Single-Dose Intravenous Simvastatin Treatment Attenuates Renal Injury in an Experimental Model of Ischemia-Reperfusion in the Rat

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Abstract. The effect of acute pretreatment with a single dose of simvastatin (1 mg/kg, i.v.; 30 min before ischemia) on renal dysfunction caused by ischemia-reperfusion (I/R) injury in the rat was investigated. 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). Simvastatin significantly improved both parameters of glomerular and tubular dysfunction (e.g., creatinine levels and fractional excretion of Na⁺, respectively) and especially improved the histological score, compared to control I/R-injured rats treated with saline or 10% DMSO only.

Keywords: simvastatin, ischemia-reperfusion renal injury, acute renal failure

Statins (3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors) were convincingly shown to produce both lipid-lowering-dependent and -independent effects (1, 2). The later effects still remain to be clarified, being attributed to their antiinflammatory, antioxidant, and/or vascular actions. In several studies, the effects of statins in ischemia-reperfusion (I/R) models of renal protection have been related to upregulation of endothelial nitric oxide synthase (eNOS) and increased production/release of NO. Statins were shown to reduce monocyte and macrophage-related expression of soluble intercellular adhesion molecule-1; lipopolysaccharide-induced secretion of tumor necrosis factor-α, interleukin-6, and inducible NOS (iNOS); and inhibition of NADPH oxidase-dependent superoxide anion production. Also, statins reduced sepsis-related vascular permeability in mice probably via increasing the activity of vascular eNOS (3–6).

The protective effects of statins in various experimental models of I/R injury were mainly shown after prolonged pretreatment lasting a few days up to four weeks (7, 8). However, a recent study by Wayman et al. (3) showed that acute pretreatment with a single dose of simvastatin (1 h prior occlusion of the left coronary artery) may significantly reduce infarct size in rats and protect the heart against I/R injury. Also, acute simvastatin pretreatment prevented postischemic renal injury in rats (9).

The aim of our study was to analyze the acute effects of a single dose of simvastatin, administered only 30 min before ischemia, on immediate post-reperfusion events in the injured rat kidney. The analysis would take into account selected biochemical and histological markers of glomerular and tubular function and reperfusion injury.

Methods have been described in detail by Chatterjee and Thiemermann (10). The experimental design is shown in Fig. 1. In brief, in vivo experiments were performed in male adult Wistar rats (n = 53) weighing 248 ± 8.1 g.

Rats were anesthetized using sodium thiopentone (Thiopental®; Nycomed Pharma, Unterschleibheim, Germany) in a dose of 120 mg/kg, i.p., and anesthesia was maintained using supplementary i.v. injections of
sodium thiopentone (approx. 10 mg/kg, i.v.) as required. All animals received a continuous infusion of 0.9% (w/v) saline (8 ml/kg per hour, i.v. during a period of adaptation (10 min) and during ischemia, and 2 ml/kg per hour during reperfusion time).

Rats were randomized into six experimental groups (n = 6 – 13 per group): Sham-operated + saline, Sham-operated + 10% DMSO (dimethylsulfoxide), Sham-operated + simvastatin (1 mg/kg), I/R + saline, I/R + 10% DMSO, and I/R + simvastatin (1 mg/kg, 30 min before ischemia). 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 the reperfusion, urine was collected (urine volumes of I/R-injured rats were increased in comparison with Sham-operated animals; not shown in figures), and after finishing the experiment, blood samples were taken and analyzed for markers of renal impairment. Also, 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 (electronic light microscope: 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 (11). 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 score per kidney was calculated by addition of all scores. Blind analysis of the histological samples was performed by two experts (Department of Pathology, School of Medicine, University of Belgrade).

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.

The following compounds were used in this study: simvastatin (Simvastatin®; Sigma-Aldrich, Poole, Dorset, UK), DMSO (Merck, Darmstadt, Germany), sodium thiopentone, and nonpyrogenic saline 0.9% w/v NaCl (Hemofarm, Belgrade, Serbia and Montenegro). 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 – 13 separate animals. Statistical analysis was carried out using GraphPad Prism/Instat 1.1 (GraphPad Software, San Diego, CA, USA) using one-way analysis of variance (ANOVA) followed by Dunnett’s or Tukey’s post-hoc test. A P value of less than 0.05 was considered significant.

The mean arterial pressure (MAP) and heart rate (HR) of anesthetized rats were statistically similar under basal conditions (i.e., before I/R injury and injection of saline or any other treatment). In rats subjected to I/R injury, renal artery occlusion caused a transient fall in MAP and HR in comparison with Sham-operated animals. I.v. bolus injection of the solution used (saline, 10% DMSO, simvastatin in 10% DMSO) did not significantly change MAP and HR (not shown).

Rats subjected to renal I/R injury demonstrated significantly increased serum levels of urea, creatinine, and fractional excretion of Na⁺ (FE₅⁺) compared to Sham-operated rats (I/R + saline and I/R + 10% DMSO vs Sham + saline and Sham + 10% DMSO) (Fig. 2: A, B, and C, respectively). There were no significant differences between Sham-operated animals treated with saline, 10% DMSO, or simvastatin (1 mg/kg) prepared in 10% v/v DMSO.

Simvastatin (1 mg/kg, i.v.; 30 min before ischemia) produced a significant reduction in serum urea and
creatinine levels and FE\textsubscript{Na} compared to rats of the I/R + 10% DMSO-group (Fig. 2: A, B, and C, respectively).

Also, renal I/R injury produced a significant increase in histological score in comparison with Sham-operated animals. Simvastatin (1 mg/kg) significantly reduced the histological score when compared to that obtained from rats subjected to renal I/R + 10% DMSO. However, it should be noted that I/R-caused renal injury was not completely abolished with simvastatin (I/R + simvastatin vs Sham + saline, P<0.05) (Fig. 2D). Representative light photomicrographs of a kidney section taken from rats subjected to renal I/R colored with PAS are shown in Fig. 3.

Our study shows the acute protective effects of a single dose of simvastatin (1 mg/kg, i.v.; 30 min before ischemia) on the selected biochemical and histological parameters of renal dysfunction and injury caused by renal I/R. In our experiments, significantly reduced histological evidence of I/R-mediated renal injury (histological score) in animals acutely pretreated with simvastatin was observed.

This observation is supported by the following key findings: in a rat model of renal I/R injury, such pretreatment with simvastatin reduced the I/R-induced increase in a) serum levels of creatinine and urea as
indicators of impaired glomerular function and b) FE$_{\text{Na}}$ as marker of proximal tubular injury (10).

Renal I/R injury causes both glomerular and tubular dysfunction (10, 12). The most prominent injury of renal proximal tubular cells of various species is likely to occur during the first 2–4 h of the reperfusion period (7, 13). In the present study, animals in both I/R groups (rats subjected to I/R injury and pretreated with saline or DMSO only) exhibited significantly impaired markers of renal function, including urine volume (not shown), serum and urine concentrations of creatinine and urea, FE$_{\text{Na}}$, and histological scores in comparison with Sham-operated animals.

There are several possible mechanisms of acute protective actions of statins in this and similar models of I/R injury. As mentioned above, the complex pharmacological profile of statins may involve both lipid-lowering-dependent and -independent effects (1, 2). The observed effects of simvastatin probably could not be explained by the cholesterol lowering effects of statins (taking at least two weeks of therapy) [a single dose of simvastatin (1 mg/kg) was not expected to significantly influence the lipid profile of rats (I/R + simvastatin or Sham-operated + simvastatin); because of this, the lipid profile was not measured] or the increased expression of eNOS (occurring after 3 days of statin pretreatment). On the other hand, certain effects of statins occurring after a few hours of pretreatment may fit into our experimental model, for example, their effects on the regulation of NO production and activity (stabilization of NOS mRNA, stabilization of NOS protein, or a direct influence on NOS activity) (8, 14). Some acute protective effects of statins in similar experimental models of I/R injury of the kidney and heart have been already shown (3, 13). The novelty of our findings, when compared to the available literature, is that acute i.v. administration of a single dose of simvastatin (1 mg/kg, i.v.; 30 min before ischemia) is sufficient to produce a significant level of protection of the I/R-injured kidney as shown both by biochemical and especially histological parameters.

Necrosis predominates over apoptosis in experimental
models of such severe I/R injury (12). This is in agreement with our results because the morphological features of apoptosis (e.g., chromatin condensation and cell shrinkage) were not extensively observed by light microscope analysis of the DMSO-treated, I/R-injured rat kidneys. In contrast, marked necrosis with tubular dilation, swelling, and luminal congestion were frequently observed, especially in tubular structures from the same preparations (Fig. 3A). Acute pretreatment with simvastatin significantly attenuated such necrotic changes, especially regarding renal tubular structures (Fig. 3: B and C).

These acute effects could be also explained by modulation of PI3k/Akt/eNO by statins (15). Since such a mechanism is related to the apoptotic cascade, this could probably explain why simvastatin in our model could not completely abolish histological markers of I/R tubular injury. Maximal concentrations of simvastatin in renal tissue were probably achieved in our experiments during the first 4 h of the reperfusion period (T_{max} of 1.4 h), but the maximal protective effects of such an agent could take more time. The other possibility is to inject simvastatin repeatedly and/or to change the time and dose of its administration. It should be pointed out that prolonged exposure to higher doses of simvastatin itself may cause both apoptosis and necrosis of renal mesangial cells (7).

In conclusion, this study provides preliminary evidence that acute administration of simvastatin in an in vivo model of ischemia and reperfusion causes a substantial reduction of kidney damage during I/R injury. Simvastatin was administered i.v. only 30 min before the occlusion of both renal arteries providing kidney protection via mechanisms independent of lipid lowering activity. In our study, we confirmed, both by measuring the biochemical markers of renal injury and by the histological score, that administration of simvastatin (1 mg/kg, i.v.) provides significant improvement in renal (predominantly tubular) function and reduces the degree of necrosis. Also, these data suggest that statins may ameliorate renal impairment and allow earlier recovery from IR injury. This may have implications for modulating renal function in a clinical setting. In particular, the potential for renal protection during aortic or transplantation surgery warrants further investigation. Mechanisms behind the renal protection afforded by acute statin administration are not clear enough and warrant further investigation.

References