Acute Protective Effects of Simvastatin in the Rat Model of Renal Ischemia-Reperfusion Injury: It Is Never Too Late for the Pretreatment

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Abstract. Acute pretreatment with a single i.v. bolus injection of simvastatin (1 mg/kg) significantly protects rat kidney injured by ischemia-reperfusion (I/R) (45 min + 6 h). We aimed to determine the optimal timing of such a pretreatment. The effects of both injections of simvastatin before ischemia and reperfusion were similar regarding total histological score. However, simvastatin injected 30 min before ischemia was 30%–75% more effective in reduction of serum creatinine levels and interstitial edema score, while its injections 5 and 30 min before reperfusion were 25%–60% more effective in reduction of tubular necrosis score and fractional excretion of Na⁺. However, the observed differences do not seem to offer significant advantage in clinical settings.

Keywords: simvastatin, acute pretreatment, ischemia-reperfusion (I/R) renal injury

Renal ischemia-reperfusion (I/R) injury with a high mortality rate and unresolved questions in pharmacotherapy remains one of the leading causes of acute renal failure (ARF) (1). Statins were convincingly shown to have intriguing therapeutic potential beyond lipid-lowering capacity (2). So called pleiotropic (lipid lowering-independent) effects of statins are attributed to their antiinflammatory, antioxidant, and/or vascular actions. In contrast to lipid lowering effects, pleiotropic actions of statins are rapid, but may be prone to tolerance (3). Protective effects of statins in experimental models of ARF were shown by different authors (4). In addition, we have recently confirmed that a single intravenous dose of simvastatin exerts such a protection in rats subjected to renal I/R injury (5). The acute protection of kidneys with pleiotropic compounds may offer a significant therapeutic approach in human renal I/R injury (e.g., during renal transplantation) (6). However, the optimal timing of acute pretreatment with statins in renal I/R injury remains to be elucidated. In order to address such a question, we have used an established rat renal I/R injury model (5).

Methods are described in detail elsewhere (7). The experimental design is shown in Fig. 1D.

In brief, in vivo experiments were performed in male adult Wistar rats (N = 51) weighing 234 ± 4 g. Rats were anesthetized using sodium thiopentone (Thiopental®; Nycomed Pharma, Unterschleibheim, Germany) (introduction, 120 mg/kg, i.p.; maintenance, approximately 10 mg/kg, i.v.). All animals received a continuous infusion of 0.9% (w/v) saline (8 ml/kg per hour, i.v., during adaptation-30 min and ischemia-45 min; 2 ml/kg per hour during reperfusion time of 4 h).

Following adaptation, I/R injury was induced by clamping both renal vascular pedicles for 45 min, followed by 4 h of reperfusion with saline (2 ml/kg per hour). In all groups during reperfusion, urine was collected, and after finishing the experiment, blood samples were taken and analyzed for markers of renal impairment.
Both kidneys of each animal were taken for histological evaluation. In all groups, post mortem samples of kidney were placed in formalin and processed through to wax. They were subsequently sectioned at 5 μm and stained with PAS (Periodic acid – Schiff). Original magnification ×20 was used. Each figure shown was randomly chosen from the series of at least 6 experiments (electronic light microscope type Leica DM LS 2, type 11020518016; Microsystems, Wetzlar, Germany). The kidney samples were then graded histologically according to the severity of injury by using a predetermined scoring system (8). The histological parameters evaluated were tubular necrosis, interstitial edema, loss of brush border, and casts formation. A minimum of 10 fields for each kidney slide were examined and assigned for severity of changes. The scoring system used was 0, absent; 1, present; and 2, marked. Total histological score per kidney was calculated by addition of all scores. Blind analysis of the histological samples was performed by two independent experts (Department of Pathology, School of Medicine, Belgrade, Serbia).

Animals were treated according to the Guide for the Care and Use of Small Laboratory Animals, School of Medicine, University of Belgrade; license number 244/9. The investigation conforms to the regulations of the European Union and USA Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health, NIH publication No. 85-23, revised 1985.

Compounds used in this study were simvastatin (Simvastatin®; Sigma-Aldrich, Poole, Dorset, UK), dimethylsulfoxide (DMSO) (Merck, Darmstadt, Germany), sodium thiopentone (Thiopental®; Nycomed Pharma, Unterschleißheim, Germany) and nonpyrogenic saline 0.9% w/v NaCl (Hemofarm, Vršac, Serbia). All values described in the text and figures are expressed as the mean ± S.E.M. of N observations. For in vivo studies, each data point represents biochemical measurements or histological scores obtained from 6 – 12 separate animals. Statistical analysis was carried out with GraphPad Prism/Instat 1.1 (GraphPad Software, La Jolla, CA, USA) using one-way analysis of variance.
ANOVA) followed by Dunnett’s post-hoc test. A P value of less than 0.05 was considered significant (NS = non significant).

I/R injury caused significant increases in serum urea and creatinine concentrations, and fractional excretion of sodium (sUr, sCre, and FENa⁺, respectively), in comparison with sham-operated rats (IR+DMSO vs Sham+DMSO; P<0.01, all) (Fig. 1: A – C).

Simvastatin similarly reduced sCre and sUr, and FENa⁺, regardless of the time of injection (Fig. 1: A – C). FENa⁺ was the most susceptible parameter to simvastatin pretreatment. Time-related differences between pretreatments were observed only in sCre (30 I vs 30 R, and 5 R; P<0.05, both; significance not shown in the figure).

I/R injury caused a marked increase in total histological score in comparison with sham-operated animals (IR+DMSO vs Sham+DMSO; P<0.01) (Fig. 2E).

Similar differences between I/R injured and sham-operated animals were observed regarding particular scores of renal injury (Fig. 2: A – D). Regarding renal histology, time-related differences between three administrations of simvastatin were observed only in tubular necrosis score (IR+DMSO vs 30 I, NS; IR+DMSO vs 30 R and 5 R, P<0.05, both) and interstitial edema score (IR+DMSO vs 30 I, P<0.01; IR+DMSO vs 30 R and 5 R, P<0.05, both).
Representative light photomicrographs of a kidney section taken from rats subjected to renal I/R injury. Periodic acid – Schiff (PAS) stain coloring. Original magnification ×20. Figures were randomly chosen from the series of at least 6 experiments. Panel A: Sham-operated animals treated with DMSO only (Sham+DMSO-group): normal kidney tissue, normal histological characteristic of glomeruli and tubules of this group. Panel B: Rats subjected to renal I/R injury, pretreated with 10% DMSO only (IR+DMSO-group): marked necrosis with tubular dilation, swelling, and luminal congestion (i.e., severe diffuse interstitial edema, severe dilatation of the tubular structure, marked tubular necrosis, and cast formation) predominates over morphological features of apoptosis (e.g., chromatin condensation and cell shrinkage). The major changes in tubules including loss of nuclei and appearance of tubular debris and casts are remarkable. Panels C – E: Rats subjected to renal I/R injury, pretreated with simvastatin at 1 mg/kg, i.v. (30 min before ischemia, 30 min before reperfusion and 5 min before reperfusion; 30I-, 30R-, and 5R-group, respectively): moderate kidney damage, focal tubular necrosis, and moderate dilatation of the tubular structure. In comparison with the IR+10% DMSO-group, in the simvastatin-treated group we observed preservation of tissue histology of the kidney.
Acute decrease of glomerular filtration rate is the result of both vascular and tubular events with subsequent apoptotic and necrotic renal cell death (1). Necrosis predominates over apoptosis in the current experimental model of ARF (e.g., IR+DMSO-group, Fig. 3C) (9). Later injection of simvastatin (i.e., 5 – 30 min before reperfusion) was necessary for a significant reduction of tubular necrosis score in the present model (30R- and 5R-group, Fig. 2A). It seems that maximal plasma concentrations of the active form of simvastatin should be achieved at the beginning of reperfusion for such an effect. In contrast, earlier protection of rat kidneys with active simvastatin (i.e., during ischemia) could not attenuate tubular necrosis. Consequently, the effects of simvastatin on tubular necrosis were rapid and short, occurring probably at the beginning of reperfusion. Acute (3 h), transient effect of a single dose of cerivastatin on endothelial responsiveness in humans has already been reported by Omori et al. (10). Rapid effects of simvastatin could be explained, at least in part, by regulation of NO production and activity (stabilization of eNOS mRNA, stabilization of eNOS protein, or a direct influence on eNOS activity) (10). The rapid effects of simvastatin on the PI3k/Akt/eNOS chain should also be considered, as well as the modulation of the Ras-ERK signaling cascade in inflammatory cells (11). Besides inhibition of small GTP-binding proteins (Rho, Ras, and Rac), which are regulated by isoprenoids, statins were shown to reduce oxidative stress in several ways: they suppress activation of NFκB (via either IkB-α or PI3k/Akt pathway), decrease parameters of in vivo LDL oxidation, and may protect paraoxonase and superoxide dismutase. Also, simvastatin decreased the production of 8-epi-PGF₂α and malondialdehyde (indicator of lipid peroxidation) in an in vivo model of myocardial I/R injury. Lipid lowering and Onset of Renal Disease (LORD) trial will assess the effects of statins on oxidative stress and inflammation in patients with chronic kidney disease (12).

Both injection of simvastatin before ischemia and before reperfusion significantly decreased interstitial edema score in comparison with the IR + DMSO-group. Interstitial edema score was significantly more improved when simvastatin was administered before ischemia compared to the later injection of the same drug (Fig. 2C). In addition, edema was almost completely abolished in the former case to the levels observed in Sham-operated animals. There are several possible explanations of such a result. First, simvastatin injected before ischemia could afford sufficient protection of peritubular blood vessels from endothelial cell damage and prevent extravasations from the beginning of ischemia; such an early protection was absent when simvastatin was injected later, that is, 5 – 30 min before reperfusion. At the same time, administration of simvastatin before ischemia possibly prevents neutrophil/endothelial interaction (13). The direct protection of injured kidneys by simvastatin was also possible when the drug was injected 30 min before ischemia because the uptake (passive diffusion) of simvastatin in the rat kidney tissues is rapid and high (C_{uptake} of 0.911 ml·min⁻¹·g⁻¹ tissue) (14). In other words, this highly lipophylic statin could easily reach the target kidney tissue before clamping of kidney pediculus if injected 30 min before ischemia. Furthermore, sufficiently high active simvastatin levels were possibly present in kidneys at the beginning of reperfusion, protecting tubular cells from the oxidative damage.

Taken together, administration of simvastatin before ischemia results in less pronounced interstitial edema, but more tubular necrosis in comparison with the injection of the same drug before reperfusion, and vice versa. Long-term follow-up of the rats subjected to I/R renal injury would help us to determine the relative importance of those acute protective actions of simvastatin and to eventually assess the optimal timing of simvastatin injection. However, it does not seem to be of a great clinical importance: first, total histological score was similar in all the experimental groups; and second, simvastatin injections could be repeated, providing the full protection of the injured kidneys. Regarding total histological score, it indicates changes in both kidney blood vessels and subsequent tubular events. Interstitial edema in reperfusion injury could be the result of both increased vascular permeability and back leakage of tubular fluid due to cast formation and tubular congestion (1, 2). The former events seem to precede tubular injury according to our results. However, modification of such a complex network could be possible only by repeated simvastatin injections at several points both during ischemia and reperfusion.

On the other hand, the acute protective effects of a single dose of simvastatin in our model of I/R injury do not seem to depend on the dose used, according to the unpublished pilot experiments from our laboratory with 3 mg of simvastatin (i.v. bolus, 30 min before ischemia, 30 min before reperfusion, and 5 min before reperfusion).

Earlier administration of simvastatin seems to result in a better protection of renal glomeruli, since sCr was significantly more reduced when the drug was injected 30 min before ischemia (Fig. 1B). However, this parameter of glomerular function has limitations as a biomarker of ARF and more investigation is needed to
clarify such an action (1). In addition, serum urea did not differ between groups (Fig. 1A).

The present study may have implications for modulating renal function in a clinical setting (e.g., renal protection during aortic or transplantation surgery). Clinical studies of the possible protective effects of statins in the acute rejection of transplanted kidneys were controversial. Some authors have suggested the possible protective effects of statins, for example, in the acute rejection of transplanted kidneys. In particular, statin therapy was associated with less acute allograft rejection in some early studies. However, trial-based clinical evidence did not support their use to lower acute rejection risk after kidney transplantation, but indicated effectiveness of statins for improvement of cardiovascular risk markers and possibly for reduction of clinical cardiac events (15).

In conclusion, acute pretreatment with a single intravenous dose of simvastatin seems to afford significant protection of rat kidneys from the I/R injury regardless of the time of injection (30 min before ischemia, 30 min before reperfusion, or 5 min before reperfusion). Certain differences in functional parameters and histological score found between the administration of the drug before ischemia and before reperfusion do not seem to be of clinical significance.

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References