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*Cell Injury, Repair, Aging and Apoptosis*

# Statins Attenuate Ischemia-Reperfusion Injury by Inducing Heme Oxygenase-1 in Infiltrating Macrophages

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**Statins induce heme oxygenase-1 (HO-1) in several cell types, such as vascular smooth muscle cells, endothelial cells, and macrophages. The present study assessed the role of statin-induced HO-1 up-regulation on circulating monocytes/macrophages and their contribution in preventing renal ischemia-reperfusion (IR) injury in a rat model. Cerivastatin was administered via gavage (0.5 mg/kg) for 3 days before IR injury; controls received vehicle. Statin pretreatment reduced renal damage and attenuated renal dysfunction ($P < 0.05$) after IR injury. The protective statin pretreatment effect was completely abolished by cotreatment with tin protoporphyrin IX (Sn-PP), a competitive HO inhibitor. IR increased HO-1 expression at the transcript and protein level in renal tissue. This effect was significantly more evident ($P < 0.05$) in the statin-pretreated animals 24 hours after IR injury. We identified infiltrating macrophages as the major source of tissue HO-1 production. Moreover, in ancillary cell culture (monocyte cell line) and in *in vivo* experiments (isolation of circulating monocytes), we confirmed that statins regulate HO-1 expression in these cells. We conclude that statin treatment up-regulates HO-1 in circulating monocytes/macrophages *in vivo* and *in vitro*. We hypothesize that local delivery of HO-1 from infiltrating macrophages exerts anti-inflammatory effects after IR injury and thereby may reduce tissue destruction.** ***(Am J Pathol 2007, 170:1192–1199; DOI: 10.2353/ajpath.2007.060782)***

Ischemia/reperfusion (IR) induces severe tissue injury mainly caused by oxidative stress. We have previously shown that statin treatment ameliorates acute IR injury. This favorable effect results from significantly reduced tissue inflammation, supporting the hypothesis that statins exert direct anti-inflammatory effects independent of serum cholesterol reduction, such as reduced cell infiltration, nitric-oxide synthase (NOS) up-regulation, mitogen-activated protein (MAP) kinase activation, and activation of redox-sensitive transcription factors.[1] The anti-inflammatory effects of statins have been linked to the induction of the cytoprotective gene encoding heme oxygenase-1 (HO-1).[2] Statins activate the HO-1 promoter in endothelial cells and cause a reduction of free radical formation. This effect may explain in part their antioxidant and anti-inflammatory actions.[3,4] The heme oxygenase enzyme family degrades heme to biliverdin, thereby providing carbon monoxide (CO) and free iron ($Fe^{2+}$). Both CO[5,6] and bilirubin (the product of biliverdin degradation)[7] may protect molecules against oxidative stress and IR injury.[8] They reduce the production of superoxide anions, inhibit lipid peroxidation, prevent apoptosis, facilitate vasodilatation, and increase local blood flow[9,10] In a transgenic mouse model, up-regulation of HO-1 provided protection from myocardial ischemia-induced oxidative damage.[11] HO-1 is highly inducible by a variety of stimuli such as oxidative stress, heat shock, cytokines, and nitric oxide.[12,13] We explored whether or not the protective effects of statins against IR tissue injury result from HO-1 induction. We studied the effect of statin pretreatment on IR injury in a model of acute renal failure. We found that infiltrating monocytes/macrophages, a hallmark of IR injury, were the major source of local HO-1 production in our model.

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## Materials and Methods

### Animal Experiments

Experiments were performed on male Sprague-Dawley rats (250 to 300 g) purchased from Charles River (Sulzfeld, Germany). The rats received a standard diet with free access to tap water. All procedures were done according to guidelines from the American Physiological Society and were approved by local authorities. One group was treated with cerivastatin (0.5 mg/kg body weight) by gavage for 3 days preoperatively (IR + statin group, $n = 10$), another group received 0.9% sodium chloride vehicle (IR group, $n = 10$), and a third group was cotreated with cerivastatin and the HO-1 inhibitor tin protoporphyrin IX (Sn-PP) (25 mg/kg body weight; Frontier Scientific, Logan, UT) by intraperitoneal injection for 3 days preoperatively (IR + statin + Sn-PP group, $n = 10$). The same cerivastatin dose has been previously used in other rat models.[14,15] We used general anesthesia with 100 mg/kg body weight ketamine (CP-Pharma, Burgdorf, Germany) and xylazine, 5 mg/kg body weight (Rompun; Bayer, Leverkusen, Germany) as described previously.[1] In brief, for IR injury the left renal pedicle was occluded for 45 minutes, and the contralateral right kidney was removed. Another group received saline and had only a right nephrectomy performed (sham-operated group, $n = 10$). Before and 24 hours after surgery, blood samples were drawn for measurement of serum creatinine concentrations by an automated method (Beckman Analyzer; Beckman Instruments GmbH, Munich, Germany). All animals were sacrificed 24 hours after IR injury. The rats were perfused via the aorta with 100 ml of ice-cold phosphate-buffered saline (PBS), and the kidney was removed.

### Histology and Immunohistochemistry

For paraffin histology, kidneys were perfused with ice-cold PBS; afterward, the kidneys were fixed for 12 hours with ice-cold fixative containing 4% paraformaldehyde in Soerensen's phosphate buffer and then paraffin-embedded. For immunohistochemistry kidneys were snap-frozen in isopentane (−35°C) and, for Western blotting, in liquid nitrogen. For morphological evaluation, 3-$\mu$m paraffin sections were stained with PAS using a standard procedure. Examination of the severity of renal tissue destruction, ie, tubular epithelial cell necrosis and cast formation, was performed without knowledge of the animal group identity.

Immunohistochemistry was performed using the following primary antibodies: monoclonal mouse anti-rat HO-1 and polyclonal rabbit anti-rat HO-1 (StressGen, Victoria, BC, Canada) and anti-rat monocytes/macrophages (ED-1; Serotec, Oxford, UK). For indirect immunofluorescence, nonspecific binding sites were blocked with 10% normal donkey serum (Jackson ImmunoResearch Laboratories, West Grove, PA) for 30 minutes. Thereafter, cryosections were incubated with the primary antibody for 1 hour. All incubations were performed in a humid chamber at room temperature. For fluorescent visualization of bound primary antibodies, sections were further incubated with Cy2- and Cy3-conjugated secondary antibodies (Jackson ImmunoResearch Laboratories) for 1 hour. Sections were analyzed using a Zeiss Axioplan-2 imaging microscope with the computer program AxioVision 3.0 (Zeiss, Jena, Germany). Semiquantitative scoring of ED-1- and HO-1-positive cells was performed using a computerized cell count program (KS 300 3.0; Zeiss). Fifteen different areas of each kidney sample were analyzed. The scoring was done without knowledge of the animal assignment to the treatment.

### Western Blotting

For Western blotting, the frozen kidneys were pulverized in liquid nitrogen and resuspended in 2 ml of lysis buffer [20 mmol/L Tris buffer, pH 7.5, containing 10 mmol/L glycerolphosphate, 2 mmol/L pyrophosphate, 1 mmol/L sodium fluoride, 1 mmol/L phenylmethylsulfonyl fluoride, 1 g/ml leupeptin, 1 mmol/L dithiothreitol, and 1 mmol/L ethylenediamine tetraacetic acid (EDTA)]. Homogenates were sonicated for three 20-second bursts on ice and centrifuged at $500 \times g$ for 1 minute to remove cell debris. Aliquots of the supernatants were stored at −80°C. The protein amount was measured using Lowry assay. Seventy micrograms of protein of each sample was suspended in loading buffer and run on a 10% polyacrylamide gel and electrophoretically transferred to nitrocellulose membrane. Membranes were blocked in 5% skim milk and 1% bovine serum albumin. Primary antibody against HO-1 was applied overnight at 4°C. After washing with TBST buffer (50 mmol/L Tris-HCl, pH 7.5, 150 mmol/L NaCl, and 0.01% Tween 20), incubation with horseradish peroxidase-conjugated goat anti-rabbit secondary antibody (Dianova, Hamburg, Germany) for 1 hour at room temperature was performed. The protein bands in the blot were detected with the use of an enhanced chemiluminescence kit (Renaissance; NEN Life Science, Zaventem, Belgium) according to the manufacturer's instructions. Relative density measurements provided quantification (Scion Image, Frederick, MD).

### RNA Extraction and Real-Time Quantitative Polymerase Chain Reaction

Frozen kidneys were ground in liquid nitrogen and total RNA was extracted using Trizol reagent (Invitrogen, Karlsruhe, Germany). For real-time quantitative polymerase chain reaction (qPCR), 1 $\mu$g of DNase-treated total RNA was reverse transcribed using Superscript II reverse transcriptase (Invitrogen), and qPCR was performed on an SDS 7700 system (Applied Biosystems, Darmstadt, Germany) using Rox dye (Invitrogen), FastStart Taq polymerase (Roche Diagnostics, Mannheim, Germany) and gene-specific primers and FAM-TAMRA-labeled probes (BioTez, Berlin, Germany). PCR amplification was performed for 10 minutes at 96°C and 40 cycles for 10 seconds at 95°C and 1 minute at 60°C. For normalization, we determined the distribution of glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The sequences of

the TaqMan sets read as follows (5′−3′): GAPDH: 5′-AAGCTGGTCATCAATGGGAAAC-3′, 5′-ACCCCATTTGATGTTAGCGG-3′, FAM-5′-CATCACCATCTTCCAGGAGCGCGCGAT-3′-TAMRA; HO-1: 5′-GCTCCTGCGATGGGTCCT-3′, 5′-TGGCATAAATTCCCACTGCC-3′, FAM-5′-ACACTCAGTTTCCTGTTGGCGACCG-3′-TAMRA. Quantification was performed using QGene software.[16]

### *Cell Culture Experiments*

We used the human monocytic cell line U937 to study the effect of cerivastatin on HO-1 expression. For cell adherence the cell culture media contained phorbol ester 12-*O*-tetradecanoylphorbol-13-acetate (TPA, $10^{-8}$ mol/L; Sigma, Seelze, Germany). We exposed the cells to cerivastatin (50 nmol/L), atorvastatin (1 $\mu$mol/L), or rosuvastatin (1 $\mu$mol/L) 6 hours before staining for HO-1. Immunocytochemistry and confocal microscopy were performed as described previously.[15] Quantification was performed with histogram function in the NIH Image software (Bethesda, MD). The subcellular regions were outlined manually, and the mean fluorescence intensity was calculated for the delineated regions.

### *Isolation of Circulating Monocytes*

Dextran sedimentation and Ficoll-Hypaque gradients were used for monocyte enrichment from whole rat blood. Briefly, 1 ml of rat blood was diluted in 1 ml of 3% dextran. After 30 minutes of incubation, the mixture was applied to 1 mol/L Ficoll (density 1.077 g/ml) and centrifuged for 20 minutes at 700 × *g* at 4°C. The buffy coat was transferred into a fresh tube. The cell pellet was washed twice with PBS and resuspended in 1 ml of sodium chloride. One hundred microliters of the cells were diluted 1:5, and cytospins were performed for 5 minutes at 700 rpm/minutes Cells were fixed with ice-cold ethanol.

### *Statistical Analysis*

We used the SPSS 12.01 software (SPSS Inc., Chicago, IL). After verifying normal distribution by the Kolmogorov-Smirnov test, we compared treatment groups by analysis of variance and post hoc Bonferroni correction as a post hoc test. Differences were considered as significant at $P < 0.05$. Data are presented as mean ± SEM.

## *Results*

### *Statin Protects from IR and Up-Regulates HO-1*

As shown in Figure 1, IR injury caused severe renal dysfunction as reflected in elevated creatinine levels (350 ± 10 $\mu$mol/L, $P < 0.005$) 24 hours after IR injury. Statin pretreatment significantly ameliorated the increase in serum creatinine concentration (201 ± 15 $\mu$mol/L, $P < 0.005$). Cotreatment with cerivastatin and the HO-1 inhibitor Sn-PP abolished the protective effect of statin pretreatment, and the increase in serum creatinine was similar to the untreated IR group (329 ± 13 $\mu$mol/L). Sham operation (right nephrectomy alone) did not result in significant serum creatinine elevation (63 ± 3 $\mu$mol/L).

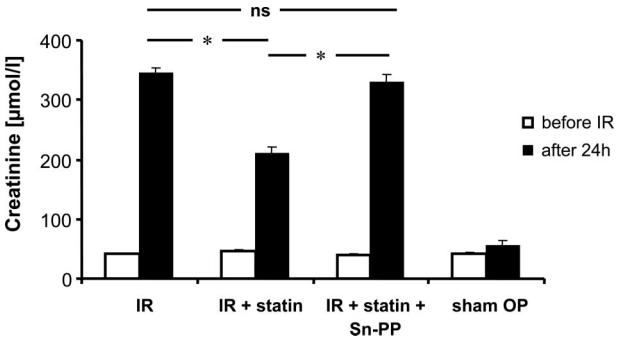


**Figure 1.** Serum creatinine before (white bars) and 24 hours after (black bars) IR injury is shown. Statin treatment attenuated the impairment of kidney function ($P < 0.005$). Cotreatment of statin with the HO inhibitor Sn-PP abolished the beneficial effect of cerivastatin. As a result, serum creatinine elevation was similar to that in the IR group. Control animals showed no significant creatinine elevation. ns, nonsignificant.

We next studied the effects of cerivastatin on ischemia-induced tissue damage. IR injury caused severe tissue damage in the outer medullary stripe that exhibited loss of the brush border and detachment of epithelial cells from the basement membrane (Figure 2A). The detach-

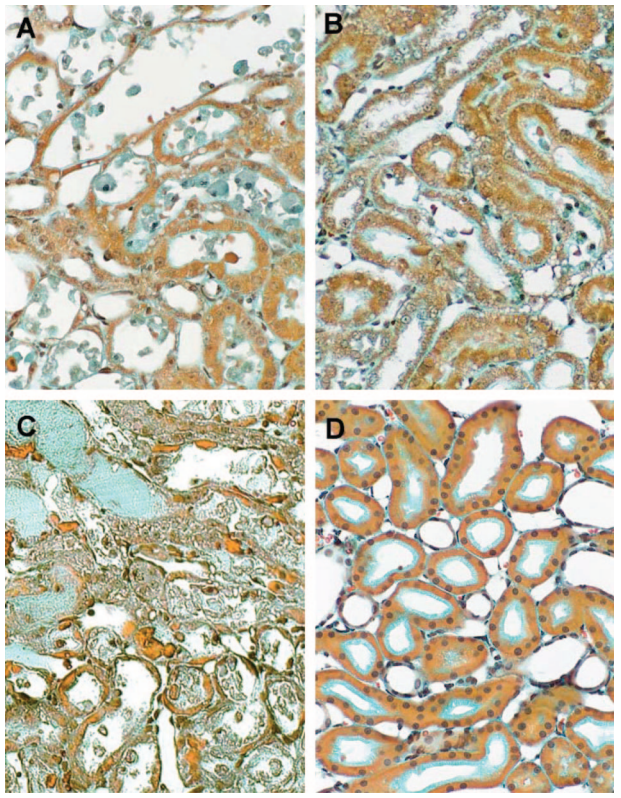


**Figure 2.** IR injury caused severe morphological damage in the outer medullary stripe (**A**). The damage was characterized by loss of the brush border, detachment of epithelial cells from the basement membrane, tubular obstruction, and denuded basement membranes. Statin treatment reduced the extent of acute tubular necrosis (**B**). Combined treatment with cerivastatin and HO inhibition (Sn-PP) caused severe morphological damage comparable with the IR group (**C**). Control kidneys showed normal renal morphology (**D**). Paraffin sections, MG stain; original magnification, ×200.

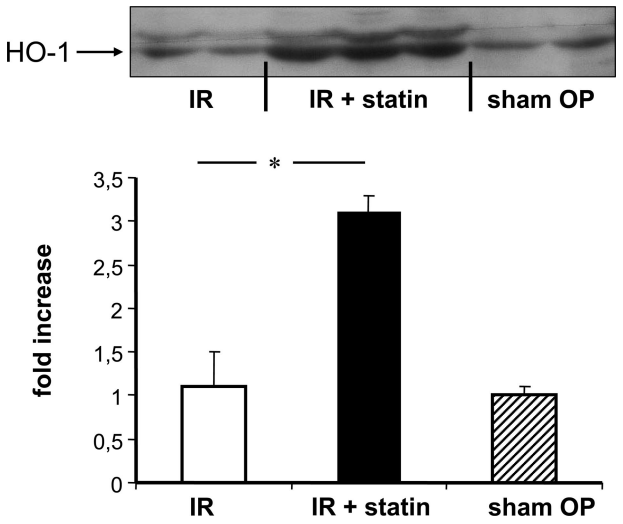


**Figure 3.** Statin up-regulated HO-1 protein in whole kidney tissue 24 hours after IR injury. The top panel shows a representative Western blot with the lower band corresponding to HO-1 (32 kd). The bottom panel shows the quantitation of the Western blot. IR injury alone did not up-regulate HO-1 quantification of the Western blot within 24 hours (white bar). In contrast, with statin treatment, IR up-regulated HO-1 almost more than threefold (black bar). Kidneys from controls showed only slight HO-1 expression (gray bar). Data represent mean ± SEM from three independent repeats; $^*P < 0.05$.

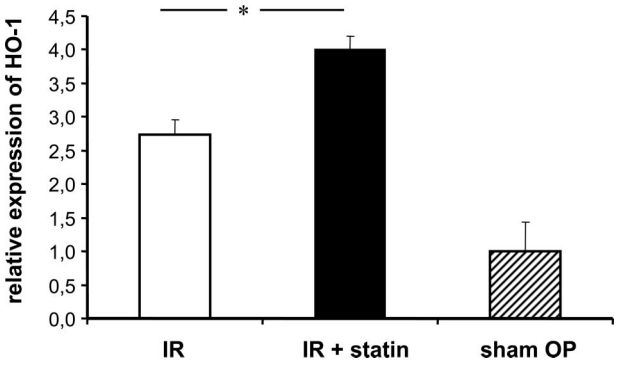


**Figure 4.** Statin treatment up-regulated HO-1 mRNA. Statin-pretreated kidneys (black bar) showed the highest up-regulation of HO-1 transcript compared with IR injury alone (white bar) or controls (gray bar). GAPDH was used as housekeeper. Data represent mean ± SD of relative HO-1 expression. Each group contained six animals; $^*P < 0.05$.

ment caused tubular obstruction and left naked basement membranes. In kidneys of statin-treated animals before IR injury, tubular necrosis was markedly reduced (Figure 2B). Most tubules were intact and showed normal brush border. Cotreatment with statin and the HO-1 inhibitor caused severe tissue damage comparable with IR injury alone (Figure 2C). Kidneys of sham-operated control animals after unilateral nephrectomy showed normal renal morphology (Figure 2D).

To test whether cerivastatin treatment induced HO-1 in renal tissue 24 hours after IR injury, we preformed Western blots. Representative Western blots of HO-1 protein expression are shown in Figure 3. IR injury caused slight but not significant up-regulation of HO-1 on the protein level (open bar), whereas with statin pretreatment, we observed significant up-regulation of HO-1 ($P < 0.05$, black bar). HO-1 expression in control kidney is shown on the right (hatched bar). Because HO-1 is regulated at the transcription level, we performed quantitative PCR of whole kidney samples shown in Figure 4. HO-1 mRNA expression of control animals was set at 1 (hatched bar). IR injury caused a 2.7-fold increase in HO-1 transcript (white bar), whereas statin pretreatment increased HO-1 expression 4.3-fold (black bar, $n = 6$ in each group). The HO-1 up-regulation with IR injury alone, compared with control animals on mRNA level, was not detected at the protein level by Western blotting. The result is explained possibly because the mRNA expression experiments preceded the protein expression determinations in these experiments. On the other hand, the mRNA expression by qPCR was more sensitive than Western blotting. In additional experiments, we investigated the effect of statin pretreatment on HO-1 mRNA and protein expression of sham-operated animals. Statin treatment alone did not cause up-regulation of HO-1 mRNA and protein levels in the kidney (data not shown).

Immunohistological staining of HO-1 is shown in Figure 5. IR injury (Figure 5A) led to peritubular and perivascular infiltration with HO-1-positive cells. Statin treatment before IR injury (Figure 5B) caused pronounced HO-1 expression, particularly in the peritubular compartment. In renal tissue of control animals (Figure 5C), only a few HO-1-positive areas were found. By further analyzing HO-1-positive areas in renal tissue sections of animals pretreated with cerivastatin, we found that primarily the infiltrating cells, rather than the resident cells, carried the HO-1 expression. We next examined the cell type of infiltrating cells expressing HO-1. We performed double staining of CD4-, CD8-, ED-1-positive monocytes/macrophages, and dendritic cells with HO-1 on kidney sections of statin-treated rats. We could identify ED-1-positive monocytes/macrophages as major source of HO-1 expression as shown in Figure 6. Infiltration of ED-1-positive monocytes/macrophages (Figure 6A) was most pronounced in the peritubular compartment. HO-1 expression is shown in the middle (Figure 6B). The right panel (Figure 6C) shows coexpression of ED-1- and HO-1-positive cells. For quantification, we compared the percentage of HO-1-positive macrophages between groups. In the IR group we counted 26 ± 3% HO-1-positive macrophages, whereas in the statin-treated group, we identified much more HO-1-positive macrophages (42 ± 3%) in the peritubular compartment. There was no relevant co-localization of HO-1-positive cells with T lymphocytes (CD4/CD8) or dendritic cells (data not shown).

## *Statin Induces HO-1 Up-Regulation in Monocytes* in Vitro *and* in Vivo

To confirm our *in vivo* findings and to test whether the effect of HO-1 up-regulation can be achieved by different statins, we performed additional *in vitro* experiments using a monocytic cell line shown in Figure 7. Stimulation of the human monocytic cell line U937 with cerivastatin (Figure 7B), atorvastatin (Figure 7C), and rosuvastatin (Figure 7D) for 6 hours caused a marked increase in

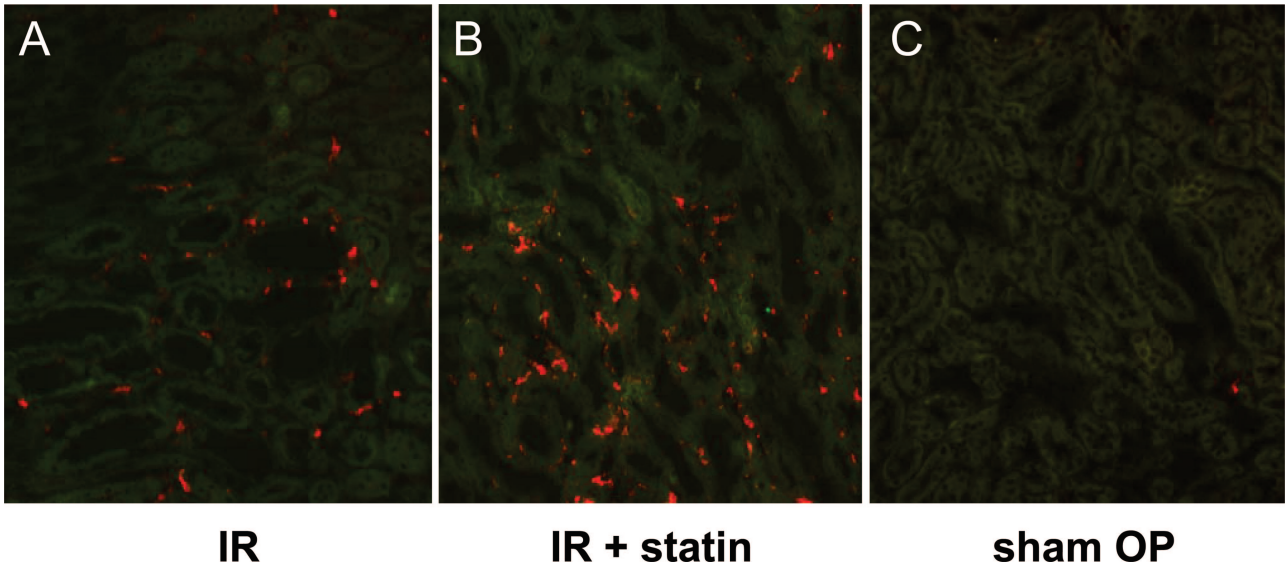


**Figure 5.** Representative immunohistochemistry for HO-1 expression in the outer stripe of the outer medulla is shown. IR injury-induced HO-1 expression in infiltrating cells (**A**). Statin-treated animals showed pronounced positive staining for HO-1 in infiltrating cells mainly in the peritubular area (**B**). Controls showed no significant HO-1 expression (**C**). Original magnification, ×200.

HO-1 expression. Semiquantitative analysis (Figure 7E) of HO-1 in 20 representative areas showed a significant increase of HO-1 protein expression by all statins tested ($P < 0.005$).

To explore whether the cell culture effects are representative for the *in vivo* situation, we treated rats for 3 days with cerivastatin and isolated circulating monocytes ($n = 6$, each group). We performed cytospins and double stained with 4,6-diamidino-2-phenylindole, indicating nuclei of monocytes, and for HO-1. The effect of statin treatment on HO-1 protein expression on circulating monocytes is shown in Figure 8. Statin treatment (Figure 8B) clearly up-regulated the number of HO-1-positive monocytic cells compared with control cells (Figure 8A). Semiquantitative analysis (Figure 8C) of HO-1-positive monocytes in 10 representative areas of each specimen showed a 4.8-fold increase of HO-1 protein expression by statin treatment compared with control ($P < 0.005$).

## *Discussion*

Our data demonstrate that a 3-day treatment course with cerivastatin before IR injury markedly reduces renal tissue damage and ameliorates renal dysfunction 24 hours after induction of renal ischemia. Moreover, we found significantly increased HO-1 transcript and protein levels in the kidneys of statin-pretreated rats. Most interestingly, we found that infiltrating monocytes/macrophages are a major source of this increased HO-1 production 24 hours after IR injury. This finding was further corroborated by our *in vivo* data, in which we showed that statin treatment increases HO-1 expression on circulating monocytes. Our results support the conclusion that after statin treatment-infiltrating monocytes/macrophages may mediate tissue protection from ischemic injury. Our data are consistent with the recently proposed concept of macrophage activation heterogeneity, and, in particular, the

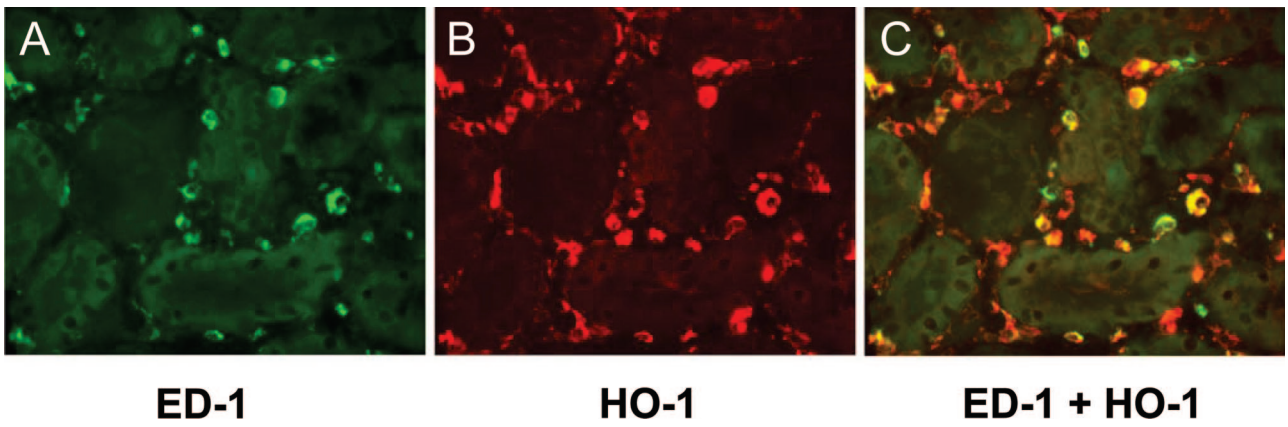


**Figure 6.** ED-1 and HO-1 expression in the kidneys of the statin-treated group 24 hours after IR injury. ED-1-positive monocytes/macrophages (**A**) in the perivascular and peritubular compartment of the outer stripe of the outer medulla show HO-1 expression (**B**); coexpression of ED-1 and HO-1 is shown in **C**. The peritubular area of the outer medullary stripe is most sensitive to hypoxia and contained the majority of infiltrating cells. Not all ED-1-positive cells were HO-1 positive. Not all HO-1-positive cells were ED-1 cells. Only a few infiltrating lymphocytes showed HO-1 up-regulation. Original magnification, ×400.

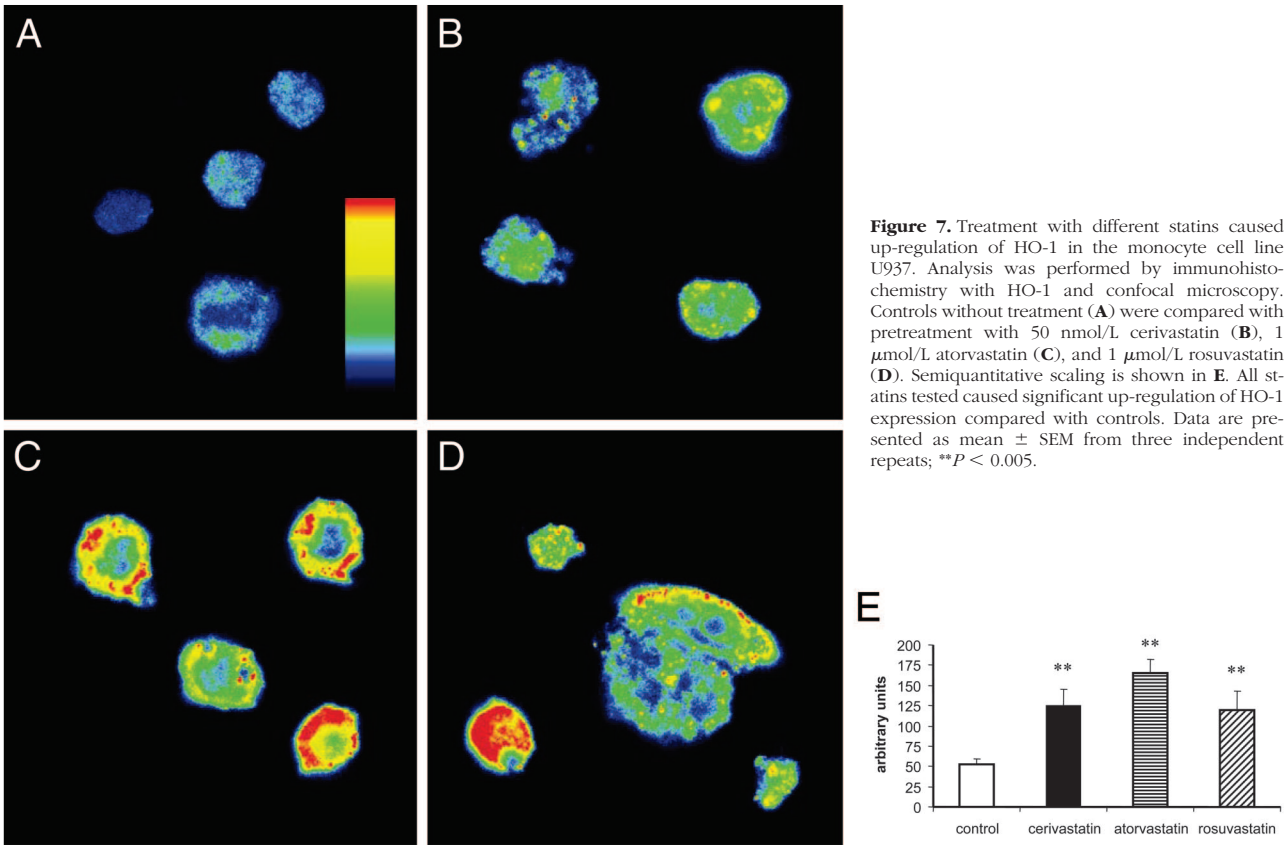


**Figure 7.** Treatment with different statins caused up-regulation of HO-1 in the monocyte cell line U937. Analysis was performed by immunohistochemistry with HO-1 and confocal microscopy. Controls without treatment (**A**) were compared with pretreatment with 50 nmol/L cerivastatin (**B**), 1 $\mu$mol/L atorvastatin (**C**), and 1 $\mu$mol/L rosuvastatin (**D**). Semiquantitative scaling is shown in **E**. All statins tested caused significant up-regulation of HO-1 expression compared with controls. Data are presented as mean ± SEM from three independent repeats; $^{**}P < 0.005$.

ability of macrophages to curtail inflammation and restore normal function after injury.[17]

Macrophages seem both to promote and to down-regulate inflammation.[18–20] We provide supportive evidence that monocytes/macrophages can be preconditioned by statins to up-regulate HO-1. Statin-stimulated and HO-1-overexpressing cells infiltrate damaged tissue and act locally by delivering HO-1. This delivery limits local tissue destruction and supports tissue repair. This infiltration also may be of relevance in tissues apart from the kidney. The inflammatory reaction after IR injury is characterized by leukocyte infiltration. In postischemic acute renal failure, the majority of infiltrating cells are ED-1-positive monocytes/macrophages.[21] Recent studies suggest that this macrophage infiltration has a heterogeneous role

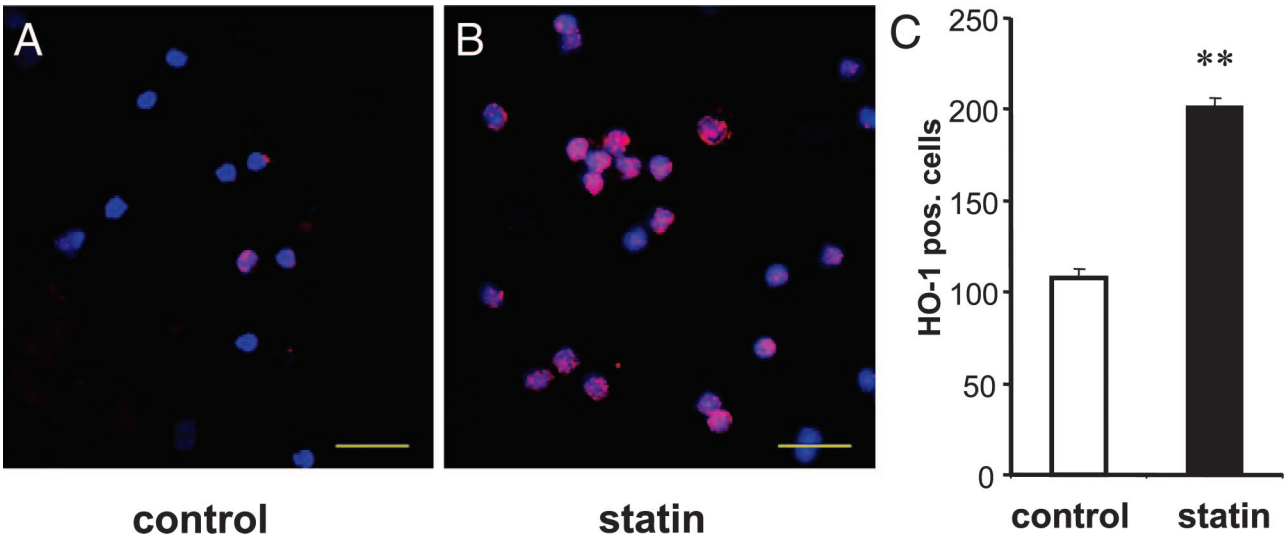


**Figure 8.** Statin induced up-regulation of HO-1 expression on circulating monocytes in statin-treated animals. Left panel shows monocytes of control rats (**A**); the middle panel shows monocytes of statin-treated rats (**B**) (scale bar = 100 $\mu$m). Semiquantitative scaling is shown in **C**. The bars represent cell counts of 10 different view fields (each group contained six animals); $^{**}P < 0.001$.

in all stages of the inflammatory process including tissue repair and healing.[18]

Beyond lipid-lowering effects, statin treatment exerts anti-inflammatory and cytoprotective effects in several atherosclerosis models.[14,15] Large clinical trials showed that statins significantly reduce cardiovascular risk.[22,23] The strong cholesterol-lowering action of statins contributes to their beneficial effects, for instance by lowering levels of proinflammatory lipids. In our earlier rat studies, we found that statins did not lower total cholesterol in the rat.[15] Rodents rely primarily on high-density lipoprotein cholesterol that is not lowered by statins. Moreover, statins were recently shown to exhibit other actions involved in endothelial function, such as cell proliferation, inflammatory response, immunological reactions, platelet function, and lipid oxidation.[24] These pleiotropic effects were not related to reduced circulating cholesterol. Moreover, statins also activated protective HO-1. As mentioned, in vascular smooth muscle cells[2] and endothelial cells,[4] up-regulation of HO-1 mRNA and protein after statin exposure has been previously demonstrated. In our model of renal IR injury, we found up-regulation of HO-1 transcript and protein in whole kidney tissue. Hypoxia, radiation, and shear stress all up-regulate HO-1 gene transcription as a response to tissue damage and the induction of repair mechanisms.[25,26] HO-1 mRNA up-regulation in response to IR injury in resident renal cells is a very early event, reaching a maximum at 6 hours and then rapidly returning to control levels.[27] Without an additional stimulus such as hypoxia, statins had no effect on renal HO-1 expression. Interestingly, we found that statin pretreatment caused significant high HO-1 activation, even after 24 hours after IR injury, stressing the fact that infiltrating cells may mediate the beneficial effect of statin treatment. At that time, the major source of HO-1 production was mainly infiltrating monocytes/macrophages in the peritubular space of the outer stripe of the outer medulla. This area is most sensitive to hypoxic damage. We have previously shown that the total number of infiltrating monocytes/macrophages is lower in the statin-pretreated group compared with IR alone.[1] In this study we revealed that even though the total number of infiltrating monocytes/macrophages was lower in the statin-treated group, the proportion of HO-1-positive monocytes/macrophages was clearly increased.

Our data support the hypothesis that monocytes/macrophages act protectively and limit further IR tissue damage via delivery of HO-1 to tissue injured by hypoxia. HO-1 degrades heme into CO and biliverdin. HO-1 converts the latter to bilirubin. These substances have powerful anti-inflammatory, antiapoptotic, and antioxidant effects and maintain the integrity of microcirculation.[28–30] The HO-1 system is one of the most important cytoprotective mechanisms activated during cellular stress resulting from hypoxia. In several disease models such as IR injury in liver,[31,] kidney,[32] solid organ transplantation,[33–35] and ischemic heart disease,[36] the beneficial effects of HO-1 up-regulation on tissue preservation were clearly demonstrated.

We believe our data may have therapeutic implications. Statins could be beneficial in the prevention of acute renal failure in patients undergoing major vascular surgery for atherosclerotic disease, particularly if they are at high risk for developing acute renal failure postoperatively, having namely advanced age, pre-existing renal impairment, and diabetes. A statin indication is often already present in such patients. However, clinical studies are needed to evaluate the role of preventive statin treatment in humans. The mechanisms of statin action in terms of HO-1 regulation are far from satisfactorily explained. Statins may result in the disruption of lipid rafts, or they may interfere with the prenylation of certain G proteins. How statins act to regulate HO-1 expression is not yet clear. The transcriptional regulation of HO-1 in our macrophages and putative influences of statins are unknown. In macrophages, HO-1 expression is mediated through accumulation of the bZIP transcription factor Nrf2 (NF-E2-related factor-2).[37,38] This transcription factor and others need to be explored. New targets could conceivably be discovered.

## *Acknowledgments*

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