

Original Article

Increased urinary losses of carnitine and decreased intramitochondrial coenzyme a in gentamicin-induced acute renal failure in rats

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Abstract

Background. This study examined whether carnitine deficiency is a risk factor and should be viewed as a mechanism during the development of gentamicin (GM)-induced ARF as well as exploring if carnitine supplementation could offer protection against this toxicity.

Methods. Adult male Wistar albino rats were assigned to one of six treatment groups: group 1 (control) rats were given daily intraperitoneal (I.P.) injections of normal saline for 8 consecutive days; groups 2, 3 and 4 rats were given GM (80 mg/kg/day, I.P.), L-carnitine (200 mg/kg/day, I.P.) and D-carnitine (250 mg/kg/day, I.P.), respectively, for 8 consecutive days. Rats of group 5 (GM plus D-carnitine) received a daily I.P. injection of D-carnitine (250 mg/kg/day) 1 h before GM (80 mg/kg/day) for 8 consecutive days. Rats of group 6 (GM plus L-carnitine) received a daily I.P. injection of L-carnitine (200 mg/kg/day) 1 h before GM (80 mg/kg/day) for 8 consecutive days.

Results. GM significantly increased serum creatinine, blood urea nitrogen (BUN), urinary carnitine excretion, intramitochondrial acetyl-CoA and total nitrate/nitrite (NO_x) and thiobarbituric acid reactive substances (TBARS) in kidney tissues and significantly decreased total carnitine, intramitochondrial CoA-SH, ATP, ATP/ADP and reduced glutathione (GSH) in kidney tissues. In carnitine-depleted rats, GM caused a progressive increase in serum creatinine, BUN and urinary carnitine excretion and a progressive decrease in total carnitine, intramitochondrial CoA-SH and ATP. Interestingly, L-carnitine supplementation resulted in a complete reversal of the increase in serum creatinine, BUN, urinary carnitine excretion and the decrease in total carnitine, intramitochondrial CoA-SH and ATP, induced by GM, to the control values. Moreover, the histopathological examination of kidney tissues confirmed the biochemical data, where L-carnitine prevents and D-carnitine aggravates GM-induced ARF.

Conclusions. (i) GM-induced nephrotoxicity leads to increased urinary losses of carnitine; (ii) carnitine deficiency is a risk factor and should be viewed as a mechanism during the development of GM-induced ARF; and (iii) carnitine supplementation ameliorates the severity of GM-induced

kidney dysfunction by increasing the intramitochondrial CoA-SH/acetyl-CoA ratio and ATP production.

Keywords: carnitine deficiency; D-carnitine; gentamicin; L-carnitine; nephrotoxicity

Introduction

Although gentamicin (GM) is a potent broad-spectrum aminoglycoside antibiotic in the treatment of life-threatening infections caused by Gram-negative and Gram-positive bacilli [1], its optimal clinical usefulness is usually limited secondary to its dose-related nephrotoxicity [2]. GM is one of the most common causes of acute renal failure (ARF) which accounts for 15–30 of all cases of ARF [1–4] and promotes both increased morbidity and greater healthcare costs [3–6]. Several mechanisms have been suggested to account for GM-induced ARF including generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) [7,8], altering the structure and function of cellular membranes [9], mesangial cells contraction [10] and inhibition of mitochondrial oxidative phosphorylation [11]. Many compounds have shown considerable protective effects against GM-induced ARF including curcumin [12], caffeic acid phenethyl ester [13], resveratrol [10], aged garlic extract [14], vitamins E and C [15] and L-carnitine [16].

L-carnitine is an essential cofactor for the translocation of long-chain fatty acids from cytoplasmic compartment into mitochondria, where beta-oxidation enzymes are located. It is synthesized mainly in kidney and liver and can be obtained exogenously from dietary source mainly red meat and dairy products [17,18]. Under normal physiological conditions, L-carnitine is highly conserved, since 95% of the filtered L-carnitine is reabsorbed at the proximal tubular level [19]. In GM-induced ARF rat model, an earlier study by Kopple *et al.* [16] was the first and the only to report that daily L-carnitine injections, particularly at 200 mg/kg/day, ameliorate the severity of GM-induced renal cortical proximal tubular necrosis and maintain greater

renal function [16]. However, results from this study should be carefully reviewed because the protection achieved by L-carnitine was not complete and its mode of action was not clear. Moreover, Kopple *et al.* found no evidence that carnitine altered the serum or renal cortical tissue concentrations of GM. Although the kidney is the main organ responsible for endogenous synthesis of L-carnitine, we could not find any study to date investigating the effects of GM on renal handling of carnitine under condition of carnitine depletion and supplementation. Therefore, this study has been initiated to investigate, for the first time, whether carnitine deficiency is a risk factor and should be viewed as a mechanism during the development of GM-induced ARF, and if so, what are the consequences of this condition on kidney function, as well as exploring if carnitine supplementation could offer protection with the possible mechanisms underlying this protection.

Methods

Animals

Adult male Wistar albino rats, weighing 230–250 g, were obtained from the Animal Care Center, College of Pharmacy, King Saud University (KSU), Riyadh, Kingdom Saudi Arabia (KSA), and were housed in metabolic cages under controlled environmental conditions (25°C and a 12 h light/dark cycle). The animals had free access to pulverized standard rat pellet food and tap water unless otherwise indicated. The protocol of this study has been approved by Research Ethics Committee of College of Pharmacy, KSU, Riyadh, KSA.

Materials

GM (Gentam 80 mg vials, Spimaco, Al-Qassim Pharmaceutical Plant, Al-Qassim, KSA) was a generous gift from King Khalid University Hospital drug store, KSU, KSA. L-carnitine and D-carnitine were kindly supplied by Dr Zaven Orfalin, Sigma-Tau Pharmaceuticals, Pomezia, Italy. It was supplied as white powder in a non-commercial plastic bottle containing 100 g and it was freshly dissolved in normal saline prior to injection. All other chemicals used were of the highest analytical grade.

Induction of carnitine deficiency using D-carnitine

Experimental animal models of carnitine deficiency were developed by Paulson and Shug [20], Whitmer [21] and Tsoko *et al.* [22]. In the current study, carnitine deficiency was induced in rats by daily I.P. injection of D-carnitine, the inactive isomer, at a dose level of 250 mg/kg/day according to Sayed-Ahmed *et al.* [23]. Depletion of L-carnitine by D-carnitine occurs via an exchange of the D- and L-isomers across the cell membrane where the intracellular L-carnitine was shown to exchange with the extracellular D-carnitine. Moreover, D-carnitine possesses an inhibitory effect upon carnitine transferase enzymes and competitive inhibitory effect upon L-carnitine uptake [20–22].

Experimental design

GM treatment protocol used in this study to develop ARF has been previously reported [5,24]. A total of 60 male Wistar albino rats were used and divided at random into six groups of 10 animals each. Rats of group 1 (control group) received I.P. injection of normal saline (2.5 ml/kg) for 8 successive days. Animals in group 2 (GM group) were daily injected with GM (80 mg/kg, I.P.) for 8 consecutive days. Animals in group 3 (carnitine-supplemented group) were given L-carnitine (200 mg/kg/day, I.P.) for 8 successive days according to Kopple *et al.* [16]. Animals in group 4 (carnitine-depleted group) were given D-carnitine (250 mg/kg/day, I.P.) for 8 successive days. Rats of group 5 (GM plus D-carnitine) received a daily I.P. injection of D-carnitine (250 mg/kg/day) 1 h prior to GM (80 mg/kg/day) for 8 consecutive days. Rats of group 6 (GM plus L-carnitine) received a daily I.P. injection of L-carnitine (200 mg/kg/day) 1 h prior to GM (80 mg/kg/day) for 8 consecutive days. Immediately after the last dose of the treatment protocol, 24-h urine was collected for

monitoring urinary carnitine excretion. Animals were then anaesthetized with ether, and blood samples were obtained by heart puncture. Serum was separated for measurement of blood urea nitrogen (BUN) and creatinine. Animals were then sacrificed by decapitation after exposure to ether in a desiccator kept in a well-functioning hood and both kidneys were isolated. The right kidneys were quickly excised, washed with saline, blotted with a piece of filter paper, decapsulated and homogenized in normal saline or 6% perchloric acid as indicated in the procedures of measurement of each parameter, using a Branson sonifier (250, VWR Scientific, Danbury, CO, USA). The left kidneys were removed for histopathological examination. They were fixed in 10% neutral buffered formalin, embedded in paraffin wax, sectioned at 3 μ m, stained with Haematoxylin and Eosin (H & E) stain and slides were prepared for light microscopic examination.

Histopathological examination of kidney tissues

To avoid any type of bias, the slides were coded and examined by a histopathologist who was blinded to the treatment groups. Grading of injury was according to the following parameters: (1) glomerular injury (% of renal parenchyma involvement): none = 0, <25% of glomeruli exhibit non-specific features of injury = +1, 25–50% of glomeruli exhibit non-specific features of injury = +2, 50–75% of glomeruli exhibit non-specific features of injury = +3, and >75% of glomeruli exhibit non-specific features of injury = +4; (2) acute tubular necrosis (% of renal parenchyma involvement): none = 0, <25% of tubules of entire renal parenchyma = +1, 25–50% of tubules of entire renal parenchyma = +2, 50–75% of tubules of entire renal parenchyma = +3, and >75% of tubules of entire renal parenchyma = +4; (3) tubulointerstitial inflammatory infiltrates: none = 0, leucocytes confined within interstitium = +1, and leucocytes infiltrating the interstitium and tubular epithelial cells = +2. The scoring system used was according to the following scale: (A) no nephrotoxicity: 0–1, (B) mild nephrotoxicity: 2–4, (C) moderate nephrotoxicity: 5–7, (D) severe nephrotoxicity: 8–10.

Assessment of BUN and serum creatinine

BUN and serum creatinine concentrations were measured spectrophotometrically according to the methods of Tobacco *et al.* [25] and Fabiny and Ertingshausen [26], respectively.

Determination of reduced glutathione and lipid peroxidation in kidney tissues

The tissue levels of the acid soluble thiols, mainly GSH, were assayed spectrophotometrically at 412 nm, according to the method of Ellman [27], using a Shimadzu (Tokyo, Japan) spectrophotometer. The degree of lipid peroxidation in kidney tissues was determined by measuring thiobarbituric acid reactive substances (TBARS) in the supernatant from tissue homogenate [28]. The homogenates were centrifuged at 3500 rpm and supernatant was collected and used for the estimation of TBARS. The absorbance was measured spectrophotometrically at 532 nm.

Determination of total nitrate/nitrite concentrations in kidney tissues

Total nitrate/nitrite (NO(x)) in kidney tissues was measured as nitrite according to the method of Miranda *et al.* [29]. The assay is based on the reduction of nitrate by vanadium trichloride combined with detection by the acidic Griess reaction. The diazotization of sulfanilic acid with nitrite at acidic pH and subsequent coupling with *N*-(10 naphthyl)-ethylenediamine produced an intensely coloured product that is measured spectrophotometrically at 540 nm.

Determination of adenosine triphosphate and adenosine diphosphate in kidney tissues

Adenosine triphosphate (ATP) and adenosine diphosphate (ADP) were determined in kidney tissues using HPLC according to the method reported by Botker *et al.* [30]. In brief, kidney tissues were homogenized in ice-cold 6% perchloric acid and centrifuged at 1000 rpm for 15 min at 0.5°C, and the supernatant fluid was injected into HPLC after neutralization to pH 6–7. Chromatographic separation was performed at a flow rate of 1.2 ml/min, using ODS-Hypersil, 150 \times 4.6 mm I.D., 5 μ m column (Supelco SA, Gland, Switzerland) and 75 mM ammonium dihydrogen phosphate as a mobile phase. The peak elution was followed at 254 nm.

Determination of total carnitine in urine and kidney tissues

Total carnitine concentrations were determined in urine and kidney tissues according to the method reported by Prieto *et al.* [31]. In brief, carnitine reacts with acetyl-CoA forming acetylcarnitine in a reaction mediated by carnitine acetyltransferase enzyme. The liberated CoA-SH reacts with 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB) to form the thiophenolate ion, whose generation is proportional to the amount of carnitine and can be measured spectrophotometrically at 412 nm. Urine and kidney tissues were deproteinized with equal volume of ice-cold 0.6 M perchloric acid and allowed to stand in an ice bath for 10 min. The mixture was centrifuged at 1000 *g* at 4°C for 5 min. The supernatant was used directly for measuring free carnitine after neutralization with 1.2 M potassium carbonate. For the assay of total carnitine, a part of supernatant was mixed with 1 M KOH and incubated at 37°C for 20 min for the hydrolysis of acylcarnitines. Carnitine level was computed from a calibration curve for carnitine hydrochloride.

Determination of CoA-SH and acetyl-CoA in isolated rat kidney mitochondria

Rat kidney mitochondria were isolated using the isolation buffer that contained 0.21 M mannitol, 0.07 M sucrose, 5 mM Tris-HCl (pH 7.4) and 1 mM EGTA. In brief, kidney tissues were homogenized in mitochondrial isolation buffer and centrifuged at 1000x *g* for 10 min at 4°C. The resulting supernatant was decanted and further centrifuged at 1000x *g* for 10 min and the resulting pellet (mitochondria) was resuspended in the isolation buffer. The protein concentration of the mitochondrial was determined by the BioRad protein assay according to the method of Bradford [32]. Free CoA-SH and acetyl-CoA were determined in isolated heart mitochondria using HPLC (Jasco Corporation, Kyoto, Japan) according to Lysiak *et al.* [33]. In brief, mitochondria were mixed with ice-cold 6% perchloric acid, centrifuged at 300x *g* for 5 min at 0.5°C, and the resulting supernatant fluid was neutralized to pH 6–7 and then injected into HPLC. Chromatographic separation was performed using ODS-Hypersil, 150 × 4.6 mm I.D., 5 μm column (Supelco SA, Gland, Switzerland). The UV detector was operated at 254 nm and set at 0.005. A mobile phase of 220 mM potassium phosphate containing 0.05% dithioglycol (A) and 98% methanol, 2% chloroform (B) was used. The flow rate was 0.6 ml/min, and the gradient used was as follows: at zero time, 94% A and 6% B; at 8 min, 92% A and 8% B; at 14 min, 87% A and 13% B; at 25 min, 80% A and 20% B; at 40 min, 55% A and 45% B; at 45 min, 55% A and 45% B; and at 60 min, 94% A and 6% B.

Statistical analysis

Differences between obtained values (mean ± SEM, *n* = 10) were carried out by one-way analysis of variance (ANOVA) followed by the Tukey–Kramer multiple comparison test. A *P*-value of 0.05 or less was taken as a criterion for a statistically significant difference.

Results

Figure 1 shows the effects of GM on nephrotoxicity indices, serum creatinine (A) and BUN (B), in carnitine-depleted and supplemented rats. Treatment with GM (80 mg/kg) for 8 successive days resulted in a significant 186% and 213% increase in serum creatinine and BUN, respectively, while administration of either D-carnitine or L-carnitine alone for 8 successive days showed non-significant change as compared to the control group. Combined treatment with GM and D-carnitine resulted in a significant 50% and 49% increase in serum creatinine and BUN, respectively, as compared to GM alone. Interestingly, administration of L-carnitine in combination with GM resulted in a complete reversal of GM-induced increase in serum creatinine and BUN to the control values.

The effects of GM, D-carnitine, L-carnitine and their combination on total carnitine level in kidney tissues are shown in Figure 2. Treatment with GM resulted in a

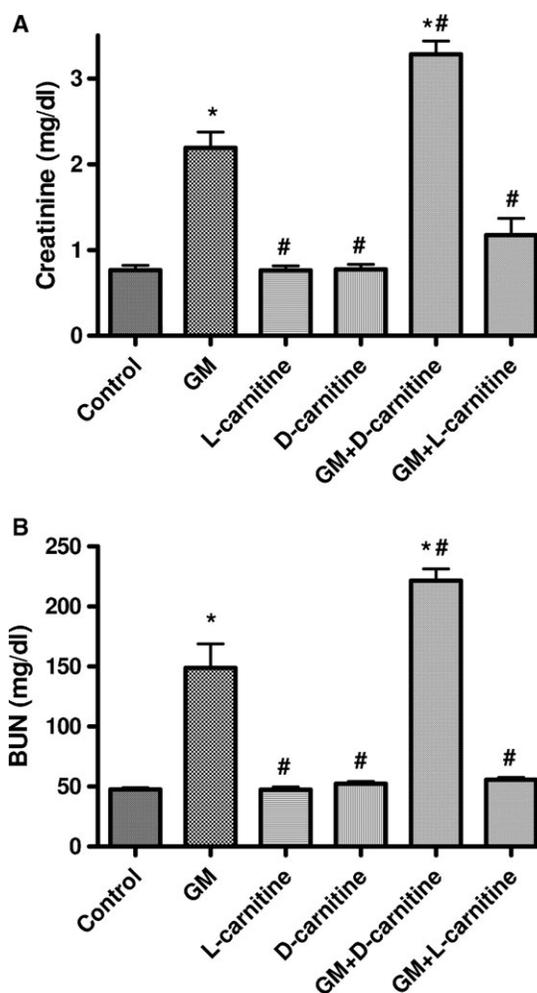


Fig. 1. Effect of GM, L-carnitine, D-carnitine and their combination on the levels of serum creatinine (A) and BUN (B) in rats. Data are presented as mean ± SEM (*n* = 10). *.# significant change from control and GM, respectively, at *P* < 0.05 using ANOVA followed by Tukey–Kramer as a post-ANOVA test.

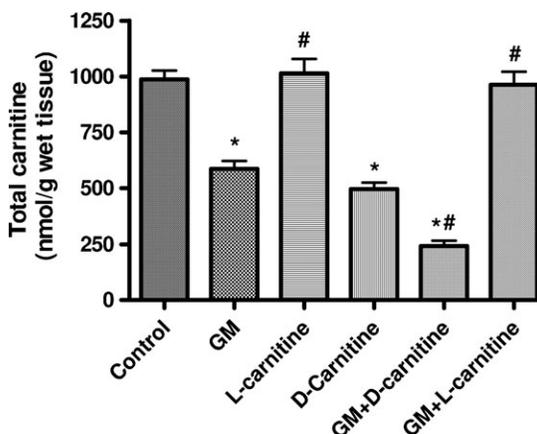


Fig. 2. Effect of GM, L-carnitine, D-carnitine and their combination on total carnitine levels in rat kidney tissues. Data are presented as mean ± SEM (*n* = 10). *.# significant change from control and GM, respectively, at *P* < 0.05 using ANOVA followed by Tukey–Kramer as a post-ANOVA test.

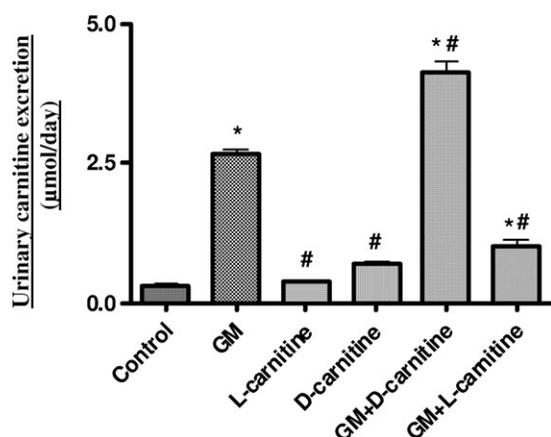


Fig. 3. Effect of GM, L-carnitine, D-carnitine and their combination on urinary carnitine excretion in rats. Data are presented as mean \pm SEM ($n = 10$). *,# significant change from control and GM, respectively, at $P < 0.05$ using ANOVA followed by Tukey–Kramer as a post ANOVA test.

significant 41% decrease in total carnitine level in kidney tissues, while administration of D-carnitine alone caused a significant 50% decrease as compared to the control group. Combined treatment with GM and D-carnitine resulted in a significant 75% and 59% decrease in the total carnitine level in kidney tissues as compared to the control and GM groups, respectively. Administration of L-carnitine to GM-treated rats resulted in a complete reversal of the decrease in total carnitine level in kidney tissues, induced by GM, to the normal levels.

The effects of GM, D-carnitine, L-carnitine and their combination on urinary carnitine excretion are shown in Figure 3. Treatment with GM resulted in a significant 8.3-fold increase in carnitine excretion as compared to the control group. Administration of either L-carnitine or D-carnitine alone resulted in a non-significant change in carnitine excretion as compared to the control group. Combined treatment with GM and D-carnitine resulted in a significant 13.0- and 1.6-fold increase in carnitine excretion as compared to the control and GM groups, respectively. Administration of L-carnitine to GM-treated rats resulted in a complete reversal of the increase in carnitine excretion, induced by GM, to the normal levels.

Table 1 shows the effects of GM, D-carnitine, L-carnitine and their combination on the intramitochondrial CoA-SH

Table 1. Effect of gentamicin, L-carnitine, D-carnitine and their combination on CoA-SH and acetyl-CoA levels in isolated rat kidney mitochondria

Treatment groups	CoA-SH (nmol/mg protein)	Acetyl-CoA (nmol/mg protein)
Control	2.20 \pm 0.10	7.93 \pm 0.38
GM	1.25 \pm 0.08*	14.37 \pm 0.34*
L-carnitine	8.30 \pm 0.43*,#	2.32 \pm 0.16*,#
D-carnitine	1.62 \pm 0.07	10.4 \pm 0.57*,#
GM + D-carnitine	0.62 \pm 0.07*	15.35 \pm 0.62*
GM + L-carnitine	2.52 \pm 0.09#	7.62 \pm 0.34#

Data are presented as mean \pm S.E.M. ($n = 10$).

*,# significant change from control and GM, respectively, at $P < 0.05$ using ANOVA followed by Tukey–Kramer as a post-ANOVA test.

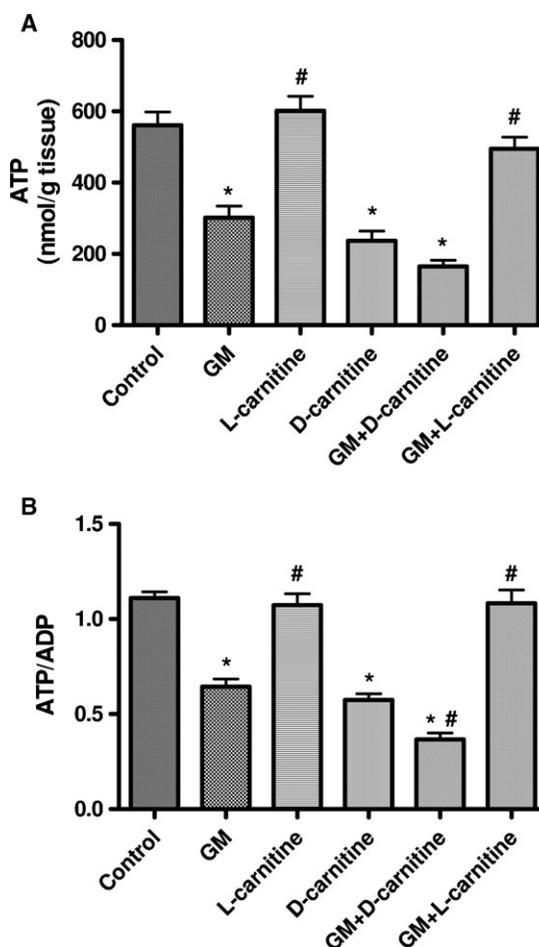


Fig. 4. Effect of GM, L-carnitine, D-carnitine and their combination on the levels of adenosine triphosphate (A) and ATP/ADP ratio (B) in rat kidney tissues. Data are presented as mean \pm SEM ($n = 10$). *,# significant change from control and GM, respectively, at $P < 0.05$ using ANOVA followed by Tukey–Kramer as a post-ANOVA test.

and acetyl-CoA. Treatment with GM resulted in a significant 43% decrease in CoA-SH, while administration of L-carnitine resulted in a significant 277% increase as compared to the control. Combined treatment with GM and D-carnitine resulted in a significant 72% decrease in the intramitochondrial CoA-SH as compared to the control. Administration of L-carnitine to GM-treated rats resulted in a complete reversal of GM-induced decrease in CoA-SH to the normal levels. On the other hand, GM caused a significant 81% increase in acetyl-CoA, whereas, L-carnitine induced a significant 71% decrease as compared to the control. Combined treatment with GM and D-carnitine resulted in a significant 94% increase in acetyl-CoA compared to the control. Interestingly, administration of L-carnitine in combination with GM resulted in a complete reversal of GM-induced increase in acetyl-CoA to the control values.

Figure 4 shows the effects of GM, D-carnitine, L-carnitine and their combination on the level of ATP (A) and ATP/ADP ratio (B), an index mitochondrial function and energy production in rat kidney tissues. Administration of GM for 8 successive days resulted in a significant 46% and 42% decrease in ATP and ATP/ADP ratio,

respectively, as compared to the control group. Treatment with D-carnitine for 8 successive days (carnitine-depleted rats) resulted in a significant 58% and 48% decrease in ATP and ATP/ADP ratio, respectively, while administration of L-carnitine alone (carnitine-supplemented rats) induced non-significant change as compared to the control group. Administration of GM to carnitine-depleted rats resulted in a significant 45% and 43% decrease in ATP and ATP/ADP, respectively, as compared to GM alone. Interestingly, administration of L-carnitine in combination with GM resulted in a complete reversal of GM-induced decrease in ATP and ATP/ADP to the control values.

The effects of GM, D-carnitine, L-carnitine and their combination on the levels of oxidative stress biomarkers, TBARS (A), GSH (B) and NOx (C), in kidney tissues are shown in Figure 5. Treatment with GM resulted in a significant 59% decrease in GSH and 85% and 63% increase in TBARS and NOx, respectively, as compared to the control group. Treatment with L-carnitine for 8 successive days showed a significant 59% increase in GSH and a significant 66% decrease in NOx, respectively. Administration of D-carnitine or L-carnitine in combination with GM completely reversed the decrease in GSH and the increase in TBARS and NOx levels, induced by GM, to the control values.

The effects of GM alone and in carnitine-depleted and supplemented rats on histopathological changes in kidney tissues are shown in Figure 6. The histopathological examination of kidney specimens from control rats revealed no nephrotoxicity (total score: 1) where mesangial cell proliferation was seen in <25% of the glomeruli (Figure 6A). Sections from rats treated with GM alone (Figure 6B) showed clear signs of moderate nephrotoxicity (total score: 5) manifested as interstitial and intraepithelial inflammation in <25% of tubules, acute tubular necrosis in 25–50% of the renal parenchyma and 25–50% focal perivascular inflammation. Sections from rats treated with GM plus D-carnitine (Figure 6C) showed clear signs of severe nephrotoxicity (total score: 8) manifested as acute tubular necrosis involving >75% of renal parenchyma and acute and chronic interstitial and intraepithelial inflammations involving 25–50% of tubules (C1) and focal mesangial proliferation in 25–50% of the glomeruli (C2). Interestingly, kidney specimens from rats treated with GM plus L-carnitine (Figure 6D) showed mild nephrotoxicity (total score: 2) manifested as significant improvement in glomeruli and renal tubules where <25% of glomeruli and renal tubules exhibited focal necrosis.

Discussion

Recent studies in our laboratory [34–36] and others [23,37] have demonstrated the progression of cisplatin and carboplatin-induced nephrotoxicity, cardiomyopathy and hepatotoxicity under condition of carnitine deficiency and that carnitine supplementation attenuates these multiple organ toxicity. Although kidney is the main organ responsible for endogenous synthesis of L-carnitine, we could not find any study to date investigating the effects of GM on renal handling of carnitine under conditions of carnitine

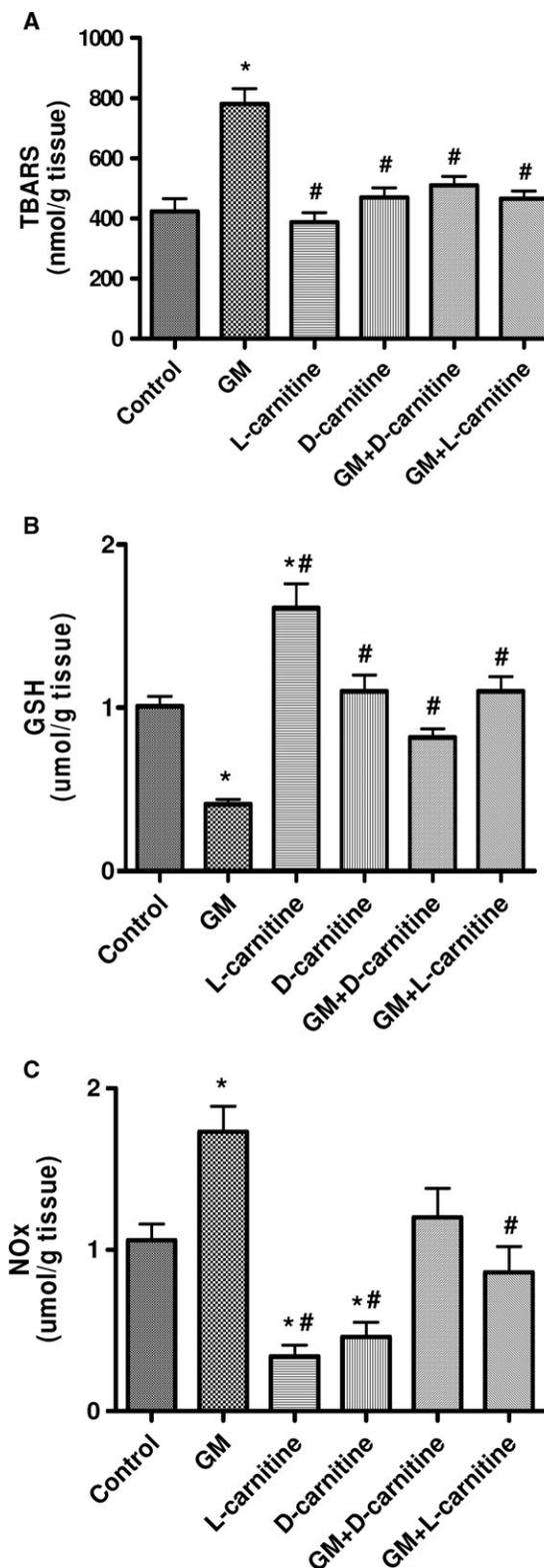


Fig. 5. Effect of GM, L-carnitine, D-carnitine and their combination on the levels of thiobarbituric acid reactive substances (A), reduced glutathione (B) and total nitrate/nitrite (C) in rat kidney tissues. Data are presented as mean \pm SEM ($n = 10$). *.# significant change from control and GM, respectively, at $P < 0.05$ using ANOVA followed by Tukey–Kramer as a post-ANOVA test.

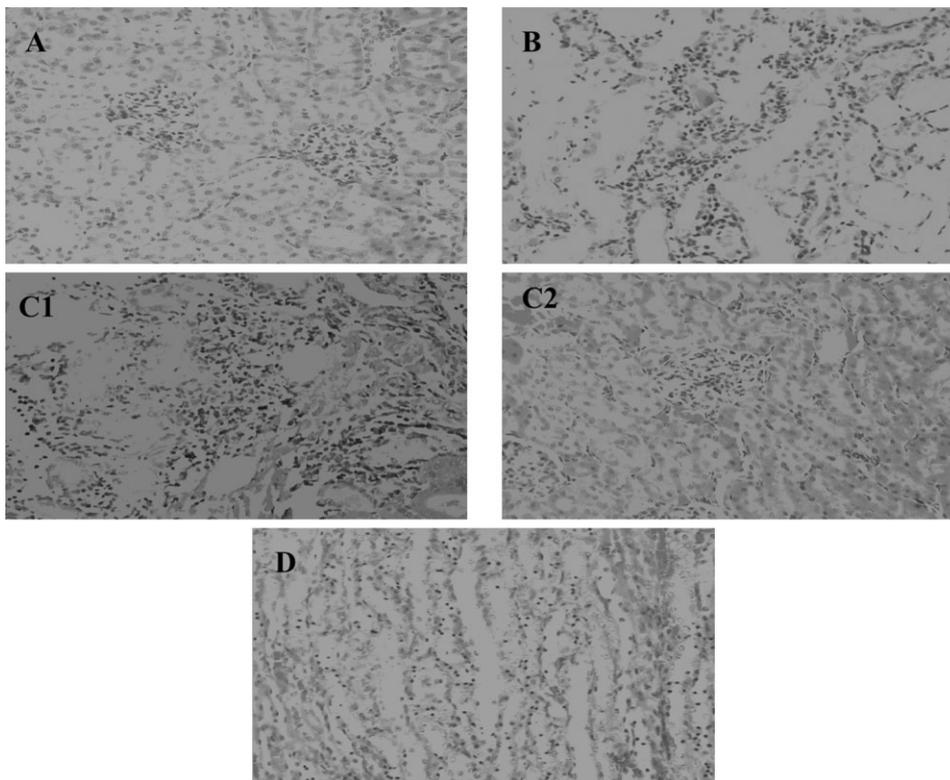


Fig. 6. Photomicrographs of the kidney specimen stained with haematoxylin and eosin. **(A)** Kidney from control rat under $\times 10$ magnification showing normal renal parenchyma and two glomeruli demonstrating mesangial cells proliferation in $<25\%$ of the glomerul. **(B)** Kidney of rat treated with GM alone under $\times 40$ magnification showing dense aggregates of inflammatory cells surrounding and infiltrating the tubules. **(C)** Kidney of rat treated with GM plus D-carnitine under $\times 20$ magnification showing an admixture of inflammatory cells surrounding the tubules and within the tubular epithelium (C1) and under $\times 40$ magnification showing a glomerulus with increased number of mesangial cells (C2). **(D)** Kidney of rat treated with GM plus L-carnitine under $\times 40$ magnification showing renal tubules lined with flattened epithelial cells exhibiting hyperchromatic nuclei, consistent with epithelial regeneration.

depletion and supplementation. Taken together, this prompted us to investigate, for the first time, whether carnitine deficiency is a risk factor and should be viewed as a mechanism during development of GM-induced ARF, and if so, what are the consequences of this condition on kidney function as well as exploring if carnitine supplementation could offer protection with the possible mechanisms underlying this protection.

Results from this study demonstrated that GM increased nephrotoxicity indices, serum creatinine (186%) and BUN (213%), and caused severe histopathological lesions in kidney tissues (Figure 6B), representing ARF. Our results confirm those previously reported [2,5,38]. On the other hand, combined treatment with D-carnitine and GM aggravated nephrotoxicity indices. This effect could be due to carnitine deficiency with subsequent impairment of fatty acid oxidation and shifting metabolism into carnitine-independent or non-lipid energy substrates. This speculation is consistent with data presented by Ahmed *et al.* which reported that carnitine supplementation to patients undergoing haemodialysis decreased protein catabolism, thereby, reducing serum concentration of the products of protein catabolism, including BUN and creatinine [39].

The observed decrease of total carnitine content in kidney tissues by GM could be a secondary event following

GM-induced inhibition of endogenous synthesis and/or inhibition of tubular reabsorption of carnitine. Since kidney is the major site for endogenous carnitine biosynthesis and 95% of filtered carnitine is reabsorbed by the proximal tubules of the nephron [19], tubular damage induced by GM [12,16] may lead to inhibition of endogenous carnitine biosynthesis and increases its urinary losses with the consequent secondary deficiency of the molecule. Moreover, hyponatraemia induced by GM [40] might hinder the action of sodium-dependent carnitine transporter, which may worsen the condition. This is in line with our data which showed that urinary carnitine excretion was increased by administration of GM. Similarly, earlier studies have reported increased urinary excretion of carnitine in patients following treatment with ifosfamide [41] and cisplatin [42]. Although, D-carnitine alone decreased kidney carnitine content more than GM, only GM increased serum creatinine and BUN. This argues against carnitine deficiency as a risk factor in GM-induced ARF. A possible explanation for this is that D-carnitine, via its previously reported antioxidant effect [23,35,36], causes stabilization of cell and mitochondrial membranes, whereas GM, via increasing ROS and RNS and decreasing GSH in kidney tissues [5,7,12,38], causes irreversible modification of membrane structures and functions. It is well documented that increased

production of ROS and RNS by GM in kidney tissues is associated with severe glomerular injury and mesangial cell proliferation and apoptosis [43]. Although our results showed that both L-carnitine and D-carnitine have similar free radical scavenging activity against generation of both ROS and RNS (Figure 5), only L-carnitine prevents while D-carnitine aggravates GM-induced ARF. Therefore, one can anticipate that oxidative stress is not the only mechanism in GM-induced nephrotoxicity, but rather carnitine deficiency plays an important role.

In the current study, the progressive decrease (75%) of carnitine level in kidney tissue following concomitant treatment with GM and D-carnitine was parallel to the marked increase in serum creatinine, BUN, urinary carnitine excretion and the massive degenerative changes in kidney tissues, which may point to the possible consideration of carnitine deficiency as a risk factor in GM-induced ARF. D-carnitine produced such tubular damage via its depletion of carnitine, as did GM partly through oxidative stress [12,38] and partly due to carnitine depletion. This aggravated nephrotoxicity could be explained on the basis of carnitine deficiency with subsequent impairment of fatty acid oxidation and ATP production. It is well known that L-carnitine is an essential cofactor for mitochondrial transport and oxidation of long-chain fatty acids [17]. Both GM and D-carnitine would deplete carnitine or both would impair the beta-oxidation of long-chain fatty acids with the consequent increase in the accumulation of toxic fatty acid intermediates and decrease in ATP production. This was supported by the marked decrease of ATP and ATP/ADP in kidney tissues observed in carnitine-depleted rats, which renders the kidney tissues vulnerable to damage by GM. Fascinatingly, L-carnitine supplementation completely reversed GM-induced decrease in ATP and ATP/ADP ratio to the control values.

Data reported here demonstrate that GM administration significantly decreased intramitochondrial CoA-SH, an indispensable activator in most of the mitochondrial energy-providing systems (TCA cycle, fatty acid beta-oxidation and pyruvate oxidation). This effect could be explained on the basis GM-induced generation of ROS and RNS with the consequent depletion of SH-containing compound including CoA-SH. It is well known that, L-carnitine acts as a buffering system by removing the accumulated acyl-CoA in mitochondria when the normal metabolic pathway of an acyl-CoA is blocked or when an acyl-CoA is formed and cannot be further metabolized [44]. Moreover, it has been reported that L-carnitine stimulates the mitochondrial efflux of acetyl-CoA in the form of acetyl-carnitine in a reaction mediated by carnitine acetyltransferase [45]. This could explain the increase in the intramitochondrial CoA-SH and the decreases in acetyl-CoA observed in our study with subsequent improvement in substrate utilization, ATP production and mitochondrial function by L-carnitine. In conclusion, data from this study suggest, for the first time, that: (i) GM-induced nephrotoxicity leads to increased urinary losses of carnitine; (ii) carnitine deficiency is a risk factor and should be viewed as a mechanism during the development of GM-induced ARF; and (iii) carnitine supplementation ameliorates the severity of GM-induced kidney dysfunction by increasing the intramitochondrial CoA-

SH/acetyl-CoA ratio and ATP production. The results from the present study warrants a detailed mechanistic study to investigate the molecular mechanisms whereby carnitine deficiency aggravates and carnitine supplementation ameliorates GM-induced ARF.

Acknowledgements. Authors of this study are grateful to Dr. Zaven Orfalin, Sigma-Tau Pharmaceuticals, Pomezia, Italy, for providing us with L-carnitine and D-carnitine. The present work was supported by operating grant from Research Center, College of Pharmacy, King Saud University (CPRC 242).

Conflict of interest statement. None declared.

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Received for publication: 28.6.09; Accepted in revised form: 12.8.09