Turkish Journal of Medical Sciences

Volume 47 | Number 3 Article 45

1-1-2017

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TÜBİTAK

Turkish Journal of Medical Sciences

http://journals.tubitak.gov.tr/medical/

Research Article

Turk J Med Sci
(2017) 47: 1019-1027
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doi:10.3906/sag-1602-136

Changes in the serum, liver, and renal cortical lipids and electrolytes in rabbits with cisplatin-induced nephrotoxicity

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Received: 22.02.2016 • **Accepted/Published Online:** 15.01.2017 • **Final Version:** 12.06.2017

Background/aim: Cisplatin is an anticancer drug that can induce nephrotoxicity. Its toxicity is associated with dyslipidemia and disturbed electrolyte balance. In the present study we investigated the changes in serum lipid profile and electrolyte levels and their contents in kidney and liver tissues of rabbits treated with cisplatin.

Materials and methods: Twenty-eight adult male New Zealand White rabbits were used in the experiment. Animals of groups C, P1, and P2 were injected with saline, cisplatin (4.0 mg/kg bw), and cisplatin (6.5 mg/kg bw), respectively, and killed 3 days after the injections. Animals of group R were given cisplatin (6.5 mg/kg bw) and killed after 7 days. All animals were killed after an overnight fast.

Results: The P2 animals showed reductions in their body weights, significant ($P < 0.001$) increases in serum creatinine and urea levels, and significant ($P < 0.001$) drops in cortical alkaline phosphatase activity and necrotic kidney histology. The treatments had no effect on liver function. Moreover, the P2 animals showed increased serum cholesterol, TAG, and elevated LDL-cholesterol, with significant accumulations of the kidney cholesterol and TAG, but no change in serum phospholipid and depleted hepatic cholesterol. Moreover, the P2 animals had depressed serum levels of potassium, calcium, and magnesium, and reduced renal cortical calcium and magnesium contents and depressed liver calcium but not magnesium. However, the P1 animals had no significant alterations in their lipid or electrolyte levels. Most of the perturbed parameters returned to normal levels in the recovery group.

Conclusion: Cisplatin nephrotoxicity in rabbits is accompanied by reductions in body weight, secondary dyslipidemia, and reduced serum potassium, calcium, and magnesium with depleted renal cortical magnesium and calcium and accumulated cortical lipids.

Key words: Cisplatin nephrotoxicity, lipids, electrolytes, kidney, liver, rabbit

1. Introduction

Cisplatin, cis-dichlorodiammine platinum (II), is a widely used agent in the therapy of a broad spectrum of malignancies (1). Its therapeutic action is by its ability to inhibit replication and transcription and induce apoptosis (2). The efficacy of cisplatin is dose dependent but use of higher doses is limited because of the risk of nephrotoxicity (3). Acute cisplatin nephrotoxicity is manifested as injury to the S3 segment of the proximal tubules, causing renal tubular dysfunction and reduced glomerular filtration (4). The ability of cisplatin and its metabolites to induce increased production of reactive oxygen species and oxidative stress in the kidney have been implicated in the pathogenesis of cisplatin nephrotoxicity (5). Moreover, cisplatin treatment was shown to induce secondary dyslipidemia, causing significantly increased serum levels of total cholesterol, low density lipoprotein (LDL)-cholesterol, and triacyglycerol (TAG) in the rat. On the other hand, hypomagnesemia and disturbance of other electrolytes have been reported in cisplatin nephrotic rats and it has been reported to enhance nephrotoxicity. In the present study we aimed to investigate the changes in serum lipid profile and electrolyte levels as well as their contents in the renal cortical tissue and liver of rabbits exposed to graded doses of cisplatin. Moreover, the changes in serum and organ tissue contents of lipids and minerals were investigated in a recovery group 7 days after the drug treatment.

2. Materials and methods

2.1. Animals

Twenty-eight healthy adult male New Zealand White rabbits weighing between 850 and 1200 g were used in the experiment. The rabbits were divided randomly into four equal groups and housed in stainless steel cages at room temperature (24 ± 2 °C) and 60% relative humidity with

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12 h light/dark cycle. The animals had free access to a nutritionally adequate pelleted diet and tap water.

2.2. Protocol of the experiment

Animals of group 1 (C) were injected intraperitoneally (ip) with 2 mL of physiologic saline and served as controls. Animals of group 2 (P1) were injected ip with cisplatin (Farmitalia, Carlo Erba, Italy) given in a single dose (4.0 mg/kg bw). Animals of group 3 (P2) were injected ip with cisplatin (6.5 mg/kg bw). Animals of group 4 (R) were injected ip with cisplatin (6.5 mg/kg bw) and served as the recovery group. All animals were weighed every alternate day during the course of the experiment. Animals of groups 1, 2 and 3 were sacrificed 3 days after the cisplatin or saline injections under light anesthesia after an overnight fast. Animals of the R group were killed 7 days after the cisplatin injection. Blood was collected from the heart in plain tubes and serum was separated by centrifugation at 2000 × *g* for 15 min at 5 °C. The whole liver and the two kidneys were removed quickly from each animal and rinsed in cold saline. The left kidney of each animal was preserved in 10% formal saline for histopathology. The rest of the tissues were stored at –70 °C awaiting analysis within 5 days.

2.3. Preparation of tissue homogenates

2.3.1. Homogenates for protein and enzyme assays

Weighed portions of kidney cortex or liver tissues were homogenized in Tris-HCl buffer (0.15 mol/L, 5 mmol/L, pH 7.8) using a glass homogenizer (Polytron, Kinematica, Kriens, Switzerland), protected by an ice jacket, at speed 6 for 30 s. The homogenates were centrifuged at 5000 × *g* for 15 min at 2 °C and the clear supernatant was used for the biochemical analysis.

2.3.2. Extraction of tissue lipids

Weighed pieces of kidney cortex or liver tissues were homogenized in chloroform:methanol (2:1 v/v) and then centrifuged at 2000 × *g* for 15 min. The solvent of the supernatants was dried off under a stream of nitrogen and the extract was used for estimation of cholesterol, TG, and phospholipids.

2.4. Biochemical assays

Serum urea, creatinine, serum and liver cholesterol, triacylglycerol (TAG), alanine transaminase (ALT), aspartate transaminase (AST), calcium, and magnesium were estimated by a spectrophotometer system (Beckman Instruments, CA, USA) utilizing kits from BioMérieux (France). The sodium and potassium were assayed by Beckman's (659500) system E2A Analyzer (Ireland) utilizing an E2A sodium/potassium reagent kit (Ireland). The high density lipoprotein cholesterol (HDL-c) was assayed after precipitation of the apo B lipoproteins using heparin and manganous chloride. The estimated values of very low density lipoprotein (VLDL) were calculated from the serum TAG concentration by the formula given by Friedewald et al. (6), and the low density lipoprotein-cholesterol (LDL-c) was calculated by subtraction from total cholesterol. The activity of kidney cortical alkaline phosphatase (ALP) was assayed by kits from Boehringer (Mannheim, Germany). The total lipids were extracted from the tissues of liver and kidney cortex with chloroform:methanol (2:1 v/v). The total phospholipids were estimated by the colorimetric method described by Barlett (7). The protein concentrations in serum or tissue homogenates were determined by the method reported by Lowry et al. (8).

2.5. Histopathological study

Portions of the kidney were removed from the formalin solution and embedded in paraffin wax, cut into 5-µm slices, and stained with hematoxylin and eosin. Five slides were randomly selected from each group and examined under a light microscope by a histopathologist unaware of the treatments. Intensity of the tubular injury was assessed as described by Clark et al. (9) as follows: 0: (normal) no cell necrosis; I (mild): usually a single cell necrosis in sparse tubules; II (moderate): more than one cell necrosis in sparse tubules; III (marked): tubules exhibiting total necrosis in almost every power field.

2.6. Statistical analysis

The presented data are means ± SD. The differences between the means of the experimental groups were computed using one-way analysis of variance. Comparison between the means was carried out using Duncan's multiple comparison procedure. P values less than 0.05 were considered significant.

3. Results

The body weight of control animals increased by 11.5% on day 3 before killing, whereas the body weights of the P1 group increased by only 1.5%. On the other hand, the weights of P2 animals decreased by 4.2% on day 3. However, the weights of the recovery group dropped by 6.4% on day 4, and then started increasing until they gained 1.7% of their initial weights by day 7 (data not shown).

3.1. Kidney function

As shown in Figure 1, the serum creatinine concentration did not show a significant increase in the P1 group, whereas it showed a highly significant ($P < 0.001$) twofold increase in the P2 animals. However, the serum creatinine concentration of the recovery group dropped back to the normal level on day 7 after the drug injection. Similarly, the serum urea level increased by 90% in the P2 group, whereas in the recovery animals it was not significantly different from that of the control group.

As shown in Figure 2, the kidney cortical protein content was not altered in the P1 or P2 groups. However, the cortical protein of the recovery animals exhibited a

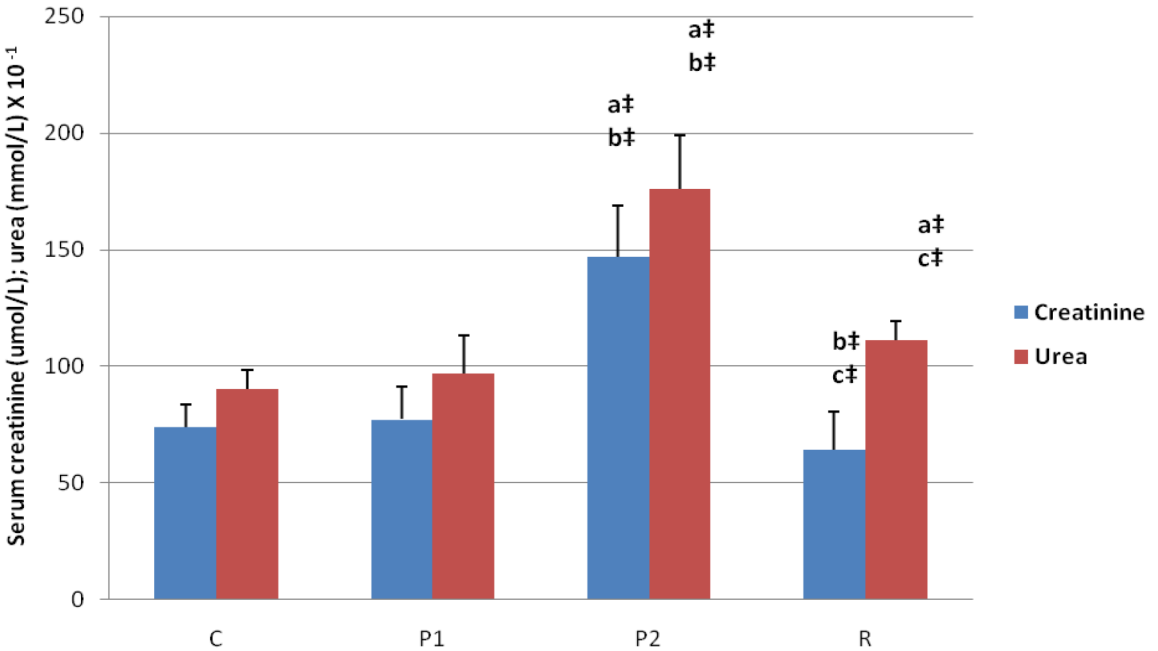

Figure 1. Serum creatinine and urea concentrations in rabbits treated intraperitoneally with cisplatin (4.0 mg/kg bw) - (P1), or with cisplatin (6.5 mg/kg bw) - (P2), or injected with similar volume of saline as controls (C) in single doses and killed 3 days after the injections, or given 6.5 mg/kg bw cisplatin and killed 7 days after the injections (R group). Columns and vertical bars are means ± SD. ‡ $P < 0.001$. a significantly different from C; b significantly different from P1; c significantly different from P2.

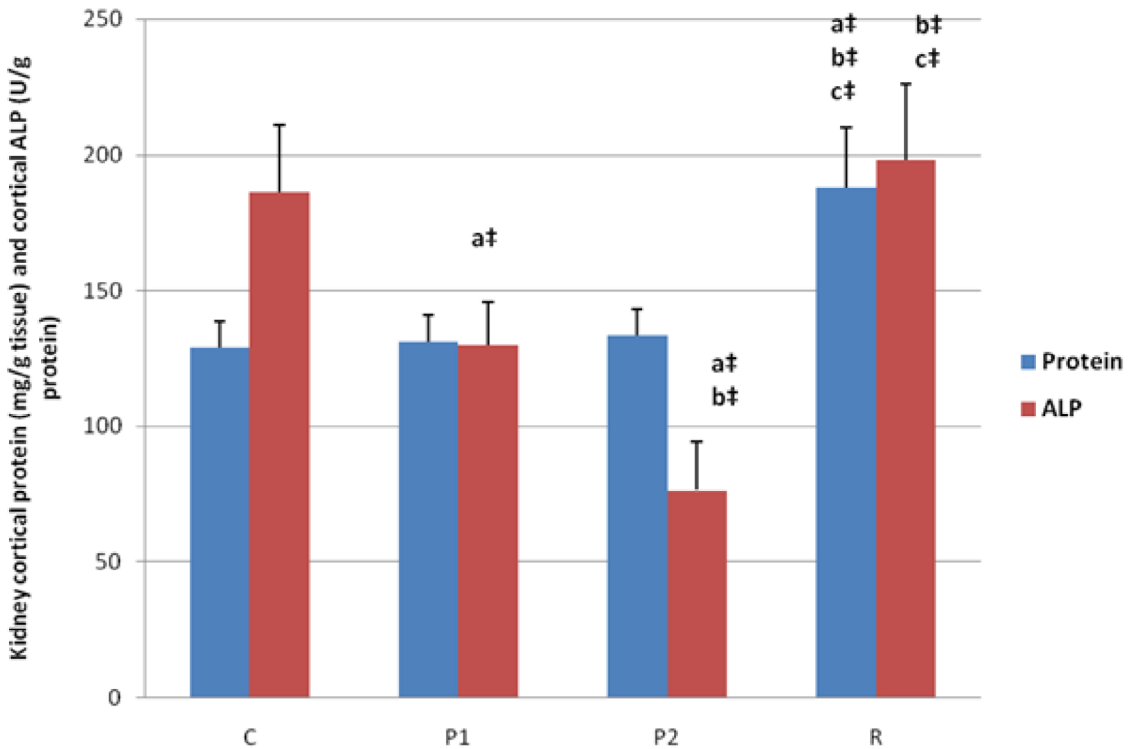

Figure 2. Renal cortical protein content and renal cortical alkaline phosphatase (ALP) activity in rabbits treated intraperitoneally with cisplatin (4.0 mg/kg bw) - (P1), or with cisplatin (6.5 mg/kg bw) - (P2), or injected with similar volume of saline as controls (C) in single doses and killed 3 days after the injections, or given 6.5 mg/kg bw cisplatin and killed 7 days after the injections (R group). Columns and vertical bars are means ± SD. ‡ $P < 0.001$. a significantly different from C; b significantly different from P1; c significantly different from P2.

significant increase of 45.87% over the controls. On the other hand, the kidney cortical ALP decreased in the P1 and P2 groups by 30.37% and 58.92%, respectively, whereas in the recovery group it was not different from that of the control group. The drug treatment caused a slight depression in the serum albumin concentration in the P2 group by 13.86%, and returned to the normal level in the recovery group (Table 1). However, the serum AST and ALT levels did not show any significant changes in any of the experimental groups.

3.2. The kidney histopathology

As shown in Figure 3, sections of the kidney from the control animals showed normal histology (Grade 0), whereas the P1 sections showed mild injury in sparse tubules (Grade 1). However, kidney sections from the P2 animals exhibited marked tubular necrosis with lumens filled with cast (Grade 3). On the other hand, sections from the R group still showed mild injury in sparse areas (Grade 1).

3.3. Serum and tissue lipids

Table 2 summarizes the changes in serum lipid concentrations in the cisplatin-treated groups. The serum total cholesterol was not significantly different in the P1 group, but exhibited a significant increase in the P2 animals of 27.56%, and returned to the control level in the recovery group. Similarly, the serum triglyceride

Table 1. Serum albumin, AST, and ALT levels in rabbits treated intraperitoneally with cisplatin (4.0 mg/kg bw) - (P1), or with cisplatin (6.5 mg/kg bw) - (P2), or injected with similar volume of saline as controls (C) in single doses and killed 3 days after the injections, or given 6.5 mg/kg bw cisplatin and killed 7 days after the injections (R group). Presented data are means ± SD. * P < 0.05. a significantly different from C.

	C	P1	P2	R
Serum albumin (g/dL)	03.03 ± 0.24	02.83 ± 0.24	02.61 ± 0.63a*	02.76 ± 0.23
Serum AST (U/L)	35.63 ± 5.55	33.74 ± 6.95	34.02 ± 5.59	38.06 ± 3.17
Serum ALT (U/L)	42.49 ± 7.88	36.24 ± 8.59	37.29 ± 7.27	45.84 ± 6.23

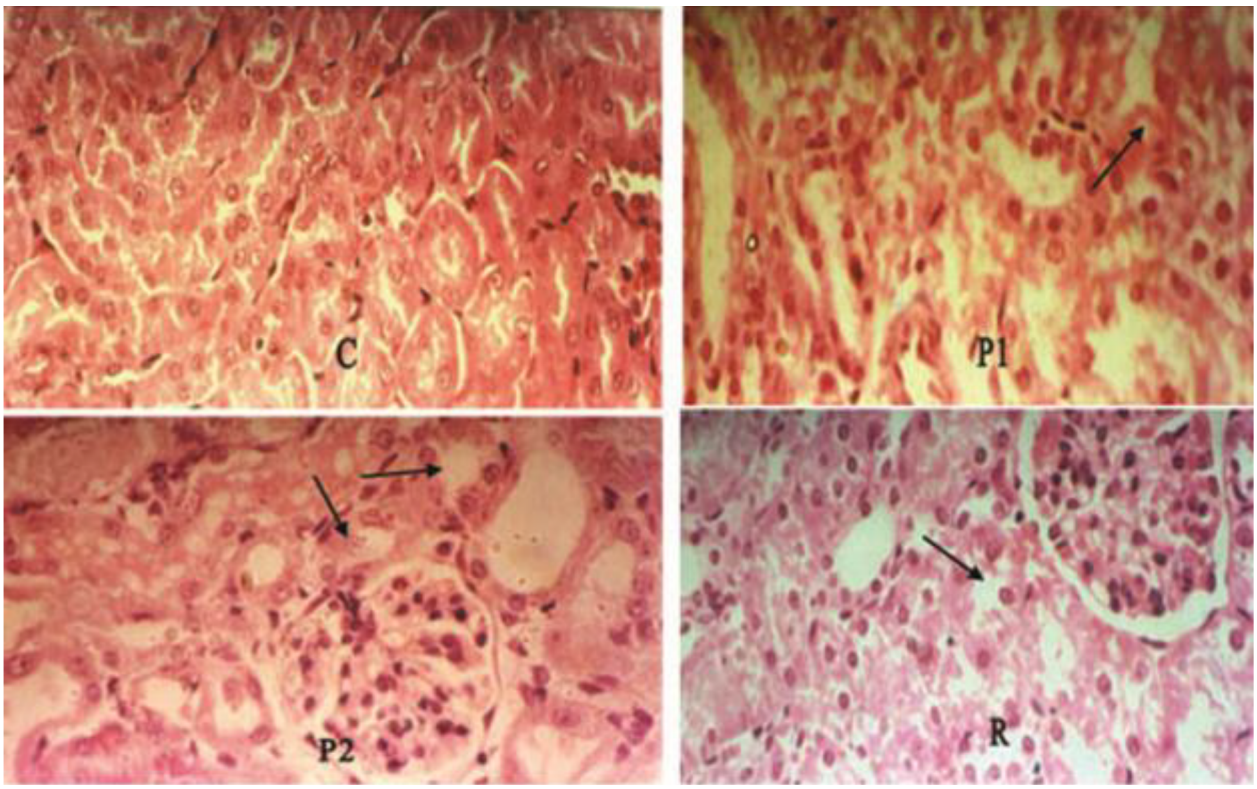

Figure 3. The light microscopic histology of kidney cortices of rabbits treated intraperitoneally with cisplatin (4.0 mg/kg bw) - (P1), or with cisplatin (6.5 mg/kg bw) - (P2), or injected with similar volume of saline as control (C) in single doses and killed 3 days after the injections, or given 6.5 mg/kg bw cisplatin and killed 7 days after the injections (R group). Arrows indicate dilated tubules and tubular lumina containing necrotic cast material in P2 and at a lower extent in P1 and R slides. (Hematoxylin & eosin ×40).

Table 2. Serum lipids and lipoproteins in rabbits treated intraperitoneally with cisplatin (4.0 mg/kg bw) - (P1), or with cisplatin (6.5 mg/kg bw) - (P2), or injected with similar volume of saline as controls (C) in single doses and killed 3 days after the injections, or given 6.5 mg/kg bw cisplatin and killed 7 days after the injections (R group).

		C	P1	P2	R
1	Serum total cholesterol (mmol/L)	4.39 ± 0.64	4.35 ± 0.66	5.60 ± 1.11a**b**	3.99 ± 0.24c**
2	Serum TG (mmol/L)	1.62 ± 0.20	1.22 ± 0.13	2.03 ± 0.63 a**	1.51 ± 0.13c*
3	Serum phospholipids (mmol/L)	0.52 ± 0.11	0.84 ± 0.26a**	02.80 ± 0.76 a‡b‡	1.40 ± 0.12 a‡b‡ c‡
4	HDL-c (mmol/L)	2.04 ± 0.30	2.38 ± 0.76	2.32 ± 1.10	1.90 ± 0.10
5	VLDL-c (TG/5) (mmol/L)	0.32 ± 0.03	0.24 ± 0.01	0.40 ± 0.03 a*b*	0.30 ± 0.01 c*
6	LDL-c (mmol/L)	1.92 ± 0.22	1.73 ± 0.30	2.88 ± 0.26 a‡b‡	1.79 ± 0.09
7	HDL/Total cholesterol	0.51 ± 0.08	0.54 ± 0.13	0.41 ± 0.12 a*b*	0.48 ± 0.03
8	LDL/Total cholesterol	0.43 ± 0.06	0.39 ± 0.04	0.51 ± 0.10 a*b*	0.44 ± 0.07

Presented data are means ± SD. * P < 0.05, ** P < 0.01, ‡ P < 0.001. a significantly different from C; b significantly different from P1; c significantly different from P2.

concentration showed a significant (P < 0.01) increase in the P2 group of 25.30% and returned to the normal level in the recovery group. On the other hand, the concentration of serum phospholipids showed significant elevations in the P1 and P2 groups of 61.53- and by 4.3-fold, respectively, and remained significantly elevated in the recovery group: 1.69-fold compared to the control group.

The HDL-cholesterol was not significantly different in the P1, P2, or recovery groups compared to the control group; however, its ratio to total cholesterol was significantly (P < 0.05) depressed in the P2 animals compared to the P1 and control groups. On the other hand, the estimated LDL-cholesterol was significantly elevated in the P2 by 50.0% compared to the control group, and dropped back in the recovery group. However, the VLDL-c in the P2 group was elevated by 25% compared to the control group.

As shown in Table 3, the hepatic cholesterol contents in the P2 and R groups were significantly decreased by 26.93% and 44.13%, respectively, whereas the hepatic phospholipid was increased in the P2 and R groups by 14.94% and 37.5%, respectively. However, the hepatic TAG was not altered in any of the experimental groups.

On the other hand, the kidney cortical cholesterol and TAG contents were increased in the P2 group by 40.60% and 2.7-fold, respectively. However, both cortical cholesterol and TAG stayed elevated in the recovery group by 22.12% and 3.2-fold, respectively, whereas the cortical phospholipid was not altered by the drug treatments.

3.4. Serum and tissue minerals

Table 4 depicts the changes in serum electrolytes. The serum potassium, calcium, and magnesium levels showed significant reductions of 20.12%, 6.83%, and 18.75%,

Table 3. Liver and kidney cortical lipids in rabbits treated intraperitoneally with cisplatin (4.0 mg/kg bw) - (P1), or with cisplatin (6.5 mg/kg bw) - (P2), or injected with similar volume of saline as controls (C) in single doses and killed 3 days after the injections, or given 6.5 mg/kg bw cisplatin and killed 7 days after the injections (R group). Presented data are means ± SD. * P < 0.05, ** P < 0.01, ‡ P < 0.001. a: significantly different from C; b: significantly different from P1; c: significantly different from P2.

		C	P1	P2	R
Liver	Cholesterol (µmol/g tissue)	20.12 ± 1.35	16.39 ± 4.79a*	14.70 ± 3.58 a**	11.24 ± 0.82 a‡b*
	TAG (µmol/g tissue)	29.53 ± 2.86	32.39 ± 2.76	33.04 ± 3.58	33.53 ± 2.89
	Phospholipid (mmol/g tissue)	0.087 ± 0.01	0.084 ± 0.01	0.10 ± 0.01 a**b**	0.11 ± 0.02 a*b*
Kidney cortex	Cholesterol (µmol/g tissue)	19.16 ± 1.35	18.56 ± 2.91	26.94 ± 4.50 a‡b‡	23.40 ± 3.40 a**b*
	TAG (µmol/g tissue)	7.88 ± 1.73	18.28 ± 1.78 a‡	29.75 ± 2.92 a‡b‡	33.80 ± 2.80 a‡b‡ c*
	Phospholipid (mmol/g tissue)	0.05 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.06 ± 0.01

Table 4. Serum minerals in rabbits treated intraperitoneally with cisplatin (4.0 mg/kg bw) - (P1), or with cisplatin (6.5 mg/kg bw) - (P2), or injected with similar volume of saline as controls (C) in single doses and killed 3 days after the injections, or given 6.5 mg/kg bw cisplatin and killed 7 days after the injections (R group).

		C	P1	P2	R
1	Serum Na (mmol/L)	140.09 ± 1.78	138.76 ± 6.20	138.56 ± 5.59	137.58 ± 4.59
2	Serum K (mmol/L)	6.51 ± 0.43	5.10 ± 0.68 a‡	5.20 ± 0.15 a‡	6.18 ± 0.93 b*c*
3	Serum Ca (mmol/L)	3.51 ± 0.44	3.32 ± 0.78 a‡	3.27 ± 0.16 a‡	4.96 ± 0.86 a‡b‡ c‡
4	Serum Mg (mmol/L)	0.96 ± 0.18	0.83 ± 0.25	0.78 ± 0.30 a‡	1.16 ± 0.18 b*c*

Presented data are means ± SD. *P <0.05, ‡ P < 0.001. a: significantly different from C; b: significantly different from P1; c: significantly different from P2.

respectively. The three electrolytes returned to the control levels in the recovery group. However, the serum sodium levels were not altered in any of the experimental groups.

The concentrations of hepatic and kidney cortical calcium and magnesium are summarized in Table 5. The liver calcium and magnesium contents were not significantly affected by the cisplatin treatments. However, the renal cortical calcium and magnesium were significantly diminished in the P2 group by 40.05% and 24.00%, respectively. On the other hand, the hepatic tissue of the recovery group showed a significant elevation in the calcium content, whereas it was significantly depleted in the kidney cortex.

4. Discussion

The single dose of cisplatin (6.5 mg/kg bw) caused severe nephrotoxicity in the rabbit, evidenced by the deranged biochemical kidney function parameters and the kidney cortical histology accompanied by reductions in body weight. A similar dose of cisplatin was also capable of inducing nephrotoxicity in other species as well (10). Cisplatin treatment is known to induce expression of proinflammatory cytokines and caspases in the renal tissue that activate the cellular inflammatory response, which mediates the process of nephrotoxicity (11). Our data indicated that the dose of cisplatin that induced nephrotoxicity had no deleterious effects on the liver. This indicates that the observed drop in serum albumin level was due to renal albumin wasting rather than depression in its synthesis. An interesting observation was the significant accumulation of kidney cortical protein in the recovery group, which paralleled the increase in the cortical ALP activity. This increase in cortical protein content coincided with the peak of body weight gain in these recovery animals. This protein expression seems to be a rehabilitation process of the proximal tubules. Many authors have indicated that several transport proteins for the efflux of cisplatin-GSH conjugates are expressed in the renal tubules following cisplatin exposure (12,13).

On the other hand, several studies have demonstrated that cisplatin and gentamicin but not amikacin can induce secondary dyslipidemia in the rat (14–16). In the present study we observed similar hypercholesterolemia and hypertriglyceridemia in the cisplatin-treated rabbits. The results indicated that more than half of the total circulating cholesterol was associated with the LDL particles. It is

Table 5. The hepatic and renal cortical calcium and magnesium contents in rabbits treated intraperitoneally with cisplatin (4.0 mg/kg bw) - (P1), or with cisplatin (6.5 mg/kg bw) - (P2), or injected with similar volume of saline as controls (C) in single doses and killed 3 days after the injections, or given 6.5 mg/kg bw cisplatin and killed 7 days after the injections (R group).

	Mineral	C	P1	P2	R
Liver	Ca (mmol/g Tis)	2.44 ± 0.25	2.24 ± 0.44	2.28 ± 0.76	2.72 ± 0.42 a‡b‡ c‡
	Mg (mmol/g Tis)	0.86 ± 0.17	0.79 ± 0.22	0.89 ± 0.28	0.92 ± 0.24
Kidney	Ca (mmol/g Tis)	3.82 ± 0.37	03.04 ± 0.42 a‡	2.29 ± 0.53 a‡b‡	1.50 ± 0.22 a‡b‡ c‡
	Mg (mmol/g Tis)	1.00 ± 0.14	0.81 ± 0.20a*	0.76 ± 0.29 a*	0.88 ± 0.11

Presented data are means ± SD. * P < 0.05, ‡ P < 0.001. a: significantly different from C; b: significantly different from P1; c: significantly different from P2

documented that the mechanism of cisplatin nephrotoxicity involves generation of free radicals and increased lipid peroxidation. One of the consequences of increased free radicals generation is oxidation of the circulating LDL particles. The oxidized LDL is not recognized by the LDL receptors and not taken up by the cells, which can lead to accumulation of the LDL in circulation. The oxidized LDL particles are associated with proinflammatory properties (17) and are involved in the initiation and progression of atherosclerosis (18).

Another finding in the present study was the severe accumulation of serum phospholipids in the cisplatin-treated animals, which paralleled the high levels of LDL and VLDL. Some reports have shown the existence of oxidized phospholipids in oxidized LDL particles. This phospholipid oxidation, which was demonstrated even in minimally oxidized LDL, was attributed to the oxidizable arachidonic acid of the surface phospholipids in the lipoprotein (19). These compounds are characterized by short polar fatty acyl chains in position 2 and a single hydrophobic fatty acid in position 1 of glycerol (20). As a consequence they are highly exchangeable between cells, tissues, and lipoproteins. These oxidized phospholipids have been detected in atheromas and are suggested to be responsible for endothelial cell phenomena including monocyte adhesion and integrin activation, leading to the progression of atherosclerosis (21,22). Our data indicated that secondary hyperlipidemia was accompanied by significant accumulations of the renal cortical cholesterol and TAG. This was in congruence with the report by Coimbra et al. (23) who found that cisplatin-induced hyperlipidemia was associated with early progressive macrophage infiltration in the rat kidney. This was suggested to cause the development of glomerulosclerosis and tubular damage. These findings support the assumption that cisplatin-induced dyslipidemia and its renal infiltration may play a role in the development and progression of cisplatin nephrotoxicity. However, the cisplatin treatment caused reductions in hepatic cholesterol with increased phospholipid content. This was in agreement with the results reported by Gevorgyn et al. (24), who found that the hepatic nuclear total cholesterol in the rat dropped by 12.38% when treated with cisplatin. This depressed hepatic cholesterol content is possibly due to the impaired hepatic receptor-mediated uptake of the LDL-cholesterol due to the oxidative modification of the lipoprotein. The modified LDL was less effectively recognized by the B/E receptor of the cell for its uptake (25). Instead, Kramer et al. (26) reported that the LDL and intermediate density lipoprotein particles were taken up by the glomerular cells. This explains the depletion of hepatic cholesterol and its accumulation in the kidney cortex. However, the recovery group had their hepatic cholesterol still significantly depressed. This indicates that although the tubular function was restored in the recovery period, the effect of renal platinum deposits on the cholesterol balance was not reversed. In a previous study we observed cisplatin-induced hyperlipidemia in the rat with significant TAG accumulation in the liver with no change in the cholesterol content (10). In contrast, in the present results no significant change was observed in the rabbit hepatic TAG content, which may be due to species variation.

Our data also indicated that cisplatin treatment caused disturbance in the serum electrolyte balance. Significant reductions were observed in the serum levels of potassium, calcium, and magnesium due to the drug treatment. The cisplatin challenge is known to hinder tubular reabsorption, resulting in a concentration defect and disturbance of the renal handling of electrolytes. Several studies have indicated the association of cisplatin toxicity with the depletion of serum magnesium, and magnesium supplementation was adopted to prevent hypomagnesemia during drug treatment (27). Hypomagnesemia was observed in humans (28) and in experimental animals (29). It was reported that in cisplatin-treated mice the urinary magnesium waste almost doubled (30), and the magnesium depletion was shown to enhance cisplatin nephrotoxicity (31). Magnesium is known to be a critical cofactor in many cellular enzyme processes including membrane transport and cellular repair (32). In a study with isolated human proximal tubules the tubular organic cation transporter 2 was implicated in cisplatin nephrotoxicity (33), and hypomagnesemia was shown to upregulate the cation transporter 2 and thereby increase the renal accumulation of cisplatin and exacerbate kidney injury (34). Our data indicated reduced serum potassium in the cisplatin-treated animals. Marklund et al. (35) suggested that the ability of cisplatin to induce apoptosis was influenced by its ability to enhance the efflux of potassium ions. This possibly indicates that the urinary potassium waste overwhelms the cellular potassium efflux, causing a negative balance. In line with our findings regarding cisplatin toxicity, the amphotericin B treatment in humans was shown to be associated with hypokalemia, hypomagnesemia, and increased renal potassium and magnesium wasting (36), and supplementation of cyclosporine-treated rats with magnesium and potassium was shown to attenuate nephrotoxicity (37). Our data also indicated significant reductions in serum calcium levels of the cisplatin-treated animals. This was in congruence with the findings reported by Maheshwari et al. (38), who stated that cisplatin nephrotoxicity was associated with hypocalcemia in the rat, and that a dose-dependent protective effect of calcium loading was observed in gentamicin nephrotoxicity (39). Calcium has been suggested to exert its nephroprotective

action by either alleviating functional hemodynamic alterations at the glomerular level or preventing structural cellular damage at the tubular level. In the present study we document significant depletion of the serum and kidney cortical tissues from the two protective minerals (calcium and magnesium), which may be a potentiating factor in the development of cisplatin toxicity. However, the contents of hepatic calcium and magnesium were not disturbed by the cisplatin challenge. This supports the idea that although the highest levels of platinum are found in the liver and kidney, the platinum accumulation in the liver is only transient (3). This transient existence of platinum in the hepatic tissue explains why the liver was not affected by the cisplatin treatment.

In conclusion, nephrotoxicity was induced by a single dose of cisplatin (6.5 mg/kg b.w.) in the rabbit evidenced by the increased serum creatinine and urea levels with drop in the cortical ALP activity and the histology of the kidney cortex. The nephrotoxicity was accompanied by reductions in serum albumin concentration and depression in body weights. None of the cisplatin treatments had an effect on the liver function. The treatment induced hypercholesterolemia and hypertriglyceridemia with elevated LDL-cholesterol. There were significant accumulations of kidney cholesterol and TAG with no change in the phospholipid content, and a drop in hepatic cholesterol. Significant depressions in the serum levels of potassium, calcium, and magnesium were also observed. This was accompanied by reductions in the renal cortical calcium and magnesium contents as well as depressed liver calcium but not magnesium. Almost all perturbed parameters returned to the normal levels during the recovery period.

References

1. Boulikas T. Molecular mechanisms of cisplatin and its liposomally encapsulated form, lipoplatin. Lipoplatin as a chemotherapy and antiangiogenesis drug. Cancer Ther 2007; 5: 349-376
2. Perez RP. Cellular and molecular determinants of cisplatin resistance. Eur J Cancer 1998; 34: 1535- 1542.
3. Hanigan MH, Devarajan P. Cisplatin nephrotoxicity: molecular mechanisms. Cancer Ther 2003; 1: 47-61.
4. Kuhlmann MK, Burkhardt G, Kohler H. Insights into potential cellular mechanisms of cisplatin nephrotoxicity and their clinical application. Nephrol Dial Transplant 1997; 12: 2478-2480.
5. Santos NAG, Catão CS, Martins NM, Curti C, Bianchi MLP, Santo AC. Cisplatin-induced nephrotoxicity is associated with oxidative stress, redox state unbalance, impairment of energetic metabolism and apoptosis in rat kidney mitochondria. Arch Toxicol 2007; 81: 495- 504.
6. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972; 18: 499-502.
7. Barlett, GR. Phosphorus assay in column chromatography. J Biol Chem 1959; 234: 466-468.
8. Lowry OH, Rosebrough NJ, Fair AI, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951; 193: 265-275.
9. Clark WF. Fish oil in lupus nephritis: clinical findings and methodological implications. Kidney Int 1993; 44: 75-86.
10. Abdel-Gayoum AA, El-Jenjan KB, Ghwarsha KA. Hyperlipidaemia in cisplatin-induced nephrotic rats. Human Exp Toxicol 1999; 18: 454-459.
11. Liu M, Chien CC, Burne-Taney M, Molls RR, Racusen LC, Colvin RB, Rabb H. A pathophysiologic role for T lymphocytes in murine acute cisplatin nephrotoxicity. J Am Soc Nephrol 2006; 17: 765-774.
12. Ishikawa T, Bao JJ, Yamane Y, Akimaru K, Frindrich K, Wright CD, Kuo MT. Coordinated induction of MRP/GS-X pump and gamma-glutamylcysteine synthetase by heavy metals in human leukemia cells. J Biol Chem 1996; 271: 14981-14988.
13. Borst P, Evers R, Kool M, Wijnholds J. A family of drug transporters: the multidrug resistance-associated proteins. J Natl Cancer Inst 2000; 92: 1295-1302.
14. Hadjzadeh MA, Rajaei Z, Keshavarzi Z, Shirazi MG, Toosi V. Effect of aqueous extract of *Rheum ribes* on cisplatin-induced nephrotoxicity in rat. J Pharm Bioallied Sci 2013; 5: 309-313.
15. Abdel-Gayoum AA, Ali BH, Ghwarsha K, Bashir AA. Plasma lipid profiles in the rat with gentamicin-induced nephrotoxicity. Human Exp Toxicol 1993; 12: 371-378.
16. Abdel-Gayoum AA, Alhasan AA, Ginawi I, Alshankyty IM. The ameliorative effect of olive oil and olive leaf extract on the amikacin-induced nephrotoxicity in the rat. Toxicol Report 2015; 2: 1327-1333.
17. Tedgui A, Mallat Z. Cytokines in atherosclerosis: pathogenic and regulatory pathways. Physiol Rev 2006; 86: 515-581.
18. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. N Engl J Med 2005; 352: 1685-1695.
19. Jerlich A, Pitt AR, Schaur RJ, Spickett CM. Pathways of phospholipid oxidation by HOCl in human LDL detected by LC-MS. Free Radic Biol Med 2000; 28: 673-682.
20. Subbanagounder G, Leitinger N, Schwenke D, Wong J W, Lee H, Rizza C, Watson A D, Faull KF, Fogelman AM, Berliner JA. Determinants of bioactivity of oxidized phospholipids. Specific oxidized fatty acyl groups at the sn-2 position. Thromb Vasc Biol 2000; 20: 2248-2254.

21. Berliner JA, Subbanagounder G, Leitinger N, Watson AD, Vora D. Evidence for a role of phospholipid oxidation products in atherogenesis. Trends Cardiovasc Med 2001; 11: 142-147.

22. Bochkov V N, Mechtcheriakova D, Lucerna M, Huber J, Malli R, Graier WF, Hofer E, Binder B, Leitinger N. Oxidized phospholipids stimulate tissue factor expression in human endothelial cells via activation of ERK/EGR-1 and Ca++/NFAT. Blood 2002; 99: 199-206.

23. Coimbra TM, Janssen U, Grone HJ, Ostendorf T, Kunter U, Schmidt H, Brabant G, Floege J. Early events leading to renal injury in obese Zucker fatty rats with type II diabetes. Kidney Int 2000; 57: 167-182.

24. Gevorgyn ES, Hovhanisyan AG, Yavroyan ZH, Hakobyan NR, Sargsyan EG. Cisplatin in vivo action on content of neutral lipids in rat liver and thymus nuclear membranes. Biological Journal of Armenia 2013; 2: 99-103.

25. Reade V, Tailleux A, Reade R. Expression of apolipoprotein B epitopes in low density lipoproteins of hemodialyzed patients. Kidney Int 1993; 44: 1360-1365.

26. Kramer A, Nauck M, Pavenstad H, Schwedler S, Wieland H, Schollmeyer P, Wanner C. Receptor-mediated uptake of IDL and LDL from nephrotic patients by glomerular epithelial cells. Kidney Int 1993; 44: 1341-1351.

27. Bodnar L, Wcislo G, Gasowska-Bodnar A, Synowiec A, Szarlej-Wcislo K. Renal protection with magnesium subcarbonate and magnesium sulphate in patients with epithelial ovarian cancer after cisplatin and paclitaxel chemotherapy: a randomised phase II study. Eur J Cancer 2008; 44: 2608-2614.

28. Willox JC, McAllister EJ, Sangster G, Kaye SB. Effects of magnesium supplementation in testicular cancer patients receiving cis-platin: a randomised trial. Br J Cancer 1986; 54: 19-23.

29. Sahin AA, Oysu C, Yilmaz HB, Topak M, Kulekci M, Okar I. Effect of oral magnesium supplementation on cisplatin ototoxicity. J Otolaryngol 2006; 35: 112-116.

30. van Angelen AA, Glaudemans B, van der Kemp AW, Hoenderop J, Bindels RJ. Cisplatin-induced injury of the renal distal convoluted tubule is associated with hypomagnesaemia in mice. Nephrol Dial Transplant 2013; 28: 879-889.

31. Lajer H1, Kristensen M, Hansen HH, Nielsen S, Frøkiaer J, Ostergaard LF, Christensen S, Daugaard G, Jonassen TE. Magnesium depletion enhances cisplatin-induced nephrotoxicity. Cancer Chemother Pharmacol 2005; 56: 535-542.

32. Lajer H, Daugaard G. Cisplatin and hypomagnesemia. Cancer Treat Rev 1999; 25: 47-58.

33. Ciarimboli G, Ludwig T, Lang D, Pavenstadt H, Koepsell H, Koepsell H, Piechota HJ, Haier J, Jaehde U, Zisowsky J et al. Cisplatin nephrotoxicity is critically mediated via the human organic cation transporter 2. Am J Pathol 2005; 167: 1477-1484.

34. Yokoo K, Murakami R, Matsuzaki T, Yoshitome K, Hamada A, Saito H. Enhanced renal accumulation of cisplatin via renal organic cation transporter deteriorates acute kidney injury in hypomagnesemic rats. Clin Exp Nephrol 2009; 13: 578-584.

35. Marklund L, Andersson B, Behnam-Motlagh P, Sandstrom P, Henriksson R, Grankvist K. Cellular potassium ion deprivation enhances apoptosis induced by cisplatin. Basic Clin Pharm Toxicol 2004; 94: 245-251.

36. Karimzadeh I, Hossein Khalili H, Dashti-Khavidaki S, Sharifian R, Abdollahi A, Hasibi M, Khazaeipour Z, Farsaei S. N-acetyl cysteine in prevention of amphotericin-induced electrolytes imbalances: a randomized, double-blinded, placebo-controlled, clinical trial. Eur J Clin Pharmacol 2014; 70: 399-408.

37. Pere AK, Lindgren L, Tuomainen P, Krogerus L, Rauhala P, Laakso J, Karppanen H, Vapaatalo H, Ahonen J, Mervaala EM. Dietary potassium and magnesium supplementation in cyclosporine-induced hypertension and nephrotoxicity. Kidney Int 2000; 58: 2462-2472.

38. Maheshwari RA, Sailor GU, Patel L, Balaraman R. Amelioration of cisplatin-induced nephrotoxicity by statins. Indian J Pharmacol 2013; 45: 354-358.

39. Patil AN, Arora T, Desai A, Tripathi CD. Comparison of the species-sensitive effects of different dosages of calcium and verapamil on gentamicin-induced nephrotoxicity in rats and rabbits. Toxicol Int 2014; 21: 225-231.