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Magnesium depletion enhances cisplatin-induced nephrotoxicity

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Abstract *Purpose:* Nephrotoxicity and magnesium (Mg)-depletion are well-known side effects to cisplatin (CP) treatment. The purpose of this present study was to investigate the role of Mg on CP induced changes in renal function. CP induced renal dysfunction was achieved by treatment with CP or vehicle (2.5 mg/kg) once weekly for 3 weeks. Since the CP-induced renal damage, including tubular reabsorption defects, is most prominent within the outer medulla (OM), changes in the expression pattern of OM aquaporins and sodium transporters including the Na,K-ATPase (α -subunit), type III Na,H-exchanger (NHE3), aquaporin 1 (AQP1) and 2 (AQP2) and the Na,K,2Cl-cotransporter (NKCC2) were investigated by semi-quantitative Western blotting. *Experimental design:* Rats had access to either a diet with standard Mg or to a Mg-depleted diet. Cisplatin was administered to female Wistar rats once a week for 3 weeks according to four regimens: (1) Cisplatin 2.5 mg/kg body weight i.p., to rats on a diet with standard Mg, (2) Cisplatin 2.5 mg/kg body weight i.p., to rats on a diet

with low Mg, (3) Isotonic NaCl 2.5 ml/kg body weight i.p., to rats on a diet with standard Mg, (4) Isotonic NaCl 2.5 ml/kg body weight i.p., to rats on a diet with low Mg. *Results:* CP had no effect on plasma creatinine or urea in rats with standard Mg intake, but the expression of all five transporters was significantly reduced when compared to vehicle treated rats on standard Mg-intake. Vehicle treated rats on low Mg-intake had a significant reduction in the expression of Na,K-ATPase, NHE3 and NKCC2, but unchanged expression levels of AQP1 or AQP2 when compared to standard treated controls. Forty percent of the CP-treated rats on low Mg-intake died during the experiment and the remaining animals had marked increased plasma creatinine and urea. Furthermore, the Western blot analysis revealed an almost complete disappearance of all four transporters, suggesting a dramatic synergistic effect of CP and Mg-depletion on renal function including the expression pattern of outer medullary sodium transporters and aquaporins. *Conclusions:* This study indicates a substantial additive effect of Mg-depletion on cisplatin induced renal toxicity as evidenced by significant changes in plasma creatinine and urea, renal failure induced mortality and loss of renal transporters. This should give cause for concern since the nephrotoxicity observed during cisplatin treatment might be substantiated by the known Mg-loss associated with cisplatin treatment especially in patients suffering from intense gastrointestinal side effects.

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Introduction

Nephrotoxicity is a well-known side effect to cisplatin treatment. Though not necessarily dose limiting renal toxicity still affects the majority of patients and a significant decrease in glomerular filtration rate is

observed during treatment [1]. The renal toxicity of cisplatin is seen as a direct cytotoxic damage on the nephrons in the outer medulla especially in the S3 segment of the proximal tubules and in the thick ascending limb of the loop of Henle (TAL) [2]. Hence, cisplatin treatment often results in tubular reabsorption defects resulting in a concentration defect. Hypomagnesemia is observed in approximately 50% of patients treated with cisplatin and is probably due to a defect in magnesium (Mg) reabsorption [3, 4]. The clinical approach towards cisplatin-induced hypomagnesemia is controversial, since serious side effects ascribed to hypomagnesemia as ventricular arrhythmia are rarely encountered. Other symptoms, especially from the skeletal muscles, like fatigue, restlessness and muscle tetanus can be difficult to separate from side effects generally related to chemotherapy [5]. Numerous studies have addressed the clinical correction of hypomagnesemia with the purpose of ameliorating a speculated risk of heart and muscle side effects in these patients, but as yet no consensus has been established concerning the clinical benefit of Mg substitution in these patients [6–8]. However, a recent study has suggested that Mg supplementation is a crucial factor in protection against the nephrotoxic actions of Cyclosporin A [9] and a possible relation between Mg-loss and cisplatin induced nephrotoxicity has briefly been addressed in one study [3]. Furthermore, Mg is known to be a critical cofactor in many cellular processes including membrane transport and cellular repair [5].

The purpose of the present study was therefore to investigate the role of Mg on changes in renal function during cisplatin treatment. Rats had access to either a diet with standard Mg or to a Mg-depleted diet. Cisplatin induced renal dysfunction was instigated by repeated cisplatin treatment under these conditions. Since the cisplatin induced renal damage, including tubular reabsorption defects, is most prominent within the outer medulla [2, 10], changes in the expression pattern of outer medullary aquaporins and sodium transporters including the Na,K-ATPase (α -subunit), aquaporin 1 (AQP1) and 2 (AQP2), the Na,K,2Cl-co-transporter (NKCC2) and the type III Na,H-exchanger (NHE3) were investigated by semi-quantitative Western blotting.

Materials and methods

Female Wistar rats from Charles River (Sulzfeld, Germany) weighing 230–270 g were used. All animals had permanent tygon catheters placed in the left femoral artery for blood sampling. The animals were housed in a temperature (22–24°C) and moisture (40–70%) controlled room with a 12-h light–dark cycle (light on from 6:00 a.m. to 6:00 p.m.). Animals were given free access to demineralised water and diet pellets. Cisplatin was administered once a week for 3 weeks according to four regimens: (1) Cisplatin (Bristol-Myers Squibb, 1 mg/ml

2.5 mg/kg body weight i.p., to rats on a diet with standard Mg, (2) Cisplatin 2.5 mg/kg body weight i.p., to rats on a diet with low Mg, (3) Isotonic NaCl 2.5 ml/kg body weight i.p., to rats on a diet with standard Mg, (4) Isotonic NaCl 2.5 ml/kg body weight i.p., to rats on a diet with low Mg. Diet with standard Mg (Altromin, Lage, Germany) contained 2,000 μ g Mg/g. That the standard diet contained excess Mg was ascertained by the finding that the fractional intestinal absorption of Mg in a metabolic pilot study was shown to be \approx 30% of the ingested amount. Mg-deficient diet (Altromin, Lage, Germany) contained 120 μ g Mg/g. The diets were otherwise comparable in contents of K, Na and protein. Feeding with appropriate study diet was initiated 24 h before the first treatment was initiated.

All animal procedures followed the guidelines for the care and handling of laboratory animals established by the Danish government.

Blood samples

Blood samples for measurement of plasma Na, K, Mg, urea and creatinine were obtained from the arterial catheter prior to each treatment cycle and at the termination of the study. Baseline samples were obtained before change to appropriate study diet. Immediately after sampling they were centrifuged for 10 min at 3,000 rpm at 4°C and the plasma was then frozen at –21°C until analysed using the standard clinical chemistry systems VITROS 950 (Ortho-Clinical Diagnostics Inc., Johnson & Johnson, NJ, USA) and Roche Hitachi Modular (Roche Diagnostics, Mannheim, Germany).

Semiquantitative immunoblotting

One week after the last cisplatin injection, the rats were anaesthetized with isoflurane/N₂O and the right kidneys were removed. The outer medulla was isolated and homogenized using a tissue homogeniser (Ultra-Turrax T8, Ika, Staufen, Germany) in 10 ml of ice-cold homogenizing buffer containing 300 mM sucrose, 25 mM imidazol, 1 mM EDTA-disodium salt, and the following protease inhibitors: Pefabloc (0.1 mg/ml buffer) and leupeptin (4 μ g/ml buffer); and phosphatase inhibitors sodium orthovanadate (184 μ g/ml), sodium fluoride (1.05 mg/ml), and okadaic acid 82 (ng/ml), pH adjusted to 7.2 with HCl. Protein concentration in the homogenate was measured using a commercial kit (Pierce BCA Protein Assay Reagent Kit, Pierce, Rockford, IL, USA). All samples were then diluted to a final protein concentration of 4 μ g/ μ l with the addition of a sample buffer (in the final solution: 486 mM Tris–HCl, pH 6.8, 7% glycerol, 104 mM SDS, 0.0875 mM bromphenol blue, and dithiothreitol 25 mM), and homogenizing buffer. Four samples from each of the four groups were randomly selected. Samples of homogenates were run on 7.5% (Na,K-ATPase, NHE3 and NKCC2) and

12% (AQP1 and 2) polyacrylamide minigels. The proteins were then transferred by electrophoresis from the gels to PVDF membranes (90 min, 100 V). After 60 min blocking in 5% milk in PBS-T buffer (AQP2 only PBS-T), membranes were probed overnight at 4°C with the appropriate primary antibody ended by wash in PBS-T.

Na,K-ATPase: Measurement of Na,K-ATPase was done by a commercial rabbit polyclonal anti- α_1 -subunit antibody (06-520; Upstate Biotechnology, Lake Placid, NY, USA). **NHE3:** Measurement of NHE3 was done by a rabbit polyclonal anti-NHE3 antibody raised against a synthetic peptide corresponding to amino acids 809–831 of rat NHE3 [11]. **AQP2:** Measurement of aquaporin-2 (AQP2) was done by commercial affinity-purified goat polyclonal anti-AQP2 antibody (sc-9882; Santa Cruz Biotechnology, Santa Cruz, CA, USA). **NKCC2:** Measurement of NKCC2 was done by a rabbit polyclonal anti-NKCC2 antibody raised to a synthetic peptide corresponding to amino acid 33–55 of rat NKCC2 [12]. **AQP1:** Measurement of AQP1 was done by a rabbit polyclonal anti-AQP1 antibody raised against the N-terminus of AQP1 [13].

The labelling was visualized with horseradish peroxidase-conjugated secondary antibody [P448 (rabbit) or P0449 (goat); Dako] using an enhanced chemiluminescence system (ECL+, Amersham). The \approx 96-kDa band corresponding to Na,K-ATPase, the \approx 86-kDa band corresponding to NHE3, the \approx 29 and 40-kDa bands corresponding to AQP1 and glycosylated AQP1, respectively, the \approx 29 and 40 kDa bands corresponding to AQP2 and glycosylated AQP2, respectively and the \approx 165-kDa band corresponding to NKCC2 were scanned by use of a FluorX multi-imager (Bio-Rad Laboratories). Densitometry of individual bands was quantified using the software program Quantity One, version 4.2.3 (Bio-Rad Laboratories). Protein labelling in samples from the different groups was expressed relative to the mean expression of the controls run on the same gel. Finally, Ponceau staining of the PVDF membranes was carried out as a control for equal loading.

Cisplatin measurement

After killing, left kidneys were removed and the outer medulla isolated producing samples of 150–200 mg wet weight. The samples were then dried to constant weight at 70°C and re-weighted to exact dry weight. An amount of 0.5 ml 65% double distilled HNO₃ was added and the sample allowed to stay overnight. 1.5 ml 65% double distilled HNO₃ and 0.5 ml double distilled H₂O (> 18.2 MOhm) was added and the sample heated in a sealed Teflon liner in a microwave oven until the sample was completely dissolved. The sample was then transferred to a plastic vial with double distilled H₂O to a total volume of 10.0 ml.

Prior to analysis with inductively coupled plasma mass spectrometry the cisplatin treated specimens were diluted 100 times by weight with 2 vol% HNO₃ and the

control samples diluted ten times with 2 vol% HNO₃ and both types of samples were spiked with 0.2 μ g/kg (ppb) Indium (In) for use as internal standard. Total dilution was 15,000–26,000 times for the cisplatin samples and 1,400–2,600 times for the control samples. A series of calibration standards from 0.01–1 ppb in 2 vol% HNO₃ with 0.2 ppb In were used for calibration as well as to estimate the detection limits of the analysis.

The analysis was performed with a Plasmatrix 2 high resolution inductively coupled plasma mass spectrometer (MicroMass Ltd, Manchester, England) equipped with an APEX sample introduction system (CPI International, Amsterdam, Netherlands). Platinum (Pt) was measured at mass to charge ratio (m/z) 194, 195 and 196 and the internal standard In was measured at m/z 115. Good agreement between the three different Pt masses was received suggesting no significant interferences and 99% and 100% recovery of two 0.2 ppb Pt spikes were observed suggesting that the analysis was done with good control of the accuracy. Detection limits calculated from three times the standard error of the estimate of the calibration sensitivity was \approx 0.04 μ g/g (ppm).

Statistics

SPSS statistical software was used. Data are presented as means \pm SEM. Differences were considered significant at the 0.05 levels. Comparisons were made using Mann–Whitney independent samples *t* test with Bonferroni correction.

Results

The overall implication of the study was that multiple doses of cisplatin had severe effects in Mg-deficient rats. Five of 14 animals died during the experimental period and the rest of the animals had significant weight losses throughout (Table 1). On the other hand, all the rats fed on a diet with standard Mg went through the experimental protocol without mortality and were in positive weight gain at the end of the experiment. In general, Mg-deficient diet caused a significant decline in plasma Mg. However, at the end of the second week four rats, which subsequently died in the third week of cisplatin treatment, had developed severe renal insufficiency, which caused a temporary increase in mean P–Mg (Table 1).

The cisplatin treatment caused significant increases in both plasma creatinine and urea in the Mg-deficient rats indicating severe renal failure, whereas, the rats on a standard Mg-intake had normal plasma concentrations of creatinine and urea throughout the 3-weeks experimental period (Fig. 1). Vehicle treated rats on the Mg-deficient diet, like the cisplatin treated rats, developed significant hypomagnesemia, but plasma creatinine and urea were unchanged throughout the experimental period (Table 1).

The vehicle treated rats on low Mg-diet had significant hyperkalemia (Table 1). There were no other significant differences in P–Na and P–K between groups.

Table 1 Plasma-electrolyte concentrations (mmol/l) at baseline and after cisplatin/NaCl administration in the four treatment groups

Series	Baseline	1 week	2 weeks	3 weeks
1. Cisplatin treated, standard Mg-diet				
<i>n</i>	6	6	5	6
Weight	257 ± 5	262 ± 5	262 ± 6	270 ± 7
P-Mg	0.81 ± 0.01	0.83 ± 0.01	0.85 ± 0.03	0.84 ± 0.02
P-K	4.7 ± 0.1	3.9 ± 0.1	4.5 ± 0.1	4.1 ± 0.1
P-Na	140 ± 1	139 ± 0	141 ± 1	134 ± 0
2. NaCl treated, standard Mg-diet				
<i>n</i>	6	5	5	6
Weight	273 ± 5	277 ± 7	278 ± 7	286 ± 5
P-Mg	0.81 ± 0.01	0.89 ± 0.04	0.86 ± 0.03	0.80 ± 0.03
P-K	4.7 ± 0.2	4.4 ± 0.2	4.3 ± 0.1	4.1 ± 0.2
P-Na	141 ± 1	141 ± 1	142 ± 1	136 ± 1
3. Cisplatin treated, Mg-deficient diet				
<i>n</i>	14	13 (1 ^b)	13	9 (4 ^b)
Weight	258 ± 5	245 ± 6	215 ± 7	218 ± 11
P-Mg	0.72 ± 0.01	0.22 ± 0.03 ^a	0.60 ± 0.14	0.28 ± 0.14 ^a
P-K	4.7 ± 0.1	5.0 ± 0.1	4.1 ± 0.1	4.4 ± 0.1
P-Na	142 ± 1	144 ± 1	137 ± 3	143 ± 2
4. NaCl treated, Mg-deficient diet				
<i>n</i>	8	8	8	8
Weight	256 ± 7	264 ± 8	268 ± 8	268 ± 9
P-Mg	0.76 ± 0.01	0.15 ± 0.02 ^a	0.15 ± 0.01 ^a	0.14 ± 0.02 ^a
P-K	4.4 ± 0.1	4.3 ± 0.1	4.1 ± 0.1	5.5 ± 0.3 ^a
P-Na	139 ± 1	142 ± 1	139 ± 0	133 ± 1

Values are mean (SEM)

^aRepresents $P < 0.05$ compared to baseline values

^bIndicates mortality since last measurement. Failure in *n* during follow-up in groups 1 and 2 represents clotting of arterial catheter

Expression of Na-transporters in renal outer medulla

Figure 2 shows western blots of membrane fractions (10 µg protein/lane) from renal outer medulla preparations. The affinity purified anti-Na,K-ATPase antibody recognizes a band at ~96 kDa corresponding to the α -subunit of the Na,K-ATPase. In the outer medulla, Na,K-ATPase is present both in the S3-segment of the proximal tubules, in the TAL and in the collecting ducts. Both cisplatin treatment in rats on standard Mg intake and vehicle treatment on low Mg-intake significantly reduced the expression of the α -subunit of the Na,K-ATPase when compared to the expression level found in vehicle treated rats on standard Mg-intake. This finding indicates that cisplatin or Mg-deficiency per se decreases the Na,K-ATPase abundance in the outer medulla. Moreover, the western blots showed a marked additive effect of cisplatin and Mg-deficiency on the expression of the Na,K-ATPase with an almost complete disappearance of the α -subunit in the cisplatin-treated rats on low Mg-intake (Fig. 2).

A similar observation was done in the western blots of membrane fractions (28 µg protein/lane) from renal outer medulla preparations for NKCC2 shown in Fig. 2. The affinity purified anti-NKCC2 antibody recognizes a broad band at ~165 kDa corresponding to the furosemide-sensitive type-2 Na-K-2Cl co-transporter exclusively expressed in the TAL and in the macula densa. Like the Na,K-ATPase both cisplatin or Mg-deficiency per se significantly decreased the abundance of NKCC2 and the combination of cisplatin and low Mg-intake resulted in an almost complete disappearance of the transporter.

The observations in NHE3 was, however, not similar to the findings in Na,K-ATPase and NKCC2 (Fig. 2). Again western blots of membrane fractions (10 µg protein/lane) from renal outer medulla were prepared. The affinity purified anti-NHE3 antibody recognizes a band at ~86 kDa corresponding to the type-3 sodium-proton exchanger expressed in the TAL and in the proximal tubules. The preparations showed that cisplatin or Mg-deficiency resulted in an almost complete disappearance of the transporter (Fig. 2). Consequently, we were unable to detect a further decrease in the cisplatin treated rats on low Mg-intake.

Expression of the aquaporins in the renal outer medulla

We also measured the expression level of the water channels AQP1 and AQP2 in the outer medulla. In the outer medulla AQP1 is present in the S3 segment of the proximal tubules and in the thin descending limb of Henle's loop. AQP2 is exclusively expressed in the principal cells of the collecting ducts. Figure 3 shows western blots of membrane fractions (28 µg protein/lane) from renal outer medulla preparations. The anti-AQP1 antibody recognizes the 29-kDa and the 35–50 kDa band, corresponding to non-glycosylated and glycosylated AQP1 protein, similarly the affinity purified anti-AQP2 antibody recognizes the 29-kDa and the 35–50 kDa band, corresponding to non-glycosylated and glycosylated AQP2 protein. Cisplatin treatment reduced both the expression of AQP1 and AQP2 in the standard Mg-fed rats when compared to the standard Mg-fed

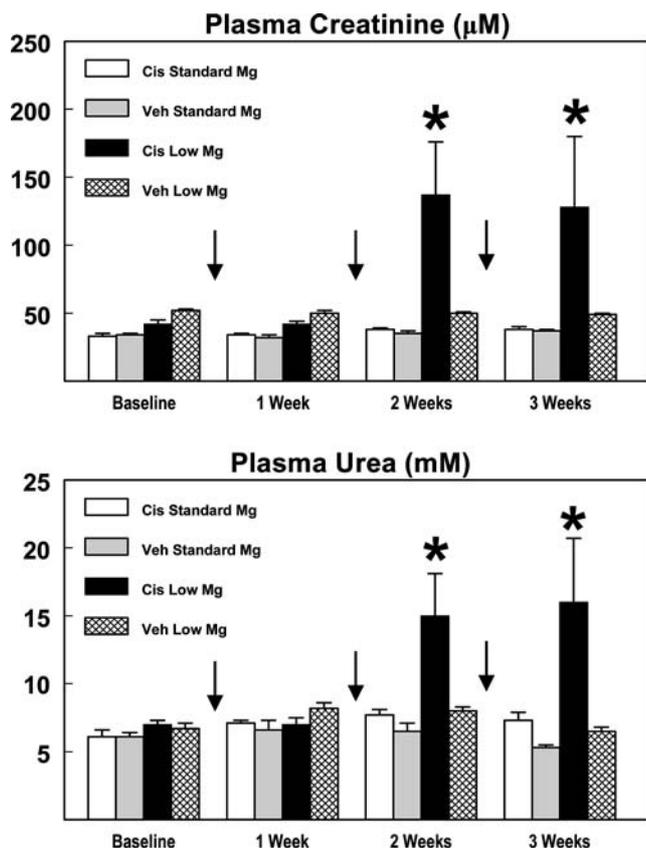


Fig. 1 P-creatinine and P-urea following repeated injections of cisplatin or NaCl to rats on standard or Mg-depleted diet. Bars represent means with SEM attached. Cis 2.5 mg/kg i.p. or NaCl 2.5 ml/kg i.p. was administered weekly (arrows) and plasma-values were measured immediately prior to next injection. *indicates $P < 0.01$ compared to baseline

vehicle treated control rats although the change was only significant in the case of AQP1. Mg-depletion did not significantly reduce the expression levels of either AQP1 or AQP2 when compared to the standard Mg-fed control rats. However, the cisplatin treated rats on low Mg-intake had an almost complete disappearance of both AQP1 and AQP2, suggesting a dramatic synergistic effect of cisplatin and Mg-depletion on the expression pattern of the two water channels (Fig.3).

Cisplatin concentration in the renal outer medulla was below the detection limit in the two groups, which did not receive cisplatin treatment. Furthermore, no significant difference in cisplatin concentration in the renal outer medulla was observed between the two groups treated with cisplatin 7.98 (1.15) ppm versus 8.23 (1.01) ppm ($P = 0.87$).

Discussion

The main finding in the present study was that cisplatin-induced renal dysfunction was significantly increased during conditions of dietary Mg-depletion when compared with rats fed a standard Mg-diet. Rats treated

with cisplatin fed on a diet with low Mg-content had marked impairment of renal function evaluated by increases in plasma creatinine and urea to a level which suggests that GFR was reduced by at least 30% [14]. Furthermore, these rats were characterized by an almost complete disappearance of the main tubular sodium transporters and aquaporins within the renal outer medulla.

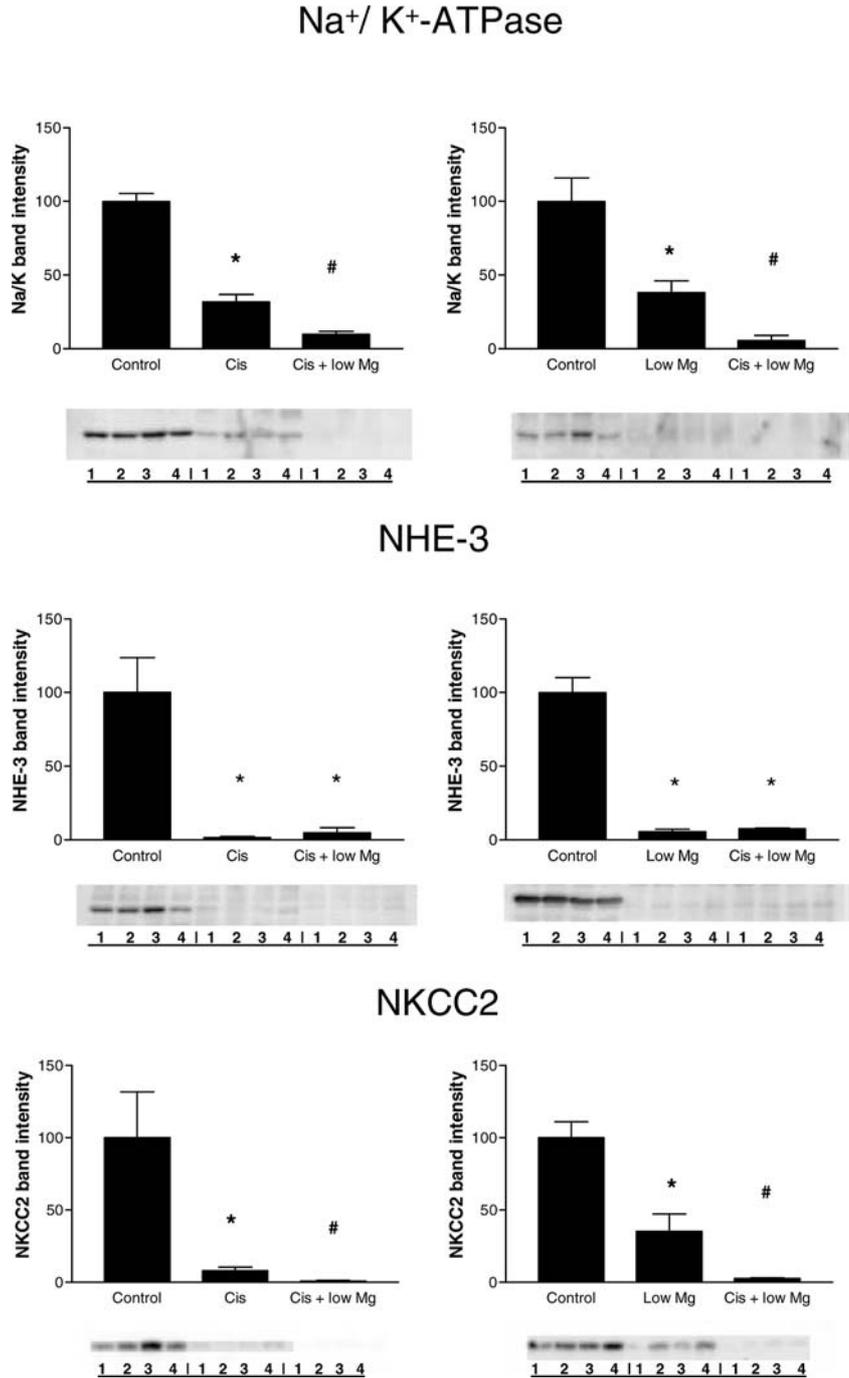
In the present study, the rats were treated with cisplatin in a dosage of 2.5 mg cisplatin/kg body weight adapted from a study by Mavichak et al. [4]. This dose was well tolerated on a standard Mg-diet containing $\approx 2,000 \mu\text{g Mg/g}$ judged from unchanged plasma creatinine and urea throughout the 3-weeks experimental period. In contrast treatment with cisplatin 2.5 mg cisplatin/kg body weight combined with a diet containing $120 \mu\text{g Mg/g}$ elicited 30% mortality from renal failure with significant renal insufficiency among survivors at the end of the experimental period.

The effect of dietary Mg-supplementation in preventing cisplatin nephrotoxicity may be related to prevention of nephrotoxic mechanisms in general. Asai et al. [9] have recently studied the role of Mg-supplementation on the development of Cyclosporine A induced nephrotoxicity in rats. They found that Mg-supplementation abolished Cyclosporine A induced precedent interstitial inflammation most likely via inhibition of chemoattractants expression and consequently attenuated tubulointerstitial fibrosis. Conversely, Mg-depletion would therefore allow a more intense inflammatory response leading to increased fibrosis and loss of renal function [9]. Other studies have implicated Mg-deprivation in oxidative stress and cell death as well as pro-inflammatory stress response [15–17]. Furthermore, compounds with antioxidant properties have been shown to suppress renal infiltration with macrophages after cisplatin treatment and subsequently suppressing renal fibrosis [18].

As would be expected, P-Mg values decreased significantly in the two groups fed a low Mg containing diet. Interestingly, this was not the case in the group treated on a standard Mg-diet and cisplatin. Mavichak et al. [4] originally used the same setup as in the present study with intraperitoneal cisplatin treatment once every week [10]. In contrast to the present finding, they found a significant decrease in P-Mg after the third dose of cisplatin on a similar dose schedule [4]. The discrepancy may be explained by differences in Mg-content in the diets since they used a Mg concentration of $300 \mu\text{g Mg/g}$. Mavichak et al. did not observe any mortality, but did observe a relative renal function decrease after the third treatment. Together these observations support an inverse association between Mg concentration in the diet and renal function impairment during cisplatin treatment, and that renal function impairment might be partly avoided by increasing oral Mg-intake.

Even though that neither cisplatin nor Mg-depletion independently elicited an increase in plasma creatinine or urea both cisplatin treatment and Mg-depletion led to

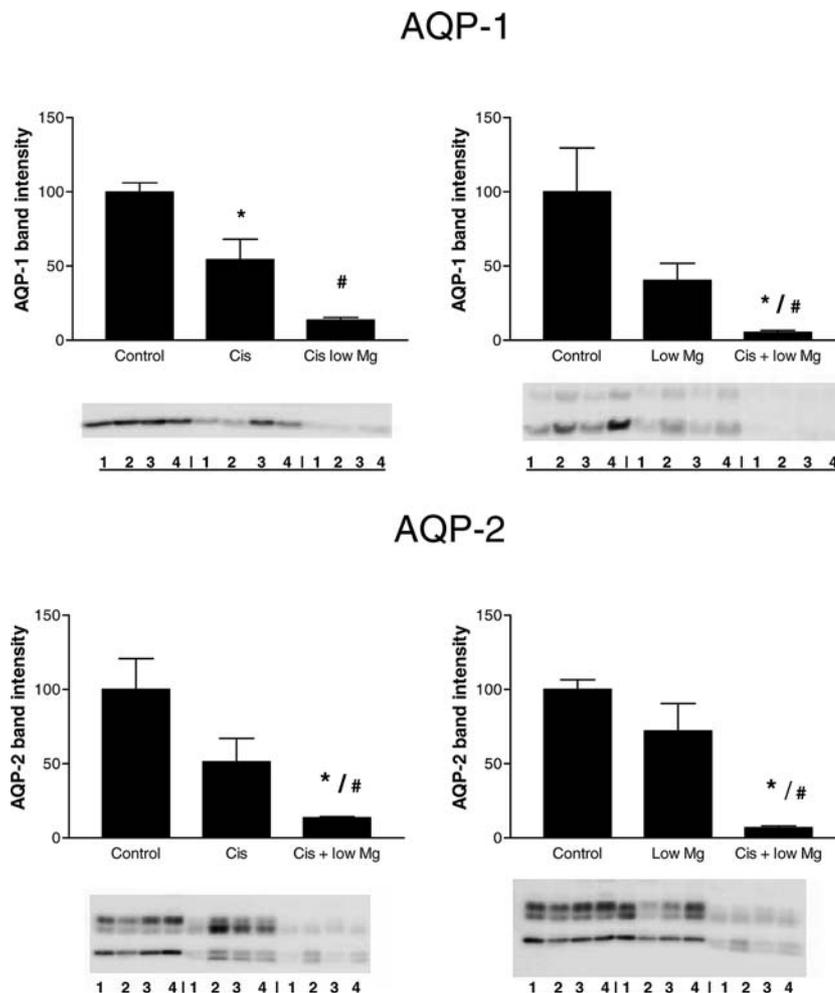
Fig. 2 Na,K-ATPase, NHE-3 and NKCC2 expression in outer medulla 1 week after the third administration of cisplatin. Control treated with isotonic NaCl 2.5 ml/kg body weight i.p, standard Mg-diet. Cis treated with cisplatin 2.5 mg/kg body weight i.p, standard Mg-diet. Low Mg treated with isotonic NaCl 2.5 ml/kg body weight i.p, low Mg-diet. Cis + low Mg denotes animals treated with cisplatin 2.5 mg/kg body weight i.p, low Mg-diet. Bars indicate mean with SEM attached. Control normalised to 100. *n* = 4. Na,K-ATPase: *Indicates *P* < 0.01 versus control. #Indicates *P* < 0.01 versus Cis or Low Mg, respectively. NHE-3: *Indicates *P* < 0.01 versus control. NKCC2: *Indicates *P* < 0.05 versus control. #Indicates *P* < 0.05 versus Cis or Low Mg, respectively



a decrease in the abundance of outer medullary sodium transporters and to a lesser degree aquaporin 1 and 2. Except for NHE3, the observed effects of cisplatin and Mg-depletion on renal transporters were additive. The observed effect of cisplatin alone might represent either a specific effect on transporters or an unspecific nephrotoxic reaction related to its cytostatic properties. Recently, Ecelbarger et al. [19] reported an unchanged expression pattern of both NKCC2, Na,K-ATPase and NHE3 within the outer medulla 1 week after a 2-fold higher dose cisplatin than used in the present study. This

suggests that cisplatin-induced downregulation of outer medullary sodium transporters is related to both follow-up time and the cumulative dosage. However, the mechanisms responsible for this effect are presently unknown. Interestingly, the present data indicates that the cisplatin induced tubular toxicity in concordance with Mg-depletion not only affects the S3 segment and the TAL, which previously have been shown morphologically for cisplatin alone [10], but also have direct effects on more distal nephron segments as indicated by the significant reduction in the expression of AQP2, which is

Fig. 3 AQP1 and AQP2 expression in outer medulla 1 week after the third administration of cisplatin. Control treated with isotonic NaCl 2.5 ml/kg body weight i.p., standard Mg-diet. Cis treated with cisplatin 2.5 mg/kg body weight i.p., standard Mg-diet. Low Mg treated with isotonic NaCl 2.5 ml/kg body weight i.p., low Mg-diet. Cis + low Mg denotes animals treated with cisplatin 2.5 mg/kg body weight i.p., low Mg-diet. Bars indicate mean with SEM attached. Control normalised to 100. $n = 4$. AQP1: *Indicates $P < 0.05$ versus control. #Indicates $P < 0.05$ versus Cis or Low Mg, respectively. AQP2: *Indicates $P < 0.01$ versus control. #Indicates $P < 0.05$ versus Cis or Low Mg, respectively



located exclusively in the collecting ducts. The observed nephrotoxicity did not seem to be mediated by an increase in renal cisplatin concentration in the Mg-depleted cisplatin treated animals, since no significant difference was observed between these animals and those treated with cisplatin on a normal diet.

The finding that Mg-depletion alone induced a marked decrease in the expression of outer medullary sodium transporters was surprising. The Na,K-ATPase is known to be Mg-dependent with Mg-depletion inducing a decrease in Na,K-ATPase concentration through secondary K-depletion [20]. In contrast, neither NKCC2 nor NHE-3 has been reported to be Mg-dependent. Thus, it seems that the effect of Mg-depletion is a diffuse tubular reaction affecting both Mg-dependent and Mg-independent transporter proteins including possible Mg transporters. The fact that combined cisplatin treatment and Mg-depletion leads to an almost complete disappearance of all the measured transporters is not surprising based on the observed independent effects of cisplatin and low-Mg. However, that the combination of cisplatin and Mg-depletion also induced acute renal failure as evidenced by the marked increase in plasma creatinine and urea was surprising. The

mechanisms responsible for this synergistic nephrotoxicity are unknown.

The possible clinical implications of these observations are obvious. Cisplatin induces Mg-depletion in patients and the present data strongly indicate that this Mg-depletion itself might enhance the nephrotoxicity of cisplatin. Thus, doses of cisplatin, which would otherwise be tolerable, may in the presence of Mg-depletion induce a significant loss of renal function leading to termination of treatment or in some cases even fatality. In this respect, it is of considerable interest that cisplatin treatment often carries extensive gastro-intestinal side effects, which might lead to further Mg-depletion through anorexia and diarrhoea. Eventually, patients with such side effects might be rendered more susceptible to the nephrotoxicity of cisplatin. In a small study by Willox et al. [3], 17 germ cell tumour patients receiving cisplatin in a dosage of 20 mg/m² per day for 5 days in four series were randomised between receiving continuous Mg-supplementation and supplementation only at serum levels below 0.45 mmol/l. Though no differences were observed in serum creatinine or creatinine clearance a significantly lower excretion of the tubular damage marker NAG (*N*-acetyl-B-D-glucosaminidase)

was observed in the urine in the group receiving Mg-supplementation. It is possible that a similar study on patients receiving 100 mg/m² would show a larger difference in nephrotoxicity between the two regimens of supplementation. Furthermore, creatinine clearance is not a very sensitive parameter for GFR in humans as opposed to ⁵¹EDTA-clearance, which is the standard today [21]. Given the fact, that a Mg-loss has always been expected in these patients combined with the commonly observed decreases in GFR during cisplatin treatment, it is surprising, that the study by Willox et al. [3] has not had much clinical impact nor been studied in conjunction with the more toxic cisplatin regimens.

Although our knowledge about the exact relation between the present animal observations and the possible human implications cannot yet be established, the observations should give cause for concern and need to be pursued in the clinical setting. Evidently, the observed effects on P-Mg and renal function are stronger than seen in most patients. However, in some patients severe loss of renal function may progress very rapidly and most patients experience some degree of lowered renal function with no simple correlation to P-Mg levels [22].

In summary, the present study therefore indicates a substantial additive effect of Mg-depletion on cisplatin induced renal toxicity. This should give cause for concern since the nephrotoxicity observed during cisplatin treatment might be substantiated by the known Mg-loss associated with cisplatin treatment especially in patients suffering from intense gastro-intestinal side effects. Therefore, further studies in both patients and experimental animals are urgently warranted in order to examine the clinical role and exact mechanisms behind a possible protective effect of Mg-supplementation on cisplatin induced renal failure.

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