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REVERSAL OF CISPLATIN-INDUCED CARNITINE DEFICIENCY AND ENERGY STARVATION BY PROPIONYL-L-CARNITINE IN RAT KIDNEY TISSUES

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SUMMARY

1. The present study examined whether propionyl-L-carnitine (PLC) could prevent the development of cisplatin (CDDP)-induced acute renal failure in rats.

2. Forty adult male Wistar albino rats were divided into four groups. Rats in the first group were injected daily with normal saline (2.5 mL/kg, i.p.) for 10 consecutive days, whereas the second group received PLC (250 mg/kg, i.p.) for 10 consecutive days. Animals in the third group were injected daily with normal saline for 5 consecutive days before and after a single dose of CDDP (7 mg/kg, i.p.). Rats in the fourth group received a combination of PLC (250 mg/kg, i.p.) for 5 consecutive days before and after a single dose of CDDP (7 mg/kg, i.p.). On Day 6 following CDDP treatment, animals were killed and serum and kidneys were isolated for analysis.

3. Injection of CDDP resulted in a significant increase in serum creatinine, blood urea nitrogen (BUN), thiobarbituric acid-reactive substances (TBARS) and total nitrate/nitrite (NO_x), as well as a significant decrease in reduced glutathione (GSH), total carnitine, ATP and ATP/ADP in kidney tissues.

4. Administration of PLC significantly attenuated the nephrotoxic effects of CDDP, manifested as normalization of the CDDP-induced increase in serum creatinine, BUN, TBARS and NO_x and the CDDP-induced decrease in total carnitine, GSH, ATP and ATP/ADP in kidney tissues.

5. Histopathological examination of kidney tissues from CDDP-treated rats showed severe nephrotoxicity, in which 50–75% of glomeruli and renal tubules exhibited massive degenerative changes. Interestingly, administration of PLC to CDDP-treated rats resulted in a significant improvement in glomeruli and renal tubules, in which less than 25% of glomeruli and renal tubules exhibited focal necrosis.

6. Data from the present study suggest that PLC prevents the development of CDDP-induced acute renal injury by a mechanism related, at least in part, to the ability of PLC to increase intracellular carnitine content, with a consequent improvement in mitochondrial oxidative phosphorylation and energy production, as well as its ability to decrease oxidative stress. This will open new perspectives for the use of PLC in the treatment of renal diseases associated with or secondary to carnitine deficiency.

Key words: carnitine deficiency, cisplatin, nephrotoxicity, propionyl-L-carnitine.

INTRODUCTION

Although cisplatin (*cis*-diaminedichloroplatinum II; CDDP) is an effective antineoplastic agent used in the treatment of various types of tumours,[1] its optimal clinical usefulness is usually limited secondary to its dose-related nephrotoxicity.[2,3] The precise mechanisms underlying this toxicity are not yet fully elucidated. However, several mechanisms have been suggested, among which oxidative stress is the best studied mechanism. Oxidative stress is caused mainly by increasing lipid peroxidation and depletion of glutathione, which, in turn, induces apoptosis of renal proximal tubule cells and consequent kidney dysfunction.[4–6] Furthermore, owing to the toxic effect of CDDP on renal tubules, CDDP inhibits specific membrane transport systems, with a consequent increase in the excretion of a number of essential endogenous substances, including L-carnitine and amino acids.[7,8]

L-Carnitine is a natural endogenous cofactor for the translocation of long-chain fatty acids from the cytoplasmic compartment into the mitochondria, where beta-oxidation enzymes are located. L-Carnitine is synthesised mainly in the kidney and liver and can be obtained exogenously from dietary sources (mainly red meat and dairy products).[9,10] L-Carnitine shows potential protective effects against many mitochondrial toxic agents.[11–13] Under normal physiological conditions, L-carnitine is highly conserved, because 90% of the filtered L-carnitine is reabsorbed at the level of the proximal tubule.[14] It has been reported that CDDP therapy is associated with secondary carnitine deficiency owing to inhibition of carnitine reabsorption, enhancement of carnitine excretion and inhibition of endogenous carnitine synthesis.[8] Earlier studies have reported that carnitine deficiency is a risk factor in CDDP-induced nephrotoxicity and that L-carnitine can protect against this toxicity without interfering with the antitumour activity of CDDP.[6,15] However, in these studies, reversal of carnitine deficiency and the protection achieved by L-carnitine was not complete. Therefore, in the present study we have selected the natural short-chain derivative of L-carnitine, propionyl-L-carnitine (PLC), which has several therapeutic advantages over L-carnitine.[16] Although recent studies in our laboratory have

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demonstrated, for the first time, that PLC supplementation prevents the development of CDDP-induced cardiomyopathy[16] and hepatotoxicity,[17] the effects of PLC supplementation on CDDP-induced nephrotoxicity have not yet been investigated. Therefore, the present study haswas undertaken to investigate whether carnitine supplementation, using PLC, could prevent the development of CDDP-induced nephrotoxicity and to investigate the mechanism(s) whereby PLC can protect against this toxicity.

METHODS

Animals

Adult male Wistar albino rats, weighing 200–230 g, were obtained from the Animal Care Center, College of Pharmacy, King Saud University (KSU). Rats were housed in metabolic cages under controlled environmental conditions (25°C and a 12 h light–dark cycle). Animals had free access to pulverized standard rat pellet food and tap water, unless indicated otherwise. The protocol of the present study was approved by the Research Ethics Committee of College of Pharmacy, KSU.

Materials

Cisplatyl vials (Laboratoire Roger Bellon, Neuilly-sur-Seine, France) were purchased from the King Khalid University Hospital drug store, KSU. Each Cisplatyl vial contained 10 mg cisplatinum, 90 mg sodium chloride and 100 mg mannitol in a dry lyophilized powder form. The contents of the vials were freshly dissolved in sterile water for injection. Propionyl-L-carnitine was kindly supplied by Dr Menotti Calvani (Sigma-Tau Pharmaceuticals, Pomezia, Roma, Italy). It was supplied as a white powder in a non-commercial plastic bottle containing 100 g and was freshly dissolved in normal saline prior to injection. All other chemicals used were of the highest analytical grade.

Experimental design

Forty adult male Wistar albino rats were used in the present study. Rats were randomly divided into four groups of 10 rats each. Rats in the first group were injected daily with normal saline (2.5 mL/kg, i.p.) for 10 consecutive days, whereas rats in the second group received PLC (250 mg/kg, i.p.) for 10 consecutive days according to Sayed-Ahmed *et al*.[18] Animals in the third group were injected daily with normal saline for 5 consecutive days before and after a single dose of CDDP (7 mg/kg, i.p.). It is well established that the selected dose of CDDP used in the present study (7 mg/kg) is the standard dose that induces nephrotoxicity.[15,19,20] Rats in the fourth group received a combination of PLC (250 mg/kg, i.p.) for 5 consecutive days before a single dose of CDDP (7 mg/kg, i.p.) and for a further 5 consecutive days after CDDP treatment. It is worth mentioning that the dose of PLC used in the present study is half the dose of L-carnitine used previously in our laboratory to protect against CDDP-induced nephrotoxicity.[15] This combined treatment protocol of PLC and CDDP has been adapted from previous studies.[15–17] On Day 6 after CDDP treatment, rats were anaesthetized with ether and blood samples were obtained by heart puncture. Serum was separated for the measurement of creatinine, blood urea nitrogen (BUN) and total carnitine. Animals were then killed by decapitation after exposure to ether in a dessicator 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 described for the measurement of each parameter, using a Branson sonifier (250; VWR Scientific, Danbury, CN, USA). The left kidneys were removed for histopathological examination. Kidneys were fixed in 10% neutral buffered formalin, embedded in paraffin wax, sectioned at 3 μm, stained with haematoxylin and eosin (H&E) stain and slides prepared for light microscopic examination.

Histopathological examination of kidney tissues

To avoid any type of bias, slides were coded and examined by a histopathologist who was blinded to the treatment groups. Grading of injury was as described below.

1. Glomerular injury (% of renal parenchyma involvement): 0, none; 1, < 25% of glomeruli exhibit non-specific features of injury; 2, 25–50% of glomeruli exhibit non-specific features of injury; 3, 50–75% of glomeruli exhibit non-specific features of injury; and 4, > 75% of glomeruli exhibit non-specific features of injury.
2. Acute tubular necrosis (% of renal parenchyma involvement): 0, none; 1, < 25% of tubules of the entire renal parenchyma are necrotic; 2, 25–50% of tubules of the entire renal parenchyma are necrotic; 3, 50–75% of tubules of the entire renal parenchyma are necrotic; and 4, > 75% of tubules of the entire renal parenchyma are necrotic.
3. Tubulointerstitial inflammatory infiltrates: 0, none; 1, leucocytes confined within the interstitium; and 2, leucocytes infiltrating the interstitium and tubular epithelial cells.

The scoring system used to describe nephrotoxicity was according as follows: 0, no nephrotoxicity; 1–3, mild nephrotoxicity; 4–7, moderate nephrotoxicity; and 8–10, severe nephrotoxicity.

Assessment of blood urea nitrogen and serum creatinine

Blood urea nitrogen (BUN) and serum creatinine concentrations were measured spectrophotometrically according to the methods of Tabacco *et al*.[21] and Fabiny and Ertingshausen,[22] respectively.

Determination of reduced glutathione and lipid peroxidation in kidney tissues

The tissue levels of the acid-soluble thiols, mainly reduced glutathione (GSH), were assayed spectrophotometrically at 412 nm, according to the method of Ellman,[23] using a Shimadzu (Tokyo, Japan) spectrophotometer. The GSH content was expressed as μmol/g wet tissue. The degree of lipid peroxidation in kidney tissues was determined by measuring thiobarbituric-acid reactive substances (TBARS) in the supernatant tissue from homogenate.[24] Homogenates were centrifuged at 1500 *g* and the supernatant was collected and used for the estimation of TBARS. Absorbance was measured spectrophotometrically at 532 nm and concentrations were expressed as nmol TBARS/g wet tissue.

Determination of total nitrate/nitrite concentrations in kidney tissues

Total nitrate/nitrite (NO_x), an index of nitric oxide (NO) production, was measured as the stable end-product nitrite according to the method of Miranda *et al*.[25] This assay is based on the reduction of nitrate by vanidium trichloride combined with detection by the acidic Griess reaction. The diazotization of sulphanilic 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. Levels of NO_x were expressed as μmol/g wet tissue.

Determination of total carnitine levels in serum and kidney tissues

Kidney homogenate was prepared in ice-cold 6% perchloric acid and centrifuged at 8000 g for 10 min. The supernatant was used for the estimation of free carnitine, whereas the remaining pellet was used for the determination of long-chain acyl carnitine after hydrolysis in 1 mol/L KOH at 65°C for 1 h according to the methods of Alhomida.[26] Carnitine was determined in serum and tissue samples using HPLC after precolumn derivatization with L-aminoanthracene, as described previously.[27] The mobile phase was prepared

by mixing 700 mL of 0.1 mol/L ammonium acetate, pH 3.5, with 300 mL acetonitrile. Chromatographic separation was performed at a flow rate of 1.5 mL/min, using a Kromasil C18, 250 × 4.6 mm ID 5 μm column (Saulentechnik Knayer, Berlin, Germany). The excitation and emission wavelengths of the spectrofluorimeter were 248 and 418 nm, respectively.

Determination of high-energy nucleotide in kidney tissues

Adenosine triphosphate and ADP were determined in kidney tissues using HPLC according to the methods of Botker *et al*.[28] Briefly, kidney tissue was homogenized in ice-cold 6% perchloric acid, centrifuged at 110 *g* for 15 min at 0.5°C and the supernatant injected into the HPLC after neutralization to pH 6–7 using KOH. Chromatographic separation was performed at a flow rate of 1.2 mL/min, using an ODS-Hypersil 150 × 4.6 mm ID 5 μm column (Supelco, Gland, Switzerland) and 75 mmol/L ammonium dihydrogen phosphate as the mobile phase. Peak elution was followed at 254 nm.

Statistical analysis

Differences between values (mean±SEM; $n = 10$) were analysed by one-way analysis of variance (ANOVA) followed by the Tukey–Kramer multiple comparison test. $P \leq 0.05$ was taken to indicate statistical significance.

RESULTS

Figure 1 shows the effects of CDDP on BUN (Fig. 1a) and serum creatinine (Fig. 1b) in normal and PLC-supplemented rats. A single dose of CDDP resulted in a highly significant increase in BUN and serum creatinine (4.5- and sixfold, respectively), whereas administration of PLC alone for 10 consecutive days did not cause any significant changes. Interestingly, administration of PLC 5 days before and after a single dose of CDDP resulted in complete reversal of CDDP-induced increases in BUN and serum creatinine to control values.

The effects of CDDP, PLC and their combination on the levels of oxidative stress biomarkers GSH, TBARS and NO_x in kidney tissues are shown in Fig. 2. A single dose of CDDP resulted in a significant 45% decrease in GSH (Fig. 2a) and a 102 and 41% increase in TBARS (Fig. 2b) and NO_x (Fig. 2c), respectively, compared with the control group. Treatment with PLC for 10 consecutive days resulted in a significant 79% increase in GSH and a significant 27 and 43% decrease in TBARS and NO_x, respectively. Administration of PLC for 5 days before and after a single dose of CDDP completely reversed the decrease in GSH and the increase in TBARS and NO_x levels induced by CDDP to control values.

Figure 3 shows the effects of CDDP on serum and kidney tissue total carnitine in normal and PLC-supplemented rats. Treatment with CDDP resulted in a significant 55% decrease in total carnitine levels in kidney tissues compared with the control group, whereas administration of PLC for 10 successive days resulted in a significant 51% increase. Administration of PLC to CDDP-treated rats resulted in a complete reversal of CDDP-induced decreases in total carnitine content in the kidney to normal levels. Worth mentioning is that none of these treatments had any significant effect on serum levels of carnitine.

Figure 4 shows the effects of CDDP, PLC and their combination on the ATP and ATP/ADP levels, indices of mitochondrial function and energy production, in rat kidney tissues. A single dose of CDDP resulted in a significant 56 and 54% decrease in ATP and ATP/ADP, respectively, compared with the control group. Conversely, treatment with PLC for 10 consecutive days resulted in a significant 43 and 143% increase in ATP and ATP/ADP, respectively. Moreover, administration of PLC to CDDP-treated rats completely reversed the decrease in ATP and ATP/ADP induced by CDDP to control values.

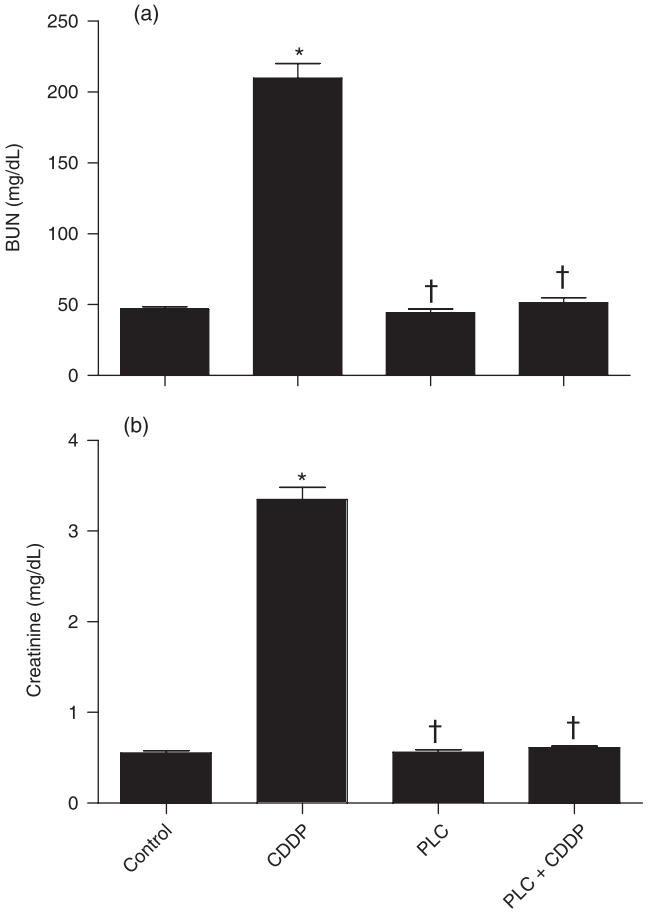

Fig. 1 Effect of cisplatin (CDDP), propionyl-L-carnitine (PLC) and their combination on the (a) blood urea nitrogen (BUN) and (b) serum creatinine levels in rats. Data are the mean±SEM ($n = 10$). *$P < 0.05$ compared with control; †$P < 0.05$ compared with CDDP (ANOVA followed by Tukey–Kramer as a post hoc test).

The effects of CDDP alone and in combination with PLC on histopathological changes in kidney tissues are shown in Fig. 5. Histopathological examination of kidney specimens from control rats revealed no nephrotoxicity (total score: 0), manifested as normal renal parenchyma and no evidence of glomerular and tubulepithelial injury (Fig. 5a). Sections from rats treated with CDDP alone (Fig. 5b–f) showed clear signs of severe nephrotoxicity (total score: 8), manifested as 50–75% of glomeruli exhibiting accentuation of lobular architecture, basement membrane thickening, mesangial cell proliferation and occasional crescent formation (Fig. 5b), 50–75% of renal tubules exhibiting tubular epithelial necrosis (Fig. 5c), apoptosis, tubulorrhexis (Fig. 5d) and tubular epithelial thyroidization (Fig. 5e) and inflammatory cells seen within the interstitium and the perivascular and subvascular areas (Fig. 5f). Interestingly, kidney specimens from rats treated with PLC and CDDP (Fig. 5g,h) showed moderate nephrotoxicity (total score: 3), manifested as significant improvement in glomeruli and renal tubules, with fewer than 25% of glomeruli (Fig. 5g) and renal tubules (Fig. 5h) exhibiting focal necrosis.

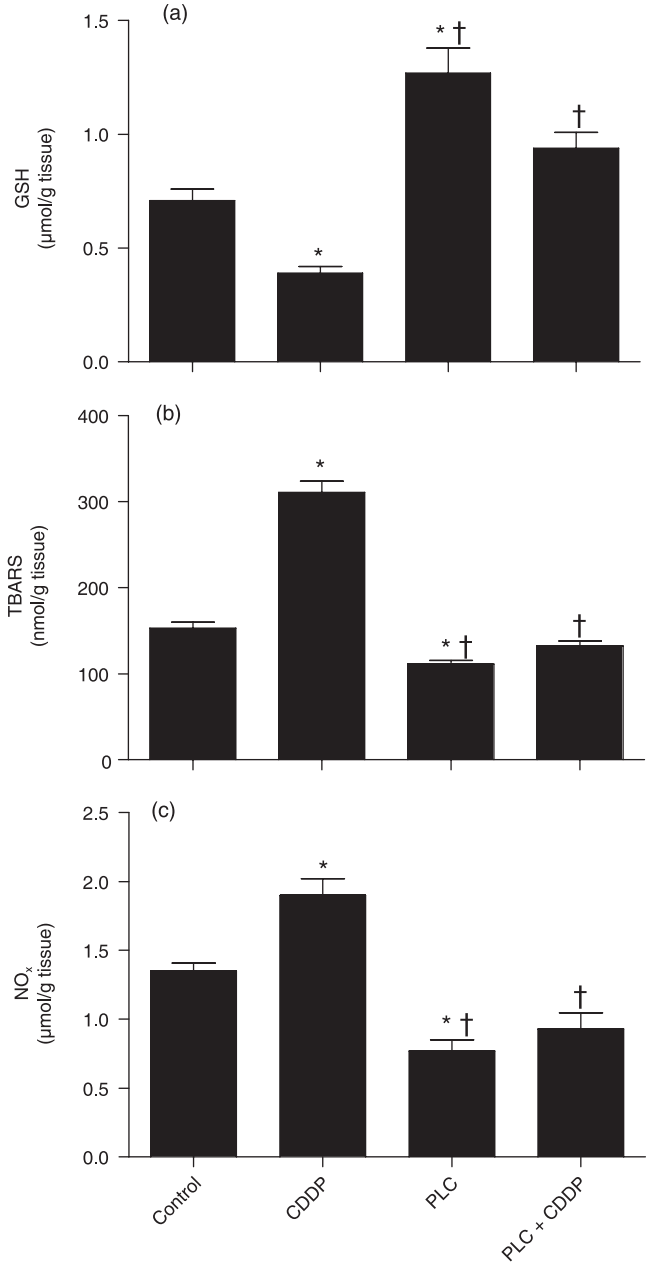

Fig. 2 Effect of cisplatin (CDDP), propionyl-L-carnitine (PLC) and their combination on (a) reduced glutathione (GSH), (b) thiobarbituric acid-reactive substances (TBARS) and (c) total nitrate/nitrite (NO_x) levels in rat kidney tissues. Data are the mean±SEM ($n = 10$). *$P < 0.05$ compared with control; †$P < 0.05$ compared with CDDP (ANOVA followed by Tukey–Kramer as a post hoc test).

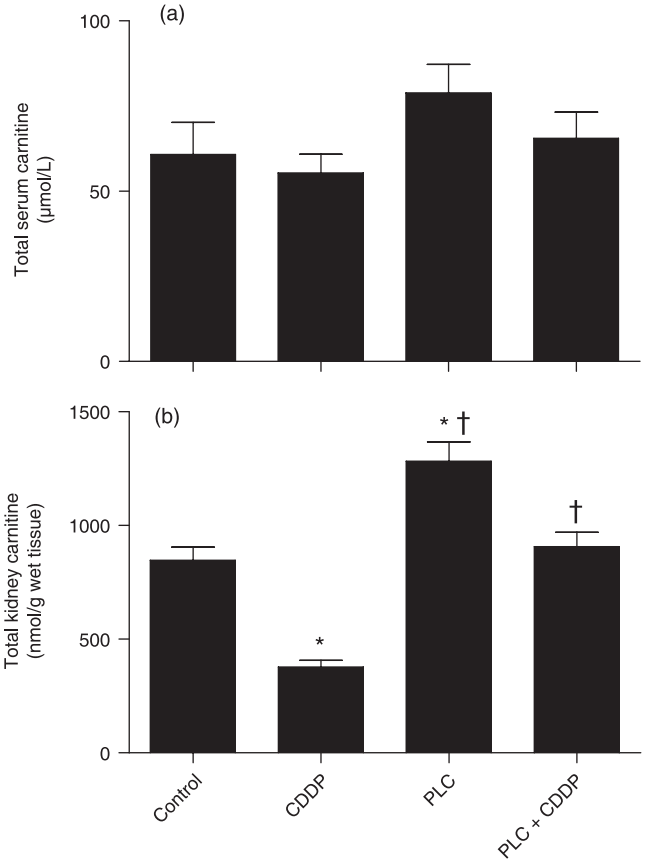

Fig. 3 Effect of cisplatin (CDDP), propionyl-L-carnitine (PLC) and their combination on total carnitine levels in (a) serum and (b) kidney tissues in rats. Data are the mean±SEM ($n = 10$). *$P < 0.05$ compared with control; †$P < 0.05$ compared with CDDP (ANOVA followed by Tukey–Kramer as a post hoc test).

DISCUSSION

The present results show that CDDP treatment is associated with changes in renal function, histopathology and other biochemical parameters, which indicate nephrotoxicity. Carnitine supplementation, using L-carnitine, was shown to attenuate CDDP-induced renal dysfunction, as reported by Sayed-Ahmed *et al.*[15] However, the effect of PLC on CDDP-induced nephrotoxicity has never been investigated. The principle finding of the present study is that PLC, which has several therapeutic advantages over L-carnitine, prevented the development of CDDP-induced nephrotoxicity.

Results from the present study demonstrated that CDDP increased nephrotoxicity indices (serum creatinine and BUN) and caused severe histopathological lesions in kidney tissues, indicating acute nephrotoxicity. These results are in accordance with those reported previously.[15,29,30] Conversely, treating animals with PLC prior to CDDP administration significantly prevented the increase in nephrotoxicity indices and histopathological lesions induced by CDDP, suggesting that PLC may have a potential protective effect against CDDP-induced renal dysfunction. The critical role of carnitine in end-stage renal disease was well explained by Ahmad *et al.*,[31] who reported that carnitine administration to patients undergoing haemodialysis decreased protein catabolism and/or enhanced protein synthesis, thereby reducing serum concentrations of the products of protein catabolism, including BUN, creatinine and phosphorus.

In the present study, the observed increase in NO_x production after CDDP administration could be a secondary event following the increase in inducible nitric oxide synthase (iNOS).[32,33] Srivastava *et al.*[34] reported that N^G-nitro-L-arginine methyl ester, an inhibitor of NOS, significantly decreased elevated BUN, serum creatinine and lipid peroxidation in the target organs following CDDP treatment, which supports our finding and confirms the role of NO in CDDP-related renal toxicity.[34] Furthermore, NO has been reported to inhibit the ability of cells to repair damaged DNA by inhibiting the DNA repair proteins.[35] In addition, Watanabe *et al.*[36] have reported that CDDP-induced totoxicity was due to increased expression of iNOS

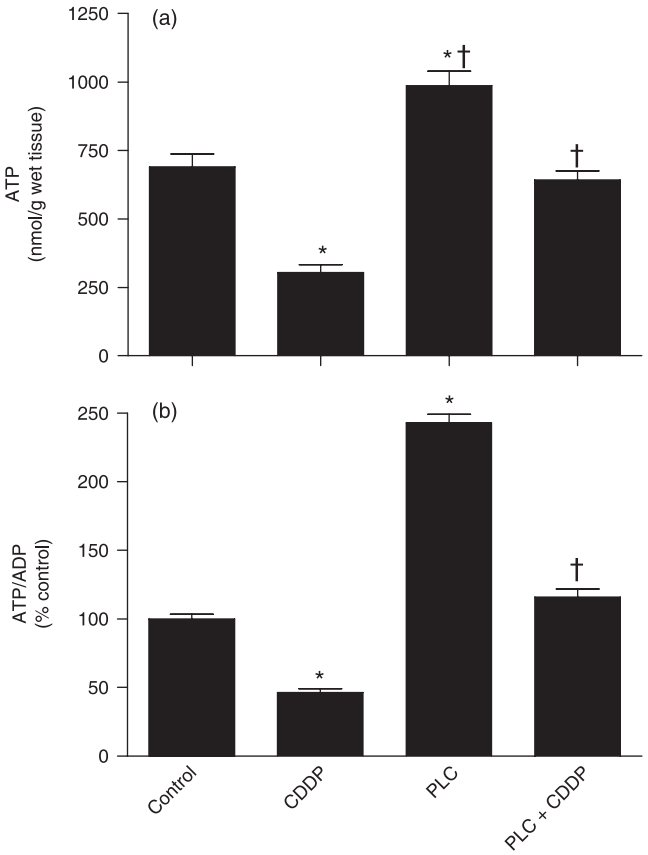

Fig. 4 Effect of cisplatin (CDDP), propionyl-L-carnitine (PLC) and their combination on (a) ATP levels and (b) the ATP/ADP ratio in rat kidney tissues. Data are the mean±SEM ($n = 10$). *$P < 0.05$ compared with control; †$P < 0.05$ compared with CDDP (ANOVA followed by Tukey–Kramer as a post hoc test).

in the guinea-pig vestibule with a consequent increase in NO production resulting in inner ear dysfunction. In the present study, we have shown that PLC treatment completely prevents CDDP-induced increases in NO production.

Lipid peroxidation in the kidney was detected by measuring levels. In agreement with previous studies,[15,19] our results showed that CDDP significantly increased TBARS levels in kidney tissues. In addition to the increase in lipid peroxidation, anti-oxidant vitamins and enzymes have been reported to decrease in kidney tissues of animals treated with CDDP.[19,37–39] Recent studies have demonstrated that CDDP increases the intracellular production of reactive oxygen and nitrogen species in the kidney, liver, cochlear and vestibular tissues by increasing the activity of cytochrome P450 enzymes,[40] NADPH oxidase,[41,42] xanthine oxidase and adenosine deaminase.[39,43,44] This imbalance between pro-oxidants and anti-oxidants, in favour of the former, causes the formation of oxidative stress (reactive oxygen species and free radicals) in kidney tissues. Thus, the observed increase in the levels of NO_x and TBARS in kidney tissues in the present study is consistent with results of previous studies, which have demonstrated the involvement of oxidative stress, lipid peroxidation and mitochondrial dysfunction in CDDP-induced nephrotoxicity.[45–48]

In the present study, the observed decrease in NO_x and TBARS production by PLC could be due to a free radical-scavenging activity. Previous studies have demonstrated that both the D and L forms of carnitine and its short-chain derivatives, including PLC, have similar non-enzymatic free radical-scavenging activity.[49,50] The anti-oxidant and free radical-scavenging activity of PLC have been reported.[16,18,51,52] Moreover, Virmani *et al*. reported that L-carnitine was able to prevent the generation of peroxynitrite and free radicals produced by methamphetamine in mice.[53]

In addition to TBARS, lipid peroxidation in the kidney can be monitored by measuring GSH. In the present study, CDDP caused a significant decrease in GSH levels, a potent factor in the control of lipid peroxidation.[53] It is well known that GSH (a tripeptide that contains thiol) has the ability to protect cells from cytotoxic damage induced by many compounds.[54,55] Treatment with PLC prevented

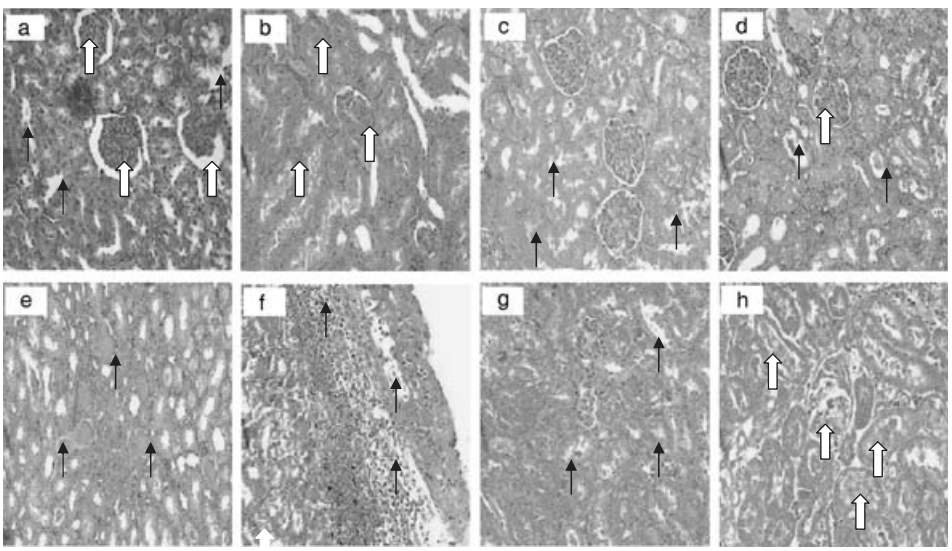

Fig. 5 Photomicrographs of kidney specimens stained with haematoxylin and eosin (original magnification ×40). (a) Kidney from a control rat showing normal renal parenchyma and no evidence of glomerular (thick white arrow) or tubuloepithelial (thin black arrow) injury. (b–f) Kidneys of rats treated with cisplatin (CDDP) alone showing glomerulus crescent formation (thick white arrow), as evidenced by a thickened parietal layer in one area (b), tubular (thin black arrow) epithelial necrosis (c), tubular epithelial necrosis (thin black arrow) with tubulorrhexis and accentuation of glomerular labular architecture (thick white arrow) secondary to basement membrane thickening and mesangial cell proliferation (d), diffuse thyroidization characterized by dilated renal tubules (thin black arrow) filled with colloid casts (e) and abundant leucocytes (thin black arrow) in the subcapsular area (f). (g,h) Kidneys of rats treated with propionyl-L-carnitine (PLC) and CDDP showing ischaemic tubular (thin arrow) necrosis (g) and a glomerulus (thick arrow) exhibiting focal necrosis (h).

CDDP-induced decreases in GSH levels, which strengthens the role of lipid peroxidation in CDDP-induced nephrotoxicity.

In contrast with CDDP, treating naïve animals with PLC significantly increased the level of GSH in kidney tissues. Chang *et al*.[6] reported that L-carnitine suppressed the release of free electrons from mitochondrial electron transport systems, a prerequisite reaction to the generation of free radicals, which may explain the protective effect of L-carnitine against CDDP-induced lipid peroxidation and apoptosis. Worth mentioning is the fact that the mitochondria are not the only source of free radicals in CDDP-induced nephrotoxicity. The contribution of cytochrome P450 enzymes, NADPH oxidase, xanthine oxidase and adenosine deaminase in CDDP-induced oxidative stress has been reported recently.[40–44]

Data presented here show that CDDP treatment significantly decreases levels of total carnitine, ATP and ATP/ADP in kidney tissues. The role of carnitine in CDDP-induced nephrotoxicity has been studied previously by Sayed-Ahmed *et al*.,[15] who showed that the risk of CDDP-induced nephrotoxicity was increased in a carnitine-depleted rat model. It has been shown that treatment with CDDP is associated with increases in the urinary excretion of carnitine, possibly due to inhibition of carnitine reabsorption by the proximal tubule of the nephron.[8] In addition, CDDP has been reported to cause severe damage to renal proximal tubules,[13] leading to impaired renal synthesis and inhibition of tubular reabsorption of carnitine, which may explain the marked decrease in the level of total carnitine in the kidney after CDDP administration.

In the present study, the marked decrease in total carnitine and the ATP/ADP ratio in CDDP-treated rats was parallel to the marked increase in nephrotoxicity indices, which may point to the contribution of carnitine deficiency and energy depletion in CDDP-induced nephrotoxicity. Interestingly, PLC supplementation completely reversed the CDDP-induced decrease in total carnitine and ATP to control values. Moreover, PLC significantly increased the ATP/ADP ratio, which is essential for mitochondrial function. These results suggest that PLC stimulates substrate utilization and/or oxidative phosphorylation, with a consequent increase in energy production and mitochondrial function. The observed increase in ATP levels following PLC supplementation can be explained on the basis that carnitine accumulates in the kidney and small intestine and stimulates the beta-oxidation of long-chain fatty acids in mitochondria, with a consequent increase in ATP production.[6] It has been shown that ATP has the ability to minimize the toxic effects of free forms of long-chain fatty acids in the mitochondria.[6]

A possible explanation for the protective effects of PLC against CDDP-induced kidney dysfunction is that, in mitochondria, PLC has a high affinity for coenzyme A (CoA-SH)/carnitine acetyltransferase and is being metabolised into two important components, namely free L-carnitine and propionyl-CoA.[56,57] The L-carnitine portion of PLC could increase the CoA-SH/acetyl-CoA ratio, which is essential for the activity of beta-oxidation enzymes, as well as many mitochondrial enzymes.[58] In isolated heart mitochondria, Sayed-Ahmed *et al*.[59] reported that L-carnitine released from PLC metabolism increased the CoA-SH/acetyl-CoA ratio by stimulating the mitochondrial efflux of pyruvate-generated acetyl-CoA in the form of acetyl carnitine in a reaction mediated by carnitine acetyl transferase. Conversely, the propionyl-CoA formed in the mitochondria from PLC can be converted into succinyl-CoA, in a reaction mediated by propionyl-CoA carboxylase, thus increasing, with an anaplerotic reaction, the flux through the Krebs cycle and the energy

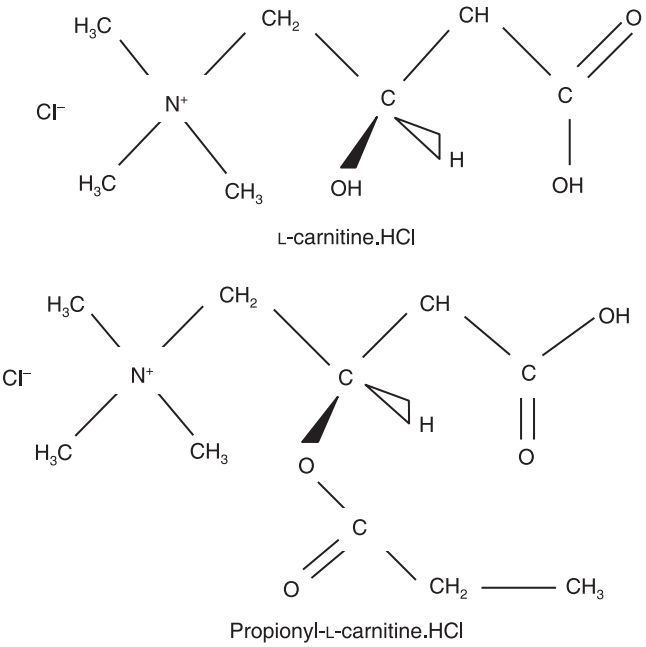

Fig. 6 Chemical structures of L-carnitine and propionyl-L-carnitine.

yielding utilization of acetyl-CoA in the absence of oxygen consumption.[60,61] Accordingly, the reversal of CDDP-induced depletion of total carnitine and ATP in kidney tissues by PLC could be a secondary event following an increase in long-chain fatty utilization, with a consequent increase in ATP production. In addition, the L-carnitine released from PLC could contribute to the stability of the mitochondrial membrane by stimulating the mitochondrial efflux of long-chain acyl-CoA accumulated by CDDP in kidney tissues.[62] Although PLC has a similar mechanism of action on metabolism as L-carnitine, the PLC molecule carries the propionyl group (Fig. 6), which enhances its uptake by cells.[63] This particular structural advantage of PLC, with a long lateral tail, over L-carnitine allows PLC to have specific pharmacological action independent of its effect on metabolism.[64]

In conclusion, data from the present study suggest that PLC prevents the development of CDDP-induced acute renal injury by a mechanism related, at least in part, to its ability to increase the intracellular carnitine content, with a consequent improvement in mitochondrial oxidative phosphorylation and energy production, as well as its ability to decrease oxidative stress. This will open new perspectives for the use of PLC in the treatment of renal diseases associated with or secondary to carnitine deficiency.

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