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ACUTE PROTECTIVE EFFECTS OF DIFFERENT DOSES OF SIMVASTATIN IN THE RAT MODEL OF RENAL ISCHEMIA-REPERFUSION INJURY

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It was previously shown that acute pretreatment with simvastatin (1 mg/kg) significantly protects rats from renal ischemia-reperfusion injury (I/R, 45 min + 4 h). The aim of our present study was to determine whether this beneficial effect of simvastatin was dose-related. A single dose of simvastatin of 1 or 3 mg/kg, i.v. bolus, dissolved in 10% DMSO (Sim_1 and Sim_3), was injected 30 min before ischemia, 30 min before reperfusion or 5 min before reperfusion (30I, 30R, and 5R, respectively). Simvastatin-treated rats were compared to the appropriate controls (I/R + DMSO and Sham + DMSO group). Sim_1 and Sim_3 groups were similar regarding serum concentrations of urea, creatinine, aspartate aminotransferase, and gamma-glutamil-transferase (sUr, sCre, ALT, and γGT, respectively), as well as total histological score. Both doses of the drug (Sim_1 and Sim_3) were more effective in the reduction of total histological score in comparison with I/R + DMSO group. Also, the higher dose of drug 3 mg/kg (Sim_3) was somewhat more effective than Sim_1 in the reduction of tubular necrosis score and loss of brush border. In conclusion, the acute protective effect of simvastatin in the experimental model of renal I/R injury does not seem to be dose-related, and the dose of 1 mg/kg should be chosen for further investigation.

Key words: acute pretreatment, dose-dependence, renal ischemia-reperfusion injury, simvastatin

INTRODUCTION

Statins were convincingly shown to have an intriguing therapeutic potential beyond their lipid-lowering capacity (Schulz, 2005). 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 (Mensah *et al.,* 2005; Jo *et al*., 2007). Protective effects of statins in experimental models of acute renal failure (ARF) were shown by different authors (Wagner *et al*., 2002;

Davignon, 2004; Yokota *et al.,* 2003). The acute protection of kidneys with pleiotropic compounds may offer significant therapeutic approach in human renal ischemic-reperfusion (I/R) injury (e.g. during renal transplantation) (Torras *et al*., 1999; Menger and Vollmar, 2007). It should be pointed out that renal I/R injury with a high mortality rate and resolved pharmacotherapy questions is of the leading causes of ARF (Lameire *et al.,* 2005). 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 (Nešić *et al*., 2006; Todorović *et al*., 2008). However, the optimal dose for acute pretreatment with statins in renal I/R injury remains to be elucidated. In this study we compared the protective effects of two different doses of simvastatin (1 and 3 mg/kg, i.v. bolus), injected 30 min before ischemia, 30 min before reperfusion and 5 min before reperfusion in the experimental model of renal I/R injury were compared.

MATERIAL AND METHODS

Animals and experimental design

In vivo studies were carried out using 68 male Wistar rats weighing 200-300 g (286 ± 11.3 g) and receiving a standard diet and water *ad libitum*. Animals were treated according to the Guide for the care and use of small laboratory animals, School of Medicine, University of Belgrade – license number 244/9/2005. The investigation conforms to the regulations of the European Union and USA Guide for the care and use of the laboratory animals published by the US National Institutes of Health, NIH publication No. 85-23, revised 1985.

Rats were randomized into eight experimental groups (N = 6-12 per group): Sham-operated + 10% DMSO (dimethylsulfoxide), I/R + 10% DMSO (I/R + DMSO), I/R + simvastatin 1 mg/kg, i.v. groups: 30 min before ischemia (I/R + Sim_1 30 min before I), 30 min before reperfusion (I/R + Sim_1 30 min before R) and 5 min before reperfusion (I/R + Sim_1 5 min before R) and I/R + simvastatin 3 mg/kg, i.v.

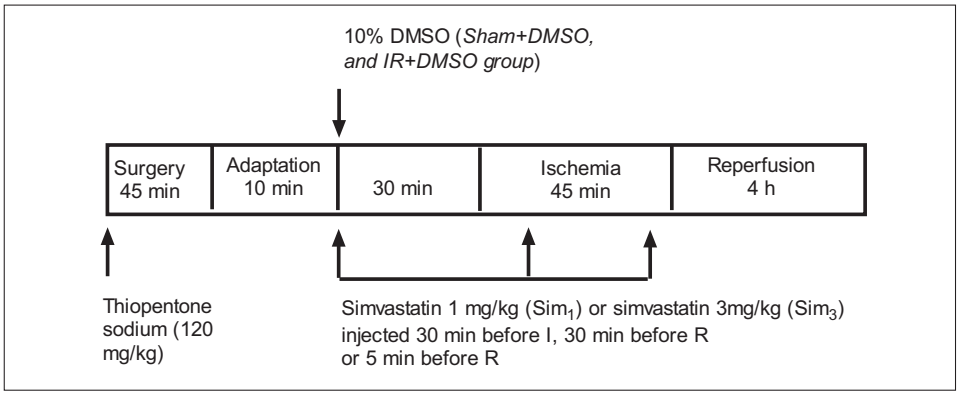


Figure 1. Experimental design

groups: 30 min before ischemia (I/R +Sim_3 30 min before I), 30 min before reperfusion (I/R + Sim_3 30 min before R) and 5 min before reperfusion (I/R + Sim_3 5 min before R). Following adaptation, I/R injury was induced by clamping both renal vascular pedicles for 45 min, followed by 4 hours of reperfusion with saline (2 ml/kg/h). In all groups during reperfusion, urine was collected and blood samples were taken and analyzed for markers of renal impairment.

Methods are described in details by Chatterjee and Thiemermann (2003), and Nešić *et al*. (2006). Experimental design is shown in Figure 1.

Materials

Compounds used in this study were: simvastatin (Simvastatin®, Sigma-Aldrich, Poole, Dorset, UK), dimethylsulfoxide (DMSO) (Merck, Darmstadt, Germany), sodium thiopentone (Thiopental®, Nycomed Pharma, Unterschleibheim, Germany) and nonpyrogenic saline 0.9% w/v NaCl (Hemofarm, Belgrade, Serbia).

Surgical procedures

Surgical preparation of rats and the protocol used to produce renal I/R were identical to those described previously by Chatterjee and Thiemermann, 2003. Briefly, anesthetized rats were placed onto a homeothermic plate to maintain a stable body temperature of 37 ± 1 ^{o}C. A tracheotomy was performed to maintain airway potency and to facilitate spontaneous respiration. The right carotid artery was cannulated (PP50, internal diameter 0.58 mm, Portex, Kent, UK) and connected to a pressure transducer (Expert Haedyn 1.0 – Sistem for hemodynamic analysis, Belgrade, Serbia) for the measurement of mean arterial blood pressure and heart rate, which were displayed on a data acquisition system installed on an IBM Personal computer (IBM Computers, Belgrade, Serbia). The jugular vein was cannulated (PP25, internal diameter 0.40 mm, Portex, Kent, UK) for the administration of anesthesia, saline, vehicle (10% DMSO) or simvastatin as required. A midline laparotomy was performed, and the bladder was cannulated (PP90, internal diameter 0.76 mm, Portex, Kent, UK). Both kidneys were located, and the renal pedicles containing the artery, vein, and nerve supplying each kidney were carefully isolated. For rats subjected to I/R, bilateral renal occlusion for 45 min was performed using 3.5 cm Dieffenbach bulldog arterial clips (Holborn Surgical and Medical Instruments, Margate, Kent, UK), which were used to clamp the renal pedicles. Reperfusion commenced once the artery clips were removed. Occlusion was verified visually by change in the color of the kidneys to a paler shade and reperfusion by subsequent blushing. Sham-operated rats were subjected to sham operation, which underwent identical surgical procedures to the rats subjected to renal I/R injury, but did not undergo bilateral renal clamping and were maintained under anesthesia for the duration of the experiment (45 min + 4 h). Throughout the experimental period, body temperature was maintained at 37 ± 1 ^{o}C by a homoeothermic plate and measured by a rectal probe (Almemo 2290-1®, Electrical Thermometer, Hugo Sachs Electronic, Germany). At the end of all experiments, rats were euthanized using an overdose of sodium thiopentone.

Measurements of biochemical parameters

At the end of the experimental period, blood samples were collected *via* the carotid artery into tubes containing serum gel (Venosafe, gel-lithium heparin, Terumo, Europe). The samples were centrifuged (6000 rpm for 3 min) to separate the serum from which biochemical parameters were measured (Institute of Psychiatry, Laboratory of Biochemistry, Belgrade, Serbia). Serum creatinine levels were used as an indicator of renal (glomerular) function (Chatterjee and Thiemermann, 2003).

Aspartate aminotransferase (AST) and γ-glutamyltransferase (γ-GT), enzymes both located in the proximal tubules, were used as indicators of reperfusion injury. Urine samples were collected throughout the reperfusion period and the volume of urine produced was recorded. Urine concentrations of Na^+ were measured and used in conjunction with serum Na^+ levels to estimate fractional excretion of Na^+ (as an indicator of tubular dysfunction), using standard formulae.

Histological evaluation

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 x 20 was used. Each figure shown was randomly chosen from the series of at least 6 experiments (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 (Solez *et al*., 1979; Doi *et al*., 2004). 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 independent experts (Department of Pathology, School of Medicine, Belgrade).

Statistical analysis

All values described in the text and figures are expressed as mean ± standard error of the mean (S.E.M.) of N observations. Each data point represents biochemical measurements or histological scores obtained from 6-12 separate animals. Statistical analysis was carried out using GraphPad Prism/Instat 1.1 (GraphPad Software, California, 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.

RESULTS

In comparison with Sham-operated animals, renal I/R injury produced significant increase in serum, urinary, and histological markers of renal dysfunction and injury, as described in details below. Also, urine volumes of I/R injured rats were increased in comparison with Sham-operated animals (not shown).

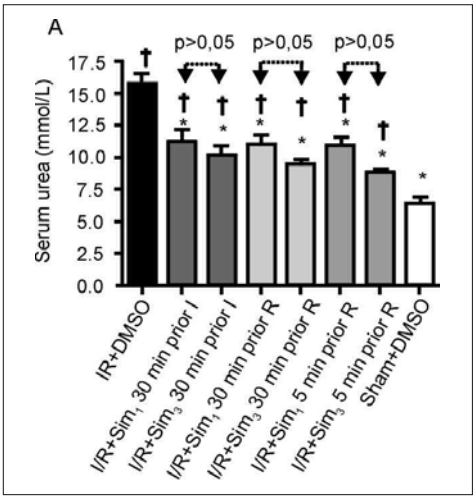
Cardiovascular responses

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 10% DMSO 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 (10% DMSO, simvastatin) did not significantly change MAP and HR (data not shown).

Effects of simvastatin (1 mg/kg or 3 mg/kg) on renal dysfunction caused by renal I/R

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 (I/R + DMSO *vs*. Sham + DMSO; $P<0.01$; Figure 2).

Acute pretreatment with a single dose of simvastatin (1 mg/kg or 3 mg/kg i.v.) significantly reduced sCre and sUr, and $FENa^+$ in three different times in comparison with the control group: 30 min before ischemia (I/R + Sim_1 30 min before I and I/R + Sim_3 30 min before I *vs.* I/R + DMSO; $P<0.05$; in both mentioned groups), 30 min before reperfusion (I/R + Sim_1 30 min before R and I/R + Sim_3 30 min before R *vs.* I/R + DMSO; $P<0.05$; in both mentioned groups) and 5 min before reperfusion (I/R + Sim_1 5 min before R and I/R + Sim_3 5 min before R *vs.* I/R + DMSO; $P<0.05$; in both mentioned groups). However, Sim_1 and Sim_3 did not completely abolish changes in sUr and sCre and $FENa^+$ caused by I/R injury.



Simvastatin similarly reduced sCre and sUr regardless of the time of injection and the dose used (Figure 2, panels A-C). Dose-related differences were observed only in $FENa^+$ injected 5 min before reperfusion (I/R + Sim_1 5 min before R *vs.* I/R + Sim_3 5 min before R; $P<0.05$). Time-related differences between pretreatments were not observed ($P>0.05$).

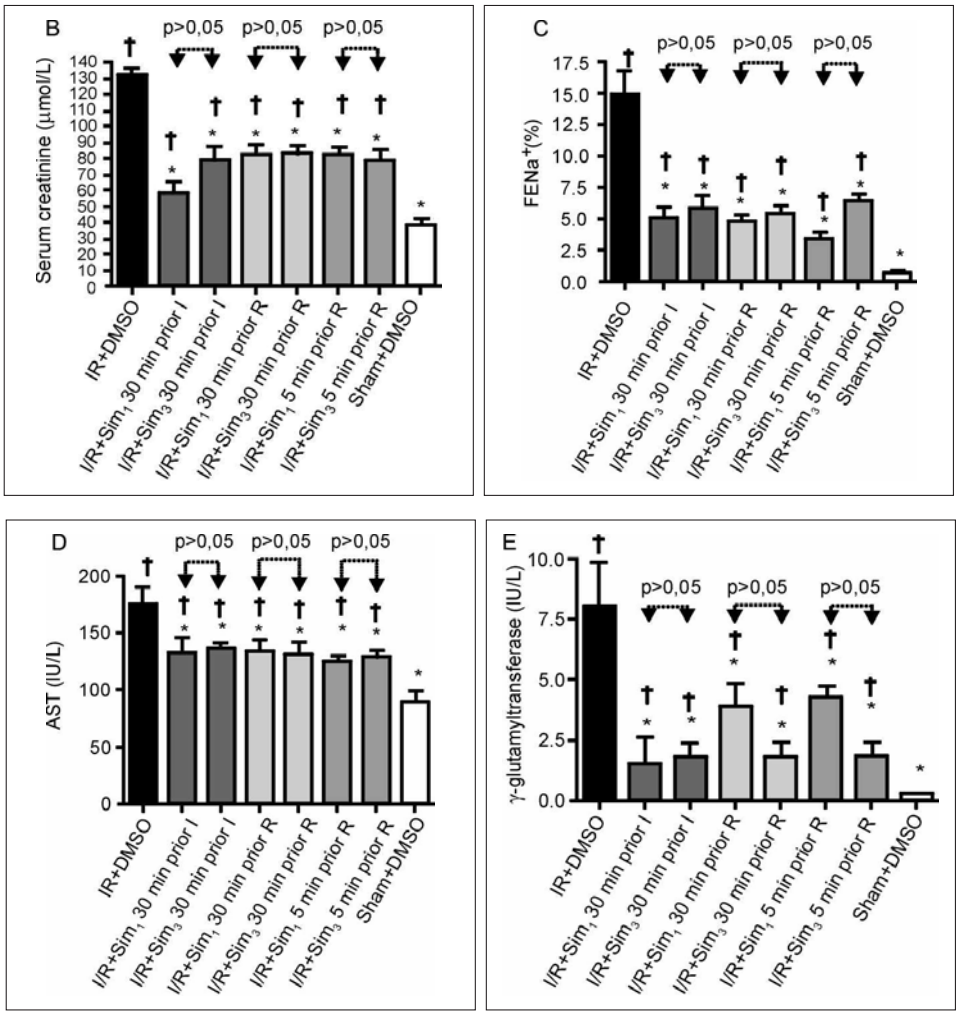


Figure 2. The effect of simvastatin (1 mg/kg or 3 mg/kg, i.v.) on renal dysfunction and injury caused by I/R. Simvastatin in both doses was injected 30 min before ischemia, 30 min before reperfusion, and 5 min before reperfusion (I/R +Sim_1 30 min before I, I/R + Sim_3 30 min before I, I/R + Sim_1 30 min before R, I/R + Sim_3 30 min before R, I/R + Sim_1 5 min before R and I/R + Sim_3 5 min before R, respectively). Control groups, I/R +10 % DMSO (dimethylsulfoxide) and Sham + 10% DMSO, received 0.5 ml of 10% DMSO only (i.v. bolus, 30 min before ischemia). Panels A, B, C, D and E: Serum urea, creatinine concentrations, fractional excretion of Na^+ ($FENa^+$), aspartat aminotransferase (AST) and γ-glutamyltransferase (γ-GT). I, ischemia; R, reperfusion. Each bar represents mean ± S.E.M. *P< 0.05 vs. I/R+DMSO-group, † P<0.05 vs. Sham + DMSO-group (N = 6-12 rats)

I/R injury produced a significant increase in serum levels of both nonspecific parameters of tubular injury AST and γ-GT (I/R + DMSO *vs.* Sham +DMSO, $P<0.05$). Both doses of simvastatin (1 mg/kg and 3 mg/kg) significantly decreased γ-GT and AST levels in comparison with I/R + DMSO ($P<0.05$, in all mentioned groups, Figure 2, panels D and E).

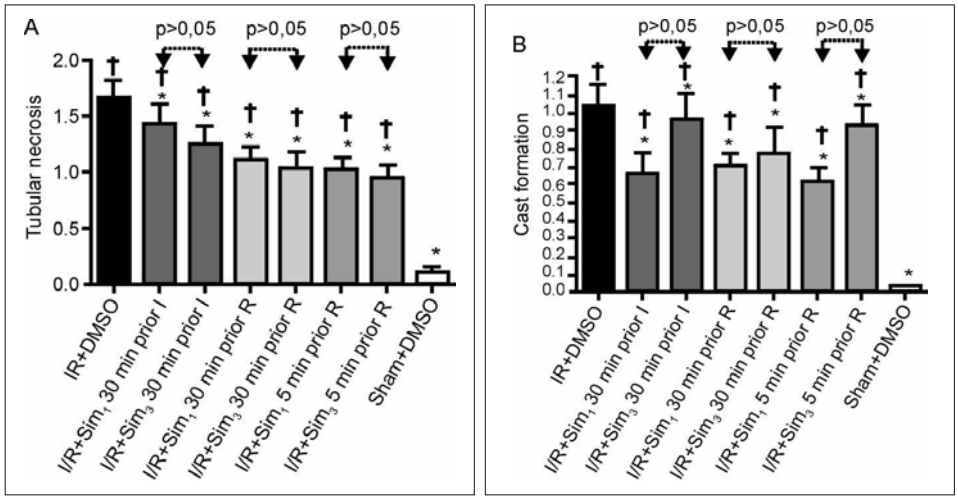
Time-related differences between pretreatments were not observed in these two parameters.

Histological analysis of simvastatin effects (1 mg/kg or 3 mg/kg) on injury caused by renal I/R

Dose-related differences between two administrated doses of simvastatin were only observed in interstitial edema score (I/R + Sim_1 30 min before I *vs.* I/R + Sim_3 30 min before I; $P<0.05$; Figure 3, panel C) and loss of brush border score (I/R + Sim_3 5 min before R *vs.* I/R + Sim_1 5 min before R; $P<0.05$; Figure 3, panel D).

I/R injury caused a marked increase in total histological score in comparison with sham-operated animals (I/R + DMSO vs. Sham + DMSO; $P<0.01$; Figure 3, panel E). Both doses of simvastatin significantly reduced the histological scores when compared to I/R + DMSO group ($P<0.01$, in all mentioned groups, shown in Figure 3, panel E). However, it should be noted that I/R-caused renal injury was not completely abolished with simvastatin (Sim_1-, Sim_3- groups *vs.* Sham + DMSO; $P<0.01$; shown in Figure 3, panel E). Regarding total histological score, dose-related differences between different groups were not observed (Figure 3, panel E). Time-related differences between pretreatments were not observed between treated groups.

Representative light photomicrographs of a kidney section taken from rats subjected to renal I/R colored with PAS are shown in Figure 4, panels A-H.



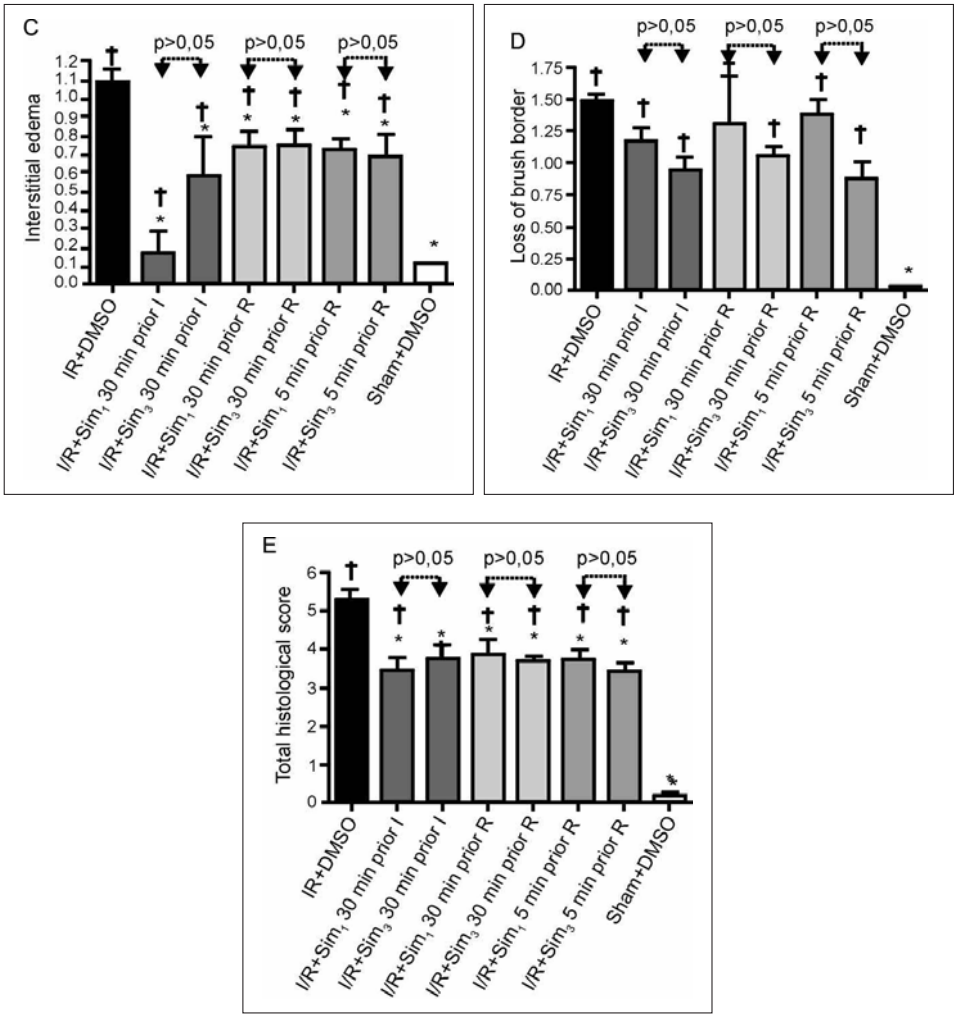


Figure 3. The effect of simvastatin (1 mg/kg or 3 mg/kg, i.v.) on histological score of renal I/R injury. Simvastatin was injected 30 min before ischemia, 30 min before reperfusion, and 5 min before reperfusion (I/R +Sim_1 30 min before I, I/R + Sim_3 30 min before I, I/R + Sim_1 30 min before R, I/R + Sim_3 30 min before R, I/R + Sim_1 5 min before R and I/R + Sim_3 5 min before R, respectively). Control groups, I/R +10 % DMSO (dimethylsulfoxide) and Sham + 10% DMSO, received 0.5 ml of 10% DMSO only (i.v. bolus, 30 min before ischemia). The histological parameters evaluated were tubular necrosis, interstitial edema, loss of brush border, and cast formation score (Panels A-D). A minimum of 10 fields for each kidney slide were examined and assigned for severity of changes. The scoring system was 0, absent; 1, present; and 2, marked. Total histological score was calculated by addition of all scores (Panel E). Each bar represents mean ± S.E.M. *P<0.05 vs. I/R+DMSO-group, † P<0.05 vs. Sham + DMSO-group (N = 6-12 rats)

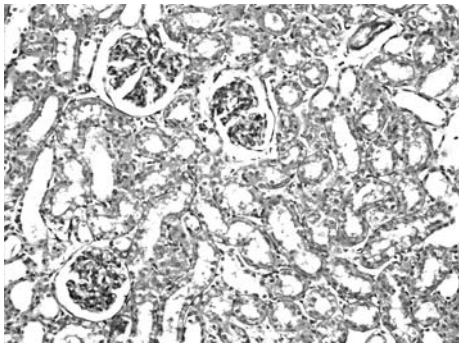


Figure 4A. Sham + 10% DMSO

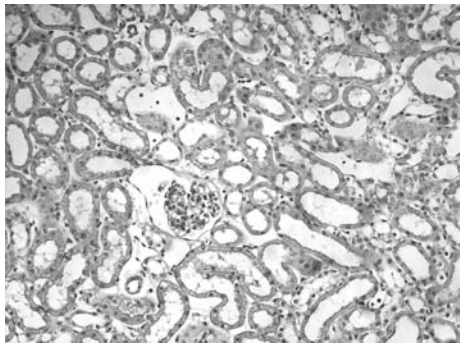


Figure 4B. I/R + 10% DMSO

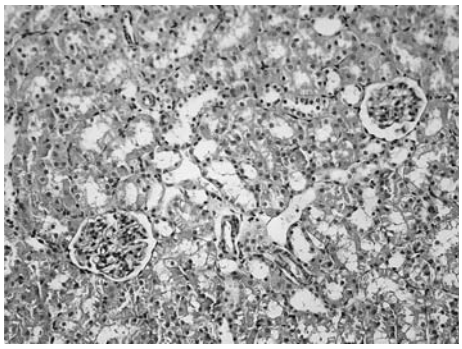


Figure 4C. I/R + simvasatin 1 mg/kg 30 min prior ischemia

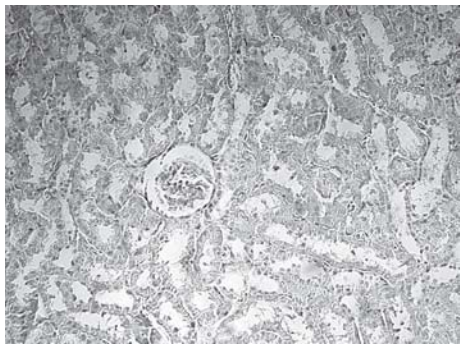


Figure 4D. I/R + simvasatin 3 mg/kg 30 min prior ischemia

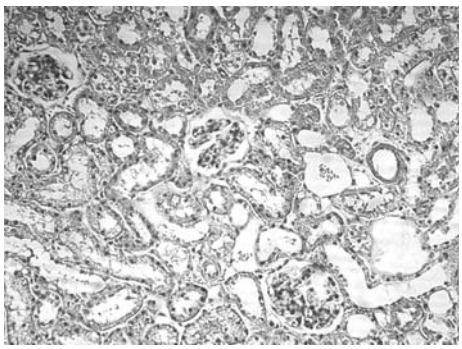


Figure 4E. I/R + simvasatin 1 mg/kg 30 min prior reperfusion

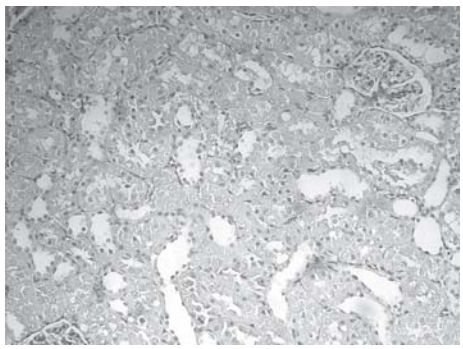


Figure 4F. I/R + simvasatin 3 mg/kg 30 min prior reperfusion

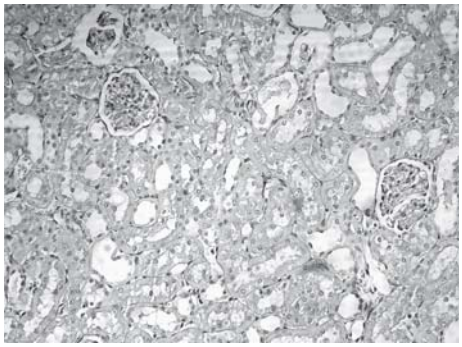


Figure 4G. I/R + simvasatin 1 mg/kg 5 min prior reperfusion

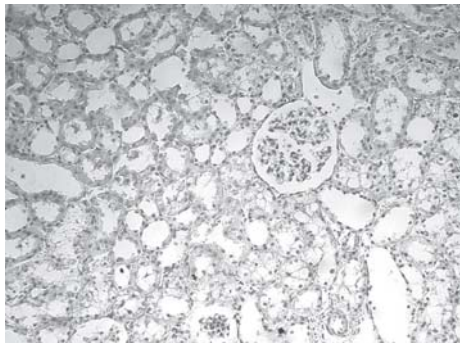


Figure 4H. I/R + simvasatin 3 mg/kg 5 min prior reperfusion

Figure 4. Histological micrographs of renal tissue. Kidney sections taken from Sham-operated rats or rats subjected to renal I/R injury. Periodic acid-Schiff (PAS) stain coloring. Original magnification x 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 (I/R+DMSO-group): marked necrosis with tubular dilatation, 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 1 mg/kg or 3 mg/kg, i.v. (I/R +Sim_1 30 min before I, I/R + Sim_3 30 min before I, I/R + Sim_1 30 min before R, I/R + Sim_3 30 min before R, I/R + Sim_1 5 min before R and I/R + Sim_3 5 min before R, respectively): moderate kidney damage, focal tubular necrosis, and moderate dilatation of the tubular structure. In comparison with the I/R + 10% DMSO group, in the simvastatin treated groups we observed preservation of tissue histology of the kidney

DISCUSSION

Acute tubular necrosis due to I/R injury in patients is associated with a high mortality rate and little progress has been made in the design of effective therapies in the past 50 years (Chatterjee and Thiemermann, 2003; Lameire *et al.,* 2005; Hölschermann *et al*., 2006; Rouschop and Leemans, 2008). In this paper, we have shown that pretreatment of rats with simvastatin (1 and 3 mg/kg, i.v. bolus) causes a substantial reduction in biochemical and histological parameters of renal I/R injury, but these effects do not seem to be dose-related.

Recently, we have shown that acute pretreatment with a single intravenous dose of simvastatin offered a significant protection of rat kidneys from I/R injury regardless of time of injection (30 min before ischemia or 30 min before reperfusion or 5 min before reperfusion) (Nešić *et al*., 2006; Todorović *et al*., 2008).

Statins in I/R injury have provided many benefits that are independent of their ability to lower blood cholesterol levels so called pleiotropic effects which include: antiinflammatory, antioxidant activities and vascular actions (Joyce *et al*., 2001a; Bonnetti *et al*., 2003; Chatterjee *et al.,* 2003; Davignon, 2004; Davignon and Leiter *et al*., 2005; Schulz, 2005; Tseng *et al*., 2005; Ikeda *et al.*, 2006; Chatterjee, 2007). Administration of statins has reduced I/R injury in several organs including the heart (Wayman *et al*., 2003; Matsuki *et al*., 2006; Ray *et al*., 2006), brain (Trinkl *et al*., 2006; Cakmak *et al*., 2007), lung (Joyce *et al*., 2001b; Yao *et al*., 2006), and gut (Naito *et al*., 2006).

In the present experiments, biochemical and histological parameters of I/R injury were influenced by different doses of simvastatin in three ways: a) Sim_3 was more protective than Sim_1 (e.g. sUr, tubular necrosis score, loss of brush border score); b) Sim_1 was more protective than Sim_3 (e.g. $FENa^+$, γ-GT, interstitial edema score, cast formation score); c) Sim_1 was similar with Sim_3 (sCre, AST, total histological score). There are several possible explanations of our findings, and both pharmacodynamic and pharmacokinetic factors should be considered. However, higher doses of simvastatin do not seem to be necessary for a significant protection of injured kidneys regarding parameters of glomerular and tubular function (sCre and $FENa^+$, respectively).

The explanation of simvastatin pharmacodynamics in the present model of I/R injury should take into consideration its effect on endothelial NO production. Enhancements of the NO level by statins potentiates vasodilatation of resistant vessels, leading to preservation of tissue perfusion after I/R. Acute (3 h), transient effect of a single dose of cerivastatin on endothelial responsiveness in humans has already been reported (Omori *et al*., 2002). Effects of simvastatin could be explained, at least in part, by the regulation of NO production and activity (stabilization of eNOS mRNA, stabilization of eNOS protein, or a direct influence on eNOS activity) (Wolfrum *et al*., 2003; Matsuki *et al*., 2006). A recent *in vitro* study confirmed that concentration of Akt phosphorylation by statins peaked at approximately 1 h and declined by 3 h after exposure in a cell culture system (Kureishi *et al*., 2000). Similar findings, i.e acute cardioprotective effect by activation of PI3-kinase/Akt were reported in a dog model of I/R (Sanada *et al*., 2004; Dillon *et al.*, 2006; Yao *et al*., 2006).

The bolus administration of simvastatin in our present study might be sufficient for the maintainenance of eNOS activation *via* the PI3-kinase/Akt pathway during reperfusion. The rapid effects of simvastatin on PI3k/Akt/eNOS chain should also be considered, as well as the modulation of Ras-ERK signaling cascade in inflammatory cells (Zipp *et al*., 2007). 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 NFkappaB (*via* either IkappaB-alpha or PI3k/Akt pathways), decrease parameters of *in vivo* LDL oxidation, and may protect paraoxonase and superoxide dismutase. Also, simvastatin decreased the production of 8-epi-$PGF_{2\alpha}$ and malondialdehyde (indicator of lipid peroxydation) in an *in vivo* model of myocardial I/R injury (Fassett *et al*., 2008). In the most recent investigation, 0.5 mg/kg cerivastatin was administered by gavage to rats for 3 days before renal I/R. Pretreatment with

cerivastatin offerd a significant protection against subsequent renal dysfunction and I/R injury that was completely abolished by tin protoporphyrin, a competitive inhibitor heme oxygenase-1 (HO-1), suggesting the involvement of this protective enzyme. Further investigations revealed that infiltrating macrophages were the major source of this upregulated HO-1 (Gueler *et al*., 2007).

Another important factor to be considered is pharmacokinetics of simvastatin. Late injection of simvastatin 5 min before reperfusion could expose the kidneys to the maximal concentration of its active form immediately at the beginning of reperfusion. 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 (Cl_{uptake} of 0.911 ml/min/g tissue) (Nezasa *et al*., 2002). In other words, this highly lipophylic statin could easily reach the target kidney tissue. Furthermore, sufficiently high active simvastatin levels were possibly present in the kidneys during ischemia and at the beginning of reperfusion, thus protecting tubular cells from oxidative damage.

CONCLUSION

Acute pretreatment with a single intravenous dose of simvastatin seems to give a significant protection to rat kidneys from I/R injury regardless of the time or dose of the injected drug. In other words, the effect of simvastatin does not seem to be dose-related. Our results may have therapeutic implications. Even a small dose of statin such as simvastatin could be beneficial in the prevention of acute renal failure in patients undergoing major vascular surgery for atherosclerotic disorders, particularly if they are at high risk for developing acute renal failure postoperatively, having namely pre-existing renal impairment, diabetes or old age. Overall, the potential for statins to provide protection against renal I/R injury and the cellular mechanisms involved remain to be clarified and warrant further investigation.

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AKUTNO PROTEKTIVNO DEJSTVO RAZLIČITIH DOZA SIMVASTATINA KOD ISHEMIJSKO-REPERFUZIJSKOG OŠTEĆENJA BUBREGA PACOVA

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SADRŽAJ

Dokazano je da akutni pretretman simvastatinom (1 mg/kg) ima značajno zaštitno dejstvo u modelu ishemijsko-reperfuzijskog oštećenja bubrega pacova (I/R, 45 min + 4 h). Cilj našeg istraživanja bio je da utvrdimo da li to protektivno dejstvo simvastatina zavisi od doze. Jedna doza simvastatina od 1 ili 3 mg/kg u i.v. bolusu, rastvorenog u 10% DMSO (Sim_1 i Sim_3), ubrizgana je 30 min pre ishemije, 30 min pre reperfuzije ili 5 min pre reperfuzije (30I, 30R i 5R). Vršeno je poređenje sa odgovarajućim kontrolnim grupama (I/R + DMSO i Sham + DMSO grupa). Serumske koncentracije ureje, kreatinina, aspartat aminotransferaze i gama-glutamiltransferaze (sUr, sCre, ALT i γGT), kao i ukupni histološki skor bile su slične u Sim_1 i Sim_3 grupi. Obe doze simvastatina smanjile su i ukupni histološki skor u poređenju sa I/R + DMSO grupom. Simvastatin u dozi od 3 mg/kg efikasnije od simvastatina u dozi od 1 mg/kg redukuje skor tubularne nekroze i gubitak četkaste ivice. U zaključku, akutno protektivno dejstvo jedne doze simvastatina u eksperimentalnom modelu I/R oštećenja bubrega ne menja se sa povećanjem doze, pa je doza od 1 mg/kg izabrana za dalja ispitivanja.