Effects of L-Carnitine on Long-Term Post Renal Ischemia and Reperfusion Injury in Rat

Sıçanlarda Uzun Süreli Böbrek İskemisi ve Reperfüzyon Hasarında L-Karnitinin Etkileri

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ABSTRACT Objective: Free radicals and nitric oxide play an important role in the pathogenesis of acute kidney injury (AKI) induced by ischemia and reperfusion (IR). Despite the protective effects of L-carnitine on renal ischemia reperfusion injury, reports are lacking on its effects on anemia and the erythrocyte antioxidant status of long-term post IR injury in the rat kidney. In the present study, we aimed to investigate the effects of L-carnitine on long-term IR injury in rat kidney. **Material and Methods:** Male Wistar Albino rats were divided into three groups (eight rats each) as Sham, IR (60 minutes of bilateral ischemia and 168 hours reperfusion) and IR+L-carnitine (intraperitoneally; 300 mg/kg/day for 7 days) groups. Anemia, kidney function, lipid peroxidation and kidney and erythrocyte antioxidant enzyme activities as well as expression of inducible nitric oxide synthase (iNOS) and endothelial nitric oxide synthase (eNOS) were examined. The kidneys were histologically evaluated. **Results:** L-carnitine treatment attenuated IR-induced histological alteration and reduced iNOS activitiy, increased eNOS activity, reduced urea and creatinine (each p=0.001), decreased malondialdehide levels (p=0.001) and increased both kidney and erythrocyte antioxidant enzymes activitiy and glutathione levels (p=0.001). Hematocrit was significantly increased (p=0.038). **Conclusion:** Our results show that L-carnitine plays a protective role in the pathogenesis of renal injury induced by IR. In addition, L-carnitine may ameliorate the anemia observed after renal IR injury.

Key Words: Reperfusion injury; oxidative stress; anemia; carnitine; kidney

ÖZET Amaç: Serbest radikaller ve nitrik oksit, iskemi reperfüzyon (IR) ile oluşturulan akut böbrek hasarının patogenezinde önemli bir rol oynamaktadır. L-karnitinin renal iskemi reperfüzyon hasarı üzerinde koruyucu etkileri gösterilmesine rağmen, sıçan böbreğinde uzun süreli IR hasarında anemi ve eritrosit antioksidan durum üzerindeki etkileri ile ilgili veri yoktur. Bu çalışmada, sıçan böbreğinde uzun süreli IR hasarında L-karnitinin etkilerini araştırmayı amaçladık. **Gereç ve Yöntemler:** Erkek Wistar Albino sıçanlar, Kontrol, IR (60 dakika her iki böbreğe iskemi ve 168 saat reperfüzyon) ve IR+L-karnitin (intraperitoneal yolla 7 gün süreyle günde 300 mg/kg L-karnitin verildi) olacak şekilde üç gruba ayrıldı. Anemi, böbrek fonksiyon testleri, lipit peroksidasyonu, böbrek ve eritrosit antioksidan enzim aktivitelerinin yanı sıra indüklenebilir nitrik oksit sentaz (iNOS) ve endotelyal nitrik oksit sentaz (eNOS) enzim ekspresyonları ile histopatolojik değişiklikler incelendi. **Bulgular:** L-karnitin tedavisinin, IR hasarının oluşturduğu histopatolojik değişiklikleri ve iNOS aktivitesini azaltırken, eNOS aktivitesini ise artırdığı gözlendi. Üre, kreatinin ve malondialdehit düzeyleri anlamlı düzeyde azaldı (p=0,001). Böbrek ve eritrosit antioksidan enzim aktiviteleri ile glutatyon düzeyleri anlamlı düzeyde arttı (p=0,001). Hematokrit düzeyi anlamlı artış gösterdi (p=0,038). **Sonuç:** Çalışmada elde edilen sonuçlar, L-karnitin tedavisinin IR ile oluşan böbrek hasarının patogenezinde koruyucu bir rol oynadığını göstermektedir. Buna ek olarak, L-karnitin, böbrekte IR hasarı sonrasında ortaya çıkan anemiyi hafifletebilir.

Anahtar Kelimeler: Reperfüzyon hasarı; oksidatif stres; anemi; karnitin; böbrek

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Acute kidney injury induced by ischemia and reperfusion (IR) develops due to various clinical conditions such as renal transplantation, during cardiopulmonary and aortic bypass surgery, accidental

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trauma, sepsis, hydronephrosis and elective urological operations.[1,2] Although reperfusion is necessary for the survival of ischemic renal tissue, the increased damage (reperfusion injury) as a result of IR injury is associated with increased renal dysfunction and worsens the prognosis.[2-4] Clinical and experimental studies have shown that free radicals play a key role in the formation of renal IR injury. Excessive formation of free radicals, lipid peroxidation in cell membranes, protein and enzyme oxidation and irreversible changes in DNA lead to cell death in the form of necrosis.[1,2,5]

Nitric oxide (NO) is synthesized by different NO synthase (NOS) isoforms and both physiological and pathophysiological conditions play an important role in the renal function of nitric oxide synthase (NOS). The enzyme is formed by one of three isoforms, namely endothelial NOS (eNOS), neuronal NOS (nNOS) and inducible NOS (iNOS).[6] In many studies, it has been reported that inhibition of iNOS activity reduces renal IR injury, whereas increased eNOS activity has a protective effect against IR injury in the kidney.[4,7]

L-carnitine plays an important role in lipid metabolism by transport of long-chain fatty acids from the cytosolic compartment into the mitochondria for beta-oxidation.[8] Apart from its main role in energy metabolism, L-carnitine is an antioxidant that prevents the accumulation of end-products of lipid peroxidation.[9-13] It also exhibits powerful protective effects against different kidney injury models including IR injury, myoglobinuric acute kidney injury, and chronic renal failure.[9-14] It has been reported that L-carnitine deficiency destabilizes the erythrocyte membrane and leads to altered function of the erythrocyte sodium potassium pump with reduced erythrocyte survival time.[15] Nemato et al. reported that anemia developed as a result of renal ischemia reperfusion injury in rats. The development of anemia in acute renal failure has been proposed to be due to decreased erythrocyte production or increased destruction of erythrocyte or the loss of erythrocytes.[16]

Despite the protective effects of L-carnitine on renal IR injury, data is lacking on its effects on anemia and erythrocyte antioxidant status in long-term post IR injury in the rat kidney. In this study, we hypothesized that L-carnitine would increase the hematocrit and prevent long-term post ischemia and reperfusion injury in the rat kidney. Therefore, we investigated the effect of L-carnitine on the kidney and red blood cell antioxidant status, serum marker of kidney function, anemia, renal iNOS and eNOS immunexpresion, and kidney histopathology in rats during long-term IR induced renal injury.

MATERIAL AND METHODS

Twenty-four male Wistar Albino rats (130 to 170 g) were used in the present study. The animals were housed in a light-controlled room with a 12-h light/dark cycle. They were given free access to tap water and were fed standard rat chow. Experimental protocols and animal care methods in the experiments were approved by the Ethics Committee at the Trakya University Faculty of Medicine.

RENAL ISCHEMIA/REPERFUSION

The rats were anesthetized with xylazine (10 mg/kg) and ketamine (80 mg/kg). Rats were subjected to bilateral renal occlusion for 60 minutes using nontraumatic vascular clamps (85 gm pressure, Harvard Apparatus Ltd., USA) to clamp the renal pedicles, followed by reperfusion for 7 days. The incision was sutured in two layers. All groups received 50 ml/kg of saline at 37°C instilled into the abdominal cavity during the entire procedure.[16,17] Animals were randomly allocated into 3 groups. The Sham group rats were subjected to the same surgical procedures as above, including dissection of the renal pedicles; however, renal clamps were not applied. Saline injections (intraperitoneal 1.5 mL/kg) were administered 30 minutes prior to the renal pedicles dissection and at 24, 48, 72, 96, 120, 144 hours after the abdominal wall was closed. The IR group rats were administered saline (intraperitoneal 1.5 mL/kg) 30 minutes prior to renal ischemia and during the reperfusion period at 24, 48, 72, 96, 120, 144 hours. The IR+LC group rats were administered 300 mg/kg L-carnitine (L-carnithine intraperitoneal 1.5 mL/kg, Sigma-Tau, Italy) 30 minutes prior to renal ischemia and during the reperfusion period at 24, 48, 72, 96, 120, 144 hours.

MEASUREMENT OF BIOCHEMICAL PARAMETERS

The rats were sacrificed after 168 hours of reperfusion and they were anesthetized with xylazine (10 mg/kg) and ketamine (50 mg/kg) before sacrification. Initially, blood samples for hematocrit (Htc) were obtained from the tail vein. Following this, blood samples were collected by cardiac puncture and bilateral nephrectomy was performed. The blood samples were centrifuged (1500xg for 10 min at 4°C) immediately and plasma and buffy coat were removed. Erythrocytes were washed three times with an ice-cold isotonic sodium chloride solution (1:10, v/v). The hemolysates, plasma and renal tissue samples were stored at -80°C until processed.

RENAL FUNCTION

Plasma urea and creatinine levels were measured using standard diagnostic kits (Chema Diagnostica, Italy).

MALONDIALDEHYDE AND GLUTATHIONE ASSAYS

The concentrations of malondialdehyde (MDA)-an indicator of lipid peroxidation- concentrations in renal tissues were assayed in the form of thiobarbituric acid reacting substances. MDA was expressed as nanomoles MDA per milligram tissue.[18] The level of glutathione (GSH) in kidney and erythrocytes was determined according to the method of Ellman.[19] The results were expressed as μmol/g tissue and μmol/gr hemoglobin.

ENZYME ASSAYS

The total (Cu-Zn and Mn) superoxide dismutase (SOD) activity in the kidney and erythrocyte was determined according to the method by Sun et al.[20] The specific activity was expressed in units per milligram protein and units per gram hemoglobin, respectively. The catalase (CAT) activity was measured according to Aebi[21] based on the determination of the rate constant (k;/s) of hydrogen peroxide decomposition rate at 240 nm. The results were expressed as the rate constant per milligram homogenate protein and gram hemoglobin. The glutathione peroxidase (GPx) activity was measured according to Lawrence and Burk by monitoring the oxidation of reduced nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) at 340 nm.[22] Results were reported as units/mg protein and gram hemoglobin. The protein content of tissue samples was determined by Lowry et al.[23] The results for hemoglobin content of the erythrocyte samples were determined by the cyanomethemoglobin method.[24]

NITRITE AND NITRATE ASSAY

Plasma and kidney total nitrite and nitrate contents were determined by the Cortas and Wakid method.[25] The results were expressed as μmol/L and μmol/mg protein, respectively.

KIDNEY HISTOLOGY

The kidney tissue was fixed in 10% neutral buffered formalin, was embedded in paraffin wax and 4-μm sections were stained with hematoxylin and eosin (HE). The extent of histological renal tubular necrosis and tubular cast formation for each animal were evaluated semi-quantitatively by a pathologist (OY) analyzing the kidney sections blinded to the treatment groups. The extension of tubular injury was evaluated in terms of the percentage area of the sections showing a level of histological damage. In this study, a special ocular (Nikon) with 400 cells field was used to detect the percentage of cast and necrosis.

IMMUNOHISTOCHEMICAL PROCEDURES

Harvested renal tissues were fixed in 10% neutral buffered formalin, were embedded in paraffin, and sectioned at 5 mm thickness. Immunocytochemical reactions were performed according to the ABC technique described by Hsu et al.[26] Specific rabbit polyclonal anti-eNOS (Cat. # RB-1711-P, Neomarkers, USA) and anti-iNOS antibodies (Cat. # RB-1605-P, Neomarkers, USA) were used at a dilution of 1:50. Immunohistochemical staining was scored in a semiquantitative manner to determine differences between the control group and experimental groups in the distribution patterns of intensity of immunolabelling of glomerular tissue. The intensity of the staining was recorded as weak (+), moderate (++), strong (+++) and very strong (++++). This analysis was performed in at least 10 glomeruli per kidney section, in two sections from each animal at x100 magnification.

STATISTICAL ANALYSIS

The results were expressed as the median (minimum-maximum) values. In two-group comparisons, Mann-Whitney U test was used. $p<0.05$ was considered statistically significant.

RESULTS

Rats underwent 60 minutes of bilateral ischemia in the kidneys followed by 168 hours of reperfusion. A steady decline in hematocrit level was observed in the IR group in comparison with the Sham group (40.00% (39.00-47.00%) vs 37.00% (34.00-39.00%), p=0.01). Thus, our experimental model can be considered to simulate the anemia of AKI. Administration of L-carnitine 300 mg/kg led to a significant rise in hematocrit at 168 hours compared with the saline-treated rats in the IR group (39.00% (36.00-42.00%) vs 37.00% (34.00-39.00%), p=0.038) (Figure 1).

The urea and creatinine levels were significantly higher among the animals from the IR group compared with those from the Sham group (for each parameter p=0.001). Treatment of rats with L-carnitine (IR+LC group) significantly reduced the IR-mediated increase in the plasma levels of urea and creatinine (for each parameter p=0.001). Increased plasma NO levels mediated by renal IR (p=0.001) were also significantly elevated in the IR+LC group (p=0.027). The kidney tissue MDA and NO levels were higher in the IR group than in the Sham group (both p=0.001). At the same time, the GSH level was lower in the IR group compared with the Sham group (p=0.002). The activities of SOD, CAT and GPx in renal tissue decreased in the IR group in comparison with the Sham group (for all parameters p=0.001). The L-carnitine treatment caused a significant reverse effect on the lipid peroxidation induced by IR (p=0.001). In addition, the decrease in GSH was attenuated in the IR+LC group compared with the IR group (p=0.003). Furthermore, SOD, CAT and GPx activities in the IR+LC group were significantly increased in comparison with the IR group (p=0.001). The erythrocyte GSH level in the IR group was significantly decreased in the IR group in comparison with the Sham group (p=0.001). Treatment of-L-carnitine significantly increased the GSH level (p=0.001). In the erythrocyte, the SOD, CAT and GPx activities decreased in the IR group in comparison with the Sham group (p=0.001, p=0.021, p=0.002, respectively). Administration of L-carnitine significantly increased the activities of SOD, CAT and GPx (p=0.001, p=0.001, p=0.012 respectively) (Table 1).

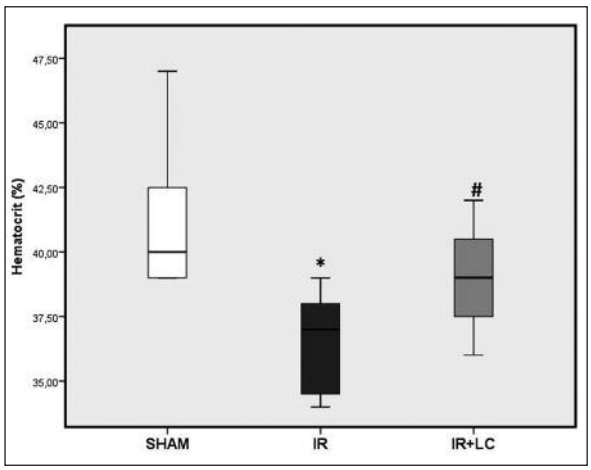

FIGURE 1: Change in hematocrit after ischemia and reperfusion injury and L-carnitine treatment.

*p<0.01 indicates the comparisons between the sham control (SHAM) and ischemia and reperfusion treated with saline (IR) groups; #p<0.05 indicates the comparisons between the IR and IR treated with L-carnitine (300 mg/kg; IR+LC) groups.

KIDNEY HISTOLOGY AND IMMUNOHISTOCHEMICAL FINDINGS

The histological changes were examined in all groups (Table 2). The Sham group did not show any histopathological changes (Figure 2a). In contrast, in the IR group, the basic histological abnormalities were tubular necrosis and cast formation (Figure 2b). Tubular cell necrosis and cast formation significantly decreased with L-carnitine treatment (Figure 2c).

The results of staining by immunohistochemistry for eNOS and iNOS were shown and were summarized semiquantitatively (Figure 3 and Table 3). In the Sham group, eNOS expression was low in glomerular endothelial cells. After IR injury, eNOS expression was moderately increased. However, after L-carnitine treatment the immunoreactivity of eNOS was obviously increased in glomerular endothelial cells. The immunoreactiv-

TABLE 1: Biochemical results of experimental groups.

Variable	SHAM (n=8) Median (min-max)	IR (n=8) Median (min-max)	IR+LC (n=8) Median (min-max)	P (Mann Whitney U test)	
Plasma					
Urea (mg/dl)	32.07	61.41	36.42	Sham-IR	0.001
	(20.65-42.39)	(53.26 - 78.26)	(23.91- 42.39)	IR-IR+LC	0.001
Creatinine (mg/dl)	0.38	1.04	0.62	Sham-IR	0.001
	(0.31-0.54)	(0.77-1.31)	(0.46-0.77)	IR-IR+LC	0.001
NO (µmol/L)	24.17	43.75	57.50	Sham-IR	0.001
	(15.83-28.33)	(33.33-55.00)	(43.33-69.17)	IR-IR+LC	0.027
Kidney					
MDA (nmol/mg tissue)	0.31	0.46	0.27	Sham-IR	0.001
	(0.24-0.34)	(0.43-0.54)	(0.21-0.30)	IR-IR+LC	0.001
GSH (µmol/g tissue)	2.91	2.15	3.10	Sham-IR	0.002
	(2.49-3.60)	(1.50-2.67)	(2.29-3.98)	IR-IR+LC	0.003
NO (µmol/mg protein)	28.19	37.96	44.26	Sham-IR	0.001
	(23.62-33.46)	(33.97-45.98)	(36.33-49.13)	IR-IR+LC	0.021
SOD (U/mg protein)	9.40	7.93	10.60	Sham-IR	0.001
	(8.90-10.17)	(7.40 - 8.53)	(10.21- 11.75)	IR-IR+LC	0.001
CAT (k/mg protein)	0.54	0.30	0.47	Sham-IR	0.001
	(0.37-0.59)	(0.22 - 0.35)	(0.40-0.57)	IR-IR+LC	0.001
GPx (U/mg protein)	1.65	0.97	1.59	Sham-IR	0.001
	(1.44- 1.89)	(0.86- 1.08)	(1.26- 2.20)	IR-IR+LC	0.001
Erythrocyte					
SOD (U/gr Hb)	755.35	617.35	777.75	Sham-IR	0.001
	(659.80- 846.00)	(514.30- 669.70)	(717.40- 807.70)	IR-IR+LC	0.001
CAT (k/gr Hb)	131.48	94.97	154.59	Sham-IR	0.021
	(86.49- 157.03)	(57.16-112.33)	(111.67-183.06)	IR-IR+LC	0.001
GPx (U/gr Hb)	371.5000	295.60	356.3500	Sham-IR	0.002
	(320.50- 449.40)	(268.00-326.70)	(186.00-396.30)	IR-IR+LC	0.012
GSH (µmol/gr Hb)	1877.20	1315.90	2120.50	Sham-IR	0.001
	(1589.20- 2462.60)	(1019.50-1517.00)	(1940.10- 2485.60)	IR-IR+LC	0.001

SHAM: Sham control group; IR: Ischemia and reperfusion treated with saline group; IR+LC: The IR treated with L-carnitine (300 mg/kg) group. All parameters were determined in the rats sacrificed at 168 hours.
NO: Nitric oxide; MDA: Malondialdehyde; GSH: Glutathione; SOD: Superoxide dismutase; CAT: Catalase; GPx: Glutathione peroxidase.

TABLE 2: Histological findings of renal IR injury and L-carnitine treated rats.

Variable	SHAM (n=8) Median (min-max)	IR (n=8) Median (min-max)	IR+LC (n=8) Median (min-max)	P (Mann Whitney U test)	
Necrosis (%)	0.00	2.69 (2.50-4.58)	1.67 (0.83-3.00)	SHAM-IR	0.001
				IR-IR+LC	0.006
Cast (%)	0.00	9.34 (7.50-15.42)	6.09 (3.75-7.33)	SHAM-IR	0.001
				IR-IR+LC	0.001

SHAM: Sham control group; IR: Ischemia and reperfusion treated with saline group; IR+LC: the IR treated with L-carnitine (300 mg/kg) group. All parameters were determined in the rats that were killed at 168 hours. Renal histology was expressed as the percentage area examined for the particular findings. Histopathological analysis of the kidney showed neither necrosis nor cast formation in the Sham group.

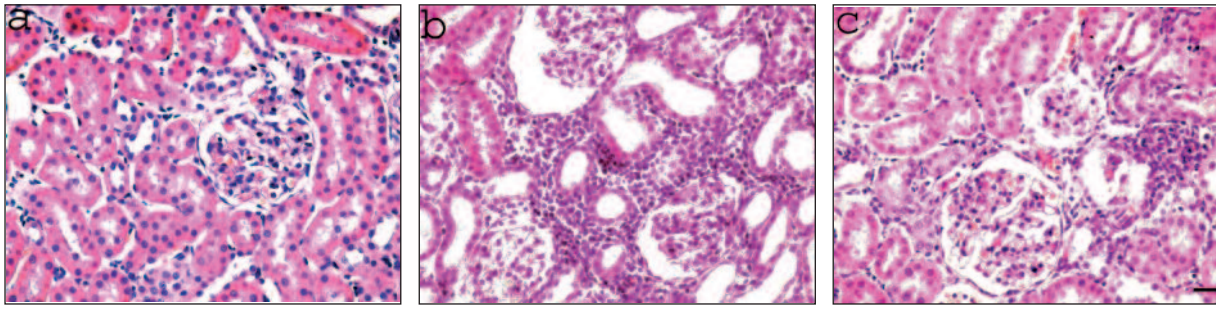

FIGURE 2: Histological appearance of the healthy kidney in the sham control group. **(a)** A haematoxylin–eosin (HE)-stained section shows neither necrosis of the tubular epithelium nor cast formation in the tubular lumina **(b)** In the ischemia and reperfusion group treated with saline (IR), note the distinct tubular necrosis and the distinct cast formation. **(c)** In the IR group administered L-carnitine (300 mg/kg; IR+LC) group, the level of tubular necrosis and cast formation is decreased compared with the saline-treated IR group (H&E, scale bar, 30 μm).

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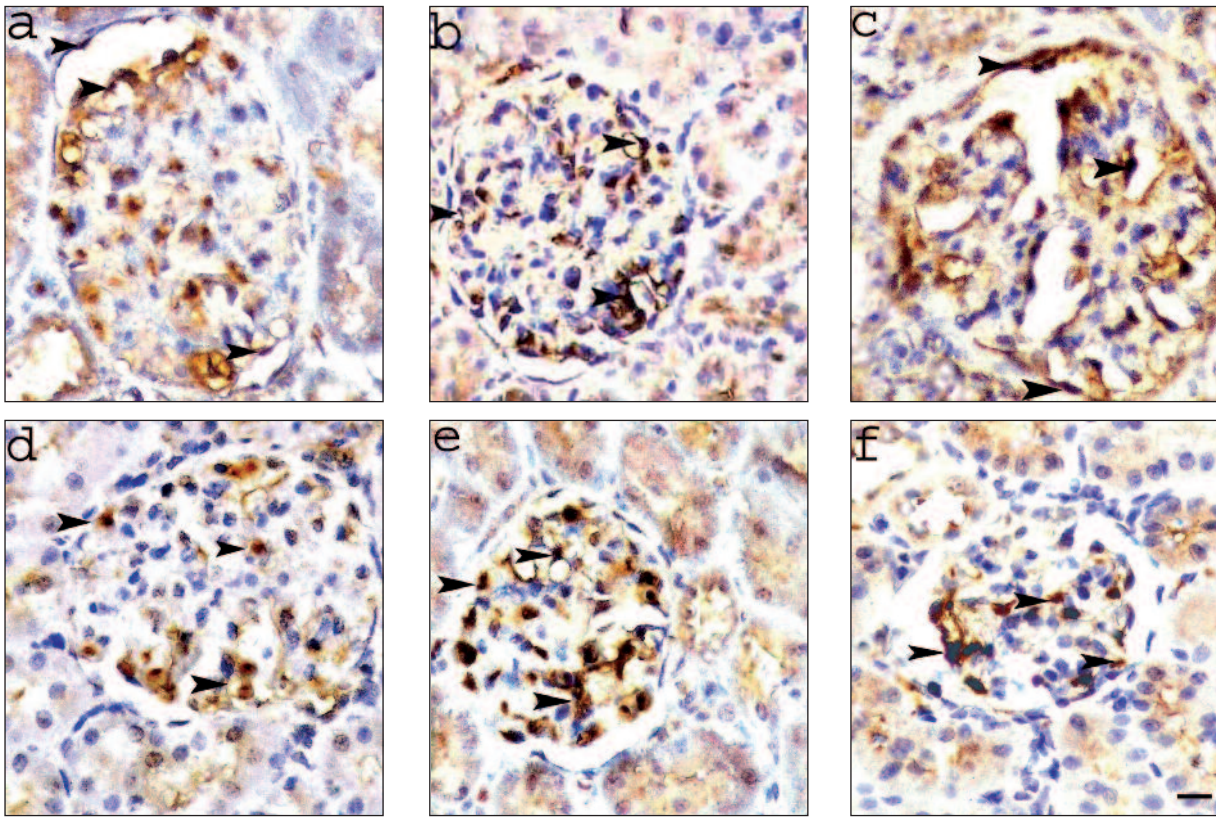

FIGURE 3: Glomerular immunostaining for endothelial nitric oxide synthase (eNOS) and inducible nitric oxide synthase (iNOS) in different groups. **a)** In the control group, weak eNOS immunostaining was noted in glomerular endothelial cells. **b)** In acute ischemia and reperfusion group treated with saline (IR), moderate eNOS immunostaining was present. **c)** L-carnitine treatment increased the intensity of eNOS immunoreactivity in glomerular endothelial cells. **d)** In the control group, weak iNOS immunostaining was noted in the glomerulus **e)** Strong iNOS immunostaining was present in the mesangium, podocytes and capillary loop of glomerulus in acute IR injury rats. **f)** L-carnitine treatment prevented the intense iNOS immunoreactivity in the glomerulus (Immunoperoxidase, haematoxylin counterstain, scale bar, 15 μm).

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ity of iNOS was rarely seen in the mesangium, podocytes and capillary loop of glomerulus, and weak expression of iNOS was detected in the Sham group. After acute IR, iNOS expression was markedly increased. L-carnitine treatment prevented the intense iNOS immunoreactivity in the mesangium, podocytes and capillary loop of the glomerulus.

TABLE 3: Semiquantitative comparison of the intensity of eNOS and iNOS immunoreactivity in renal tissues for each group. SHAM, IR and IR+L-carnitine groups

	SHAM	IR	IR+ L-carnitine
eNOS	+	++	++++
iNOS	+	++++	++

The intensity of the staining was recorded as weak (+), moderate (++), strong (+++) and very strong (++++) (n: 8 for each group).
SHAM, Sham control group; IR, ischemia and reperfusion treated with saline group; IR+LC, the IR treated with L-carnitine (300 mg/kg) group.
eNOS: endotelyal Nitrik Oksit Sentaz; iNOS: indüklenebilir Nitrik Oksit Sentaz.

DISCUSSION

Free radicals and nitric oxide play crucial roles in the pathophysiology of ischemia-reperfusion injury in the kidney. The nitric oxide produced by iNOS has been reported to increase renal ischemia-reperfusion injury. In addition, reaction of NO with superoxide leads to the formation of peroxynitrite, which also contributes to renal damage.[4,6,7,27] In the present study, we demonstrated that renal IR injury caused by bilateral occlusion (60 minutes) and reperfusion (168 hours) resulted in a reduction in renal function as demonstrated by increased plasma levels of urea and creatinine. A significant decline in hematocrit was observed. Thus, this experimental model can be used to create the anemia of renal IR injury, which reduced the antioxidant enzyme activities of both erythrocytes and the kidney and increased lipid peroxidation. Both iNOS and eNOS expression and histological evidence of increased necrosis and tubular cast formation was observed. Administration of L-carnitine significantly attenuated the (1) renal dysfunction, (2) anemia, (3) both kidney and erythrocytes antioxidant enzyme activities and GSH level, (4) decreased iNOS activities and increased eNOS activities, and (5) histological findings of IR-mediated renal injury.

Histological findings were also consistent with the results of renal function and significantly higher in the IR group than in the Sham group. L-carnitine treatment improved renal function compared with those in the IR group, with decreased tubular necrosis and cast formation. The results of the present study are consistent with those of previous studies.[11,12]

The importance of the free radicals in kidney disease was shown in several experimental models.[9-14] Previous studies suggested that renal IR injury increased lipid peroxidation and decreased GSH levels and activities of antioxidant enzymes in both erythrocytes and the kidney. The current study findings are also in accordance with the results of previous studies.[9-12] Treatment with L-carnitine decreases renal MDA levels and increases GSH levels as well as erythrocyte and kidney antioxidant enzyme activities in renal IR injury. The protective effects of L-carnitine in renal IR injury have been shown in previous studies.[9-12] Ergun et al. produced IR injury by 60 min of ischemia and 15 min reperfusion in a rabbit model, which increased serum and kidney lipid peroxidation levels, which then significantly decreased with L-carnitine administration.[10] Their study showed that in case of short-term reperfusion L-carnitine had no effect on hematocrit. Serum L-carnitine and SOD activity were shown to decrease and MDA level to increase after renal IR injury. Pretreatment with L-carnitine protected the kidney from IR injury.[12]

Liangos O. et al. report that "anemia is a common clinical problem in patients with AKI and there is a clear temporal relationship between cessation of kidney function, decline of erythropoietin production, and development of anemia, which has been documented in several studies of patients with AKI".[28] Nemoto et al. showed a decrease in hematocrit level in a rat model of unilateral renal artery occlusion and contralateral nephrectomy, following moderate (30 min clamp) and severe (45 min clamp) ischemic injury and 7 days reperfusion.[16] The administration of erythropoietin normalized the hematocrit. The main cellular function of L-carnitine is to facilitate the entry of long-chain fatty acids into mitochondria for oxidation and provision of energy in the form of adenosine triphosphate (ATP). The impressive effects of L-carnitine on anemia center on the improvement of erythrocyte survival, specifically through enhanced erythrocyte membrane stability.[15,28] Furthermore, Kitamura et al. reported that L-carnitine stimulated erythropoiesis in mouse bone marrow cell cultures.[29] Sener et al. showed that L-carnitine treat-

ment increased the SOD, CAT and GPx activities in erythrocytes as well as plasma GSH levels and decreased MDA levels in chronic renal failure.[13] In the current study, L-carnitine treatment reversed the depletion of erythrocyte antioxidant activities, which may protect the membrane stability of erythrocytes. The net effects of L-carnitine on anemia in renal IR injury need to be elucidated in future studies.

Nitric oxide plays an important role in modulating tissue injury and renal blood flow in the healthy kidney as well as several pathological kidney conditions. Several *in vivo* and *in vitro* studies have demonstrated that inhibition of iNOS activity or expression can ameliorate or prevent NO-mediated injury, suggesting that NO generated by iNOS contributes to renal IR injury. In contrast, increased eNOS activity has a protective effect in IR injury in the kidney.[4,7,30] We demonstrated that with L-carnitine treatment, the immunoreactivity of eNOS was obviously increased in glomerular endothelial cells. In the IR group, iNOS expression was markedly increased. L-carnitine treatment prevented the intensity of iNOS immunoreactivity in the mesangium, podocytes and capillary loop of the glomerulus. Furthermore, L-carnitine treatment enhanced the NO levels significantly, most probably from the endothelial cells and this increase is suggested to lead to reduced IR injury in the kidney tissue.

In conclusion, the present results indicated that L-carnitine treatment protected against long-term post IR injury in the rat kidney. Thus, L-carnitine may have a potential as a therapeutic drug for various clinical conditions, including renal IR injury and anemia.

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