Clinical and Experimental Pharmacology and Physiology (2006) **33**, 119–124

PROTECTIVE EFFECTS OF L-CARNITINE ON MYOGLOBINURIC ACUTE RENAL FAILURE IN RATS

Nurettin Aydogdu,* Gulizar Atmaca,* Omer Yalcin,† Recep Taskiran,* Ebru Tastekin* and Kadir Kaymak*

*Departments of *Physiology and †Pathology, Faculty of Medicine, University of Trakya, Edirne, Turkey*

SUMMARY

1. Muscle injury (rhabdomyolysis) is one of the causes of acute renal failure (ARF). Iron, free radicals and nitric oxide (NO) play a critical role in the pathogenesis of glycerol-induced myoglobinuric ARF. L-Carnitine is an anti-oxidant and prevents the accumulation of end-products of lipid peroxidation. Therefore, the aim of the present study was to investigate the effects of L-carnitine on myoglobinuric ARF induced by intramuscular (i.m.) hypertonic glycerol injection.

2. Sprague-Dawley rats were divided into three groups. Rats in group 1 ($n = 8$) were given saline, whereas those in groups 2 ($n = 10$) and 3 ($n = 10$) were injected with glycerol (10 mL/kg, i.m.). Concomitant with and 24 h after glycerol injection, L-carnitine (200 mg/kg, i.p.) was administered to group 3 rats. Forty-eight hours after glycerol injection, blood samples and kidney tissues were taken from anaesthetised rats.

3. Plasma creatine kinase (CK) activity, urea, creatinine and NO levels, as well as kidney tissue superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) enzyme activity and malondialdehyde (MDA) and glutathione (GSH) levels, were determined. In the kidney tissue, histopathological changes and iron accumulation in the tubular epithelium were also investigated.

4. Glycerol treatment caused severe ARF: a marked renal oxidative stress, significantly increased CK activity, urea and creatinine levels and decreased plasma NO levels. Histopathological findings in group 2 rats confirmed that there was renal impairment by cast formation and tubular necrosis and a marked increase in iron accumulation in the tubular epithelium. All these factors were significantly improved by L-carnitine supplementation.

5. These results may indicate that L-carnitine treatment protects against functional, biochemical and morphological damage and iron accumulation in glycerol-induced myoglobinuric ARF in rats. In this model, the protective effect of L-carnitine treatment may provide a new insight into the treatment of rhabdomyolysis-related ARF.

Key words: acute renal failure, anti-oxidant enzymes, L-carnitine, iron, lipid peroxidation, nitric oxide.

INTRODUCTION

The term 'rhabdomyolysis' refers to the disintegration of skeletal muscle, which results in the release of muscular cell constituents into the extracellular fluids and the circulation.[1] Rhabdomyolysis is associated with both traumatic (natural disasters such as earthquakes, car accidents and mine collapse) and non-traumatic (hyperthermia, muscle ischaemia, exposure to toxins, such as alcohol or drug overdose, etc.) cases.[2–4] Rhabdomyolysis is one of the causes of acute renal failure (ARF). The occurrence of ARF following untreated rhabdomyolysis has been put at between 17 and 33% of cases and accounts for between 3 and 15% of all cases of ARF.[5]

The most common *in vivo* model of myoglobinuric ARF is produced by intramuscular (i.m.) injection of hypertonic glycerol, which causes myolysis, haemolysis and intravascular volume depletion and exposes the kidney to a large burden of heme proteins, myoglobin and haemoglobin. It has been suggested that heme proteins or their degradation products (including haematin and iron) display tubular nephrotoxic properties, partially mediated by the generation of free oxygen radicals, and induce vasoconstriction, further enhancing ischaemic damage.[2–4] Because iron is a transition metal that readily accepts and donates electrons, it greatly facilitates free radical production and may itself become a free radical. The results of these processes are massive lipid peroxidation and, ultimately, cell death.[2] Nitric oxide (NO) scavenging induced by heme proteins could directly contribute to renal hypoperfusion and tissue injury in the setting of rhabdomyolysis. Myoglobinuric renal injury is largely secondary to the ischaemic, as well as toxic, renal insults induced by heme proteins.[2] The role of free oxygen radicals in renal failure induced by muscle injury has been examined using the glycerol model. In this model, the administration of anti-oxidants and iron chelation therapy have been shown to provide partiall protection against renal failure.[2–4,6,7]

Carnitine is a vitamin-like substance that is structurally similar to amino acids. Carnitine is obtained mostly from the diet. It can also be synthesised endogenously by skeletal muscle, heart, liver, kidney and brain from the essential amino acids lysine and methionine.[8] The major role of L-carnitine is in the transport of long-chain fatty acids into the mitochondrial matrix for β-oxidation and the provision of energy in the form of ATP. L-Carnitine is an anti-oxidant that prevents the accumulation of end-products of lipid peroxidation.[9] It has been used successfully in the treatment of a variety of diseases. The protective effect of L-carnitine on kidney tissue has been proven in various models, such as cisplatin-induced injury of the kidney and small intestine, gentamycin-induced nephrotoxicity, ischaemia–reperfusion injury of the kidney and chronic renal failure.[10–13] Carnitine can also act as a chelator by decreasing the

Correspondence: Dr Nurettin Aydogdu, Trakya Universitesi, Tip Fakultesi, Fizyoloji AD, 22030 Edirne, Turkey. Email: naydogdu@hotmail.com

Received 22 November 2004; revision 25 July 2005; accepted 9 October 2005.

concentration of cytosolic iron, which plays a very important role in free radical chemistry.[9,14]

In light of the findings described above, the present study examined the effects of exogenous L-carnitine supplementation on renal function, anti-oxidant status, lipid peroxidation production, NO, histopathological changes of the kidney and iron accumulation in glycerol-induced ARF.

METHODS

Induction of ARF and L-carnitine treatment

Female Sprague-Dawley rats (160–210 g) were housed in an air-conditioned room with 12 h light–dark cycles, where the temperature (21 ± 2°C) and relative humidity (60–65%) were kept constant. All animal experiments were approved by Trakya University School of Medicine Animal Care and Use Committee. Rats were allowed free access to standard rat chow, but were deprived of water for 16 h prior to the study. Rats were randomly divided into three groups. The first group (n = 8; sham) served as a sham control and was administered 10 mL/kg, i.m., saline; the second group (n = 10; ARF) served as the ARF control; and the third group (n = 10; ARF-LC) was administered 10 mL/kg, i.m., glycerol (50% v/v in sterile saline). Concomitantly, in the sham and ARF groups, saline was administered i.p., whereas in the ARF-LC group, L-carnitine (200 mg/kg, i.p.; Carnitene®; Sigma-Tau, Rome, Italy) were administered. The i.p. injections were repeated after 24 h. All groups received the same volume of injection. All i.m. injection volumes were divided equally into two and were injected into each hindlimbs; this was performed under the light ether anaesthesia. Rats were followed up for 48 h without any diet or water restriction. Animals were killed after the follow-up period. At the time of death, rats were anaesthetized with 10 mg/kg xylazine and 50 mg/kg ketamine. Blood samples were collected by cardiac puncture and bilateral nephrectomy was performed. Blood samples were centrifuged immediately (1500 g for 10 min at 4°C) and plasma samples were stored at −85°C until assayed. Renal tissue samples were also stored at −85°C.

Renal function and creatine kinase activity assays

Plasma urea and creatinine levels were measured using standard diagnostic kits (Chema Diagnostica, Jesi, Italy). Plasma creatine kinase (CK) activity, which is an indicator of muscle damage or myopathy, was determined using a Synchron LX20 analyser (Beckman, Fullerton, CA, USA).

Malondialdehyde and glutathione assays

Kidney tissue samples were homogenized with 150 mmol/L ice-cold KCl for the determination of malondialdehyde (MDA) and glutathione levels. Homogenates were centrifuged at 2600 g for 10 min at 4°C. The MDA concentrations in renal tissue, an indicator of lipid peroxidation, were assayed in the form of thiobarbituric acid-reacting substances.[15] Supernatant (200 μL) was added to 0.2 mL of 8.1% sodium dodecyl sulphate (SDS), 1.5 mL 20% acetic acid (pH 3.5), 1.5 mL of 0.8% thiobarbituric acid and 0.6 mL distilled water. This mixture was heated to 95°C for 60 min. After cooling with tap water, 1.0 mL distilled water and 5.0 mL of a mixture of n-butanol : pyridine (15 : 1, v/v) was added and the mixture was shaken vigorously and centrifuged at 2600 g for 10 min at 25°C. The absorbance of the organic layer was read at 532 nm. Malondialdehyde was quantified using an extinction coefficient of 1.56×10^5 L/mol per cm and is expressed as nmol MDA/mg tissue.

The glutathione (GSH) level was determined according to the method of Ellman.[16] The concentration of GSH was monitored spectrophotometrically at 412 nm. Results are expressed as μmol/g tissue.

Enzyme assays

All enzyme activities were determined after renal tissue homogenization with phosphate-buffered saline (PBS) at pH 7.4. Total (Cu/Zn and Mn) superoxide dismutase (SOD) activity was determined according to the method of Sun *et al*.[17] This is based on the inhibition of nitroblue tetrazolium (NBT) reduction by the xanthine–xanthine oxidase system as a superoxide generator. One unit of SOD was defined as the amount of enzyme causing 50% inhibition in the NBT reduction rate. Specific activity is expressed in units/mg protein. Catalase (CAT) activity was measured according to the method of Aebi.[18] This procedure is based on the determination of the rate constant (k; /s) of the hydrogen peroxide decomposition rate at 240 nm. Results are expressed as the rate constant/mg homogenate protein. Glutathione peroxidase (GPx) activity was measured according to the methods of Lawrence and Burk[19] by monitoring the oxidation of reduced NADPH at 340 nm. Enzyme units were defined as the number of μmol NADPH oxidized/min and calculated using the extinction coefficient of NADPH at 340 nm (6.22×10^3 L/mol per cm). Results are reported as units/mg protein. The protein content of tissue samples was determined according to the method of Lowry *et al*.[20]

Nitrite and nitrate assay

Nitrite and nitrate are the primary oxidation products of NO subsequent to its reaction with oxygen and, therefore, the nitrite/nitrate concentration in plasma was used as indicator of NO synthesis. Quantification of nitrate and nitrite was based on the Griess reaction, in which a chromophore with a strong absorbance at 545 nm is formed by the reaction of nitrite with a mixture of naphthylethylenediamine and sulphanilamide.[21] Samples were deproteinized with Somogyi reagent. The nitrate was reduced to nitrite by copper-coated cadmium in glycine buffer (pH 9.7). The total nitrite/nitrate concentration was calculated using sodium nitrate standard solution. Results are expressed as μmol/L.

Kidney histology

Right kidneys were cut longitudinally and fixed in 10% formalin solution. Tissues were embedded in paraffin wax and 4 μm sections were stained with haematoxylin and eosin (HE). The extent of histological renal tubular necrosis and tubular cast formation for each animal were evaluated semiquantitatively by a pathologist (OY) analysing the kidney sections without prior knowledge of the treatment group. The extent of tubular injury was evaluated in terms of the percentage area of sections showing a particular level of histological damage. In addition, 4 μm sections of parafin-embedded tissues stained with iron stain (Procedure No. HT 20; Sigma Diagnostics, St Louis, MO, USA). The percentage iron accumulation for each group was calculated after semiquantitative evaluation of the granular blue-coloured areas. In the present study, a special ocular (Nikon, Tokyo, Japan), which has 400 cells, was used to detect the percentage of casts, necrosis and iron accumulation.

Statistical analysis

Results are expressed as the mean±SD. The Kruskal-Wallis test was used to compare the three groups. In two-group comparisons, the Mann-Whitney U-test was used. $P < 0.05$ was considered statistically significant.

RESULTS

In the present study, all animals were water restricted for 16 h prior to glycerol injection and, later on, had free access to food and water. The rats in the ARF group lost an average of 8–12% of their bodyweight during the study period. The ARF-LC group also lost an average of 5–7% of bodyweight. The bodyweight of rats in the sham group did not change significantly. One rat died in the ARF group and was not included in the analysis.

As shown in Table 1, plasma CK activity, urea and creatinine concentrations were significantly increased in the ARF group compared with the sham group. Treatment with L-carnitine (ARF-LC group) significantly decreased plasma CK activity, creatinine and urea levels.

Table 1 Biochemical results of experimental groups

	Sham ($n = 8$)	ARF ($n = 9$)	ARF-LC ($n = 10$)
Plasma			
Urea (mg/dL)	41.73 ± 6.02	617.56 ± 32.40*	487.99 ± 21.09‡
Creatinin (mg/dL)	0.68 ± 0.08	7.33 ± 0.67*	4.67 ± 0.61‡
CK (U/L)	375.50 ± 86.96	975.88 ± 167.89*	685.70 ± 246.45†
NO (μmol/L)	28.50 ± 2.62	13.42 ± 1.78*	26.49 ± 4.21‡
Kidney tissue			
MDA (nmol/mg tissue)	0.43 ± 0.02	0.92 ± 0.15*	0.63 ± 0.08‡
GSH (μmol/g tissue)	3.47 ± 0.36	1.80 ± 0.32*	3.14 ± 0.36‡
SOD (U/mg protein)	9.44 ± 0.39	6.90 ± 0.41*	9.70 ± 0.63‡
CAT (*k*/mg protein)	0.62 ± 0.08	0.17 ± 0.03*	0.43 ± 0.09‡
GPx (U/mg protein)	2.17 ± 0.21	1.08 ± 0.05*	1.65 ± 0.46‡

Data are the mean±SD. *$P < 0.001$ compared with sham control; †$P < 0.05$, ‡$P < 0.001$ compared with the acute renal failure group treated with saline (ARF).

All parameters were determined in the rats that were killed at 48 h.

ARF-LC, ARF rats treated with L-carnitine (200 mg/kg, i.p.); CK, creatine kinase; NO, nitric oxide; MDA, malondialdehyde; GSH, glutathione; SOD, superoxide dismutase, CAT, catalase; GPx, glutathione peroxidase.

Plasma NO concentrations were significantly decreased in the ARF group compared with the sham group. The NO level was significantly improved in the ARF-LC group compared with the ARF group.

In the ARF group, kidney tissue MDA levels were significantly increased compared with those in the sham group. In the ARF-LC group, there was a significant reduction in MDA levels when they were compared with levels in the ARF group.

The ARF group had significantly decreased enzyme activities (SOD, CAT and GPx) compared with the sham group. This reduction in enzyme activity was significantly improved by treatment with L-carnitine. The tissue levels of GSH in the ARF group were decreased compared with those in the sham group. The GSH levels were significantly increased by L-carnitine treatment.

The histological changes in all groups were examined and results are given in Table 2. The sham group did not show any histopathological changes (Fig. 1). In contrast, in the ARF group, the basic histological abnormalities were tubular necrosis and cast formation. Necrosis was most severe in the cortical segments of the proximal tubules and less extensive changes were observed in medullar segments of the kidney. Moreover, blue granular deposits showed iron accumulation in the tubular epithelium of the kidney (Fig. 1). Tubular cell necrosis cast formation and iron accumulation significantly decreased with L-carnitine treatment (Fig. 1). In the kidney, iron deposits were observed in the cytoplasm of tubular epithelial cells and cast formation was observed in the tubular lumina. In the ARF group, the deposition of iron was more excessive in intraluminal cast formations (82.80%) than in the cytoplasm of tubular epithelial cells (17.20%). Conversely, in the ARF-LC group, iron deposition in the intraluminal cast formations was decreased (59.31%; $P < 0.01$) but increased in the cytoplasm of tubular epithelial cells (40.69%; $P < 0.01$).

Table 2 Histological findings in control and treated rats

	Sham ($n = 8$)	ARF ($n = 9$)	ARF-LC ($n = 10$)
Necrosis (%)	0	32.77 ± 6.47	19.30 ± 6.45**
Cast formation (%)	0	50.77 ± 3.83	38.30 ± 5.39**
Iron accumulation (%)	0	25.08 ± 5.38	14.87 ± 8.10*
Iron accumulation in cast (%)	0	82.80 ± 7.70	59.31 ± 14.15*
Iron accumulation in cytoplasm (%)	0	17.20 ± 7.70	40.69 ± 14.15*

Data are the mean±SD. *$P < 0.01$, **$P < 0.001$ compared with the acute renal failure group treated with saline (ARF).

All parameters were determined in the rats that were killed at 48 h. Renal histology is expressed as the percentage area examined for the particular findings. Iron accumulation is expressed as the percentage of the granular blue-coloured area. Histopathological analysis of the kidney showed neither necrosis and cast formation nor iron accumulation in the sham control group.

ARF-LC, ARF rats treated with L-carnitine (200 mg/kg, i.p.).

DISCUSSION

The pathophysiology of myoglobinuric acute ARF has been studied extensively in the animal model of glycerol-induced ARF.[2–4,6,7] At the kidney level, the main pathophysiological mechanisms in the genesis of myoglobinuric ARF have been identified as intense renal vasoconstriction, tubular obstruction by intraluminal cast formation and direct heme-protein induced cytotoxicity.[2]

In the present study, we observed increased plasma CK activity, urea and creatinine levels in the ARF group. This group also had increased kidney tissue lipid peroxidation products, elevated MDA levels and decreased SOD, CAT and GPx enzyme activities and GSH levels. In addition, NO, which is an important mediator in the pathophysiology of the glycerol-induced ARF model, was also decreased in the same group. Histopathological findings of this group confirmed renal impairment by cast formation and tubular necrosis and distinct granular deposits showing iron accumulation in the tubular epithelium. These findings were similar to those of previous studies performed using the same model.[3,6,7,22–27]

Interestingly, the present findings demonstrated that L-carnitine treatment produced a significant reduction in CK activity, urea and creatinine levels, inhibited MDA production and reversed the depletion of GSH and NO levels, as well as SOD, CAT and GPx activities. In addition, we observed a marked reduction in both tubular necrosis and cast formation and granular deposits of iron accumulation in the morphological findings of the carnitine-supplemented group.

In previous studies, L-carnitine has been shown to have antioxidant and protective effects against oxidative damage in different organs or tissues, including the kidney.[9–11,13,14,28] It has been demonstrated that L-carnitine administration inhibits both serum and kidney tissue MDA formation in response to renal ischaemia–reperfusion injury.[11] Kalaiselvi and Panneerselvam[9] showed that carnitine supplementation enhances the activities of anti-oxidant enzymes, such as SOD, CAT and GPx, and GSH levels and decreases the MDA concentration in kidney tissues of 24-month-old rats. Sener *et al*.[13] have shown that L-carnitine administration prevents MDA formation and enhances GSH levels in the kidney, heart, aorta and corpus cavernosum and decreases plasma urea and creatinine levels in rats with chronic renal failure. In the present study, we have shown that L-carnitine treatment restores reduced plasma SOD, CAT and

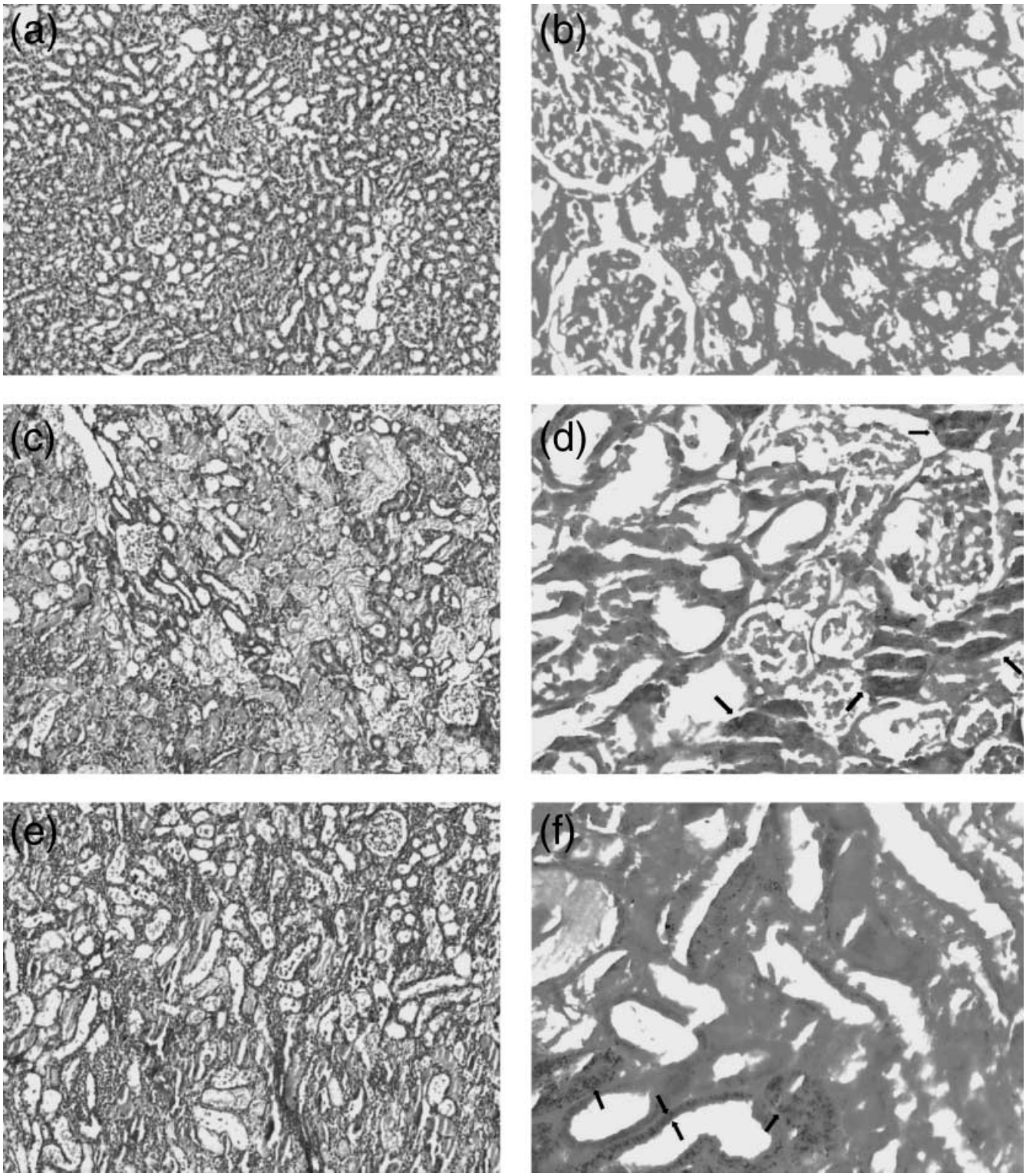

Fig. 1 Histological appearance of the healthy kidney in the sham control group. (a) A haematoxylin–eosin (HE)-stained section shows neither necrosis of the tubular epithelium nor cast formation in the tubular lumina (HE; original magnification ×50). (b) There are no granular deposits in the iron stained section (iron stain; original magnification ×200). (c) In the acute renal failure (ARF) group treated with saline, note the distinct tubular necrosis and the distinct cast formation in the HE-stained section (HE; original magnification ×50). (d) Note the distinct granular deposits showing iron accumulation (arrows) in intraluminal cast formations and a slight accumulation in the cytoplasm of epithelial cells in myoglobinuric ARF (iron stain; original magnification ×200). (e) In the ARF group administered L-carnitine, the level of tubular necrosis and cast formation is decreased compared with the saline-treated ARF group (HE; original magnification ×50). (f) Note also the decrease in granular deposits (arrows) compared with the saline-treated ARF group (iron stain; original magnification ×200). Although the iron that was present in the intraluminal cast formations decreased, it increased in the cytoplasm of tubular epithelial cells.

GPx activities as well as GSH levels and decreases MDA levels in the chronic renal failure group. In the present study, the effects of L-carnitine on the anti-oxidant enzymes, MDA and GSH levels were found to agree with reports published previously.

Nitric oxide is synthesised by different NO synthases (NOS): endothelial (e) NOS, neuronal (n) NOS and inducible (i) NOS. Nitric oxide plays an important role in modulating tissue injury and renal blood flow in the healthy kidney, as well as in several pathological kidney conditions. The contribution of NO in the pathogenesis of ARF has been unclear; however, 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 I/R injury. Furthermore, eNOS-derived NO is believed to be responsible for maintaining physiological renal haemodynamics and function.[29] The NO system has been studied in renal impairment associated with heme protein. One of these studies showed that the administration of arginine protected against renal dysfunction, whereas the administration of an inhibitor of NOS worsened renal function in the glycerol model of ARF.[30] We have reported previously that the decreasing effect of glycerol-induced ARF on NO production may play an important role in the impairment of renal function.[23,24] In these studies, both caffeic acid phenethyl ester and melatonin inhibited NO production and, therefore, no protective effect was demonstrated for glycerol-induced ARF.[23,24] In the present study, L-carnitine treatment enhanced NO levels significantly, most probably from endothelial cells, and this increase may lead to a reduction in the intensity of the ischaemia in the kidney tissue. Arslan *et al*.[28] reported that L-carnitine treatment significantly enhanced the levels of NO in plasma and this evaluation may have provided enhanced protection against ischaemia in the flap. This supports our finding that plasma NO levels were elevated with L-carnitine treatment.

Cells acquire iron from carrier proteins (transferrin) or from cell surface iron transporters. Intracellular iron is controlled by the actions of the iron-responsive proteins, the ferritin complex and heme oxygenase-1.[31] Recently, a protein was identified called neutrophil gelatinase-associated lipocalin (Ngal) or lipocalin 2, also known as siderocalin, which forms a complex with iron-binding siderophores (Ngal : siderophore : Fe).[32] This complex is more likely to transfer iron to proximal tubule cells and could chelate iron.[31,32] In rhabdomyolysis, the cytotoxic iron moiety is derived from heme, a product of myoglobin metabolism. Intracellular iron is an important mediator of tissue damage. The iron that is derived from renal tubules catalyses the free radicals reaction, which is associated with lipid peroxidation and renal injury.[33] In glycerol-induced ARF, there is enhanced generation of hydrogen peroxide, which is a substrate for hydroxyl radical formation via the iron-catalysed Fenton and Haber–Weiss reactions.[34] Paller[6] has shown that iron chelator (deferoxamine) therapy mitigated the model of glycerol-induced ARF. Moreover, it has been demonstrated that an iron chelator (deferoxamine) and hydroxyl radical scavengers each protected against the glycerol-induced ARF model.[7]

In the present study, in the kidney of glycerol-induced ARF, iron deposits were observed. In contrast, L-carnitine treatment significantly decreased the total iron content in the kidney tissue. In addition, although the iron that was present in intraluminal cast formations decreased, iron increased in the cytoplasm of tubular epithelial cells in kidney tissue. These effects of L-carnitine on iron homeostasis, in addition to its chelating property,[9,14] may be dependent on the modulatory role of the substances responsible for the transport, storage or metabolism of iron. The net effect of L-carnitine on iron homeostasis in the kidney tissue needs to be elucidated in future studies.

Plasma or serum CK activity correlates with the degree of muscle injury and is a valid gross indicator of the extent of muscle damage.[5] In the present study, the finding of decreased CK activity may be a result of less muscle damage following treatment with L-carnitine. Therefore, L-carnitine presumably has a protective role against muscle damage in addition to its ameliorating effects on kidney tissue. Akar *et al*.[35] reported that the administration of L-carnitine was effective in reducing reperfusion injury in rabbit skeletal muscle. This finding supports our approach.

In conclusion, the present study may indicate that free radicals, lipid peroxidation, iron accumulation in the kidney, depletion of the anti-oxidant status, glomerular dysfunction, decreased NO levels and impaired renal morphology may be involved in the pathophysiological mechanisms of glycerol-induced ARF. Although our data do not reveal the mechanism of the action of L-carnitine, the prevention of lipid peroxidation, the scavenging of reactive oxygen species and/or a reduction in anti-oxidant depletion, enhancing NO levels and decreasing iron accumulation may be the putative mechanisms in this model.

REFERENCES

1. Vanholder R, Sever MS, Erek E, Lameire N. Rhabdomyolysis. *J. Am. Soc. Nephrol.* 2000; **11**: 1553–61.
2. Zager RA. Rhabdomyolysis and myohemoglobinuric acute renal failure. *Kidney Int.* 1996; **49**: 314–26.
3. Baliga R, Zhang Z, Baliga M, Shah SV. Evidence for cytochrome p-450 as a source of catalytic iron in myoglobinuric acute renal failure. *Kidney Int.* 1996; **49**: 362–9.
4. Baliga R, Ueda N, Walker PD, Shah SV. Oxidant mechanisms in toxic acute renal failure. *Drug Metab. Rev.* 1999; **31**: 971–97.
5. Beetham R. Biochemical investigation of suspected rhabdomyolysis. *Ann. Clin. Biochem.* 2000; **37**: 581–7.
6. Paller MS. Hemoglobin- and myoglobin-induced acute renal failure in rats: Role of iron in nephrotoxicity. *Am. J. Physiol.* 1988; **255**: F539–44.
7. Shah SV, Walker PD. Evidence suggesting a role for hydroxyl radical in glycerol-induced acute renal failure. *Am. J. Physiol.* 1988; **255**: F438–543.
8. Rebouche CJ, Seim H. Carnitine metabolism and its regulation in microorganisms and mammals. *Annu. Rev. Nutr.* 1998; **18**: 39–61.
9. Kalaiselvi T, Panneerselvam C. Effect of L-carnitine on the status of lipid peroxidation and antioxidants in aging rats. *J. Nutr. Biochem.* 1998; **9**: 575–81.
10. Chang B, Nishikawa M, Sato E, Utsumi K, Inoue M. L-Carnitine inhibits cisplatin-induced injury of the kidney and small intestine. *Arch. Biochem. Biophys.* 2002; **405**: 55–64.
11. Ergun O, Ulman C, Kilicalp AS, Ulman I. Carnitine as a preventive agent in experimental renal ischemia-reperfusion injury. *Urol. Res.* 2001; **29**: 186–9.
12. Kopple JD, Ding H, Letoha A *et al.* L-Carnitine ameliorates gentamicin-induced renal injury in rats. *Nephrol. Dial. Transplant.* 2002; **17**: 2122–31.
13. Sener G, Paskaloglu K, Satiroglu H, Alican I, Kacmaz A, Sakarcan A. L-Carnitine ameliorates oxidative damage due to chronic renal failure in rats. *J. Cardiovasc. Pharmacol.* 2004; **43**: 698–705.
14. Rani APJ, Panneerselvam C. Effect of L-carnitine on brain lipid peroxidation and antioxidant enzymes in old rats. *J. Gerontol.* 2002; **57A**: B134–7.
15. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbutiric acid reaction. *Anal. Biochem.* 1979; **95**: 351–8.
16. Ellman GL. Tissue sulfhydryl groups. *Arch. Biochem. Biophys.* 1959; **82**: 70–7.

17. Sun Y, Oberley LW, Li Y. A simple method for clinical assay of superoxide dismutase. *Clin. Chem.* 1988; **34**: 497–500.
18. Aebi H. Catalase *in vitro* assay methods. *Methods Enzymol.* 1984; **105**: 121–6.
19. Lawrance RA, Burk RF. Glutathione peroxidase activity in selenium-deficient rat liver. *Biochem. Biophys. Res. Commun.* 1976; **71**: 952–8.
20. Lowry OH, Rosebrough NJ, Farr AL, Randall RI. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* 1951; **193**: 265–75.
21. Cortas NK, Wakid NW. Determination of inorganic nitrate in serum and urine by kinetic cadmium-reduction method. *Clin. Chem.* 1990; **36**: 1440–3.
22. Abul-Ezz SR, Walker PD, Shah SV. Role of glutathione in an animal model of myoglobinuric acute renal failure. *Proc. Natl Acad. Sci. USA* 1991; **88**: 9833–7.
23. Aydogdu N, Atmaca G, Yalcin O, Batcioglu K, Kaymak K. Effects of caffeic acid phenethyl ester on glycerol-induced acute renal failure in rats. *Clin. Exp. Pharmacol. Physiol.* 2004; **31**: 575–9.
24. Aydogdu N, Atmaca G, Yalcin O, Batcioglu K, Kaymak K. Effects of exogenous melatonin on myoglobinuric acute renal failure in the rats. *Renal Failure* 2004; **26**: 479–86.
25. Nath KA, Balla G, Vercellotti GM *et al.* Induction of heme oxygenase is a rapid, protective response in rhabdomyolysis in the rat. *J. Clin. Invest.* 1992; **90**: 267–70.
26. Singh D, Chander V, Chopra K. Protective effect of naringin, a bioflavonoid on glycerol-induced acute renal failure in the rat kidney. *Toxicology* 2004; **201**: 143–51.
27. Akpolat T, Akpolat I, Ozturk H *et al.* Effect of vitamin E and pentoxifylline on glycerol-induced acute renal failure. *Nephron* 2000; **84**: 243–7.
28. Arslan E, Milcan A, Unal S *et al.* The effects of carnitine on distally-burned dorsal skin flap: An experimental study in rats. *Burns* 2003; **29**: 221–7.
29. Goligorsky MS, Brodsky SV, Noiri E. NO bioavailability, endothelial dysfunction, and acute renal failure: New insights into pathophysiology. *Semin. Nephrol.* 2004; **24**: 316–13.
30. Maree A, Peer G, Schwartz D *et al.* Role of nitric oxide in glycerol-induced acute renal failure in rats. *Nephrol. Dial. Transplant.* 1994; **9**: 78–81.
31. Mori K, Lee HT, Rapoport D *et al.* Endocytic delivery of lipocalin–siderophore–iron complex rescues the kidney from ischemia–reperfusion injury. *J. Clin. Invest.* 2005; **115**: 610–21.
32. Yang J, Goetz D, Li JY *et al.* An iron delivery pathway mediated by a lipocalin. *Mol. Cell* 2002; **10**: 1045–56.
33. Larbi EB. Drug-induced rhabdomyolysis. *Ann. Saudi Med.* 1998; **18**: 525–30.
34. Guidet B, Shah SV. Enhanced *in vivo* H_2O_2 generation by rat kidney in glycerol-induced renal failure. *Am. J. Physiol.* 1988; **255**: F440–45.
35. Akar H, Sarac A, Konuralp C, Yildiz L, Kolbakir F. Comparison of histopathologic effects of carnitine and ascorbic acid on reperfusion injury. *Eur. J. Cardiothorac. Surg.* 2001; **19**: 500–6.