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Research article

COMBINED ANGIOTENSIN II TYPE-1 RECEPTOR BLOCKADE AND SUPEROXIDE ANION SCAVENGING AFFECT THE POST-ISCHEMIC KIDNEY IN HYPERTENSIVE RATS

IVANOV Milan[1]*, MIHAILOVIĆ-STANOJEVIĆ Nevena[1], MARKOVIĆ-LIPKOVSKI Jasmina[2], JOVOVIĆ Đurđica[1], KARANOVIĆ Danijela[1], MILORADOVIĆ Zoran[1], GRUJIĆ-MILANOVIĆ Jelica[1]

[1]Institute for Medical Research University of Belgrade, Belgrade, Serbia;
[2]Medical School University of Belgrade, Institute of Pathology, Belgrade Serbia

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Ischemic acute kidney injury is characterized by renal vasoconstriction, filtration failure, tubular obstruction, tubular backleak and overproduction of angiotensin II and reactive oxygen species. Considering this complexity, the aim of our study was to investigate the effects of angiotensin II type-1 receptor blocker - Losartan and superoxide anion scavenger - Tempol, in a combined treatment on acute kidney injury in postischemic hypertensive rats.

The experiment was performed in anesthetized, adult male spontaneously hypertensive rats. The right kidney was removed and the left renal artery was occluded for 40 minutes. Experimental groups received combined treatment (Losartan + Tempol) or saline in the femoral vein 5 minutes before, during and 175 minutes after clamp removal.

Hemodynamics and biochemical parameters were measured and kidney specimens were collected 24h after reperfusion. Histological examination was performed by optical microscopy.

Combined treatment improves renal haemodynamics parameters which were exacerbated due to acute kidney injury. Acute kidney injury significantly decreased creatinine and urea clearance and increased lipid peroxidation in the plasma. Treatment with Losartan and Tempol induced a significant increase of creatinine and urea clearance. Lipid peroxidation in the plasma decreased and glutathione peroxidase enzyme activity in the erythrocytes increased after Losartan + Tempol treatment. This combined treatment reduced cortico-medullary necrosis and tubular dilatation in the kidney.

Our results indicate that synergism of Losartan and Tempol treatment could have beneficial effects on blood pressure and kidney function, during postischemic acute kidney injury development in experimental hypertension.

Key words: Acute kidney injury; Hypertension; Losartan; Reactive oxygen species; Tempol

*Corresponding author: e-mail: ivmilan@imi.bg.ac.rs

INTRODUCTION

Ischemia/reperfusion (IR) acute kidney injury (AKI) is a complex phenomenon with structural and functional changes in the kidney, which ensue mainly due to increased generation of reactive oxygen species (ROS) during reperfusion [1]. The manifestation of this oxygen radical-mediated injury are exacerbation of glomerular filtration and renal haemodynamic, damage of kidney tissue due to lack of antioxidative defence and free radical-mediated lipid peroxidation, as well as increased level of vasoactive Angiotensin II. [2-5]

Angiotensin II (AngII), as one of the main vasoactive signaling molecules, is involved in the generation of ROS. Overproduction of AngII during ischemic AKI [3] may cause an increased expression and activity of one of the major ROS generators, NADPH oxidase [6]. Also,due to intrarenal vasoconstriction, high level of AngII has harmful effects on morphological changes in the kidney tissue [2,4].

Beside IR injury, the pathology of hypertension is closely related to oxidative stress. There are many reports about this relation in humans (humans with essential hypertension, *preeclampsia* during pregnancy) and animal studies (spontaneously hypertensive,Dahl saltsensitive, or AngII–infused rat) [7].

This clearly implicates the important role of oxidants in the pathophysiology of the hypertensive postischemic kidney. This fact directed the focus of our study towards the influence of these two pathogenetic factors, hypertension and ROS, in the development and progression of AKI.

The protective effect of supplementation with antioxidant enzymes [1] and blockade of generators of ROS, such as NADPH oxidase [8], against IR induced oxidative stress provide unequivocal evidence that ROS has a great impact on the degree of tissue damage. Our previous studies showed positive effects of Losartan on oxidative stress, as well as on renal function and structure in the postischemic injured hypertensive kidney [5]. On the other side, superoxide dismutase mimetic, Tempol, also showed some beneficial effects on renal haemodynamic and oxidative status in the postischemic hypertensive kidney [9].

The goal of our study was to examine the effects of the combination (LT) - antihypertensive (Losartan) and antioxidative (Tempol) supplementation on kidney function and morfological changes in spontaneously hypertensive rats (SHR), during ischemic AKI development.

MATERIAL AND METHODS

In our study we used 24 weeks old male SHR, weighting about 300 g, which were bred at the Institute for Medical Research, University of Belgrade, Serbia, and fed with a standard chow for laboratory rats (Veterinarski zavod, Subotica, Serbia).

Ethics Statement

The experimental protocol was approved by the Ethic Committee of the Institute for Medical Research, University of Belgrade, Serbia (No. 0148.1/10), according to the National Law on Animal Welfare ("Službeni Glasnik" no. 41/09) that is consistent with guidelines for animal research and principles of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Purposes (Official Daily N. L 358/1-358/6, 18, December 1986) and Directive on the protection of animals used for scientific purposes (Directive 2010/63/EU of the European Parliament and of the Council, 22.9.2010.).

Experimental protocol

Animals were divided into the following groups: sham-operated rats (SHAM, n=7), rats with AKI (AKI, n=7) and animals which received the combination of AT1R antagonist - Losartan (DUP 153, Du Pont, Wilmington, DE, USA) and 4-Hydroxy-2, 2, 6, 6 tetramethylpiperidine-*N*-oxyl – Tempol (Sigma Chemical Co. St. Louis, MO, USA) after AKI induction (AKI+LT, n=9).

For surgical procedure, all rats were anaesthetized with 35 mg/kg b.m. sodium pentobarbital. AKI was induced by right kidney nephrectomy and atraumatic occlusion of the left renal artery for 40 minutes. SHAM and AKI rats received the vehicle (saline, 4ml), while AKI+LT group received a combination of Losartan (10 mg/kg b.m.) and Tempol (40mg/kg/h b.m.) dissolved in 4 ml saline, in the femoral vein 5 minutes before, during and 175 minutes after clamping. After infusion, abdominal incision was closed with several sutures and SHR were placed into metabolic cages for 24h urine collection, having free access to water and chow.

Haemodynamic measurements after 24h

Haemodynamic parameters were measured in anaesthetized rats, through a femoral artery catheter (PE–50, Clay-Adams Parsippany, NY, USA), connected to a physiological data acquisition system (9800TCR Cardiomax III-TCR Thermodilution Cardiac Output for mice, rats and large subjects, Columbus Instruments', Columbus, OH, USA). A jugular vein was cannulated with polyethylene tubing PE-50 for the injection of cold saline. The right carotid artery was catheterized with PE-50 tubing and attached to a thermo sensor, which was coupled to the Cardiomax III for the determination of cardiac output (CO). The other end of the thermocouple was placed in cold saline. Following 20 min. for stabilization after surgery, cold saline (0.2ml) was supplied through the jugular vein and mean arterial pressure (MAP) and CO were recorded. Total peripheral vascular resistance (TPVR) was calculated by dividing MAP with CO normalized for body weight and expressed as mmHg x min x kg/ml.

The prepared left renal artery was utilized for renal blood flow (RBF) recording, using a Transonic T106 Small Animal Flowmeter (Transonic System Inc., Ithaca, NY, USA).

Vascular resistance in this vascular bed (RVR) was calculated by dividing MAP with RBF, normalized for body weight and expressed as mmHg x min x kg/ml.

Biochemical measurements 24 h after reperfusion

After haemodynamic measurements, blood samples were taken for determination of creatinine (P_{Cr}), urea (P_U) and phosphates (P_{Phos}) in plasma. Lithium-heparin (Li-heparin, Sigma, USA) was used as an anticoagulant. 24h urine samples were used for the determination of urine creatinine (U_{Cr}) and urea (U_U) concentrations. All biochemical parameters were measured using an automatic COBAS INTEGRA 400 plus (Hoffmann-La Roche, Germany) analyzer. Creatinine (C_{Cr}) and urea (C_U) clearances were calculated according to standard formula and normalized to body weight. After blood samples collection, animals were sacrificed by pentobarbital overdose injection.

Oxidative stress parameters

To determine the degree of lipid peroxidation, the concentration of thiobarbituric acid reactive substances (TBARS) in the plasma was measured [10]. Antioxidant enzymes activities of the erythrocytes were measured by spectrophotometry. Catalase (CAT) activity was measured by the depletion of H_2O_2 [11]. Glutathione reductase (GR) activity was measured using the method developed by Glatzle et al.[12] Superoxide dismutase (SOD) activity was measured according to McCord and Fridovich, where we evaluated a decrease of cytochrome c reduction [13]. Activity of glutathione peroxidase (GSH-Px) was determined by the method previously described by Paglia and Valentine [14].

Morphological and immunohistological examination

For the morphological analysis of the left kidney, renal tissue was prepared as described previously [4], and stained by hematoxilin eosine (H&E) and periodic acid-Schiff (PAS). Intensity and spread of tubular necrosis, number of intra-luminal cast formations, swelling and vacuolization of cells, loss of luminal membrane or brush borders, tubular dilatation, interstitial oedema and separation of cells from the tubular basal membrane were semi-quantitatively evaluated as described previously [4]. The level of each manifestation was graded with 1 for low, 2 for moderate, 3 for high, and 0 for the lack of manifestation. The sum of these changes represented the histopathological score.

Statistical analysis

The results are shown as the mean, with the standard error of the mean. We used the single-sided Student's t-test for two-samples of equal variance, and value $p<0.05$ was considered notable (Microsoft Excel 2010).

RESULTS

Haemodynamic parameters

Haemodynamics parametrs, 24 h after reperfusion, are shown in Table 1. The MAP was significantly decreased in AKI group, in comparison to SHAM. Furthermore, in the AKI+LT group, MAP was significantly decreased in comparison to AKI. There were no differences in CO and TPVR between experimental groups. The significant decline of RBF in AKI group (p<0,01) and concomitant increase of RVR (p<0,01;compared to SHAM), were completely abolished with LT infusion. Namely, LT treatment markedly increased RBF (AKI+LT vs. AKI; p<0,05) and decreased RVR in hypertensive rats with AKI (AKI+LT vs. AKI; p<0,01) (**Fig. 1**).

Table 1. Haemodynamics parametrs in experimental groups 24 hours after reperfusion

	SHAM (n=7)	AKI (n=7)	AKI+LT (n=9)
MAP mmHg	147.1±5.6	119.2±3.5***	60.3±7.4###
CO ml/min/kg	281.7±45.8	289.8±45.2	231.3±32.7
TPVR mHg x min x kg/ml	0.74±0.1	0.51±0.11	0.34±0.05

MAP-mean arterial pressure; CO- cardiac output; TPVR- total peripheral vascular resistance. n-number of animals. *** p < 0.001 compared to SHAM; ### p < 0.001 compared to AKI.

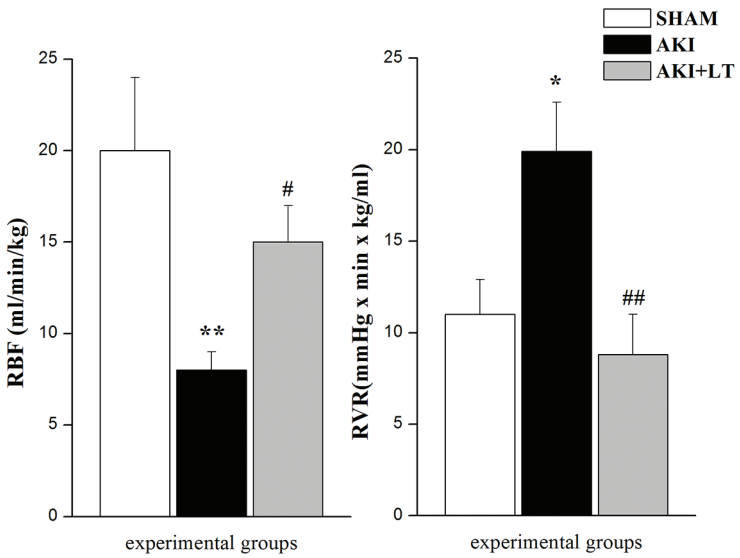

Figure 1. Renal haemodynamic parameters of SHR. RBF- renal blood flow, RVR – renal vascular resistance, SHAM - sham operated SHR (n=7), AKI - SHR with acute renal failure (n=7), AKI+LT - SHR received Losartan and Tempol after AKI (n=9). *p < 0.05, **p < 0.01 vs. SHAM; #p < 0.05, ##p < 0.01 vs. AKI; n-number of animals.

Biochemical parameters

Significantly higher levels of P_{Cr}, P_U and P_{Phos} were found in AKI group in comparison to SHAM (AKI vs. SHAM; $p<0{,}001$). LT treatment markedly decreased P_{Cr} (AKI+LT vs. AKI; $p<0{,}01$) , as well as P_U and P_{Phos} (AKI+LT vs. AKI; $p<0{,}05$) concentrations in comparison to AKI (**Fig. 2**).

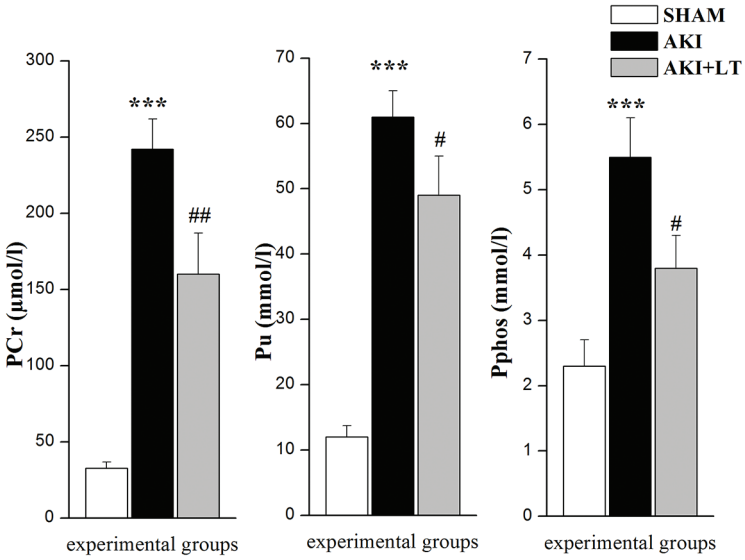

Figure 2. Plasma creatinine (Pcr), urea (Pu), and phosphates (Pphos) concentrations of SHR. SHAM - sham operated SHR (n=7), AKI - SHR with acute renal failure (n=7), AKI+LT - SHR received Losartan and Tempol after AKI (n=9). ***p < 0.001 vs. SHAM; #p < 0.05, ##p < 0.01 vs. AKI; n-number of animals.

In the urine samples, due to AKI induction, both creatinine and urea levels were significantly decreased compared to SHAM. The treatment with LT improved urea concentration in urine, but did not effect creatinine concentration (Table 2). C_{Cr} and C_U were markedly lower due to the induction of AKI. However, C_{Cr} and C_U were significantly improved after LT treatment, compared to AKI group. (Table 2).

Table 2. Biochemical parameters in experimental groups 24 hours after reperfusion

	SHAM (n=7)	**AKI (n=7)**	**AKI+LT (n=9)**
U_{Cr} mmol/l	6.31±1.05	2.39±0.45**	3.00±0.35
U_U mmol/l	908±173	227±43**	333±40$^{\#\#}$
C_{Cr} ml/min/kg	6.50 ± 0.99	0.29 ± 0.13***	0.76±0.12$^{\#\#}$
C_U ml/min/kg	2.38 ± 0.28	0.10 ± 0.05***	0.27±0.04$^{\#}$

U_{Cr}- urine creatinine; U_U-urine urea; C_{Cr}-creatinine clearance; C_U-urea clearance. n-number of animals. ** p < 0.01, *** p < 0.001 compared to SHAM; $^{\#}$ p < 0.05, $^{\#\#}$ p < 0.01 compared to AKI.

Oxidative stress parameters

AKI injured rats showed a significant increase in lipid peroxidation compared to SHAM operated animals. LT treatment significantly decreased plasma TBARS compared to the rats with induced AKI. The levels of antioxidative enzyme activity in erythrocytes: catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and glutathione reductase (GR) were shown in Table 3. GSH-Px activity shows moderate, but not significant decrease in AKI vs. SHAM operated group. LT significantly increased activity of GSH-Px in comparison to AKI group. SOD, CAT and GR activity, were not different between groups.

Table 3. Oxidative stress parameters in experimental groups 24h after reperfusion

	SHAM (n=7)	AKI (n=7)	AKI+LT (n=9)
pTBARS (nmol/ml)	7.28 ± 0,76	10.54 ± 0.52**	8.06 ± 1.1#
eCAT (mmol H_2O_2/min)	22.15 ± 6.05	14.32 ± 2.91	14.3 ± 4.71
eGR (µmol NADPH/min/g Hb) *10^3	5.6 ± 1.8	4.9 ± 1.2	12.1 ± 3.9
eGSH-Px (µmol NADPH/min/g Hb)	185.1 ± 59.4	152.44 ± 27.1	270.6 ± 40.1#
eSOD (U/g Hb)*10^3	1.6 ± 0.4	1.5 ± 0.5	1.7 ± 0.4

TBARS - thiobarbituric acid reactive substances; CAT - catalase activity; GR -glutathione reductase activity; GSH-Px - glutathione peroxidase activity; SOD - superoxide dismutase activity. Data are presented as mean ± SEM; n-number of animals; p-plasma; e- erythrocyte; **p<0.01 compared with SHAM; #p<0.05 compared with AKI.

Histological studies

There were significant differences in pathomorphological parameters between experimental groups. **Fig. 3a** shows the normal appearance of glomeruli, interstitium, tubules and blood vessels in SHAM operated animals. Only in a few kidney specimens we observed smaller number of PAS positive casts in the lumen of the tubules. The kidneys of animals with AKI showed dilatation of certain segments of the proximal and distal tubules, with or without loss of brush-border. The most notable changes were present in the cortico-medullary zone, where the broad areas of tubular necrosis and a large number of PAS positive casts in the collecting ducts were observed. The intensity of interstitial edema in this group varies from sample to sample (**Fig. 3b**). In LT treated animals, less damages were noticed in comparison to renal tissue of AKI animals. Tubular dilatations were smaller, and tubular necrosis in the cortico-medullary zone, was reduced. Interstitial edema is rarely observed. In addition, the number of tubular casts in the renal medulla is lower, compared to AKI animals (**Fig. 3c**). The histopathological score (**Fig. 3d**) shows the sum of these changes (AKI vs. SHAM, p<0,001; AKI+LT vs. AKI, p<0,01).

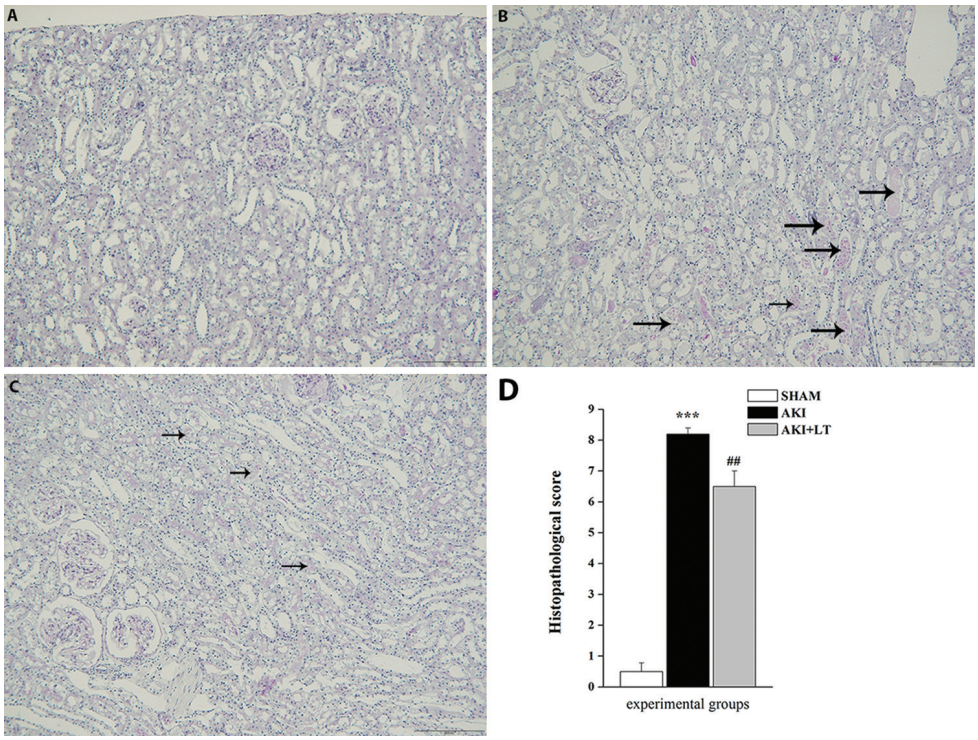

Figure 3A. Normal appearance of glomeruli, interstitium, tubules and blood vessels in SHAM operated animals. Very rare PAS positive casts in the lumen of the tubules
Figure 3B. Massive cortico-medullary tubular necrosis. Intensive interstitial edema. Numerous PAS positive casts in the collecting ducts and dilatation of certain segments of the proximal and distal tubules, (with or without loss of brush-border) in the AKI control group.
Figure 3C. Moderately intensive tubular necrosis in LT treated rats, reduced tubular dilatation and less number of PAS-positive casts.
Figure 3D. Histopathological score in experimental groups 24h after reperfusion. SHAM, n=7; AKI, n=7; AKI+LT, n=9 (n-number of animals). Data are presented as mean ± SEM (***p<0.001 compared with SHAM; ##p<0.01 compared with AKI)

DISCUSSION

In the presented study we investigated the effects of concomitant AT1R blockade and O_2^- scavenging in hypertensive rats with ischemic AKI. The significance of their roles is evaluated on the basis of biochemical and histological parameters, as well as the activities of antioxidative enzymes and lipid peroxidation as a parameters of oxidative stress.

After AKI induction, we noticed mild MAP reduction, similar to the results of Bowmer at al. [15] performed in the model of glycerol induced AKI. These authors considered high uremia (plasma urea was significantly elevated in the AKI group in our study as well) and its influence on the autonomic nervous system (diminished α_1 adrenoreceptors sensitivity) as a cause of MAP reduction after AKI. Dobrian et al [7]

showed that only the combined treatment with LT (and not a single treatment with Losartan or Tempol) decreases MAP to control values in the model of 1K1C induced hypertension. These results are similar to ours where combined treatment with LT decreases MAP to the physiological level in the group of SHR with IR induced AKI.

Renal dysfunction in this model was characterized by the rapid fall in renal blood flow and increase of renal vascular resistance, accompanied with the decrease of both urea and creatinine clearances. In our study, RBF was significantly reduced, followed by enhanced RVR in postishaemic SHR twenty-four hours after AKI induction. Intrarenal vasoconstriction is one of the main factors involved in the initiation and maintenance of AKI [16]. It is well known that Losartan and Tempol could have beneficial effects on renal haemodynamics [4,9,17,18]. Miloradović et al. showed that Losartan (10 mg/kg b.w.) strongly increases RBF after moderate NO deficiency in the kidney of postischemic Wistar rats [4]. On the other side, Schnackenberg et al. pointed that Tempol (12.4 mg/kg t.m.) significantly reduces RVR, without influencing RBF in SHR [17]. Considering that IR injury is a well known generator of ROS, Tempol treatment can be useful for the regulation of renal haemodynamic in increased oxidative stress [18]. In our model of ischemic AKI induced in SHR, treatment with Tempol in combination with Losartan, beside auspicious effects on RVR [17], had beneficial effects on RBF, too. That implies significant roles of Ang II and superoxide radicals on renal haemodynamic in the present model of ischemia-reperfusion.

Our study shows that postischemic kidney damage decreases creatinine clearance twenty time fold, indicating adjunctive reduction of glomerular filtration rate (GFR) in postischemic hypertensive kidney. GFR (expressed by clearence creatinine) is one of the most important markers of AKI. It is well known that Losartan has beneficial effects on renal function. Kontogiannis and Burns showed that blockade of AT1 receptors with Losartan accelerates the recovery of renal function, leading to a significant decrease in serum creatinine, after 60 minutes bilateral occlusion of the male Sprague-Dawley rats renal hilum [3]. In another study, Miloradović et al. showed that Losartan led to a slight GFR improvement in postischemic kidney of Wistar rats, after moderate blockade of NO synthase [4]. On the other side, our previous results showed that Tempol did not improve GFR in SHR with ischemic AKI [9]. Oposite to our results, Chatterjee at al. showed that Tempol treatment improves GFR in normotensive surroundings [19]. Our results correlate with these findings because combined treatment with anti-hypertensive Losartan, and SOD mimetic Tempol, doubled creatinine clearance in SHR with AKI.

Hyperphosphatemia is an important marker of tubular damage in AKI. Rubinger at al., showed that hyperphosphataemia is a result of reduced expression of sodium-dependent phosphate cotransporter in tubules, which is induced by ischemic damage of kidney tissue [20]. In our previos studies, Losartan produced a significant decrease in plasma phosphate [21], due to a better preservation of tubular structures after RAS blockade [5] in hypertensive rats with induced AKI, and thus better excretion of phosphate was observed. On the other hand, treatment with SOD mimetic, Tempol,

did not result in a significant decrease in plasma phosphate in such conditions [9]. Yamada at al. showed beneficial effects of Tempol during renal failure in high uremic rats, despite the fact that Tempol did not improve hyperphosphatemia [22]. We showed that beside the improvement in haemodynamics and GFR, the hyperphosphatemia is markedly lower in LT treatment SHR with postischemic kidney injury, as well.

In our model there are two important facts considering ROS generation. It is well known that, during ischemic AKI, AngII concentration goes up [3], and Ang II activates NADPH oxidase (via AT1 receptors) which can in return generate superoxide anion [6]. The second fact is the nature of I/R injury. I/R injury in moment of reperfusion generates high concentrations of ROS [23].

Dobrian et al indicated that treatment with Losartan or Tempol alone did not effect O_2^- generation in a rat model of renal hypertension (1K1C), but on the other side combined treatment with Losartan and Tempol decreases oxidative stress in Sprague-Dawley rats [7]. Our results are complementary with this study. Blockage of AT1R with Losartan and scavenging O_2^- at the same time decrease lipid peroxidation in SHR with AKI. This combined treatment increases GSH-Px activity,i.e. the enzyme which decomposes a very potent ROS, hydrogen peroxide, down to the water molecule.

Inal at al. showed that renal I/R injury in Sprague-Dawley rats decreases GSH-px activity, and treatment with substances which have antioxidant potential could increase the activity of this enzyme [24]. According to these results, a slight reduction in GSH-Px activity in our study is significantly higher in the LT treated group in SHR with AKI. Our previous results suggest that only AT1R blockade could not improve GSH-Px activity in our experimental model [5]. On the other side, LT treatment, beside decreasing lipid peroxidation, increases GSH-Px activity and thus improves antioxidant defence.

Histopathological examination is the best way to analyze morphological changes in the kidney tissue after AKI development. Broad areas of necrosis, the most common in the cortico-medullary zone, and a large number of PAS positive casts in the renal medulla [25] are the most notable changes in the ischemic kidney that can be seen by light microscopy. Morphological changes in the kidneys isolated from our study in the control AKI group, entirely correspond to the previous description. Besides the mentioned changes, we observed a significant number of dilated proximal and distal tubules.

Numerous studies showed a successful recovery potential of Losartan and Tempol in AKI [4,5,19]. On the other side, our previous study showed that hypertension suppressed Tempol recovery capacity [9]. In this study, with a combined treatment due to blockage of AT1 receptor in the early stages of ischemic AKI in hypertension, Tempol could express beneficial effects on renal morphological structure. Beyond decreasing MAP to the physiological level, the combined LT treatment also decreased the number of PAS positive casts, and smaller fields of necrosis were observed, as well.

These histological findings suggest a positive effect of LT treatment are consistent with the improvement of both systemic and renal artery hemodynamics parameters, as well with better biochemical parameters of kidney function.

CONCLUSION

This study, for the first time, demonstrated that combined treatment with AT1R blocker and O_2^- scavenger could have beneficial effects in concomitant model of postischemic AKI and hypertension. This study also showed that kidney injuries are strongly mediated by Ang II and partly by O_2^-. AT1R blockade and O_2^- scavenging improve glomerular filtration and renal haemodynamic, decrease lipid peroxidation and increase antioxidative defense. Furthermore, treatment with LT combination mitigates kidney damage in SHR during AKI development, but we did not get expected additive protective effects in comparison to our previous study, where animals were treated only with Losartan [5]. In comparison to the former results, we conclude that the most positive effects in this study are the consequence of AT1R blockade. These findings could be useful in hypertensive patients who develop AKI, but further investigations, especially in pre-clinical and clinical studies, are necessary.

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Authors' contributions

IM and MZ have made substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data, GMJ and MSN have been involved in drafting the manuscript and revisiong it critically for important intellectual content, JĐ and MLJ have given final approval of the version to be published, KD, IM and GMJ carried out biochemical studies, MZ and MSN participated in the surgery of small experimental animals. Histological studies were done by MLJ. All authors read and approved the final manuscript.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

REFERENCES

1. Dobashi K, Ghosh B, Orak JK, Singh I, Singh AK: Kidney ischemia-reperfusion: modulation of antioxidant defenses. Mol Cell Biochem 2000, 205(1-2):1-11.
2. Gobe G, Zhang XJ, Willgoss DA, Schoch E, Hogg NA, Endre ZH: Relationship between expression of Bcl-2 genes and growth factors in ischemic acute renal failure in the rat. J Am Soc Nephrol 2000, 11(3):454-67.
3. Kontogiannis J, Burns KD: Role of AT1 angiotensin II receptors in renal ischemic injury. Am J Physiol 1998, 274(1 Pt 2):F79-F90.
4. Miloradovic Z, Jerkic M, Jovovic D, Mihailovic-Stanojevic N, Milanovic JG, Stosic G, Markovic-Lipkovski J: Bosentan and losartan ameliorate acute renal failure associated with mild but not strong NO blockade. Nephrol Dial Transplant 2007, 22(9):2476-84.
5. Ivanov M, Mihailovic-Stanojevic N, Grujic MJ, Jovovic D, Markovic-Lipkovski J, Cirovic S, Miloradovic Z: Losartan improved antioxidant defense, renal function and structure of postischemic hypertensive kidney. PLoS One 2014, 9(5):e96353.
6. Rajagopalan S, Kurz S, Munzel T, Tarpey M, Freeman BA, Griendling KK, Harrison DG: Angiotensin II-mediated hypertension in the rat increases vascular superoxide production via membrane NADH/NADPH oxidase activation. Contribution to alterations of vasomotor tone. J Clin Invest 1996, 97(8):1916-23.
7. Dobrian AD, Schriver SD, Prewitt RL: Role of angiotensin II and free radicals in blood pressure regulation in a rat model of renal hypertension. Hypertension 2001, 38(3):361-6.
8. Dodd O, Pearse DB: Effect of the NADPH oxidase inhibitor apocynin on ischemia-reperfusion lung injury. Am J Physiol Heart Circ Physiol 2000, 279(1):H303-H312.
9. Miloradovic Z, Ivanov M, Mihailovic-Stanojevic N, Grujic MJ, Jovovic D, Vajic UJ, Markovic-Lipkovski J: Acute superoxide radical scavenging reduces blood pressure but does not influence kidney function in hypertensive rats with postischemic kidney injury. Biomed Res Int 2014, 2014:512619.
10. Ohkawa H, Ohishi N, Yagi K: Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979, 95(2):351-8.
11. Beutler E: Catalase. In: *Red cell metabolism, a manual of biochemical methods*. New York, USA: Grune and Stratton: 1982, 105-106.
12. Glatzle D, Vuilleumier JP, Weber F, Decker K: Glutathione reductase test with whole blood, a convenient procedure for the assessment of the riboflavin status in humans. Experientia 1974, 30(6):665-7.
13. McCord JM, Fridovich I: The utility of superoxide dismutase in studying free radical reactions. I. Radicals generated by the interaction of sulfite, dimethyl sulfoxide, and oxygen. J Biol Chem 1969, 244(22):6056-63.
14. Paglia DE, Valentine WN: Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 1967, 70(1):158-69.
15. Bowmer CJ, Nichols AJ, Warren M, Yates MS: Cardiovascular responses in rats with glycerol-induced acute renal failure. Br J Pharmacol 1983, 79(2):471-6.
16. Nissenson AR: Acute renal failure: definition and pathogenesis. Kidney Int Suppl 1998,66:S7-10.
17. Schnackenberg CG, Welch WJ, Wilcox CS: Normalization of blood pressure and renal vascular resistance in SHR with a membrane-permeable superoxide dismutase mimetic: role of nitric oxide. Hypertension 1998, 32(1):59-64.

18. Majid DS, Kopkan L: Nitric oxide and superoxide interactions in the kidney and their implication in the development of salt-sensitive hypertension. Clin Exp Pharmacol Physiol 2007, 34(9):946-52.
19. Chatterjee PK, Cuzzocrea S, Brown PA, Zacharowski K, Stewart KN, Mota-Filipe H, Thiemermann C: Tempol, a membrane-permeable radical scavenger, reduces oxidant stress-mediated renal dysfunction and injury in the rat. Kidney Int 2000, 58(2):658-73.
20. Rubinger D, Wald H, Gimelreich D, Halaihel N, Rogers T, Levi M, Popovtzer MM: Regulation of the renal sodium-dependent phosphate cotransporter NaPi2 (Npt2) in acute renal failure due to ischemia and reperfusion. Nephron Physiol 2005, 100(1):1-12.
21. Ivanov M, Mihailovic-Stanojevic N, Grujic MJ, Jovovic D, Miloradovic Z: Prevention of systemic and regional haemodynamic alterations, hypercreatininemia, hyperuremia and hyperphosphatemia by losartan in hypertension with acute renal failure. Acta Physiol Hung 2011, 98(1):1-7.
22. Yamada S, Taniguchi M, Tokumoto M, Toyonaga J, Fujisaki K, Suehiro T, Noguchi H, Iida M, Tsuruya K, Kitazono T: The antioxidant tempol ameliorates arterial medial calcification in uremic rats: important role of oxidative stress in the pathogenesis of vascular calcification in chronic kidney disease. J Bone Miner Res 2012, 27(2):474-85.
23. Paller MS, Hoidal JR, Ferris TF: Oxygen free radicals in ischemic acute renal failure in the rat. J Clin Invest 1984, 74(4):1156-64.
24. Inal M, Altinisik M, Bilgin MD: The effect of quercetin on renal ischemia and reperfusion injury in the rat. Cell Biochem Funct 2002, 20(4):291-6.
25. Jerkic M, Miloradovic Z, Jovovic D, Mihailovic-Stanojevic N, Elena JV, Nastic-Miric D, Grujic-Adanja G, Rodriguez-Barbero A, Markovic-Lipkovski J, Vojvodic SB, Manero MV, Prieto MP, Lopez-Novoa JM: Relative roles of endothelin-1 and angiotensin II in experimental post-ischaemic acute renal failure. Nephrol Dial Transplant 2004, 19(1):83-94.

UTICAJ BLOKADE RECEPTORA TIP 1 ZA ANGOTENZIN II UDRUŽENE SA UKLANJANJEM SUPEROKSIDNOG ANJONA NA POSTISHEMIČNI BUBREG KOD PACOVA SA UROĐENOM HIPERTENZIJOM

IVANOV Milan, MIHAILOVIĆ-STANOJEVIĆ Nevena, MARKOVIĆ-LIPKOVSKI Jasmina, JOVOVIĆ Đurđica, KARANOVIĆ Danijela, MILORADOVIĆ Zoran, GRUJIĆ-MILANOVIĆ Jelica

Glavne karakteristike ishemične akutne bubrežne slabosti su: renalna vazokonstrikcija, pad glomerulske filtracije, tubularna opstrukcija, vraćanje glomerulskog filtrata u intersticijum tubula i prekomerna produkcija angiotenzina II i reaktivnih vrsta kiseonika. Uzimajući u obzir kompleksnost ovog poremećaja, cilj ove studije je bio da istraži efekte kombinovanog tretmana blokatora angiotenzin II receptora tip 1 – Losartana i sakupljača superoksidnog anjona – Tempola u modelu ishemične bubrežne slabosti kod hipertenzivnih pacova.

Eksperiment je urađen na odraslim anesteziranim mužjacima hipertenzivnih pacova. Desni bubreg je uklonjen, dok je na levoj renalnoj arteriji urađena okluzija u trajanju od 40 minuta. Eksperimentalne grupe su primile kombinovani tretman (Losartan+Tempol) ili fiziološki rastvor u femoralnu venu, 5 minuta pre i 175 minuta nakon uklanjanja kleme sa renalne arterije. Hemodinamski i biohemijski parametri su izmereni, a uzorci bubrega uzimani su 24 časa nakon reperfuzije. Histološka ispitivanja su rađena pomoću svetlosnog mikroskopa.

Kombinovani tretman poboljšava renalnu hemodinamiku, poremećenu usled ishemične akutne bubrežne slabosti. U ovom modelu bubrežne slabosti dolazi do pada klirensa kreatinina i uree, i povećanja lipidne peroksidacije u plazmi.Tretman Losartanom i Tempolom dovodi do značajnog povećanja klirensa kreatinina i uree. Lipidna peroksidacija je smanjena, a aktivnost glutation peroksidaze u eritrocitima je povećana nakon kombinovanog tretmana sa Losartanom i Tempolom. Takođe, ovakav kombinovan tretman smanjuje kortiko-medularnu nekrozu i tubularnu dilataciju u bubregu.

Naši rezultati ukazuju na to da sinergizam Losartana i Tempola može imati povoljan efekat na krvni pritisak i bubrežnu funkciju tokom razvoja postishemične akutne bubrežne slabosti u eksperimentalnoj hipertenziji.