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Original Article

Role of nitric oxide in kidney and liver (as distance organ) function in bilateral renal ischemia-reperfusion: Effect of L-Arginine and NG-nitro-L-Arginine methyl ester

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Abstract

Background: Renal ischemia-reperfusion (RIR) is a major cause of renal dysfunction that acts through different mechanisms. We investigated the role of L-Arginine as an endogenous nitric oxide (NO) precursor and NG-nitro-L-Arginine methyl ester (L-NAME) as an NO inhibitor on kidney and liver function in RIR model.

Materials and Methods: Fifty-eight Wistar rats were randomly assigned to four groups. Groups 1 (sham-operated, $n = 13$) received a single dose of saline (4 ml/kg, i.p.) and 2 (Ischemia [Isch], $n = 14$) received a single dose of saline (4 ml/kg, i.p.). Groups 3 (Isch + L-NAME, $n = 15$) received a single dose of L-NAME (20 mg/kg, i.p.) and 4 (Isch + L-Arginine $n = 16$) received a single dose of L-Arginine (300 mg/kg, i.p.), After 2 h, renal failure was induced by clamping both renal pedicles for 45 min, followed by 24-h reperfusion in Groups 2–4. Finally, blood samples were obtained, and kidney tissue samples were subjected for pathology investigations.

Results: The body weight decreased, and the serum levels of blood urea nitrogen (BUN) and creatinine (Cr), and kidney tissue damage score (KTDS) increased significantly in the Isch and Isch + L-NAME groups compared with the sham group while L-Arginine improved weight reduction ($P < 0.05$), and it reduced the serum levels of BUN and Cr, and KTDS when compared with the Isch and Isch + L-NAME groups. Kidney weight increased significantly in all groups compared with the sham group. L-Arginine reduced the liver tissue level of malondialdehyde and increased alkaline phosphatase.

Conclusion: L-Arginine as an NO precursor can improve kidney function against RIR. It also improves oxidative stress in liver tissue.

Key Words: L-Arginine, liver, NG-nitro-L-Arginine methyl ester, rat, renal ischemia

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Received: 28.04.2015, **Accepted:** 04.07.2015

INTRODUCTION

Renal ischemia-reperfusion (RIR) is a common cause of renal dysfunction in partial nephrectomy, renal transplantation, surgical revascularization of the renal artery, and treatment of suprarenal aortic aneurysms.[1-3] The main pathophysiologic

Access this article online

Quick Response Code:

Website:
www.advbiores.net

DOI:
10.4103/2277-9175.167954

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How to cite this article: Ghasemi M, Nematbakhsh M, Daneshmand F, Moeini M, Talebi A. Role of nitric oxide in kidney and liver (as distance organ) function in bilateral renal ischemia-reperfusion: Effect of L-Arginine and NG-nitro-L-Arginine methyl ester. Adv Biomed Res 2015;4:233.

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effect in RIR injury is depletion of intracellular energy reserve that leads to a series of complex biochemical pathological and physiological injuries.[4] One of the important mechanisms is generation of reactive oxygen species (ROS) such as superoxide anion (O_2^-.), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH.) that can cause oxidative injury of cellular macromolecules.[5,6] RIR injury can cause reduction in glomerular filtration rate (GFR),[7] intracellular antioxidant index such as glutathione (GSH) and enzymes such as glutathione reductase, superoxide dismutase (SOD), and catalase;[8,9] and disturbance in the release of nitric oxide (NO). On the other hand, there is some evidence that ischemia-reperfusion (IR) injury affects remote organs.[10] For example, it may lead to the failure of other systems like lungs, brain, and liver.[11] It seems that some effects are mediated by an imbalance in the oxidant and antioxidant systems.[12] NO is a free radical that can easily pass through the cell membranes. Thus, together with relaxation of smooth muscles, this leads to vascular dilation[13] and improves blood flow in the arteries. Different isoforms of NO synthase (NOS) have been identified in the kidney; namely, endothelial NOS (eNOS), inducible NOS (iNOS), and neuron NOS (nNOS). Among the isoforms, eNOS is mainly found in the vasa recta, inner medullary collecting duct, and glomeruli; iNOS can be expressed by vascular smooth muscle cells, renal tubular cells, and immune cells such as monocytes, macrophages, and neutrophils;[14] and nNOS is expressed in cells of the macula densa and seems to participate in tubuloglomerular feedback but has minimal effects on medullary perfusion.[15] L-Arginine is a non essential amino acid that is produced in the kidneys and acts as a substrate for NOS. NG-nitro-L-Arginine methyl ester or (L-NAME) has characteristics similar to L-Arginine in binding to NOS and inhibits NO production.[16] Thus, it can be used as an antagonist of L-Arginine. Atanasova *et al*. discovered L-NAME worsens Ischemia (Isch) effects,[17] and Klahr reported L-Arginine protects renal disease by increasing GFR.[18] However, further information is still required on the alteration of antioxidant parameters after RIR pretreated with NO precursor and blocker. As mentioned IRI affects remote organs such as lungs, brain, intestines and liver[3,11] via migration cytokines, ROS, and other inflammatory agents in the circulation to distal.[3,19,20] Liver is a big gland and plays a major role in metabolism with numerous functions, including regulation of glycogen storage[21] decomposition of red blood cells, plasma proteins synthesis[22] hormone production, and amino acids metabolism,[23,24] detoxification,[25] and it produces bile.[26] Oxidative stress is a common mechanism of liver injury[27] and the role of L-Arginine and L-NAME on liver function after RIR also should be determined. Accordingly, this study was designed to determine the effect of L-Arginine and L-NAME on renal and liver biomarkers and tissue injury in rats.

MATERIALS AND METHODS

Animals

Fifty-eight male and female Wistar rats (weighing 214.72 ± 3.19 g and 185.72 ± 2.70 g, respectively) were used in the current study. The rats were kept at a temperature of 23–25°C and 12 h light/12 h dark cycle and had free access to water and chow for at least 1 week prior to experiment. The study was in advance approved by the Isfahan University of Medical Sciences Ethics Committee.

Experimental protocol

The male and female rats were randomly divided into four experimental groups.

Group 1 (n = 13) received a single dose of saline (4 ml/kg body weight [BW] i.p.) and after 2 h underwent surgery without Isch process as the sham-operated group. Group 2 (n = 14) as the Isch group received a single dose of saline (4 ml/kg BW i.p.) after 2 h experienced Isch. Groups 3 (n = 15) received a single dose of L-NAME (20 mg/kg, i.p.) underwent Isch after 2 h as Isch + L-NAME and 4 (n = 16) received a single dose of L-Arginine (300 mg/kg BW i.p.) as Isch + Arginine group and underwent Isch after 2 h similar to Groups 2 and 3. All groups intervention followed by 24-h reperfusion.

The animals were anesthetized by ketamine (75 mg/kg BW i.p.) and Groups 2–4 were operated and underwent bilateral kidney Isch for 45 min and then reperfusion. After 24 h of reperfusion, the animals were re-anaesthetized, ventilation tube was inserted into the trachea, and the catheters were implanted into the carotid artery to obtain a blood sample. Finally, the rats were sacrificed, their kidneys and two pieces of liver tissue were removed and weighed immediately. The right kidneys were divided into two parts. One part of the kidney and a part of the liver were homogenized in phosphate buffered saline (PBS) (10 ml/g tissue) separately and centrifuged for measurement of GSH and other biochemical parameters. Other pieces were homogenized in sucrose buffer (10 ml/g tissue) for measurement of SOD. Then, the supernatant was removed and frozen at −20°C to measure renal levels of biochemical parameters. The left kidneys were placed in formalin to be fixed for staining procedures.

Measurements

Super oxide dismutase assay

The activity of SOD was assayed according to the method modified by Kakkar *et al*.[28] Briefly,

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200 µl sample was mixed with 1.2 ml sodium pyrophosphate buffer (pH = 8.3, 0.052 M), 0.1 ml phenazine methosulfate (186 µM), and 0.3 ml nitroblue tetrazolium (300 µM) and 1 ml water. The reaction started as 0.2 ml reduced nicotinamide adenine dinucleotide (780 µM) was added. The reaction mixture was then incubated at 30°C for 90 s. Then, the reaction was stopped by addition of 1 ml of glacial acetic acid. Absorbance of the chromogen formed was measured at 560 nm. One unit of SOD activity is defined as the enzyme concentration required for inhibiting chromogen production by 50% in 1 min under the assay condition.

Glutathione assay

GSH was measured by the reaction of sulfhydryl groups with 5,5'-dithio-bis-(2-nitrobenzoic acid), (the Ellman's reagent) by quantitating sulfhydryl groups based on the molar absorptivity.[29] Tissues were homogenized in 10% w/v PBS (0.1 M PH = 8) containing 1 mM ethylene diamine tetraacetic acid. After centrifuging, the supernatant was removed. To perform protein denaturation, 1000 µL cold meta phosphoric acid (5%) was added to 500 µL sample and shaken for 5 s, finally the mixture was centrifuged at >1000 ×*g* for 5 min and the supernatant was carefully removed. Then, 100 µL of the samples or PBS as the blank were added to a test tube containing 20 µL of Ellman's Reagent Solution (4 mg Ellman's Reagent/1 ml PBS) and 1 mL of PBS. The solution in both tubes were mixed and incubated at the room temperature for 15 min. Finally, the sample absorbance was read at 412 nm by a spectrophotometer. The concentration of sulfhydryl in the sample was calculated from the molar extinction coefficient of TNB by C = A/bE equation where A = absorbance, b = path length in centimeters (=1), c = concentration in moles/liter (=M), E=14.150/M cm (for PBS in this condition).

Assessment of other factors

Serum liver and kidney levels of nitrite (stable metabolite of NO) were assayed using an assay kit (Promega Corporation, USA). Serum levels of creatinine (Cr), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP); and blood urea nitrogen (BUN) were measured using quantitative kits (Pars Azmoon, Iran) by autoanalyzer (Technicon Ireland LTD). Assessment of malondialdehyde (MDA) level in the serum and kidney was performed by the manual method. Briefly, a mixture of 500 µl of the sample and 1000 µl of 10% trichloroacetic acid was centrifuged at 2000 g for 10 min, then 500 µl of the supernatant was plused with 500 µl of 0.67% thiobarbituric acid. After 10 min of incubation in the boiling water and then cooling, the absorbance was measured at 532 nm. Concentrations of MDA for serum and kidney samples were reported in µmol/L and nmol/g tissue, respectively.

Histopathological procedures

The left kidneys were fixed in 10% formalin solution and embedded in paraffin for hematoxylin and eosin staining to test the tubular damage. The damage was evaluated by a pathologist who was totally blind to the study. Kidney tissue damage score (KTDS) was graded from 1 to 4 based on the intensity of tubular lesions (hyaline cast, debris, vacuolization, flattening and degeneration of tubular cells, and dilatation of tubular lumen), while score zero was assigned to normal tubules without any damage.

Statistical analysis

Data are expressed as mean ± standard error of the mean. The BW loss, kidney weight (KW); and levels of BUN, Cr, MDA, NO, liver and antioxidant enzymes were analyzed by one-way analysis of variance followed by least significant difference as *post hoc*. The groups were compared with regard to the pathological damage score by the Kruskal–Wallis and Mann–Whitney tests.

RESULTS

Effect of NG-nitro-L-Arginine methyl ester and L-Arginine on serum levels of blood urea nitrogen and creatinine, and BW, kidney weight, and kidney tissue damage score

The serum levels of Cr significantly increased in the Isch and Isch + L-NAME treated groups when compared with the sham group ($P = 0.006$) and L-Arginine decreased these parameters when compared with the Isch group ($P = 0.065$) and Isch + L-NAME treated group ($P = 0.063$). The serum levels of BUN significantly increased in the Isch alone and Isch + L-NAME treated groups when compared with the sham group ($P = 0.001$). However, L-Arginine decreased these parameters significantly when compared with the Isch ($P = 0.044$) and Isch + L-NAME treated groups ($P = 0.038$) [Figure 1]. Isch also induced weight loss in the Isch ($P = 0.03$) and Isch + L-NAME treated groups ($P = 0.053$) compared with the sham group. Moreover, administration of L-Arginine ameliorated ischemia-induced weight loss insignificantly ($P = 0.112$). The KW in the Isch alone, Isch + L-NAME, and Isch + L-Arginine groups elevated significantly when compared to the sham group ($P = 0.003$, 0.002 and 0.003, respectively). KTDS in the Isch alone, Isch + L-NAME, and Isch + L-Arginine groups elevated significantly when compared to the sham group ($P = 0$). However, administration

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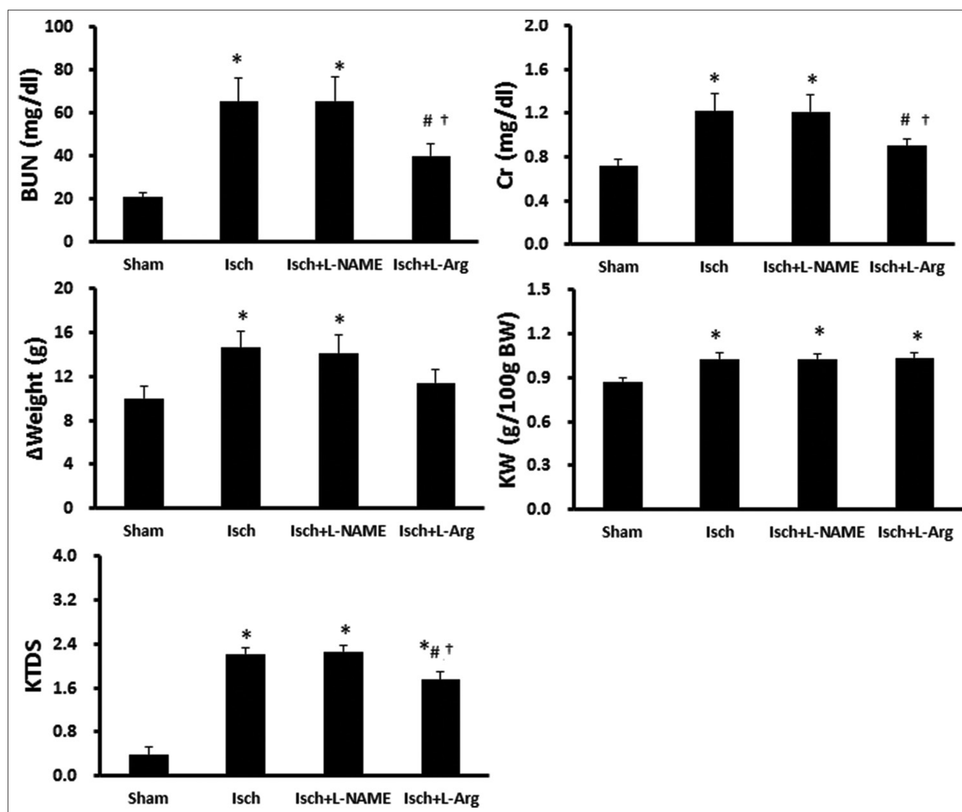

Figure 1: Comparison of the groups with regard to the serum levels of blood urea nitrogen and creatinine, kidney weight, body weight change (ΔW), and kidney tissue damage score (*), (#), and (†) indicate significant difference from the sham, the ischemia, and the NG-nitro-L-Arginine methyl ester groups, respectively

of L-Arginine reduced KTDS in comparison with the Isch (P = 0.014) and Isch + L-NAME treated group (P = 0.006) [Figure 1]. The samples images of kidney tissue are demonstrated in Figure 2.

Effect of NG-nitro-L-Arginine methyl ester and L-Arginine on serum, kidney, and liver tissue levels of nitrite, malondialdehyde, glutathione, and super oxide dismutase

The serum levels of MDA decreased significantly in the Isch group (P = 0.011) and Isch + L-NAME groups (P = 0.001) when compared with the sham group, while the serum level of MDA was increased in L-Arginine treated group compared with the Isch group (P = 0.051) and Isch + L-NAME group (P = 0.003). These data also indicated that L-Arginine improved liver tissue MDA level compared with the sham (P = 0.03), Isch alone, (P = 0.08) and Isch + L-NAME (P = 0.04) groups. No significant differences were observed in the kidney MDA levels among the groups [Table 1].

The serum level of SOD elevated in the Isch + L-NAME group compared with the sham (P = 0.002) and Isch (P = 0.004) groups. However, L-Arginine decreased it significantly compared with the Isch (P = 0.03) and Isch + L-NAME (P = 0) groups. No significant changes were observed in kidney and liver tissue levels of

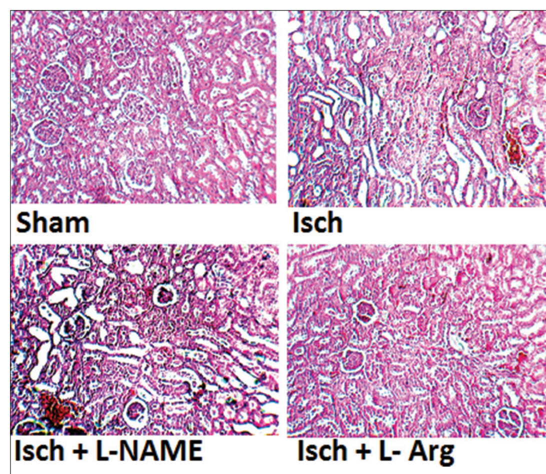

Figure 2: Sample s images of kidney tissue in 4 experimental groups. More damage is shown in ischemia and ischemia + NG-nitro-L-Arginine methyl ester groups

SOD among the groups. The liver nitrite level in the L-Arginine treated group decreased significantly compared with the sham (P = 0.038), Isch (P = 0) and Isch + L-NAME groups (P = 0.002). However, the groups were not significantly different in terms of kidney and serum nitrite levels. It was observed no significant difference in renal, liver and serum levels of GSH [Table 1].

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Effect of NG-nitro-L-Arginine methyl ester and L-Arginine on serum and liver tissue levels of alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase

Serum AST concentrations increased significantly in the Isch + L-NAME group compared with the sham group ($P = 0.01$). Liver ALP concentrations increased significantly in the Isch + L-Arginine group compared with the sham group ($P = 0.034$). The groups were not significantly different with regard to the serum and tissue levels of ALT, kidney and liver tissue levels of AST, and serum level of ALP [Table 1].

DISCUSSION

In this study, we demonstrated that L-Arginine administration improved renal function and tissue damage against RIR. Serum Cr and BUN levels are considered as indexes of renal function; thus, increase in their concentration indicates kidney dysfunction.[30] It was reported that GFR decreases during hypoxia[31] and NO production is disturbed by endothelial cells injury.[32] Endothelial cells, neutrophils, macrophages, Kupffer cells, and hepatocytes synthesize NO from L-Arginine,[33] and NO improves renal blood flow[34] and GFR by dilation of vessels.[35] It seems that improvement in renal function and histology by administration of L-Arginine in our study is because of NO generation and GFR increase. In addition, our results showed the damages did not alter by administration of nonselective NOS inhibitor. NO generated by iNOS is harmful, leading to tissue damage.[36,37] Several *in vivo* and *in vitro* searches have demonstrated that inhibiting the expression or activity of iNOS[38,39] can prevent renal I/R injury. Generation of proxy nitrite ($ONOO^-$) causing endothelial dysfunction[40] and the inhibition of eNOS to provide vasoconstrictive agents[41,42] are the two possible pathways. However, eNOS-derived NO is a good one by which may protect the tissue from I/R induced injury via platelet aggregation and adhesion and attenuation of endothelium leukocyte interactions.[43] Another mechanism by which eNOS-derived NO may exert protection in our model of I/R is vasodilation and enhanced perfusion of the tissue.[44] L-NAME is a nonselective NOS inhibitor for both eNOS and iNOS, so the consequence of this paradox did not alter kidney injury in our study. KW was increased by RIR. Different conditions such as the imbalance between vasodilatory and vasoconstrictive agents, endothelial congestion, and endothelial injury may enhance endothelial permeability.[45,46] Increased vascular endothelial permeability in kidney allows passing of macromolecules and water across vessel walls to kidney tissue, which leads to interstitial edema.[45] Kaneko *et al*. reported increased vascular permeability in the kidney during IR.[47] In the current study, KW possibly increased because of vascular endothelial permeability, followed by the development of edema.

Table 1: Serum (A), kidney (B), and liver (C) levels of MDA, GSH, SOD, ALT, ALP, AST, and nitrite in four experimental groups

Serum (A)							
Group	SMDA (μmol/L)	SGSH (μmol/L)	SSOD (U/ml)	SALT (U/L)	SALP (U/L)	SAST (U/L)	Serum nitrite (μmol/L)
Group 1: Sham	0.29±0.03	470.16±50.30	45.21±1.20	88.85±17.60	289.54±57.40	298.69±31.73	11.99±3.29
Group 2: Ischemia	0.18±0.02*	453.37±42.54	46.95±3.20	77.83±6.32	343.16±66.60	373.50±36.89	08.92±1.05
Group 3: Ischemia + L-NAME	0.14±0.02*	642.24±84.47	61.15±4.14*,#	86.80±13.66	312.23±53.11	445.20±46.67*	10.73±1.34
Group 4: Ischemia + L-Arginine	0.26±0.02†,#	580.13±83.09	36.67±3.46†,#	62.93±5.12	320.60±49.69	350.63±34.54	09.27±1.49
P	0.002	0.194	0.000	0.380	0.931	0.068	0.663

Kidney (B)						
	KMDA (μmol/g)	KGSH (μmol/g)	KSOD (U/g)	KALT (U/g)	KAST (U/g)	Kidney nitrite (μmol/g)
Group 1: Sham	0.23±0.03	13.94±0.89	842.09±53.53	7.60±1.58	13.51±2.99	0.18±0.01
Group 2: Ischemia	0.21±0.03	12.18±0.66	753.37±52.70	5.54±1.39	08.77±1.71	0.14±0.01
Group 3: Ischemia + L-NAME	0.22±0.04	13.43±0.72	736.25±47.90	6.70±1.70	11.46±3.53	0.15±0.01
Group 4: Ischemia + L-Arginine	0.15±0.02	12.51±0.82	808.33±27.54	5.70±1.47	11.07±2.71	0.14±0.01
P	0.143	0.379	0.349	0.785	0.724	0.248

Liver (C)							
	LMDA (μmol/g)	LGSH (μmol/g)	LSOD (U/g)	LALT (U/g)	LALP (U/g)	LAST (U/g)	Liver nitrite (μmol/g)
Group 1: Sham	0.17±0.04	19.32±0.96	681.16±54.41	16.30±1.89	0.19±0.02	21.92±3.70	0.17±0.02
Group 2: Ischemia	0.14±0.05	18.40±1.33	648.72±62.51	13.23±1.50	0.39±0.07	17.71±2.40	0.21±0.01
Group 3: Ischemia + L-NAME	0.16±0.03	19.57±1.00	529.28±46.97	13.64±2.48	0.31±0.04	18.32±3.21	0.20±0.01
Group 4: Ischemia + L-Arginine	0.05±0.0*,†,#	18.65±0.61	612.21±56.90	14.43±1.82	0.42±0.10*	15.76±1.71	0.13±0.00*,#,†
P	0.095	0.819	0.263	0.740	0.146	0.500	0.001

*,#,†Significant difference from the sham, the ischemia, and the L-NAME groups, respectively. MDA: Malondialdehyde, SOD: Super oxide dismutase, ALT: Alanine aminotransferase, ALP: Alkaline phosphatase, AST: Aspartate aminotransferase, SMDA: Serum malondialdehyde, SSOD: Serum super oxide dismutase, SALT: Serum alanine aminotransferase, SALP: Serum alkaline phosphatase, SAST: Serum alkaline phosphatase, KMDA: Kidney malondialdehyde, KSOD: Kidney super oxide dismutase, KALT: Kidney alanine aminotransferase, KALP: Kidney alkaline phosphatase, KAST: Kidney alkaline phosphatase, LMDA: Liver malondialdehyde, LSOD: Liver super oxide dismutase, LALT: Liver alanine aminotransferase, LALP: Liver alkaline phosphatase, LAST: Liver alkaline phosphatase, L-NAME: NG-nitro-L-Arginine methyl ester, GSH: Glutathione, SGSH: Serum glutathione, LGSH: Liver glutathione, KGSH: Kidney glutathione

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It was reported that RIR initiate some reactions in the organ and elicit a systemic inflammatory response by release of cytokines and inflammatory mediators; including tumor necrosis factor, interleukin 6, platelet-activating factor, leukotrienes, and NO. This would prompt development of ROS with consequent oxidative stress.[12,19] Enhancement of oxidative stress leads to changes in the activity of the enzyme, cytoskeletal structure, membrane transport, and antioxidant defense in cells.[48] MDA as the end product of lipid peroxidation and GSH and SOD levels are the three indexes of oxidative stress. SOD and GSH levels decrease in oxidative stress.[49] Cytokines, ROS, and other inflammatory agents in the circulation can migrate to distal organs such as the liver and lung and initiate injury.[3,19,20] Kim *et al*. reported that acute kidney injury may lead to severe hepatic and intestinal injury.[11] Elevations in liver enzymes including AST, ALT, and ALP are indicators of hepatocellular injury that are released from the liver following a stressful insult and enter the circulation.[48,50,51] In the present study, we demonstrated that the serum level of SOD increases in Isch + L-NAME group whereas this factor was decreased by L-Arginine. This is in contrast with the MDA serum level. López-Neblina *et al*. concluded that exogenous NO has beneficial and protective effects on ischemia-induced kidney damages in rats. However, this protection is independent of lipid peroxidation.[52] This means that despite the increase lipid peroxidation and oxidative stress, NO can improve kidney function. Savas *et al*. reported that MDA and nitrite levels increased in L-Arginine treatment in a rat model of spinal cord IR injury.[53] As regarded, iNOS derived NO damage NO increase vascular dysfunction through the generation of peroxynitrite ($ONOO^-$)[40] and inhibition of eNOS-derived NO.[41,42] Therefore in conditions of this study iNOS derived NO (induced by L-Arginine) increases lipid peroxidation and oxidative stress because we observed that in L-Arginine group the serum level of SOD decreased and in Isch + L-NAME group increased in contrast with the MDA serum level. Furthermore, serum nitrite did not elevate in L-Arginine treatment in this study; therefore, it is possible that increasing in oxidative stress in Isch + L-Arginine treated rats is due to formation of ONOO– (not serum nitrite) in the presence of the NO precursor. The results obtained in the current study showed that serum level of AST increased in the Isch + L-NAME group but did not change in the liver tissue. It is known that AST is predominantly present in the liver. However, it is found in other organs such as muscle, heart, kidneys, red blood cells, brain, and small bowel.[54] Thus, it seems that the increase in serum level of AST is due to injury of other remote organs. The results of the present study indicate that L-Arginine treatment increase ALP, but not other enzymes. It is reported that liver damage is characterized by an increase in all the three hepatic enzymes not one of them.[55] It is observed that L-Arginine could improve the liver proliferation and elevate ALP in the rats underwent partial hepatectomy and the increase in the ALP level is considered as a liver cell regeneration index.[56] Therefore, it seems that increased ALP induced by L-Arginine is not associated with liver damage. Ischemia-induced BW loss and L-Arginine treatment enhanced the enzyme level. It is reported that Isch and lack of intercellular oxygen may cause adenosine triphosphate (ATP) depletion.[57,58] This consequently increases the cellular glucose consumption and uptake, which lead to glycogenolysis and gluconeogenesis[59] and consequently the reduction of body weight (BW). This is while L-Arginine prevents depletion of cellular ATP storage by the improvement of oxygen delivery. Thus, it is possible that L-Arginine ameliorate BW loss via the above-mentioned mechanism.

CONCLUSION

We concluded that L-Arginine protects renal tissue function and histology against RIR injury. However, L-Arginine did not improve stress oxidative in kidney whereas this effect was opposite in the liver.

Acknowledgment

This research was supported by Isfahan University of Medical Sciences.

Financial support and sponsorship

This research was supported by Isfahan University of Medical Sciences.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Bird JE, Milhoan K, Wilson CB, Young SG, Mundy CA, Parthasarathy S, *et al.* Ischemic acute renal failure and antioxidant therapy in the rat. The relation between glomerular and tubular dysfunction. J Clin Invest 1988;81:1630-8.
2. Walker LM, York JL, Imam SZ, Ali SF, Muldrew KL, Mayeux PR. Oxidative stress and reactive nitrogen species generation during renal ischemia. Toxicol Sci 2001;63:143-8.
3. Moeini M, Nematbakhsh M, Fazilati M, Talebi A, Pilehvarian AA, Azarkish F, *et al.* Protective role of recombinant human erythropoietin in kidney and lung injury following renal bilateral ischemia-reperfusion in rat model. Int J Prev Med 2013;4:648-55.
4. Bastin J, Cambon N, Thompson M, Lowry OH, Burch HB. Change in energy reserves in different segments of the nephron during brief ischemia. Kidney Int 1987;31:1239-47.
5. Paller MS, Hoidal JR, Ferris TF. Oxygen free radicals in ischemic acute renal failure in the rat. J Clin Invest 1984;74:1156-64.
6. McCord JM. Oxygen-derived free radicals in postischemic tissue injury. N Engl J Med 1985;312:159-63.
7. Wiseman MJ, Saunders AJ, Keen H, Viberti G. Effect of blood glucose control on increased glomerular filtration rate and kidney size in

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insulin-dependent diabetes. N Engl J Med 1985;312:617-21.
8. Liu F, Ng TB. Effect of pineal indoles on activities of the antioxidant defense enzymes superoxide dismutase, catalase, and glutathione reductase, and levels of reduced and oxidized glutathione in rat tissues. Biochem Cell Biol 2000;78:447-53.
9. Szymonik-Lesiuk S, Czechowska G, Stryjecka-Zimmer M, Slomka M, Madro A, Celinski K, *et al.* Catalase, superoxide dismutase, and glutathione peroxidase activities in various rat tissues after carbon tetrachloride intoxication. J Hepatobiliary Pancreat Surg 2003;10:309-15.
10. Esme H, Fidan H, Koken T, Solak O. Effect of lung ischemia – Reperfusion on oxidative stress parameters of remote tissues. Eur J Cardiothorac Surg 2006;29:294-8.
11. Kim M, Park SW, Kim M, D'Agati VD, Lee HT. Isoflurane activates intestinal sphingosine kinase to protect against renal ischemia-reperfusion-induced liver and intestine injury. Anesthesiology 2011;114:363-73.
12. Yassin MM, Harkin DW, Barros D'Sa AA, Halliday MI, Rowlands BJ. Lower limb ischemia-reperfusion injury triggers a systemic inflammatory response and multiple organ dysfunction. World J Surg 2002;26:115-21.
13. Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 1980;288:373-6.
14. Tojo A, Gross SS, Zhang L, Tisher CC, Schmidt HH, Wilcox CS, *et al.* Immunocytochemical localization of distinct isoforms of nitric oxide synthase in the juxtaglomerular apparatus of normal rat kidney. J Am Soc Nephrol 1994;4:1438-47.
15. Kone BC, Baylis C. Biosynthesis and homeostatic roles of nitric oxide in the normal kidney. Am J Physiol Ren Fluid Electrolyte Physiol 1997;41:F561.
16. Moore PK, Oluyomi AO, Babbedge RC, Wallace P, Hart SL. L-NG-nitro arginine methyl ester exhibits antinociceptive activity in the mouse. Br J Pharmacol 1991;102:198-202.
17. Atanasova I, Burke TJ, McMurtry IF, Schrier RW. Nitric oxide synthase inhibition and acute renal ischemia: Effect on systemic hemodynamics and mortality. Ren Fail 1995;17:389-403.
18. Klahr S. Can L-arginine manipulation reduce renal disease? Semin Nephrol 1999;19:304-9.
19. Kaçmaz A, User EY, Sehirli AO, Tilki M, Ozkan S, Sener G. Protective effect of melatonin against ischemia/reperfusion-induced oxidative remote organ injury in the rat. Surg Today 2005;35:744-50.
20. Carden DL, Granger DN. Pathophysiology of ischaemia-reperfusion injury. J Pathol 2000;190:255-66.
21. Taylor R, Magnusson I, Rothman DL, Cline GW, Caumo A, Cobelli C, *et al.* Direct assessment of liver glycogen storage by 13C nuclear magnetic resonance spectroscopy and regulation of glucose homeostasis after a mixed meal in normal subjects. J Clin Invest 1996;97:126-32.
22. Miller LL, Bly CG, Watson ML, Bale WF. The dominant role of the liver in plasma protein synthesis; a direct study of the isolated perfused rat liver with the aid of lysine-epsilon-C14. J Exp Med 1951;94:431-53.
23. Dave G, Johansson-Sjöbeck ML, Larsson A, Lewander K, Lidman U. Metabolic and hematological effects of starvation in the European eel, *Anguilla anguilla* L. I. Carbohydrate, lipid, protein and inorganic ion metabolism. Comp Biochem Physiol A Comp Physiol 1975;52:423-30.
24. Boulware S, Tamborlane W, Matthews L, Sherwin R. Diverse effects of insulin-like growth factor I on glucose, lipid, and amino acid metabolism. Am J Physiol Endocrinol Metab 1992;262:E130-3.
25. Mitzner SR, Stange J, Klammt S, Peszynski P, Schmidt R, Nöldge-Schomburg G. Extracorporeal detoxification using the molecular adsorbent recirculating system for critically ill patients with liver failure. J Am Soc Nephrol 2001;12 Suppl 17:S75-82.
26. Clayton PT, Whitfield P, Iyer K. The role of phytosterols in the pathogenesis of liver complications of pediatric parenteral nutrition. Nutrition 1998;14:158-64.
27. Czaja MJ. Cell signaling in oxidative stress-induced liver injury. Semin Liver Dis 2007;27:378-89.
28. Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. Indian J Biochem Biophys 1984;21:130-2.
29. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys 1959;82:70-7.
30. Aktoz T, Aydogdu N, Alagol B, Yalcin O, Huseyinova G, Atakan IH. The protective effects of melatonin and vitamin E against renal ischemia-reperfusion injury in rats. Ren Fail 2007;29:535-42.
31. Oostendorp M, de Vries EE, Slenter JM, Peutz-Kootstra CJ, Snoeijs MG, Post MJ, *et al.* MRI of renal oxygenation and function after normothermic ischemia-reperfusion injury. NMR Biomed 2011;24:194-200.
32. Davignon J, Ganz P. Role of endothelial dysfunction in atherosclerosis. Circulation 2004;109 23 Suppl 1:III27-32.
33. Cantoni L, Di Nicolantonio F, Barelli D, Rizzardini M, De Matteis F. Modulation of cerebellar and hepatic nitric oxide synthase by exogenous arginine and endotoxin. Nitric Oxide 2001;5:198-207.
34. Engelman DT, Watanabe M, Maulik N, Cordis GA, Engelman RM, Rousou JA, *et al.* L-arginine reduces endothelial inflammation and myocardial stunning during ischemia/reperfusion. Ann Thorac Surg 1995;60:1275-81.
35. Hall JE, Coleman TG, Guyton AC, Kastner PR, Granger JP. Control of glomerular filtration rate by circulating angiotensin II. Am J Physiol Regul Integr Comp Physiol 1981;241:R190-7.
36. Guan Z, Gobé G, Willgoss D, Endre ZH. Renal endothelial dysfunction and impaired autoregulation after ischemia-reperfusion injury result from excess nitric oxide. Am J Physiol Renal Physiol 2006;291:F619-28.
37. Goligorsky MS, Brodsky SV, Noiri E. NO bioavailability, endothelial dysfunction, and acute renal failure: New insights into pathophysiology. Semin Nephrol 2004;24:316-23.
38. Chatterjee PK, Patel NS, Kvale EO, Cuzzocrea S, Brown PA, Stewart KN, *et al.* Inhibition of inducible nitric oxide synthase reduces renal ischemia/reperfusion injury. Kidney Int 2002;61:862-71.
39. Wangsiripaisan A, Gengaro PE, Nemenoff RA, Ling H, Edelstein CL, Schrier RW. Effect of nitric oxide donors on renal tubular epithelial cell-matrix adhesion. Kidney Int 1999;55:2281-8.
40. Schild L, Reinheckel T, Reiser M, Horn TF, Wolf G, Augustin W. Nitric oxide produced in rat liver mitochondria causes oxidative stress and impairment of respiration after transient hypoxia. FASEB J 2003;17:2194-201.
41. Cowley AW Jr, Mori T, Mattson D, Zou AP. Role of renal NO production in the regulation of medullary blood flow. Am J Physiol Regul Integr Comp Physiol 2003;284:R1355-69.
42. Noiri E, Peresleni T, Miller F, Goligorsky MS. *In vivo* targeting of inducible NO synthase with oligodeoxynucleotides protects rat kidney against ischemia. J Clin Invest 1996;97:2377-83.
43. Lü P, Liu F, Wang CY, Chen DD, Yao Z, Tian Y, *et al.* Gender differences in hepatic ischemic reperfusion injury in rats are associated with endothelial cell nitric oxide synthase-derived nitric oxide. World J Gastroenterol 2005;11:3441-5.
44. Wink DA, Miranda KM, Espey MG, Pluta RM, Hewett SJ, Colton C, *et al.* Mechanisms of the antioxidant effects of nitric oxide. Antioxid Redox Signal 2001;3:203-13.
45. Legrand M, Mik EG, Johannes T, Payen D, Ince C. Renal hypoxia and dysoxia after reperfusion of the ischemic kidney. Mol Med 2008;14:502-16.
46. Lum H, Malik AB. Mechanisms of increased endothelial permeability. Can J Physiol Pharmacol 1996;74:787-800.
47. Kaneko H, Koshi S, Hiraoka T, Miyauchi Y, Kitamura N, Inoue M. Inhibition of post-ischemic reperfusion injury of the kidney by diamine oxidase. Biochim Biophys Acta (BBA) Mol Basis Dis 1998;1407:193-9.
48. Acquaviva R, Lanteri R, Li Destri G, Caltabiano R, Vanella L, Lanzafame S, *et al.* Beneficial effects of rutin and L-arginine coadministration in a rat model of liver ischemia-reperfusion injury. Am J Physiol Gastrointest Liver Physiol 2009;296:G664-70.
49. Li C, Jackson RM. Reactive species mechanisms of cellular hypoxia-reoxygenation injury. Am J Physiol Cell Physiol 2002;282:C227-41.
50. Alavian SM. Occult hepatitis B virus infection among hemodialysis patients. Hepat Mon 2012;12:242-3.
51. Rezaie A, Fazlara A, Haghi Karamolah M, Shahriari A, Najaf Zadeh H, Pashmforosh M. Effects of *Echinacea purpurea* on hepatic and renal toxicity induced by diethylnitrosamine in rats. Jundishapur J Nat Pharm Prod 2013;8:60-4.
52. López-Neblina F, Paez AJ, Toledo AH, Toledo-Pereyra LH. Role of nitric

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oxide in ischemia/reperfusion of the rat kidney. Circ Shock 1994;44:91-5.
53. Savas S, Savas C, Altuntas I, Adiloglu A. The correlation between nitric oxide and vascular endothelial growth factor in spinal cord injury. Spinal Cord 2008;46:113-7.
54. Thapa BR, Walia A. Liver function tests and their interpretation. Indian J Pediatr 2007;74:663-71.
55. Halevy A, Gold-Deutch R, Negri M, Lin G, Shlamkovich N, Evans S, *et al.* Are elevated liver enzymes and bilirubin levels significant after laparoscopic cholecystectomy in the absence of bile duct injury? Ann Surg 1994;219:362-4.
56. Montenegro WS, Malafaia O, Nassif PA, Moreira LB, Prestes MA, Kume MH, *et al.* Evaluation of liver regeneration with use of diet supplemented with L-arginine. Acta Cir Bras 2014;29:603-7.
57. Kamiike W, Watanabe F, Hashimoto T, Tagawa K, Ikeda Y, Nakao K, *et al.* Changes in cellular levels of ATP and its catabolites in ischemic rat liver. J Biochem 1982;91:1349-56.
58. Singh P, Ricksten SE, Bragadottir G, Redfors B, Nordquist L. Renal oxygenation and haemodynamics in acute kidney injury and chronic kidney disease. Clin Exp Pharmacol Physiol 2013;40:138-47.
59. Michael MD, Kulkarni RN, Postic C, Previs SF, Shulman GI, Magnuson MA, *et al.* Loss of insulin signaling in hepatocytes leads to severe insulin resistance and progressive hepatic dysfunction. Mol Cell 2000;6:87-97.