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The influence of spironolactone on the serum electrolytes balance and renal cortical magnesium and calcium contents in cisplatin – treated rabbits

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ABSTRACT

Background: Cisplatin is a widely used anticancer drug. Its use is known to induce nephrotoxicity and may be associated with disturbance of the electrolyte balance. Spironolactone, an antagonist of aldosterone, is frequently prescribed as a potassium sparing diuretic. **Aims and Objective:** The present study aims to investigate whether spironolactone can correct the cisplatin-induced electrolyte disturbance in the rabbit. **Materials and Methods**: Thirty two New Zealand rabbits were distributed into four groups. Animals of control (C); spironolactone (S); cisplatin (P); spironolactone and cisplatin (SP) groups were injected ip with saline; given spironolactone (20 mg/kg bw/day) orally for 5 days; injected ip with cisplatin (6.5 mg/kg bw); given both spironolactone and cisplatin, respectively. Serum, liver and kidney cortical homogenates were used for biochemical analysis. **Results**: Serum creatinine and urea levels of P were significantly ($P<0.001$) elevated and were severely raised in SP by 7-fold and 2.7-fold. The serum magnesium was increased in S and SP groups by 93.75% and 112%, respectively and was depressed in P by 18.75% compared to C. Serum potassium and calcium of P were significantly ($P<0.001$) depressed, whereas, serum calcium levels in the S and SP were significantly elevated. The liver magnesium and calcium of SP were elevated by 38.37% and 40.7%, respectively, whereas, kidney magnesium showed reduction in the S, P and SP by 36%, 24% and 19%, respectively and elevated in the SP group by 26.56% compared to S group. The kidney calcium was depressed in the S, P and SP by 30.89%, 40.0% and 28.2%, respectively compared to C.**Conclusion**: Cisplatin treatment depleted the serum and renal cortical magnesium. The serum calcium and potassium were reduced secondary to the magnesium depletion. The co-administration of spironolactone reversed the cisplatin-induced magnesium and calcium depletions in serum and renal tissue.

Key words: Cisplatin nephrotoxicity, Spironolactone, Serum electrolytes, Kidney, Liver, Rabbit

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INTRODUCTION

Cisplatin is an anticancer drug widely used in the treatment of a broad spectrum of solid malignancies. The drug is known to induce nephrotoxicity in human and experimental animals[1] and it`s use is associated with disturbance of the electrolyte balance.[2] It is known to cause urinary magnesium wasting leading to hypomagnesemia and hypocalcemia.[3] Spironolactone, an antagonist of the adrenal mineralocorticoid (aldosterone), is known to have hypotensive properties and has been used in the treatment of congestive heart failure.[4] In primary hyperaldosteronism it rapidly corrects the biochemical abnormalities and usually restores the blood pressure to normal.[5] It acts by competitively binding and displacing aldosterone from its receptor in the distal convoluted

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renal tubular cells thus, inhibits aldosterone's regulatory effects on sodium and potassium balances.[4] Spironolactone was found to significantly lower arterial stiffness and left ventricular mass in patients with stage 3 chronic kidney disease.[6] Since spironolactone is a potassium sparing diuretic and is frequently prescribed with the nephrotoxic drugs, we aimed to investigate the possible influence of spironolactone administration on the serum balances of four essential electrolytes; sodium, potassium, calcium and magnesium, and the contents of calcium and magnesium in the liver and renal cortices of rabbit with cisplatin-induced nephrotoxicity.

MATERIALS AND METHODS

Animals

Thirty two adult healthy male New Zealand White rabbits weighting between 800 and 1150 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 12 hr light/dark cycle. The animals had free access to a nutritionally adequate pelleted diet and tap water.

Protocol of the experiment

Animals of groups: C, S, P and SP were injected intraperitoneally (ip) with 2ml of physiologic saline, given spironolactone (20 mg/kg bw/day) orally for 5 consecutive days, injected ip with cisplatin (6.5 mg/kg bw), or given spironolactone (20 mg/kg bw/day) orally for 5 days and injected ip with cisplatin (6.5 mg/kg bw) on day 3, respectively. All animals were sacrificed three days after the cisplatin or saline injections or 24 Hr after the last dose of spironolactone following an overnight fast. Blood was collected from the heart in plain tubes and serum was separated by centrifugation at 2000 x g for 15 min at 5° C. The whole liver and left kidney were removed quickly from each animal, rinsed in cold saline and stored at -70° C awaiting analysis within 5 days. All experimental procedures were carried out in accordance to the Helsinki declaration for animal experiments and the protocol was approved by the Research Ethical Committee, Faculty of Medicine, University of Benghazi, Benghazi, Libya.

Biochemical assays

Serum urea and creatinine concentrations were estimated by a spectrophotometer system (Beckman Instruments, CA). Serum, liver and kidney cortical magnesium and calcium, serum alanine transaminase (ALT) and aspartate transaminase (AST) were assayed by a spectrophotometer system (Beckman Instruments, CA) utilizing kits from BioMeriecux- France. The serum sodium and potassium concentrations were assayed by Beckman`s (659500) system E2A Analyzer, Ireland utilizing E2A sodium/potassium reagent kit-Ireland. The activity of alkaline phosphatase (ALP) enzyme in serum, liver and kidney cortex was assayed by kits from Boehringer Mannheim, Germany. The protein concentrations in serum or tissue homogenates were determined by the dye-binding method described by Noble and Bailey.[7] The concentration of serum albumin was estimated utilizing commercial BCG-albumin estimation kit supplied by Sigma-Aldrich Co. USA.

Statistical analysis

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

RESULTS

The kidney function

The S group showed a slight increase in the serum creatinine concentration by 12.63% compared to control. Whereas, animals of the P group had their serum creatinine increased by 99.07% compared to control, and by 76.75% compared to the S group. However, the SP animals had their serum creatinine severely elevated by 700%, 610% and 300% compared to C, S and P, respectively. Similarly, the serum urea concentration in the P group was increased by 94.36% compared to control. Whereas, that of SP group was increased by 272% compared to control and by 91.41% compared to P group (Figure 1). On the other hand, the renal cortical ALP activity showed significant reductions by 34.29%, 58.92% and 71.33% in the S, P, and SP groups, respectively compared to control (Figure 2). As shown in Table 1, the serum albumin was significantly ($P<0.05$) reduced in the P group by 13.84%, whereas in the SP group it was depressed by 17.49% compared to control. However, the serum ALT, AST and liver tissue ALP activities, measured as liver function parameters, did not show any significant changes in any of the experimental groups compared to control.

Effects on serum and tissue electrolytes

The effects of cisplatin and spironolactone treatments on the serum electrolyte concentrations are summarized in Table 2. The serum magnesium was significantly elevated in the S and SP groups by 93.75 and 112%, respectively compared to control. Whereas that of the P group was significantly depressed by 18.75% and 58.06% compared to C and S, respectively. However, the serum magnesium of the SP was significantly higher by 158% compared to P group. On the other hand, the serum potassium in animals

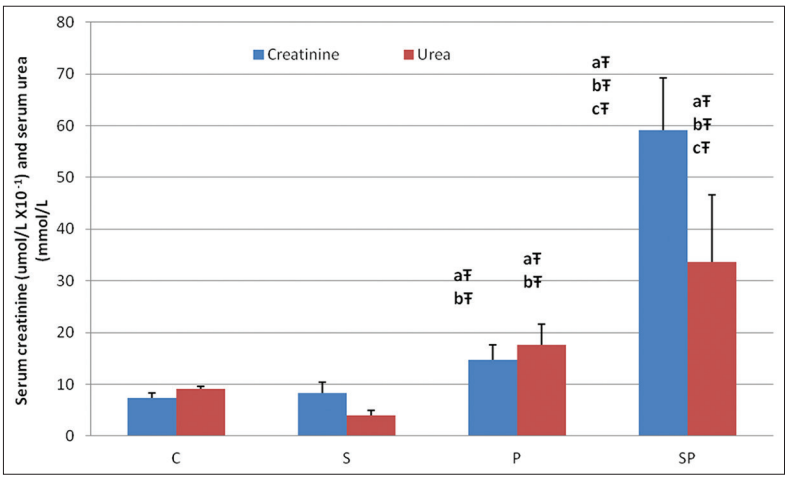

Figure 1: Serum creatinine and urea levels in rabbits injected intraperitoneally (ip) with saline (C), given spironolactone (20 mg/kg bw/ day) orally for 5 days, injected ip with cisplatin (6.5 mg/kg bw) in a single dose (P) or given spironolactone (20 mg/kg bw/ day) orally for 5 days then injected with cisplatin (6.5 mg/kg bw) on day 3 (SP). Columns and vertical bars represent means ± SD. ₮ P < 0.001. a significantly different from C, b significantly different from S, c significantly different from P.

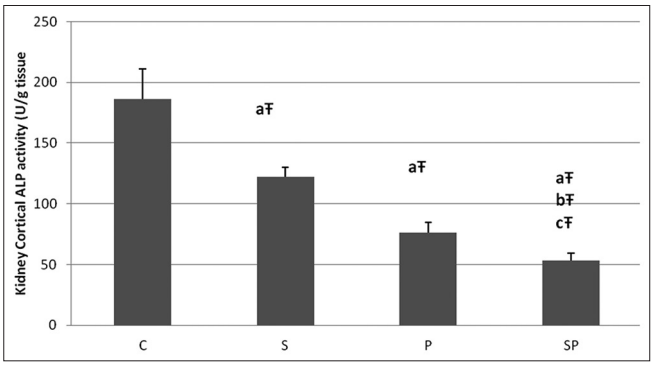

Figure 2: Renal cortical alkaline phosphatase (ALP) activity in rabbits injected intraperitoneally (ip) with saline (C), given spironolactone (20 mg/kg bw/ day) orally for 5 days, injected ip with cisplatin (6.5 mg/kg bw) in a single dose (P) or given spironolactone (20 mg/kg bw/ day) orally for 5 days then injected with cisplatin (6.5 mg/kg bw) on day 3 (SP). Columns and vertical bars represent means ± SD. ₮ P < 0.001. a significantly different from C, b significantly different from S, c significantly different from P.

of the P group was significantly depressed by 20% and 13.6% compared to C and S groups, respectively. However, the serum potassium in the SP group was significantly higher than P group by 25.76% and was not different from that of the control group. Similarly, the serum calcium in the S group was significantly elevated by 96.8% compared to control. Whereas, it was significantly reduced in the P group by 52.6% compared to the S group. However, the serum calcium level in the SP group was significantly elevated by 200%, 55.2% and 228% compared to C, S and P, respectively.

As shown in Table 3, the liver tissue calcium content in the S group was significantly elevated by 77.8% compared to control and slightly depressed in the P group by 6.55% compared to control. However, the liver calcium was significantly elevated in the SP group by 40.57%, 20.96% and 50.43% compared to C, S and P, respectively. Whereas, the liver magnesium was not different in the S or P groups compared to control. However, it was significantly elevated in the SP group by 38.37%, 36.76 and 33.7% compared to C, S and P, respectively. On the other hand, the kidney cortical calcium content was significantly depressed in the S and P groups by 30.89% and 40.0%, respectively compared to control and in the P group by 13.25% compared to S.Whereas in the SP group, it was lower than control by 28.2%, higher than P by 19.65% and not different from that of S group. Similarly, the cortical magnesium was reduced in the S, P and SP groups by 36%, 24% and 19%, respectively compared to control, and significantly higher in the SP group by 26.56% compared to S group.

DISCUSSION

In the present study cisplatin given in a single injection at a dose of 6.5 mg/kg bw induced significant nephrotoxicity in the rabbits to investigate the changes in electrolyte levels and to elucidate the effect of spironolactone administration on these electrolyte balances. Magnesium is a divalent cation that acts as an important cofactor for over 300 metabolic and cellular transmembrane enzymes, and its deficiency has been implicated in the development of several metabolic diseases.[8,9] In the present study treatment with cisplatin caused significant reduction in the serum magnesium levels. This was in congruence with the reports that indicated the association of a negative magnesium balance with cisplatin treatment.[10] The authors attributed this drug-induced negative effect to reduction in the intestinal absorption of magnesium.[3] Whereas, other investigators believe that cisplatin induces hypomagnesemia through its renal toxicity

Table 1: Serum albumin levels and liver function parameters in rabbits injected intraperitoneally (ip) with saline (C), given spironolactone (20 mg/kg bw/day) orally for 5 days, injected ip with cisplatin (6.5 mg/kg bw) in a single dose (P) or given spironolactone (20 mg/kg bw/day) orally for 5 days then injected with cisplatin (6.5 mg/kg bw) on day 3 (SP)

		C	S	P	SP
1	Serum albumin (g/dl)	3.03±0.24	2.69±0.74	2.61±0.63a*	2.50±0.41a**
2	Serum ALT (U/L)	42.49±7.88	46.05±12.28	37.28±7.27	39.11±12.25
3	Serum AST (U/L)	35.63±5.55	28.47±13.23	34.02±5.59	40.94±10.83
4	Liver ALP (U/g protein)	43.43±9.46	39.22±12.38	38.02±8.65	45.65±13.37

The presented data are means±SD. *P<0.05, ** P<0.01. a significantly different from C

Table 2: Serum electrolyte levels in rabbits injected intraperitoneally (ip) with saline (C), given spironolactone (20 mg/kg bw/day) orally for 5 days, injected ip with cisplatin (6.5 mg/kg bw) in a single dose (P) or given spironolactone (20 mg/kg bw/day) orally for 5 days then injected with cisplatin (6.5 mg/kg bw) on day 3 (SP)

		C	S	P	SP
1	Serum Sodium (mmol/L)	140.00±1.78	138.79±6.67	138.56±2.76	140.43±6.67
2	Serum Potassium (mmol/L)	6.51±0.43	6.32±0.93	5.20±0.15a₮b₮	6.54±1.13c₮
3	Serum Calcium (mmol/L)	3.51±0.44	6.91±3.34 a₮	3.27±0.16 b₮	10.73±2.51 a₮b₮c₮
4	Serum Magnesium (mmol/l)	0.96±0.18	1.86±0.59 a₮	0.78±0.30 a* b₮	2.04±0.27 a₮c₮

The presented data are means±SD. *P<0.05, ₮ P<0.001. a significantly different from C, b significantly different from S, c significantly different from P.

Table 3: Liver and kidney cortical calcium and magnesium contents in rabbits injected intraperitoneally (ip) with saline (C), given spironolactone (20 mg/kg bw/day) orally for 5 days, injected ip with cisplatin (6.5 mg/kg bw) in a single dose (P) or given spironolactone (20 mg/kg bw/day) orally for 5 days then injected with cisplatin (6.5 mg/kg bw) on day 3 (SP)

	C	S	P	SP
Liver				
Ca (mmol/g tissue)	2.44±0.25	4.34±0.93 a₮	2.28±0.76 b₮	3.43±1.22 a₮b₮c₮
Mg (mmol/g tissue)	0.86±0.17	0.87±0.27	0.89±0.28	1.19±0.35 a₮b₮c₮
Kidney Cortex				
Ca (mmol/g tissue)	3.82±0.37	2.64±0.56 a₮	2.29±0.53 a₮	2.743±1.06 a₮ c*
Mg (mmol/g tissue)	1.00±0.14	0.64±0.26 a₮	0.76±0.29 a₮	0.81±0.29 a₮ b*

The presented data are means±SD. *P<0.05, ₮ P<0.001. a significantly different from C, b significantly different from S, c significantly different from P

that hinders magnesium reabsorption in the loop of Henle causing urinary wasting.[11]

The mechanism of renal magnesium handling is known to involve a membrane protein known as; Transient Receptor Potential Melastatin subtype 6 (TRPM6) and the epidermal growth factor (EGF). TRPM6 is believed to be a magnesium- channel linked to an α-kinase domain.[12] and a mutation in the TRPM6 gene was shown to cause hypomagnesemia with secondary hypocalcemia.[13] The mechanism underlying this hypomagnesemia was shown to involve impairment of intestinal magnesium absorption as well as renal magnesium wasting.[14] Tacrolimus, a calcineurin inhibitor, caused hypomagnesemia through down-regulation of TRPM6 channels, whereas, cetuximab, an EGF- receptor inhibitor, was shown to abolish the stimulatory effect of EGF on TRPM6 channel activity.[15,16] Ledeganck et al.[17] indicated that cisplatin treatment had a similar effect that down-regulated the expression of EGF and TRPM6 causing renal magnesium wasting. However, the lowered serum magnesium levels observed in our cisplatin- treated rabbits seem to be caused primarily by renal wasting and to a lower extent by reduced intestinal absorption. This was evidenced by the depleted renal cortical magnesium content in the cisplatin-treated group, with unaltered liver magnesium content. However the percentage of reduction in the serum magnesium of the cisplatin treated animals was close to its reduction percentage in the kidney cortical tissue. This indicates that the major part of magnesium reduction was due to the renal tubular wasting. Our data also revealed significant reductions in the serum and cortical calcium levels in the cisplatin- treated group. Magnesium is known to contribute in the parathyroid hormone (PTH) release in response to hypocalcemia. Thus, the observed reduction in serum calcium can be attributed to the magnesium depletion. The mechanism underlying the effect of magnesium deficiency has been related to an activation of the α-subunit

of G-protein causing inhibition of the PTH secretion. The PTH in turn is believed to regulate the magnesium homeostasis by influencing the renal magnesium reabsorption.[18] The activation of calcium receptors in the renal tubules was shown to inhibit the renal magnesium reabsorption,[19] which would further reduce the magnesium level and inhibit the PTH release ending up in depletion of the serum calcium level. Thus, the observed reduction in serum calcium could be secondary to the cisplatin-induced magnesium depletion. Interestingly, the depletion of renal cortical magnesium paralleled with the depression in its calcium content, whereas, the unaltered liver magnesium content was accompanied with unaffected liver calcium concentration, indicating a primary role for magnesium in the homeostasis of calcium.

On the other hand, magnesium is known to act as a cofactor for Na^+, K^+-ATPase and hypomagnesemia can impair its activity. The Na^+, K^+-ATPase is a membrane-bound enzyme that actively transfers potassium and sodium ions across the cell membrane maintaining the ionic gradient.[20] Several earlier articles have suggested that impairment of Na^+, K^+-ATPase caused by magnesium deficiency contributes to urinary potassium wasting.[21,22] Our data indicated that serum potassium levels were significantly reduced in the cisplatin-treated animals which paralleled with the depleted serum magnesium. This was in congruence with several reports. In humans, Arunkumar and co-workers[2] observed significant depletions of both magnesium and potassium in the majority of patients treated with cisplatin. It has been indicated that concomitant magnesium deficiency can aggravate hypokalemia and render it refractory to treatment by potassium supplementation. The magnesium deficiency is believed to potentiate the tubular potassium wasting. This occurs by releasing the magnesium-mediated inhibition of the potassium channels located in the tubular membrane.[23,24] The present findings are in line with this theory, where the cisplatin-treated animals with reduced serum potassium had their renal cortical tissue depleted from magnesium that may have contributed in enhancing the urinary potassium wasting. It has also been reported that magnesium deficiency needs to be accompanied with elevated aldosterone levels for exacerbating potassium wasting.[23] In the present study this was evidenced by the effect of spironolactone, the aldosterone antagonist, which reversed the hypokalemia in the cisplatin- treated animals back to the control levels. Moreover, some investigators have indicated that expression of the tubular Na^+, K^+-ATPase was up-regulated by the aldosterone treatment and was suppressed by spironolactone.[25] Similar ameliorative effects of spironolactone were also observed with other nephrotoxic drugs. Spironolactone was shown to prevent hypokalemia by reducing urinary potassium loss in patients treated with amphotericin B.[26]

Although, spironolactone alone doubled the serum magnesium level, it failed to increase the serum potassium beyond the control levels, whereas, in the cisplatin-treated animals spironolactone effectively reversed the cisplatin-induced potassium depletion. This indicates that the effect of spironolactone in potassium sparing is enhanced when there is potassium deficiency, whereas its effect is only minor when the serum potassium is sufficient. In line with this, authors have indicated that spironolactone by itself has only slight diuresis, requiring concomitant administration of another diuretic that inhibits sodium reabsorption.[4] It was reported that hypertensive patients with high renin activity have significantly lower serum magnesium levels and plasma renin activity was inversely associated with serum magnesium levels.[27] This explains the observed effect of spironolactone that antagonizes the effect of renin and aldosterone to raise the serum magnesium level. Moreover, patients treated with spironolactone for six months had increased plasma and erythrocyte magnesium concentrations and decreased the erythrocyte efflux.[28] In our results this magnesium sparing effect of spironolactone was significant even when administered along with cisplatin. However, this finding of spironolactone to reverse the cisplatin induced depletion of serum magnesium by about two-fold did not prevent the aggravating effect of spironolactone on the cisplatin-induced nephrotoxicity. Some investigators believe that magnesium depletion may contribute in enhancing the development of cisplatin nephrotoxicity and that its supplementation can protect from the renal toxicity.[29] This probably indicates that the protective effect of magnesium sufficiency on the kidney was not effective in counteracting the aggravating effect of spironolactone on cisplatin toxicity. Our data have indicated that correction of the magnesium balance by spironolactone concomitantly corrected the serum levels of calcium and potassium in the animals treated with both drugs. The present findings and earlier reports emphasize that the magnesium state in the body influences the homeostasis of sodium, potassium and calcium. Therefore, the pathophysiology aspects of potassium and calcium metabolism become important if magnesium depletion is suspected during treatment with nephrotoxic drugs, and magnesium deficiency may need to be corrected and be ruled out as an underlying cause when diagnosing potassium and calcium disorders.

CONCLUSION

Cisplatin treatment depleted the serum and renal cortical magnesium. There was significant reduction in serum calcium and potassium shown to be secondary to the magnesium depletion. The spironolactone significantly raised the magnesium levels in serum and renal cortices

and consequently ameliorated the serum and cortical calcium concentration. Spironolactone corrected the electrolyte balances in cisplatin treated animals, but failed to moderate the cisplatin induced nephrotoxicity. Lower doses of spironolactone may be beneficial in correction of the cisplatin induced electrolyte imbalances with minimum adverse effects on the kidney.

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Authors Contribution:
AA Abdel-Gayoum- Concept and design and execution of experiments, acquisition and statistical analysis of data, manuscript preparation, critical review of the manuscript; **MH Ahmaida-** Concept and design of study, animal treatments, analysis of the data, critical evaluation of the manuscript.

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