by a point-counting technique as for the trichome quantiﬁcation. For each section at least 30 consecutive renal cortical 200× ﬁelds were examined.

RT–PCR

Total RNA was extracted by guanidinium thiocyanate–phenol–chlo roform method [16]. The cDNA was synthesized
from 2 μg of total RNA using an oligo dT primer (Promega, San Luis, USA) and the MMLV-RT enzyme (Promega). PCR was carried out to amplify the following specific cDNAs: collagen I (primers: sense 5'-CTT CGT GTA AAC TTC CTC C-3' and antisense 5'-CAC TGG TTT TTG GTC TTC AC-3', product of 224 bp), collagen III (primers: sense 5'-GGC GCT TTT CAC CAT ATT AG-3' and antisense 5'-GCA TGT TTC TCC GGT TTC-3', product of 266 bp), fibronectin (primers: sense 5'-TTA TGA CGA CGG GAA GAC CTA-3' and antisense 5'-GGC TGG ATG GAA AGA TTA CTC-3', product of 266 bp), transforming growth factor-β1 (TGF-β1) (primers: sense 5'-GGA CTA CTA GCC CAA AGA AG-3' and antisense 5'-TCA AAA GAC AGC CAC TCA GG-3', product of 293 bp), basic fibroblast growth factor (bFGF) (primers: sense 5'-AAG CAG AAG GAG GAG AGG TTG 0-3' and antisense 5'-CTC CCC CAA AAA TTC AAG TGT ACT CTC G-3', product of 249 bp).

PCR was performed mixing 2 μl of RT reaction product (cDNA), 10 μl of 10× buffer [Tris–HCl 20 mM pH 8.0, potassium chloride 100 mM, EDTA 0.1 mM, DTT 1 mM, 50% glycerol, 0.5% Tween-20 and 0.5% Nonidet-P40], 2 μl of 10 mM dNTP mix, 10 μM of each primer and 5 U of Taq DNA polymerase (Promega) and water up to 100 μl. Magnesium chloride concentration was adjusted for each pair of primers, and cDNAs were amplified according to the following conditions: 94°C for 50 s, 55°C (the annealing temperature varied for each pair of primers) for 60 s, and 72°C for 2 min. The number of cycles was predetermined for each pair of primers in order to avoid the PCR plateau phase. The PCR products were analysed in 2% agarose gel along with a 100 bp DNA ladder, and the bands were semi-quantified using a software (ImageMaster version 2.0; Pharmacia Biotech, Buckinghamshire, UK). Negative controls were routinely done by omitting the primers in the PCR. All PCRs resulted in the amplification of a single product of the predicted size. The results are expressed as optical density (OD), and the β2-microglobulin expression was used as a control of the reaction to assure equal cDNA among samples.

**Western blot**

Kidney sections stored at −75°C were homogenized in lysis buffer (10 mM HEPES pH 7.6, 25 mM potassium chloride, 3 mM magnesium chloride, 5 mM EDTA, 10% glycerol, 1 mM DTT, 0.1% SDS and 1:100 protease inhibitor cocktail; Sigma) and centrifuged at 10 000 g. The supernatant was used as molecular weight standard. Thirty micrograms of total protein was loaded in a stacking polyacrylamide gel and then resolved on 12% polyacrylamide gel. The gel was stained with Coomasie blue to assess the equal loading of protein. Then samples were wet-transferred to a 0.2 μ nitrocellulose membrane (Amersham Pharmacia Biotech, Piscataway, USA). Thereafter blots were blocked for 1 h with 5% nonfat dry milk in TBST buffer (20 mM Tris–HCl pH 7.6, 0.8% NaCl and 0.05% Tween-20) and incubated overnight at room temperature with a 1:500 mouse anti-TGF-β1 primary antibody (Genzyme, Cambridge, USA). After washing in TBST, the blots were incubated with secondary anti-mouse IgG-horseradish peroxidase conjugate antibody at 1:5 000 (Santa Cruz Biotechnology, CA, USA) for 1 h. Blots were then washed again in TBST and bands detected by enhanced chemiluminescence (Amersham Bioscience, Buckinghamshire, UK). ODs for quantification were obtained using a software (ImageMaster version 2.0; Pharmacia Biotech, Buckinghamshire, UK). A prestained-protein marker (New England Biolabs Inc., Beverly, USA) was used as molecular weight standard.

**Statistical analysis**

Data are reported as mean ± SEM. Comparisons between groups were done by one-way analysis of variance (ANOVA) followed by the Bonferroni’s multiple comparison post-test. The level of statistical significance was P < 0.05.

**Results**

There was no difference between groups concerning the gain of weight of rats. Body weight at the end of study was 260 ± 2 g and 264 ± 4 g in the VH and SIMV groups, respectively. Cholesterol serum levels did not differ between groups (74.1 ± 3.9 in VH vs 72.9 ± 3.5 mg/dl in SIMV). Contralateral (CL) kidneys were pooled since there was no morphological, cellular or molecular difference between contralateral kidneys of both VH- and SIMV-treated rats (data not shown).

**Renal morphology**

Nonobstructed contralateral kidneys from rats of both groups did not show any morphological alteration. The kidneys of rats subjected to ureteral obstruction developed a conspicuous tubulointerstitial injury consisting of tubular dilatation and atrophy, interstitial inflammation and a marked interstitial fibrosis. Glomeruli and vessels were well preserved. Analysis of interstitium by the trichrome staining showed that SIMV significantly attenuated interstitial fibrosis (8.2 ± 1.3 % vs 13.2 ± 0.6%, P < 0.01 SIMV vs VH) (Figures 1 and 2).

**Inflammatory findings: MCP-1, macrophages and lymphocytes**

CL kidneys displayed rare cells positive for either macrophages or lymphocytes antigen (2.7 ± 0.3 and 3.3 ± 0.4 cells/0.5 mm², respectively) (Figure 1). Ureteral obstruction induced an inflammatory cell influx in renal cortex, which predominated in the interstitial compartment. Interestingly, we observed peritubular inflammatory cells disrupting tubular basal membrane, and macrophages were even seen invading tubular lumen (Figure 2E). SIMV treatment led to a significant attenuation on the macrophage (14.8 ± 0.7 vs 21.5 ± 0.8 cells/0.5 mm², P < 0.01) and
Simvastatin attenuation in rates with unilateral ureteral obstruction

Simvastatin significantly attenuated inflammatory cell infiltration.showed a striking interstitial fibrosis and a marked cell infiltration. whereas VH-treated rats subjected to UUO CL kidneys presented low interstitial expansion and showed rare

Fig. 1. Semiquantitative analysis of (A) interstitial fibrosis (%IF) and quantification of (B) ED1 positive cells (macrophage) and (C) CD3 positive cells (lymphocyte) in the interstitial renal cortex. CL kidneys showed low interstitial expansion and showed rare inflammatory cells, whereas VH-treated rats subjected to UUO showed a striking interstitial fibrosis and a marked cell infiltration. SIMV significantly attenuated IF and inflammatory cell infiltration. *P < 0.01 vs VH. #P < 0.05 vs VH.

lymphocyte (10.1 ± 1.5 vs 16 ± 1.5 cells/0.5 mm², P < 0.05) recruitment compared to non-treated animals (Figures 1 and 2). Analysis of MCP-1 mRNA expression in the kidneys showed a marked induction of this chemokine in the VH-treated animals compared with contralateral kidneys (64.3 ± 3.1 vs 19.2 ± 7.5 OD, P < 0.05), which was significantly downregulated with SIMV treatment (48.9 ± 2.5 vs 64.3 ± 3.1. OD, P < 0.01) (Figure 3).

Extracellular matrix compounds, TGF-β and bFGF

We compared mRNA expression of some extracellular matrix compounds in the obstructed kidneys of VH- and SIMV-treated rats in order to confirm the results obtained with trichrome staining. The UUO model is notoriously characterized by a striking upregulation of some matrix compounds in the obstructed kidneys of VH-treated animals compared to CL kidneys. In the SIMV group, the upregulated vimentin expression in tubular cells significantly decreased (17.6 ± 2.0 vs 29.4 ± 2.5 positive tubules/200× field, P < 0.005) (Figures 6 and 7). α-SMA, constitutively expressed in smooth muscle cells of renal arterioles, was rarely seen in the interstitium of contralateral non-obstructed kidneys (0.69 ± 0.07% of interstitial cortical area), whereas ureteral obstruction caused a striking increase in the expression of α-SMA in the interstitium compartment. SIMV significantly reduced interstitial α-SMA expression at day 14 (8.0 ± 0.76 vs 12.34 ± 0.23% in the VH group, P < 0.001) (Figures 6 and 7). CL kidneys demonstrated only scant proliferating tubular and interstitial cells. However, UUO induced a severe tubular and interstitial proliferative response that occurred as early as 3 days (tubular cells 5.2 ± 0.2 in VH vs 2.5 ± 0.6 cells/0.5 mm² in CL; and interstitial cells 6.8 ± 3.5 in VH vs 1.99 ± 0.8 cells/0.5 mm² in CL, P < 0.05 for both parameters) and was maintained at day 14 (tubular cells 3.4 ± 0.5 cells/0.5 mm² in VH; interstitial cells 9.8 ± 1.8 cells/0.5 mm² in VH). Nevertheless, SIMV treatment did not significantly change proliferation at any timepoint (tubular

Vimentin, α-SMA and PCNA

CL kidneys normally expressed vimentin in epithelial visceral cells and smooth muscle cells of arterioles. This basal constitutive expression was not influenced by UUO. In contrast, tubular cells, which were negative for vimentin in contralateral kidneys, showed a dramatic overexpression for this protein in obstructed kidneys of VH-treated rats. In the SIMV group, the upregulated vimentin expression in tubular cells significantly decreased (11.2 ± 2.7 vs 21.1 ± 5.0 OD, P = 0.1) (Figure 5B).
cells 4.4 ± 0.1 and 4.2 ± 0.9 cells/0.5 mm², at 3 and 14 days, respectively. Interstitial cells 5.8 ± 0.5 and 6.1 ± 1.5 cells/0.5 mm², at 3 and 14 days, respectively, (P = NS) (Figure 7).

**Discussion**

Several features of HMG-CoA reductase inhibitors, better known as statins, have been revealed, most of them regarding anti-inflammatory actions [1,3,4]. Moreover, these effects, which appear to depend largely on the inhibition of the transcription factor NF-κB, are independent of their lipid-lowering properties [4,12,17]. Initially described in the cardiovascular scenario, these effects of statins have also been tested in renal diseases. Others have shown a protective effect of statins on renal injury associated with hypertension, either renin- or salt-dependent [5,6]. Moreover, statins showed beneficial biological effects in the Heymann passive nephritis, a model of nephrotic syndrome [12]. More recently, the
anti-inflammatory effects of statins were shown to attenuate progressive renal disease in a model of diabetes, and the fibrosis associated with cyclosporine nephrotoxicity [7,18].

In this study, we investigated the effects of SIMV on a model of renal fibrosis that is independent of hypertension, vascular injury, renal insufficiency or the systemic oxidative stress that accompanies diabetes. To that end, we chose the UUO model, which is characterized by intense renal inflammation and striking fibrosis limited to the tubulointerstitial compartment [19]. We clearly demonstrated that SIMV treatment attenuated the inflammation and the resulting interstitial fibrosis in the obstructed kidneys.

MCP-1 is a protein largely produced by tubular cells in response to renal injury. Eddy and Giachelli [20] have shown that proteinuria very early elicits a proximal tubular activation and MCP-1 secretion, which could account for the interstitial inflammation that follows glomerular proteinuria. Furthermore, Morigi et al. [21] demonstrated that proximal tubule cells subjected in vitro to albumin respond with NFκB activation, and that SIMV treatment decreases NFκB-dependent MCP-1 production [12]. In our study we demonstrate that in the UUO model there is an upregulation of the MCP-1 renal expression that is independent of proteinuria. SIMV not only clearly decreased MCP-1 mRNA levels in the kidney but also led to less severe renal interstitial inflammation, as demonstrated by a decrease in the lymphomononuclear cells in that compartment.

Recently, Wolf et al. [22] have described a regulatory loop between MCP-1 and TGF-β in renal injury independent of macrophage recruitment.
Here, the relationship between the decrease in the inflammatory cells and the resulting protection in the interstitial fibrosis afforded by SIMV could also be explained by downregulation of the profibrotic cytokine TGF-\(\beta\). Although the abundance of TGF-\(\beta\) mRNA in the injured kidney was not modified with statin in this model, the expression of some extracellular matrix compounds was clearly downregulated and the regulation of TGF-\(\beta\) renal expression clearly occurred at the translational level. Alternatively, it has recently been demonstrated \textit{in vitro} that MCP-1 has the ability to induce fibroblast to increase production of collagen through a mechanism independent of TGF-\(\beta\) [23]. Thus, it is conceivable that the MCP-1 expression could itself directly upregulate ECM compounds production by fibroblasts or activate tubular cells to transdifferentiate into fibroblast-like cells.

EMT is a process that has been described in progressive scarring renal diseases [9–11]. In the present study we show that SIMV interfered with the expression of surrogate markers of transdifferentiation of tubular cells, suggesting that SIMV might, either through its antinflammatory or a direct effect, decrease EMT. The overexpression of interstitial \(\alpha\)-SMA that we found in the obstructed kidneys can be partly explained by migration of dedifferentiated tubular cells through the basal membrane into the interstitium. The expression of vimentin that we demonstrated in obstructed tubular cells corresponds with an undifferentiated, mesenchymal state. This undifferentiated state might precede and allow the proliferation and migration of tubular cells to take place. Nevertheless, further \textit{in vitro} studies are necessary to confirm the role of statin in preventing tubular EMT. It has been demonstrated that UUO is a model of renal disease characterized by increased proliferative activity of tubular cells [19]. We confirmed this feature in this model. However, we did not demonstrate a significant anti-proliferative action of SIMV, which argues against a direct effect of SIMV on either proliferation or apoptosis.
Fig. 6. Representative photomicrographs of renal immunohistochemistry for α-SMA (A, B and C) and vimentin (C, D and E). (A) CL kidneys showed positive arteriolar α-SMA and a weak staining in the interstitium. UUO induced a marked α-SMA interstitial expression in the VH-treated rats (B), which was reduced with SIMV treatment (C) [original magnification: (A–C), 200×]. (D) Vimentin expression normally occurred in the arterioles (arrow) and visceral epithelial cells (arrowhead) as seen in the CL kidney (100×). No tubular staining was found in CL kidneys. (E) Abnormal tubular expression was markedly induced with ureteral obstruction in VH-treated rats (400×), which was attenuated with SIMV. (F) Larger view of the undifferentiated tubular cells expressing vimentin in an obstructed kidney from a VH-treated rat (original magnification 1000×).

Fig. 7. Semiquantitative analysis of (A) α-SMA, (B) vimentin and (C and D) PCNA immunostaining. (A) α-SMA is greatly expressed in the obstructed kidney, and SIMV treatment significantly attenuates its interstitial expression. (B) There is no expression of vimentin in normal, non-obstructed tubular cells. UUO leads to a high expression of this marker of undifferentiated state. SIMV partially prevented this de novo expression. (C) Tubular proliferation (PCNA staining). UUO induced a marked tubular proliferation that occurred as early as day 3 after UUO. However, SIMV did not prevent tubular cell proliferation. (D) PCNA positive interstitial cells were progressively increased in the obstructed kidneys. SIMV groups showed a trend toward a decrease in proliferation of interstitial cells, although without reaching significance. #P<0.001 vs VH. *P<0.005 vs VH.
In contrast, SIMV treatment diminished both renal interstitial inflammation and markers of EMT. Therefore, we hypothesize that part of the beneficial effects of statin could be accounted for by a decrease in renal interstitial inflammation and the resulting tubular activation. We observed that the inflammation that follows UUO is accompanied by features of tubulitis, which might conceivably contribute to tubular activation. In experimental renal transplantation, tubulitis is a finding associated with the renal fibrosis that characterizes chronic rejection [24], and tubulitis that is found in human subclinical rejection correlates with chronic interstitial fibrosis and long-term renal dysfunction [25].

It has been demonstrated that EMT appears to be mediated by upregulation of some cytokines such as TGF-β, bFGF and connective tissue growth factor (CTGF) [10,11,26]. We confirmed an overexpression of TGF-β at least at the post-transcriptional level in the UUO kidneys, which was attenuated by SIMV, whereas the bFGF message was also upregulated in the obstructed VH-treated animals although not significantly decreased with SIMV. We did not discount that other cytokines such as CTGF would possibly be more relevant to transdifferentiation and renal fibrosis of the UUO model [26].

Further studies are necessary to clarify the exact role of statins on renal fibrosis and EMT, but it is possible that statins have either direct effects on the extracellular matrix production/turnover or indirect effects through their anti-inflammatory actions. The interference with renal EMT process could be an alternative explanation for the anti-fibrotic effects of this class of drugs with a marked anti-inflammatory activity.

In conclusion, in this model of renal interstitial fibrosis that is independent of vascular injury or hypertension, SIMV significantly attenuated interstitial fibrosis. These anti-fibrotic effects are likely relevant to transdifferentiation and renal fibrosis of the UUO model [26].

Acknowledgements. This work was supported in part by FAPESP, grant no. 01/02932-0.

Conflict of interest statement. None declared.

References
25. Shimizu A, Yamada K, Sachs DH, Colvin RB. Persistent rejection of peritubular capillaries and tubules is associated...


Received for publication: 22.10.04
Accepted in revised form: 6.4.05