

Comparative effects of L-arginine and vitamin C pretreatment in SHR with induced postischemic acute renal failure

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Abstract. Postischemic acute renal failure is worsened when occurs in a various conditions with impaired nitric oxide (NO) synthesis, such as arterial hypertension. Reoxygenation itself increases ischemic injury through the massive production of oxygen-free radicals. Therefore, we have directed our investigations to effects of both NO donor and antioxidant treatment on course of acute renal failure in experimental hypertension. Experiments were performed in anesthetized, adult male spontaneously hypertensive rats. In ARF groups the right kidney was removed, and rats were subjected to renal ischemia by clamping the left renal artery for 40 min. Experimental group received NO donor L-arginine (2 g/kg b.m.) (L-Arg group), or oxidant scavenger vitamin C (100 mg/kg b.m.) (Vit C group) during 3 days before the period of ischaemia. All parameters were measured 24 h after reperfusion. The mean arterial pressure was markedly reduced and renal vascular resistance significantly dropped in the ARF+L-Arg group vs. ARF group. Tubular injuries were similar between the ARF+L-Arg and ARF groups. Intensity of tubular necrosis and dilatation was markedly reduced in ARF+Vit C group in comparison to ARF. L-arginine failed to reduce tubular injury, despite its evident improvement of systemic and renal haemodynamic, thus NO seems to act as a double-edged sword, but reduction of tubular injury promotes vitamin C as an effective chemoprotectant against ischemia-reperfusion tubular injury in hypertension.

Key words: Acute renal ischemia — SHR — Nitric oxide — Vitamin C

Introduction

Postischaemic acute renal failure frequently occurring in clinical practice is associated with significant morbidity and mortality, despite advances in pharmacology and renal replacement therapy (Bonventre and Weinberg 2003).

There are several factors involved in the initiation and maintenance of the acute renal failure: decrease in glomerular capillary permeability, back-leak of glomerular filtrate, tubular obstruction and intrarenal vasoconstriction (Nissenson 1998), but their causality has never been selective and rarely preventable. On the other hand, long-lasting hypertension damages medium size and small-size renal blood vessels, dis-

ables adequate tubuloglomerular responses, and predisposes nephroangiosclerosis patients to acute renal failure (Welch et al. 2000). Also, patients with pre-existing hypertension are at a particular risk of fatal outcome (Rihal et al. 2002; Aronson and Blumenthal 1998). Recently, one of the most investigated mechanisms in the context of tone control is the nitric oxide (NO)-dependent vasodilatation. There is a decrease in the basal production of NO and expression of endothelial NO synthase in spontaneously hypertensive rats (SHR) (Crabos et al. 1997; Chou et al. 1998). NO participates in several vital processes in the kidney which encompass regulation of glomerular and medullar haemodynamic, tubuloglomerular feedback response, renin release, extracellular fluid volume (Kone 1997) and regulation of Na⁺,K⁺-ATPase, Na⁺/H⁺ exchangers, and paracellular permeability of proximal tubular cells (Liang and Knox 2000). Several studies have suggested that NO bioactivity is reduced in models of postischemic acute renal failure as assessed by a blunted response to endothelium-

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dependent vasodilators such as acetylcholine and bradykinin and increased constrictor responses to renal nerve stimulation and angiotensin II (Cherla and Jaimes 2004).

Oxidative stress appears to be the main mechanism causing tissue ischemia-reperfusion damage. Reperfusion injury generates significant amount of free oxygen radicals, the effect of which could not be „buffered“ by endothelial cells exposed to ischemia (Radović 2006). Water-soluble vitamin C has frequently been used in experimental studies to interfere in mechanisms of oxidative injury. In addition, vitamin C has consistently proved to be a potent antioxidant in certain experimental and clinical conditions (Lloberas et al. 2002).

Therefore, we developed an experimental model that mimicked the clinical situation where kidneys which suffered from long-lasting hypertension have been subjected to ischemia-reperfusion injury. The main goal of this study was to compare the efficiency of an attempt to diminish reperfusion injury in SHR by NO donor or antioxidant pretreatment.

Materials and Methods

Materials

Male adult 3-month-old SHR, weighing about 300 g, were bred in the Institute for Medical Research, Belgrade and fed with a standard chow for laboratory rats (Veterinarski zavod, Subotica, Serbia) *ad libitum*. All animal experiments were conducted in accordance with local institutional guidelines for the care and use of laboratory animals. The investigation also conformed to the principles and guidelines of Conseil de l'Europe (published in the Official Daily N. L358/1-358/6, 18th December 1986), the U.S. National Institutes of Health (Guide for the Care and Use of Laboratory Animals, NIH publication No. 85-23), and the Canadian Council on Animal Care.

We used: i) L-arginine, substrate for NO (Sigma), ii) soluble vitamin C (Galenika a.d., Serbia), antioxidant molecule.

Experimental groups and model of acute renal failure

The animals were divided into two experimental groups: L-arginine treated animals with acute renal failure (ARF+L-Arg; $n = 10$) and vitamin C-treated animals with induced acute renal failure (ARF+Vit C; $n = 10$). There were two control groups, control sham operated rats (SHAM; $n = 9$) and control rats with acute renal failure (ARF; $n = 9$). In all ARF groups the right kidney was removed, and the rats were subjected to renal ischemia by clamping the left renal artery for 40 min. The SHAM group consisted of right nephrectomized rats and received vehicle *via* the same route as all other groups. All rats were placed in individual metabolic cages immediately after clamp removal and surgery procedures.

Experimental animals were pretreated by gavages with L-arginine (2 g/kg b.m.) or vitamin C (100 mg/kg b.m.) during 3 days before the period of ischaemia.

Haemodynamic measurements 24 h after reperfusion

Haemodynamic parameters were measured after the 24 h urine collection period. All animals were anaesthetized (35 mg/kg sodium pentobarbital; i.p.). Mean arterial pressure (MAP) was determined directly through a femoral artery catheter PE-50 (Clay-Adams, Parsippany, NY, USA), using a low-volume displacement transducer P23 Db (Statham, Oxnard, CA, USA), and recorded on a direct writing recorder.

For the blood flow measurement, the left renal artery was gently separated. An ultrasonic flow probe (1 RB, internal diameter = 1 mm) was placed around the artery for the measurement of renal blood flow, using a transonic small animal flowmeter T106 (Transonic System Inc., Ithaca, NY, USA). Renal vascular resistance (RVR) was calculated by dividing MAP by renal blood flow and expressed as mmHg-min-kg/ml.

Biochemical measurements 24 h after reperfusion

Urinary and plasma concentrations of creatinine and urine protein concentrations were determined using a COBAS INTEGRA® 400 plus (Hoffmann-La Roche, Germany) analyzer.

Urine volume was collected in a graduated tubes after the 24 h metabolic cage collection period.

Histological examination

Left kidney was examined morphologically, 24 h after the period of reperfusion. The renal tissue was fixed in 10% buffered formalin solution. Later, the kidney was dehydrated in alcohol, blocked in paraffin wax, and 5 μ m thick sections were sliced and stained by periodic acid-Schiff (PAS) reaction. Using light microscopy, the following parameters were semi-quantitatively evaluated on the scale from 0 to 4 according to the degree of lesions: intensity and spread of tubular necrosis, number of intraluminal cast formations, swelling and vacuolization of cells, loss of luminal membrane or brush borders, tubular dilatation, interstitial oedema, separation of cells from tubular basal membrane. The severity of congestion, i.e. the accumulation of red blood cells in glomeruli, peritubular capillaries, and intrarenal veins, was graded on a scale from 1 to 3, as described by Mandal et al. (1977). The sum of these changes represented the histopathological score for comparison between groups.

Statistical analysis

The results are expressed as mean \pm S.E.M. One-way analysis of variance (ANOVA) was applied. When the ANOVA results

were significant, Bonferroni's *t*-test was used to determine the level of significance and $p < 0.05$ was considered to be significant (Primer of Biostatistics, by Stanton A. Glanz).

Results

Hemodynamic parameters

The MAP was markedly, but not significantly, decreased in the both ARF+L-Arg group and ARF+Vit C group in comparison to ARF group (130.25 ± 3.75 mmHg and 128.60 ± 4.20 mmHg vs. 141.86 ± 12.04 mmHg). Also, in the both experimental groups, MAP was significantly decreased in comparison to SHAM (Figure 1).

The renal blood flow was not different between experimental groups, but markedly rose in ARF+L-Arg group in comparison to the controls (Figure 2). The RVR significantly dropped in the ARF+L-Arg group vs. ARF (67.81 ± 7.04 vs. 148.87 ± 26.85 mmHg·min·100 g/ml) (Figure 2).

Biochemical parameters

Glomerular filtration rate (GFR), presented as an endogenous creatinine clearance, significantly fell in all ARF groups 24 h after the period of ischemia. There were no significant differences between the ARF groups, but GFR was moderately increased in ARF+L-Arg group in comparison to ARF group (Table 1).

The value of the urine protein excretion showed no relevant differences between experimental groups (Table 1). The urine volume was significantly higher in ARF+L-Arg group in comparison to controls (Table 1).

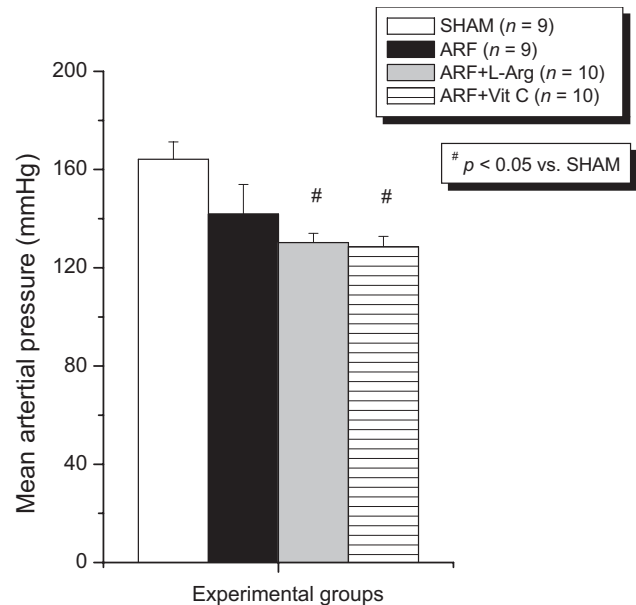


Figure 1. Mean arterial pressure in experimental groups.

Histological studies

Morphological examination of the renal tissue revealed significant differences between experimental groups of animals.

Glomeruli, tubulointerstitium and blood vessels of the sham-operated animals were without any changes on the light microscopy examination. In a few kidney specimens, only a small number of PAS positive casts were observed in the tubular lumen (Figure 3A).

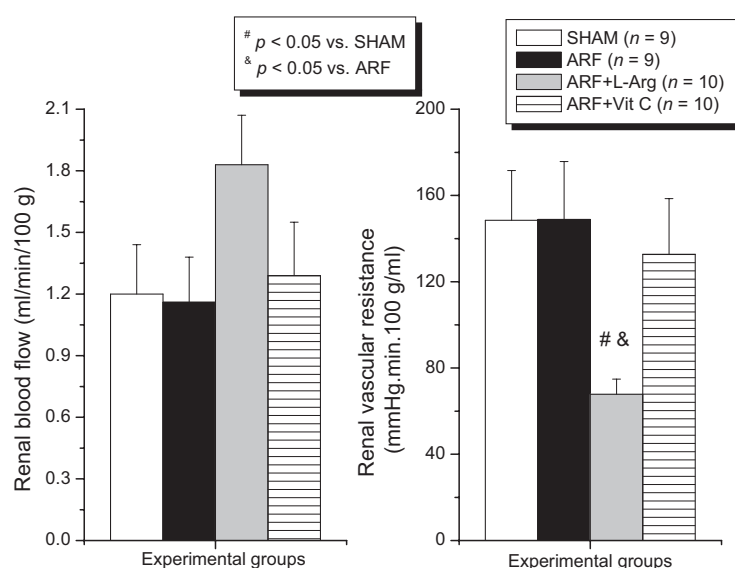


Figure 2. Renal blood flow and renal vascular resistance in experimental groups.

Kidneys in the ARF control group showed dilatation of some segments of proximal and distal tubules with or without loss of brush border of proximal tubular epithelium. Swelling of some proximal tubular epithelial cells was present. The most prominent lesions were widespread tubular necrosis in

the corticomedullary zone and a huge number of PAS positive casts in the lumina of distal tubuli and collecting ducts. The intensity of interstitial oedema varied among specimens in this group. Glomeruli and blood vessels were the same as in the SHAM group (Figure 3B).

Table 1. Biochemical parameters and histopathological score

Groups	Creatinine clearance (ml/min/kg)	Plasma urea (mmol/l)	Urine protein excretion (mg/24 h/kg)	Urine volume (ml/24 h/kg)	Histopathological score
SHAM (<i>n</i> = 9)	5.44 ± 0.27	10.23 ± 0.29	468.55 ± 38.54	33.7 ± 3.8	0.58 ± 0.2
ARF (<i>n</i> = 9)	1.29 ± 0.38 [#]	40.90 ± 3.75 [#]	494.98 ± 113.30	40.00 ± 4.2	9.87 ± 0.66 [#]
ARF+L-Arg (<i>n</i> = 10)	2.05 ± 0.81 [#]	48.66 ± 5.28 [#]	446.17 ± 53.40	67.3 ± 7.6	9.50 ± 0.43 [#]
ARF+Vit C (<i>n</i> = 10)	0.95 ± 0.03 [#]	47.62 ± 3.77 [#]	353.53 ± 50.73	45.9 ± 7.1	6.71 ± 0.36 [#] & [*]

[#] *p* < 0.05 compared to SHAM group; & *p* < 0.05 compared to ARF control; * compared to ARF+L-Arg group; *n*, number of animals.

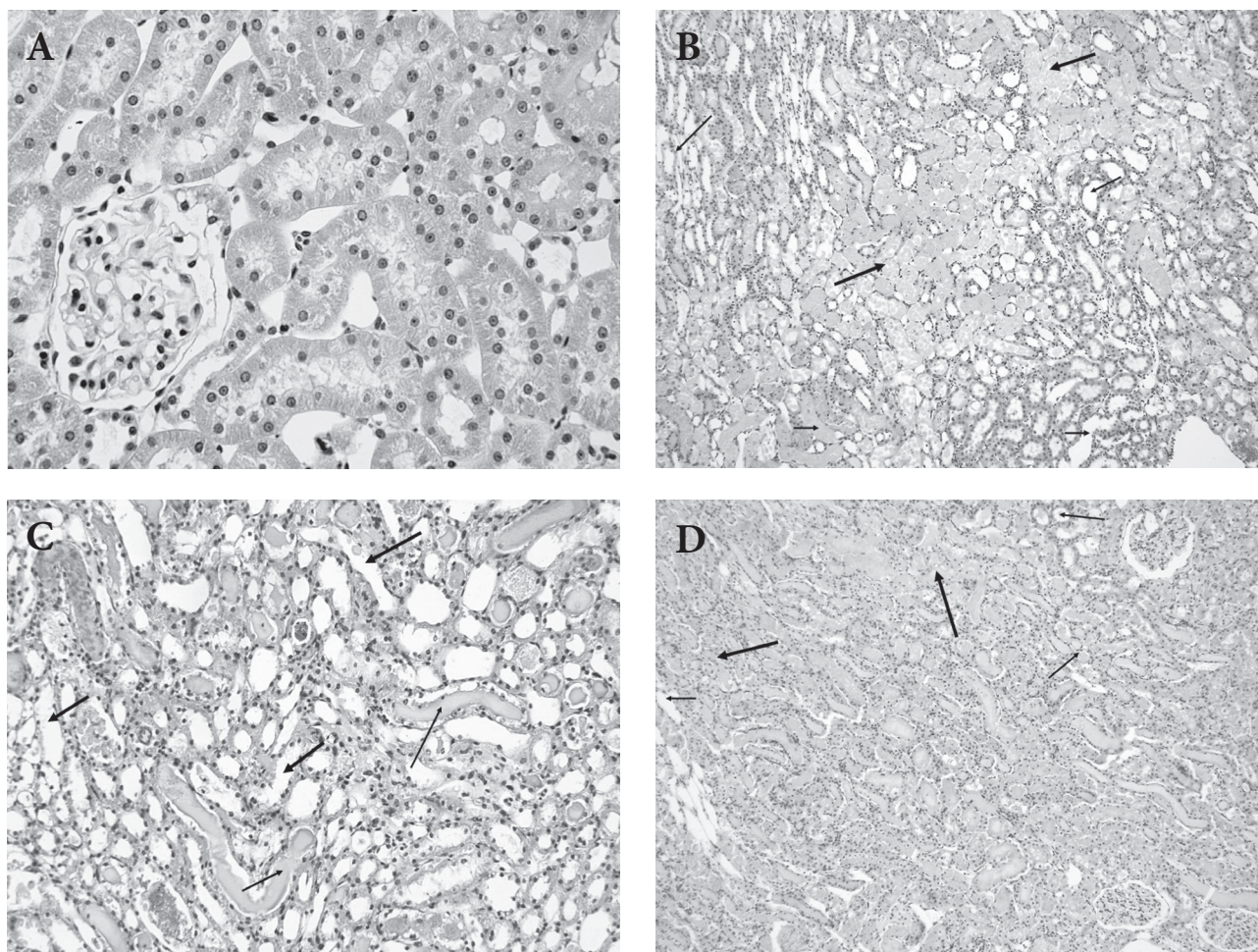


Figure 3. Histology of the kidney 24 h after reperfusion. **A.** A normal shape of the glomerulus and tubulointerstitium in the sham-operated animals (PASX320). **B.** Intensive corticomedullary tubular necrosis (bull arrow) and dilatation (thin arrow), PAS positive casts in the ARF group (PASX250). **C.** Intensive tubular dilatation and necrosis (bull arrow), PAS positive casts (thin arrow) and interstitial edema in ARF+L-Arg group (PASX250). **D.** Glomeruli, PAS positive casts, mild tubular necrosis (bull arrow) and slightly prominent proximal tubular dilatation (thin arrow) in ARF+Vit C group (PASX250).

Widespread tubular necrosis in the corticomedullary zone, huge number of PAS positive casts in distal tubuli and intensity of interstitial oedema, were similar in the ARF and ARF+L-Arg group (Figure 3C).

In some specimens of the ARF+Vit C group tubular necrosis in cortico medullary junction was less intense and less widespread in comparison to the control animals with acute renal failure. Also, intensity of tubular dilatation was markedly reduced in ARF+Vit C group in comparison to ARF group (Figure 3D).

Discussion

Recently, multiple strategies including L-arginine supplementation have been attempted to ameliorate course of acute renal failure. In several studies administration of exogenous L-arginine has been shown to protect the kidney against toxic or ischemic injury (Cherla and Jaimes 2004). Besides, decreased action or synthesis of NO has been implicated in diseases such as hypertension, hypercholesterolemia, diabetes or atherosclerosis (Moncada and Higgs 1995). In the present study, L-arginine markedly, but not significantly, decreased MAP, similarly to long-term L-arginine application in the study of Jerkić and co-workers, performed in Wistar rats (Jerkić et al. 1999). Our results indicate two possibilities, that NO synthesis is moderately decreased in SHR or that in models of ischemic acute renal failure changes in inducible NO synthase expression are accompanied by increased production of superoxide anion and peroxynitrite leading to nitrosative stress and vasoconstriction. By our opinion, second possibility seems realistically. In experimental models of hypertension, vitamin C, alone or in combination with vitamin E, accelerates degradation of S-nitrosoglutathione, increases synthesis of NO, and reduces blood pressure (Chen et al. 2001). In the present study, although MAP had tendency to decrease in the ARF+Vit C group in comparison with ARF group, this result is not statistically significant. Some other studies, investigating the importance of endogenously generated oxygen radicals in the regulation of blood pressure in SHR have shown modest or scant results also (Yoshioka et al. 1985; Nakazono et al. 1991).

This study has shown that renal haemodynamic was improved by L-arginine treatment and resulted in a significant decrease in RVR 24 h after ischemia. Chintala (Chintala et al. 1993) summarized that synthesis of endothelium-derived relaxing factor is probably turned on maximally by the ischemic insult, because treatment with L-arginine resulted in only a modest improvement of renal perfusion pressure and glomerular filtration rate in the ischemic kidney. However, beneficial effects of L-arginine treatment in the present study strongly suggest, that release of NO does not reach its maximum 24 h after reperfusion injury in SHR.

This is similarly to study of Jerkić et al. (1999) performed in Wistar rats, and in accordance with Schramm (Schramm et al. 1996) who suggested that renal ischemia, which injures endothelial cells, reduced the production of NO in them also. In the present study, administration of vitamin C had no influence on renal haemodynamic parameters, leading us to conclude that free radical scavenging has no dominant influence on renal vasomotor control in SHR with induced postischemic acute renal failure.

Twenty four hours after acute renal failure induction, GFR was drastically reduced. It was not significantly different among groups with acute renal failure irrespective of their treatment, but had tendency to increase in L-arginine-treated animals. On the other hand, it's well known that creatinine clearance is not optimal indicator of glomerular filtration in rats treated with L-arginine (and only in them) since creatinine arises as a waste product in the decay of L-arginine. However, it seemed almost inconceivable to add a more appropriate method, e.g. determination of inulin clearance, to our procedure, which was already very difficult for the animals. Plasma urea rose due to ischemic injury and was not significantly different among groups with acute renal failure irrespective of their treatment, indicating irrelevant role of NO bioavailability and radical scavenging in reperfusion-induced uremia in SHR.

Increase in urine volume in animals treated with L-arginine could be a consequence of restoration of the pressure-dependent increases in renal medullar haemodynamic in association with restoration of pressure natriuresis in SHR (Larson and Lockhart 1995). Urine protein excretion was not different between groups, what is in accordance with findings of Basile et al. (2001) that during the first 14-week postinjury, protein excretion is low in both sham-operated and postischemic animals.

Our previous study (Jerkić et al. 1999) performed in Wistar rats has shown that treatment with L-arginine reduces tubular cell injury in acute post ischemic renal failure and that NO acts cytoprotectively in rat kidney. In the present study, our results show that widespread tubular necrosis in the corticomedullary zone, huge number of PAS positive casts in distal tubuli and intensity of interstitial oedema, were similar in the ARF and ARF+L-Arg group. The absence of tubular improvement could be explained by different rat strain and by production of other vasoconstrictors (such as endothelin 1 and angiotensin II) in post-ischemic condition in hypertension and particularly by pre-existing oxidative stress in SHR. On the other hand, long-lasting hypertension damages medium size and small-size renal blood vessels and disables adequate tubuloglomerular responses (Radović et al. 2006), leading to worsen reperfusion injuries.

Recently, it's well known that hypoxia, as a result of ischemia and subsequent reperfusion, is characterized by increased reactive oxygen species and decreased efficacy

of the antioxidant system, which lead to tubular cell injury and death (Meji'a-Vilet et al. 2007). In many studies, antioxidant ascorbic acid has been shown to attenuate renal damage caused by a variety of insults, such as postischemic stress, cisplatin, aminoglycosides, and potassium bromate in animals and has an extensive safety record as a dietary supplement in humans (Spargias et al. 2004). Less intense and less widespread tubular necrosis in corticomedullary junction and reduced tubular dilatation in ARF+Vit C group in our study are in concordance with these findings. This effect suggests that free radical mediating vasoconstriction may represent an important pathophysiological mechanism of renal vasoconstriction and that vitamin C could be protective against acute ischemia-reperfusion injury in SHR.

In summary, our results suggest that short-term L-arginine supplementation improves systemic and renal haemodynamic in combined model of experimental hypertension and acute renal failure, probably due to direct vasodilatory action of NO on systemic and renal vasculature and also, well known, natriuretic and diuretic effects of L-arginine. In opposite to previous, L-arginine supplementation failed to improve glomerular function and to reduce tubular injury. Thus, our results lead us to conclude that the deleterious effects abolish the beneficial during excess NO exposure in this model. This also implies other vasoconstrictors could be involved in post-ischemic condition in hypertension that impede NO donor to improve tubular injury. On the other hand, prior administration of natural antioxidant, vitamin C moderately improves renal hemodynamic, but reduction of tubular necrosis and dilatation promotes vitamin C as an effective chemoprotectant against ischemia-reperfusion tubular injury in the combined model of experimental hypertension and acute renal failure. Therefore, future studies in which vitamin C would be given after the induction of acute renal failure may mimic better clinical situation for possible promotion of antioxidants as therapeutic agents in ischemic acute renal failure with hypertension.

Acknowledgement. This work was supported by grant (project No. 145054) from the Ministry of Science and Technological Development of Serbia.

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