Postischemic Acute Renal Failure Is Reduced by Short-Term Statin Treatment in a Rat Model

FAIKAH GUELER,* SONG RONG,* JOON-KEUN PARK,* ANETTE FIEBELER,* JAN MENNE,† MARLIES ELGER,* DOMINIK N. MUELLER,‡ FRANZISKA HAMPICH,§ RALF DECHEND,‡ UTA KUNTER,§ FRIEDRICH C. LUFT,‡ and HERMANN HALLER*

*Department of Internal Medicine-Nephrology, Hannover Medical School, Hannover, Germany; †Phenomiques GmbH, Hannover, Germany; ‡Franz Volhard Clinic HELIOS Klinikum-Berlin, Max Delbrueck Center of Molecular Medicine, Medical Faculty of the Charité, Humboldt University of Berlin, Berlin, Germany; and §Division of Nephrology, Department of Medicine, Rheinisch-Westfälische Technische Hochschule (RWTH) Aachen, Aachen, Germany.

Abstract. Postischemic acute renal failure (ARF) is common and often fatal. Cellular mechanisms include cell adhesion, cell infiltration and generation of oxygen free radicals, and inflammatory cytokine production. Hydroxy-3-methylglutaryl coenzyme A reductase inhibitors ("statins") directly influence inflammatory mechanisms.

Renal ischemia-reperfusion injury is a major cause of acute renal failure (ARF) after major surgery or renal transplantation (1,2). Several experimental ameliorative strategies have been tested (3). However, there is still a remarkable lack of definitive evidence supporting specific prophylactic therapies in any setting (4). Postischemic ARF seems to be a consequence of hypoxia attributable to impaired perfusion, with subsequent reperfusion leading to acute inflammatory changes. Pathophysiologic mechanisms include intracellular damage, with ATP depletion (5), and intracellular Ca2+ accumulation (6). Cellular activation leads to reactive oxygen species generation, phospholipase activation, and membrane lipid alterations (7). Inflammatory reactions are characterized by surface adhesion molecule expression, followed by leukocyte infiltration, protease activation, and cytokine production (8). Reorganization of integrins from basal to apical surfaces of injured tubular epithelial cells has been demonstrated to facilitate detachment, contributing to tubular obstruction (9). These changes are most pronounced in the outer medullary stripe, i.e., a part of the kidney that is extremely susceptible to hypoperfusion and hypoxic damage (10). Other compartments, such as the corticomедullary junction, are also injured, however. We and others have demonstrated that inhibition of inflammatory pathways, such as inhibition of redox-sensitive nuclear factor-κB (NF-κB) or blockade of intercellular adhesion molecule-1.
(ICAM-1) expression, is a beneficial strategy in ARF models (11,12). Recent evidence suggests that statins can prevent vascular inflammatory reactions in human subjects and in animal models, independently of their LDL cholesterol-lowering effects (13). In earlier studies involving a hypertensive rat model of angiotensin II-induced renal and cardiac damage, we observed that statin treatment was protective and NF-κB and activator protein-1 (AP-1) activation was reduced (14,15). Because statins could possibly have prophylactic utility among patients at risk for ischemic ARF, we tested whether similar amelioration might occur in an animal model.

**Materials and Methods**

Experiments were performed with 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 performed according to the guidelines of the American Physiological Society and were approved by local authorities. One group of rats was treated with cerivastatin (0.5 mg/kg body wt) by gavage for 3 d before surgery. Surgery was performed with general anesthesia with ketamine (100 mg/kg body wt; CP-Pharma, Burgdorf, Germany) and xylazine (5 mg/kg body wt; Rompun Bayer, Leverkusen, Germany). On the third day of treatment, surgery was performed as described previously (10). The left renal pedicle was occluded for 45 min, during which time a right nephrectomy was performed. A control group received saline vehicle, by gavage, and underwent a clipping operation and contralateral nephrectomy as described above. The third group received saline treatment and underwent a sham operation, in which the abdomen was opened, the left renal pedicle was dissected but not clamped, and the right kidney was removed. All animals were anesthetized for 45 min and euthanized 24 h later. Each group contained 18 animals. Blood samples were obtained before surgery and at the time of euthanasia. Creatinine levels were measured by using an automated method (Beckman analyzer; Beckman, Germany). The rats maintained 18 animals. Blood samples were obtained before surgery and at the time of euthanasia. Creatinine levels were measured by using an automated method (Beckman analyzer; Beckman, Germany). The rats were perfused, via the aorta, with 100 ml of cold phosphate-buffered saline, and their kidneys were removed.

For measurement of inulin and p-aminohippurate (PAH) clearance, rats were anesthetized and placed on a heating table for maintenance of body temperature (37°C). A catheter was placed in the carotid artery for measurement of systemic BP. Catheters were also inserted into the jugular and femoral veins and the bladder, for infusion, blood sampling, and urine collection, respectively. Inulin and PAH (in saline solution with 1% bovine serum albumin) were infused at a constant rate of 50 μl/min. After equilibrium was reached (30 min), four consecutive urine samples (30 min each) were collected. Blood samples were collected in the middle of each period. Inulin and PAH concentrations were measured colorimetrically.

For paraffin histologic assessments, kidneys were perfused with cold phosphate-buffered saline and then with cold fixative containing 4% paraformaldehyde in Soerensen’s phosphate buffer. The kidneys were fixed for an additional 4 h and then embedded in paraffin. For immunohistochemical analyses, the kidneys were snap-frozen in isopentane (−35°C). For Western blotting, the kidneys were snap-frozen in liquid nitrogen. For morphologic evaluations, 3-μm paraffin sections were stained with trichrome Masson-Goldner stain, by using standard procedures.

Immunohistochemical analyses were performed with the following primary antibodies: monoclonal mouse anti-rat ICAM-1 (1A29; Serotec, Oxford, UK), anti-rat monoclones/macrophages (ED-1; Serotec), polyclonal rabbit anti-inducible nitric oxide synthase (iNOS) (ABR, Golden, CO), rabbit anti-rat fibronectin (Paesel and Lolei, Frankfurst, Germany), and goat anti-collagen type IV (Southern Biotechnology Associates, Birmingham, AL). For indirect immunofluorescence assays, nonspecific binding sites were blocked with 10% normal donkey serum (Jackson ImmunoResearch, West Grove, PA) for 30 min. The cryosections were then incubated with the primary antibody for 1 h. All incubations were performed in a humidified chamber at room temperature. For fluorescence observation of bound primary antibodies, sections were subsequently incubated with Cy3-conjugated secondary antibodies (Jackson ImmunoResearch) for 1 h. Immunostaining for monocytes/macrophages (with ED-1) was performed by using the avidin-biotin complex-peroxidase system (Vector Laboratories, Burlingame, CA). The sections were treated with 0.6% H2O2 in methanol for 10 min, to quench endogenous peroxidase activity. Subsequently, 10% goat serum was used to block nonspecific binding sites. Endogenous biotin was quenched by using an avidin-biotin blocking kit (Vector Laboratories). Observation was performed with an aminothio-carbazole/H2O2 mixture. Nuclei were counterstained with Delafield’s hematoxylin solution (Fluka, Buchs, Switzerland). Specimens were analyzed by using a Zeiss Axiosoplan-2 imaging microscope, with the computer program AxioVision 3.0 (Zeiss, Jena, Germany). Semi-quantitative scoring of ED-1-positive cells was performed by using a computerized cell-counting program (KS 300 3.0; Zeiss). Fifteen different areas of each kidney sample were analyzed. Semi-quantitative analysis of iNOS expression was performed by counting the numbers of glomeruli with high, medium, and low levels of expression. Scoring was performed without knowledge of the identity of the animal group.

For Western blotting, the frozen kidneys were pulverized in liquid nitrogen and resuspended in 2 ml of lysis buffer (20 mM Tris buffer, pH 7.5, containing 10 mM glycerophosphate, 2 mM pyrophosphate, 1 mM sodium fluoride, 1 mM phenylmethylsulfonyl fluoride, 1 g/ml leupeptin, 1 mM dithiothreitol, and 1 mM ethylenediaminetetraacetate). Homogenates were sonicated with 20-s bursts, on ice, and centrifuged at 500 g for 1 min, to remove cell debris. Aliquots of the supernatants were stored at −80°C. Protein amounts were measured by using the Lowry assay. Seventy micrograms of protein from each sample were suspended in loading buffer, separated on a 10% polyacrylamide gel, and electrophoretically transferred to a nitrocellulose membrane. Membranes were blocked with 5% skim milk/1% bovine serum albumin for 1 h at room temperature. A primary antibody against phosphorylated extracellular activated kinase-1/2 (ERK1/2) [p42/44 mitogen-activated protein (MAP) kinase; NE Biolabs, UK] was applied, with gentle rocking, overnight at 4°C. After three 10-min washing steps with TBST buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.01% Tween 20), incubation with horseradish peroxidase-conjugated goat anti-rabbit secondary antibody (Dianova, Hamburg, Germany) was performed for 1 h at room temperature. After three additional TBST washes, the membrane was incubated with Renaissance reagent (NEN Life Science, Zavantem, Belgium), according to the instructions provided by the manufacturer, and then exposed to x-ray film (Kodak, Rochester, NY). Quantification was performed with relative density measurements (Scion Image, Frederick, MD).

Tissue preparation for electrophoretic mobility shift assays was performed as described previously (16). Nuclear extracts (5 μg) were incubated in binding reaction medium with 0.5 ng of [32P]dATP-end-labeled oligonucleotide, containing the NF-κB binding site from the MHC enhancer (H2K, 5'-GATCAGGGGCTGGGATTCCTCCATCTCCACAGG-3'). For AP-1, double-stranded oligonucleotides containing the consensus sequence for AP-1 (5'-CGGTGATGACTCAGCCGGAA-3') (Santa Cruz Biochemicals, Santa Cruz, CA) were
Serum creatinine levels before (Figure 1). As demonstrated in Figure 1, 45 min of ischemia and subsequent reperfusion caused severe loss of renal function, as reflected in a 7.5-fold elevation in creatinine levels (P < 0.005), compared with the sham-operated group. Renal function decreases were ameliorated by statin treatment, which reduced the elevation by 40%. Statin-treated rats demonstrated threefold creatinine level increases 24 h after surgery, compared with sham-operated animals (P < 0.005). Right nephrectomy alone (sham operation) did not increase creatinine concentrations. To substantiate the effects of statin treatment on renal function after ischemia, we analyzed GFR (inulin clearance) and PAH clearance. These results are presented in Figure 2: GFR and PAH clearance in sham-operated animals were 0.95 ± 0.09 ml/min and 4.4 ± 0.33 ml/min (n = 13), respectively. Ischemia (45 min) reduced these values to 0.06 ± 0.02 ml/min and 0.07 ± 0.02 ml/min (n = 9, P < 0.001), respectively. Pretreatment with a statin significantly ameliorated the decreases in both GFR and PAH clearance (0.21 ± 0.03 ml/min and 0.36 ± 0.09 ml/min, respectively; n = 11, P < 0.001).

We next studied the effects of statins on ischemia-induced tissue damage. Representative examples of these experiments are presented in Figure 3. Ischemia-reperfusion injury caused severe tissue damage in the S3 segment of proximal tubules. The outer medullary stripe exhibited loss of the brush border and detachment of epithelial cells from the basement membrane. This effect left naked basement membranes (Figure 3D, arrow) and caused tubular obstruction (Figure 3D, arrowhead). In kidneys of statin-treated animals, the tubular necrosis was markedly reduced. Most tubules were intact and demonstrated normal brush borders (Figure 3, B and E). Kidneys from sham-operated animals are presented in Figure 3, C and F.

Figure 1. Serum creatinine levels before (□) and 24 h after (■) ischemia-reperfusion injury. Ischemia-reperfusion injury (IR) and treatment with saline vehicle resulted in significant creatinine level elevation after 24 h. Treatment with a statin before ischemia-reperfusion injury (IR + statin) dramatically reduced the impairment of kidney function. A right nephrectomy without ischemia of the contralateral kidney (sham OP) caused mild creatinine elevation after 24 h.

Figure 2. Inulin clearance (A) and PAH clearance (B) after 45 min of ischemia without treatment (IR) (n = 9) or with statin pretreatment (IR + statin) (n = 11), compared with sham-operated control animals (sham OP) (n = 13). Ischemia induced major decreases in both inulin clearance and PAH clearance (P < 0.001, compared with control animals). Pretreatment with a statin significantly increased GFR (*P < 0.001, compared with ischemia-reperfusion) and PAH clearance (*P < 0.05, compared with ischemia-reperfusion).
upregulation of adhesion molecules. Representative immuno-histologic specimens stained for ICAM-1 are presented in Figure 5. Figure 5, A to C, demonstrates representative glomeruli, and Figure 5, D to F, presents representative arteries. Ischemia-reperfusion injury induced massive upregulation of ICAM-1 in the glomeruli and cortical peritubular interstitium (Figure 5A). The adhesion molecule ICAM-1 was dramatically upregulated in the intima and in the perivascular interstitium of arteries after ischemia-reperfusion injury (Figure 5D). Statins blocked ICAM-1 upregulation in the glomeruli (Figure 5B), as well as perivascular and endothelial upregulation of ICAM-1 expression (Figure 5E). Figure 5, C and F, presents control sections from the corresponding areas in kidneys of sham-operated rats, with low ICAM-1 expression.

Next, we analyzed the effects of statins on the upregulation of iNOS expression in glomeruli. Representative immunostainings for iNOS are presented in Figure 6, A to C. Ischemia-reperfusion injury caused significant elevation of iNOS expression in the glomeruli of the corticomedullary junction (Figure 6A); the staining pattern was mainly focal. The statin blocked iNOS upregulation in the glomeruli (Figure 6B). Sham-oper-
ated animals demonstrated low levels of iNOS expression, comparable to that of statin-treated animals (Figure 6C). Figure 6, lower, presents the semiquantitative analysis of iNOS expression; iNOS expression was defined as high, medium, or low. Ischemia-reperfusion yielded a significantly greater number of glomeruli with high levels of iNOS expression, compared with the statin-treated and sham-operated animals ($P < 0.005$).

We then examined fibronectin expression. Fibronectin is a multifunctional protein that is upregulated in the early phase of ischemia-reperfusion injury and remains at high levels for several weeks. Representative immunostaining for fibronectin

Figure 4. Perivascular infiltration by ED-1-positive monocytes and macrophages. (A) Ischemia-reperfusion (IR) caused severe infiltration of ED-1-positive cells. (B) The statin (IR + statin) reduced the influx of monocytes/macrophages into renal tissue. (C) The sham operation (sham OP) resulted in solitary infiltrating cells. (Lower) Semiquantitative scoring of ED-1-positive cells was performed. Results are depicted as mean ± SEM for each group. *$P < 0.005$. Magnifications, ×200 in A through C.
in the outer medullary stripe is presented in Figure 7, A to C. We observed that tubulointerstitial fibronectin was upregulated in ischemia-reperfusion injury (Figure 7A). Statin treatment completely prevented the marked expression of fibronectin (Figure 7B). Control animals demonstrated similar staining patterns, compared with statin-treated animals (Figure 7C). Collagen IV is a marker for matrix accumulation in the kidney. After ischemia-reperfusion injury, there was a broadening of the peritubular interstitium, with increased expression of collagen IV. Representative staining for collagen IV is presented in Figure 7, D to F. Ischemia-reperfusion injury led to marked upregulation of collagen IV expression in the periglomerular and peritubular interstitium (Figure 7D). We observed that the statin partially prevented matrix expansion (Figure 7E), compared with sham-operated control animals (Figure 7F).

We next assessed possible intracellular statin-related effects. MAP kinases are involved in inflammatory processes and can be upregulated by ischemia. Therefore, we studied the activation of the MAP kinase ERK1/2. The p42/44 antibody directed against the phosphorylated form of ERK1/2 detected activation. Representative Western blots are presented in Figure 8. Figure 8A presents the Western blot results; each lane corresponds to results for one animal. Figure 8B presents the densitometric analysis results. Ischemia-reperfusion injury caused marked activation of ERK1/2 (Figure 8A, left). We observed that the statin was effective in decreasing ERK1/2 activation after ischemia-reperfusion injury (Figure 8A, middle). The sham operation caused only slight activation of ERK1/2 (Figure 8A, right).

In Figure 9, we demonstrate activation of the DNA-binding
nuclear factor NF-κB in electrophoretic mobility shift assays. Two lanes are representative of the group. NF-κB DNA-binding activity was upregulated in the kidney after ischemia-reperfusion injury. The sham operation resulted in slight NF-κB activation. The statin blocked NF-κB activation in a time-dependent manner. Three days of statin treatment before ischemia were more effective in blocking NF-κB activation, compared with 2 d of treatment. Similar effects on AP-1 activity were observed (data not shown).

**Discussion**

We investigated the effects of cerivastatin on acute ischemia-reperfusion injury. We observed that 3-d treatment significantly ameliorated the decrease in renal function with post-
ischemic acute tubular necrosis and considerably limited the structural damage after ischemia. Histologically, we observed that statin treatment reduced damage in the S3 segment of proximal tubules in the outer medullary stripe, the area that is most susceptible to hypoperfusion and hypoxia. Untreated animals demonstrated typical changes in the S3 segment, namely loss of the brush border, destruction of epithelial cells, naked basement membranes, and tubular obstruction. Statin treatment clearly reduced the morphologic damage. Inflammatory reactions attributable to ischemia-reperfusion injury are characterized by leukocyte infiltration. In postischemic ARF, the majority of infiltrating cells are ED-1-positive monocytes and macrophages (10). We observed that the statin reduced ED-1-positive cell infiltration by >50%, compared with no treatment. Inflammatory cell infiltration in ischemia-reperfusion injury is accompanied by significant upregulation of adhesion molecules (17). We observed that activation of the adhesion cascade, as reflected by upregulation of ICAM-1 on the endothelium of arteries and in the perivascular space, was inhibited by the statin. The upregulation of ICAM-1 expression in glomeruli, the periglomerular area, and the peritubular interstitium was also prevented. Other strategies to prevent ICAM-1 expression after ischemia-reperfusion injury during rat renal transplantation have been demonstrated to reduce chronic graft dysfunction (11). The statins might also be beneficial in such situations.

Enhanced NO production, presumably attributable to macrophage-type iNOS, participates in hypoxic/ischemic proximal tubular injury (18). Experiments in gene-disrupted mice demonstrated that iNOS blockade attenuates renal ischemia-reper-

**Figure 7.** Representative immunohistochemical evaluations of fibronectin expression (A to C) and collagen IV expression (D to F). (A) Ischemia-reperfusion injury (IR) caused matrix accumulation in the peritubular interstitium, with increased expression of fibronectin. (B) The statin (IR + statin) partially prevented the matrix expansion. (C) Sham-operated animal (sham OP). (D) Ischemia-reperfusion injury caused matrix accumulation in the periglomerular and peritubular interstitium, with markedly elevated collagen IV expression. (E) The statin partially prevented the matrix expansion. (F) Sham-operated animal. Magnification, ×200 in A through F.
fusion injury (19). We observed that statin treatment prevented increased iNOS expression in glomeruli. This statin-related effect could lead to a decrease in free radical oxygen levels during ischemia and reperfusion. In other models of renal disease, inhibition of oxidative systems was demonstrated to have marked beneficial effects and to prevent tissue damage (20).

Fibronectin is a major glycoprotein in plasma and in the extracellular matrix. Fibronectin plays a role as a chemoattractant for several cell types and generates a scaffold to which other matrix components can attach. In response to ischemia-reperfusion injury, fibronectin is markedly upregulated in the renal interstitium (21). We observed that cerivastatin effectively blocked the increased expression of fibronectin in the tubulointerstitium of the outer medullary stripe. We also examined collagen IV expression, because abnormal collagen turnover is a major histologic feature in many forms of renal disease. Collagen IV expression is also predictive of final outcomes. In ischemia-reperfusion injury, increased deposition of collagen IV occurs in the renal interstitium, Bowman’s capsule, and tubular basement membranes (22). We observed that the increased collagen IV deposition in the peritubular interstitium after ischemia-reperfusion injury was decreased with statin treatment.

We focused on MAP kinase ERK1/2 activation because, in our earlier angiotensin II-induced glomerulosclerosis model, we observed that these signaling molecules were probably involved in renal injury (14). Many extracellular signals, including oxidative stress, are transduced to the nucleus via activation of the MAP kinase cascade (5). Statins can directly interfere with intracellular signaling mechanisms (23). The most likely mechanism involves statin inhibition of G protein prenylation, via reductions of farnesylation and geranylgeranylation. In vivo studies have demonstrated that these statin-related effects on intracellular signaling mechanisms can be overcome with the addition of either mevalonate or farnesol (24,25). Small G proteins of the Ras family regulate MAP kinase activation. Ras binds to Raf, thus activating MEK, which in turn phosphorylates ERK1/2 (26). As in our previous in vitro studies, we observed that cerivastatin downregulated the ischemia-induced activation of ERK1/2 (14). We have now extended these observations to the in vivo setting and to a different model, namely posts ischemic ARF.

NF-κB and AP-1 are redox-sensitive transcription factors that are activated by many stimuli, including atherogenic events (16). NF-κB and AP-1 regulate the expression of genes encoding inflammatory adhesion molecules, cytokines, and chemokines, leading to inflammation (27). We demonstrated that statin treatment partially prevented the upregulation of the DNA-binding activity of NF-κB and AP-1 in ischemia-reperfusion injury. NF-κB belongs to a family of transcription factors that are recognized by the κB enhancer element. Numerous proinflammatory genes have binding sites for NF-κB, and the products of these genes are integral parts of cellular activation and inflammatory response systems. There are close relationships between NF-κB and mediators of cell activation

Figure 8. Western blots of phosphorylated extracellular activated kinase-1/2 (ERK1/2) (42/44 kD). Each lane represents 70 μg of protein from a single kidney. (A) Ischemia-reperfusion (IR) led to marked phosphorylation of ERK1/2. The statin (IR + statin) decreased the ischemia-reperfusion injury-induced activation of ERK1/2. Kidneys from sham-operated rats (sham OP) demonstrated only slight ERK1/2 phosphorylation. (B) The results of densitometric analysis of phospho-ERK1/2 intensities are depicted as mean values.

Figure 9. Activation of DNA-binding nuclear factors by ischemia-reperfusion injury. (Lanes 1 and 2) Ischemia-reperfusion injury (IR) caused strong upregulation of nuclear factor-κB (NF-κB) DNA-binding activity. (Lanes 3 and 4) Sham-operated animals (sham). (Lanes 5 to 8) Statin pretreatment before ischemia-reperfusion injury (IR + statin) could partially prevent the upregulation of NF-κB DNA-binding activity. Three-day treatment (lanes 7 and 8) was more effective than 2-d treatment (lanes 5 and 6).
We and others previously demonstrated that the DNA-binding activity of NF-κB and AP-1 could be blocked by statin treatment in rat models (15). Direct inhibition of this transcription factor prevents tissue damage in other forms of renal disease as well (29).

We do not think that our findings are related to a reduction in LDL cholesterol levels. We did not measure cholesterol levels in this study, because in our earlier study we demonstrated that treatment of Sprague-Dawley rats with cerivastatin (0.5 mg/kg per d for 3 wk) did not decrease cholesterol levels (15). We performed our study from clinical interest, because prophylactic treatment with a statin drug for 3 d would clearly be welcome for patients at risk for developing ARF. Elective transplant recipients, patients electively undergoing major surgery, and perhaps patients undergoing radiologic interventions might be candidates. We can only speculate regarding the clinical relevance of our findings. We selected cerivastatin because this drug penetrates nonhepatocyte cells, in which it also inhibits hydroxy-3-methylglutaryl coenzyme A reductase (30). Other lipid-soluble statins also exhibit this property (31). Whether our findings are characteristic of relatively lipid-soluble statins only or can be extrapolated to hydrophilic statins cannot be determined on the basis of our data. Our speculations regarding intracellular mechanisms also require further testing. However, the data we report here and those from other studies (32,33) support the concept that statins have novel pleiotropic effects on intracellular signaling, independent of circulating cholesterol levels.

In conclusion, we demonstrated that a statin ameliorated postischemic ARF. Inflammatory mechanisms were significantly affected, supporting the hypothesis that the statin exerted direct anti-inflammatory effects in vivo. Cell infiltration, ICAM-1 and iNOS upregulation, matrix molecule expression, MAP kinase ERK1/2 activation, and transcription factor activation were all reduced. Our results indicate that certain statins may be useful in the prevention of ARF.

Acknowledgments
We thank Kerstin Bankes, Yvonne Nikolai, Michaela Beese, Petra Berkefeld, and Karin Dressler for technical assistance. This study received support from the Bayer Corporation. Drs. Muller, Elger, Luft, and Haller were supported by grants-in-aid from the Deutsche Forschungsgemeinschaft. This study was also supported by the Klinisch-Pharmakologischer Verbund, Berlin-Brandenburg.

References