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Effects of the diuretics mannitol or acetazolamide on nephrotoxicity and physiological disposition of cisplatin in rats

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Summary. *The anticancer drug cisplatin has been known to produce severe renal lesions characterized by high levels of blood urea nitrogen (BUN), toxic nephrosis, and platinum (Pt) retention in the kidney. The effect of IV pretreatment with acetazolamide (ACZ) 30 min before or mannitol (MAN) immediately prior to IP administration of 5 mg/kg cisplatin on Pt excretion, tissue distribution, and nephrotoxicity was investigated in male F344 rats.*

ACZ pretreatment reduced the cisplatin-induced nephrotoxicity, as indicated by only a slight elevation of BUN, a milder histopathologic lesion, and a more rapid recovery of renal function and structure. Although MAN-pretreated animals exhibited similar changes in BUN to ACZ-pretreated animals, the renal damage was similar to that seen in aninals treated with cisplatin alone. A reduction of kidney Pt content was observed with both diuretics, although there was significantly less retention after ACZ pretreatment.

The diuretic ACZ was more effective than MAN in reducing the renal lesions induced by cisplatin and it might be clinically useful in preventing cisplatin nephrotoxicity.

Introduction

Renal toxicity is an important potential complication following tumor chemotherapy with cisplatin (DDP). A number of methods for reducing the renal toxicity have been investigated [15]. In patients, the most commonly used is large-volume hydration with diuresis [6]. In animals, furosemide or mannitol-induced diuresis concurrent with DDP administration has been reported to decrease DDP renal toxicity in some studies, but to aggravate renal toxicity in others [5, 13]. The exact mechanism of diuretic-induced reduction of nephrotoxicity is not yet known, although the reduction of Pt concentration in the tubular fluid caused by the diuretics is assumed to account for the protection.

Because of questions regarding the value of furosemide or mannitol as diuretics for use with DDP and because of the recognized value of hydration with diuresis for preventing DDP nephrotoxicity, acetazolamide (ACZ) has recently been studied and shown to posses several advantages that recommend it for use with DDP [11]. No direct comparison, however, has been done between ACZ and commonly used diuretics such as mannitol (MAN). The present study was conducted to compare the effects of ACZ and MAN on the nephrotoxicity and tissue distribution of DDP.

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Materials and methods

Male Fisher 344 rats (Taconic Farms, Germantown, NY) weighing 175–200 g were used. A 3- to 4-day acclimation period was allowed before initiation of the experiment. Animals had free access to Purina rat chow and tap water.

Cisplatin (*cis*-dichlorodiammineplatinum) was obtained from the Division of Cancer Treatment, National Cancer Institute, and was prepared immediately before injections at a concentration of 1 mg/ml in 0.9% NaCl solution (NS). Acetazolamide (Lederle Laboratories, Pearl River, NY) was prepared in NS and used at 20 mg/kg [17]. Mannitol was purchased from Sigma Chemical Co., St. Louis, MO, and was used at a dose of 1.8 g/kg prepared in 0.45% NaCl. Both diuretics were used at doses that previously have been shown to produce an optimal diuresis but with no adverse effects on either body weight or BUN levels [11; unpublished data). Perfix fixative was obtained from Fisher Scientific Co., NJ.

Animals were divided into four groups. The rats were lightly anesthetized with ether for all injections. The first group (CONTROL) received the same volume of NS alone as was received by the drug-treated groups. The second group (DDP) received 5 mg/kg cisplatin IP. The third group (ACZ) received ACZ IV in the tail vein 30 min prior to the administration of 5 mg/kg cisplatin IP. The fourth group (MAN) received MAN IV just prior to administration of 5 mg/kg DDP IP. Five animals from each group were kept in plastic cages and killed by ether overdose 4 days after treatment. Blood was collected from the aorta into a heparinized syringe and plasma was immediately separated. Liver and kidneys were removed and weighed. One kidney was fixed in Perfix solution, sectioned at 5 μm, and stained with hematoxylin and eosin for histologic evaluation. A small portion of each tissue was digested in 10 volumes of concentrated nitric acid for evaluation of tissue platinum concentration.

Five additional animals from each group were placed into individual metabolic cages immediately after dosing. Urine was collected and its volume measured at 30, 60, and 120 min, 24 h, and then daily for 7 days, and the platinum concentration was determined. On day 7 these animals were killed by ether overdose and blood and tissues were collected and analyzed as described above. Additional animals were killed 4 and 24 h after treatment and kidneys were removed and homogenized in NS. A portion of the homogenate was combined with an equal volume of 10% trichloroacetic acid to precipitate tissue proteins. Platinum concentration was determined in the

homogenate and in the supernatant for estimation of the amount of Pt that was bound to tissue proteins. Plasma from all cisplatin-treated animals was used for platinum analysis and for determination of BUN according to the diacetyl monoxime method described by Crocker [2]. Platinum concentrations in plasma, urine, and tissue digests were estimated by atomic absorption spectrophotometry [9]. Differences among the four treatment groups were statistically evaluated using an analysis of variance with significance determined at $P \leq 0.05$.

Results

By day 4, body weights of animals from the DDP group had dropped to 50% of the weight at day 0 (Fig. 1). On day 4 animals in the ACZ and MAN groups showed less weight loss than was seen in the DDP group, reaching 84% and 87% of the weight recorded on day 0, respectively. On day 7 the three groups had regained weight up to 83%, 108%, and 97% of control in the DDP, ACZ, and MAN groups, respectively.

Figure 2 shows the results of BUN determinations on day 4 and 7 after drug administration. Rats treated with cisplatin only had BUN values of 43.5 ± 8.7 mg/dl and 26.4 ± 5.5 mg/dl on days 4 and 7, respectively. These values were significantly higher than the control values (17.9 ± 1.7 mg/dl). On day 4, BUN values in the ACZ and MAN groups were 24.4 ± 2.7 mg/dl and 26.5 ± 4.7 mg/dl, respectively, which were significantly higher than control but significantly lower than in the DDP group. On day 7, the BUN values of the ACZ and MAN groups had both returned to control values. BUN values in the DDP group were obviously returning to normal but were still higher than BUN values in the control group (day 0 values, Fig. 2).

Figure 3 shows urine volume in the different treatment groups. The DDP group showed two peaks of urine output, one on day 1 and one on day 6 after treatment. In the ACZ group there was an increase in urine volume on days 1, 2, and 5. In the MAN group, urine volume was increased significantly on all days except day 2.

Figure 4 shows the cumulative percent of the administered dose of cisplatin that was excreted in the urine. The pattern of elimination of platinum was biphasic in all treatment groups, with the highest amount being excreted during the first 24 h. There were no significant differences in the pattern of platinum excretion among the three groups, although there was a tendency for less platinum to be excreted in animals pretreated with mannitol.

Table 1 shows the total platinum concentrations in plasma, liver, and kidney. On day 4, kidney and liver concentrations of platinum were similar in animals given DDP only or DDP plus either ACZ or MAN. However, the platinum concentration was significantly higher the in the ACZ group than in the DDP or MAN groups.

On day 7, the platinum concentrations in the plasma were the same in the three groups. The platinum concentration in the liver was significantly lower in the ACZ group than in the DDP and MAN groups. The renal platinum concentration in both the ACZ and MAN groups was significantly less than in the DDP group. Furthermore, the renal platinum concentration in the ACZ group was significantly lower than that in the MAN group.

Table 2 shows the concentration of free and protein-bound platinum in plasma and kidney 4 h and 24 h after treatment. After 4 h renal content of free and bound platinum was the same in all three groups. However, total plasma platinum was significantly higher in the ACZ group than in the DDP or MAN groups. The total plasma platinum concentration was the same in the three groups after 24 h, but the renal concentrations of both free and protein-bound platinum were significantly lower in the ACZ and MAN groups than in the DDP group. As noted in the preceding paragraph, renal platinum content in the ACZ group was significantly less after 7 days than in the MAN group.

Four days after drug administration, the kidneys from animals in the DDP group showed a moderately severe acute tubular nephrosis, which appeared at the corticomedullary junction (CMJ). This was characterized by cellular swelling and degeneration, karyorrhexis, aberrant mitotic figures, multifocal proximal tubular necrosis, tubular dilation, and slight protein leakage into the tubular lumen. The renal lesions in the MAN group were similar to those in the DDP group

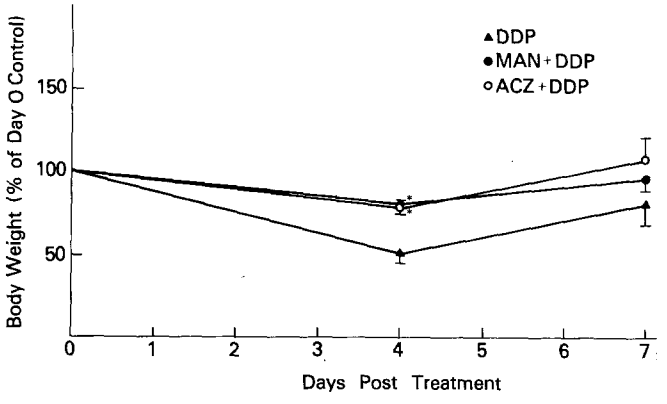

Fig. 1. Effect of ACZ (20 mg/kg IV) or MAN (1.8 g/kg IV) on DDP (5 mg/kg IP)-induced changes in body weight relative to day 0. Values are means ± SD. * Values significantly higher than in DDP group

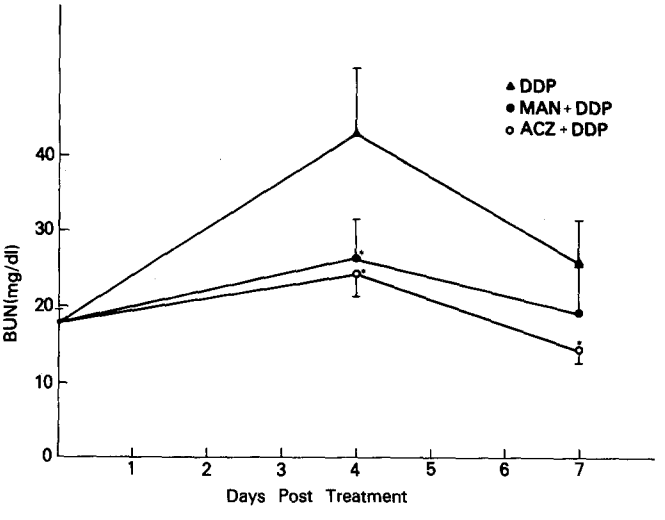

Fig. 2. Effect of ACZ (20 mg/kg IV) or MAN (1.8 g/kg IV) on DDP (5 mg/kg IP)-induced changes in BUN. Values are means ± SD. * Values significantly lower than in DDP group

except that there was also an extremely flattened epithelium and exposed basement membrane. However, in the ACZ group the lesions were only of moderate intensity and were characterized by cellular swelling, degeneration, multifocal proximal tubular necrosis, and minimal tubular dilation. By day 7 (Fig. 5), the CMJ nephrosis in the DDP group had become severe and was characterized by protein casts, enlarged cells with foamy cytoplasm and large pale nuclei, dilated tubules with flattened epithelium, occasional luminal debris, and aberrant mitotic figures. The multifocal necrosis and dilated tubules extended to the renal capsules in some areas. The lesion in the MAN group was similar to that in the

Table 1. Concentration of total platinum in plasma and tissues from rats receiving cisplatin alone (DDP) or after pretreatment with either acetazolamide (ACZ + DDP) or with mannitol (MAN + DDP)

Treatment	Pt (μg/ml)		Pt (μg/g wet tissue weight)			
	Plasma		Liver		Kidney	
	Day 4	Day 7	Day 4	Day 7	Day 4	Day 7
DDP	0.16 ± 0.03	0.18 ± 0.05 (4)	1.8 ± 0.74	1.6 ± 0.5 (4)	6.9 ± 1.1	10.8 ± 2.3 (4)
ACZ + DDP	0.23* ± 0.04	0.13 ± 0.05	2.0 ± 0.61	0.94* ± 0.05	7.8 ± 2.3	4.6** ± 0.8
MAN + DDP	0.13 ± 0.06	0.13 ± 0.02 (3)	1.6 ± 0.39	1.5 ± 1.0 (3)	7.9 ± 1.5	7.2* ± 0.4 (3)

Values are mean ± SD of five rats unless otherwise indicated in parentheses
* Significant difference from DDP group ($P < 0.05$)
** Significant difference from MAN + DDP group ($P < 0.05$)

Table 2. Relative amount of protein-bound platinum in kidneys of rats following cisplatin alone or with acetazolamide or mannitol

Treatment	Plasma total Pt (μg/ml)		Kidney Pt (μg/g wet weight)					
			Total		Free		Bound	
	4 h	24 h	4 h	24 h	4 h	24 h	4 h	24 h
DDP	1.4 ± 0.1	0.5 ± 0.2	23.3 ± 2.9	23.4 ± 1.4	2.7 ± 0.1	8.0 ± 0.8	20.6 ± 3.0	15.4 ± 0.7
ACZ + DDP	1.8* ± 0.1	0.4 ± 0.2	21.7 ± 5.2	15.3** ± 0.1	2.7 ± 0.2	5.5* ± 0.9	19.0 ± 5.4	9.9** ± 0.0
MAN + DDP	1.4 ± 0.1	0.9 ± 0.3	21.5 ± 3.4	17.8* ± 0.4	2.5 ± 0.1	6.1* ± 0.2	18.0 ± 3.9	11.7* ± 0.7

Values are mean ± SD ($n = 3$)
* Significantly different from DDP group ($P < 0.05$)
** Significantly different from DDP and from MAN + DDP groups ($P < 0.05$)

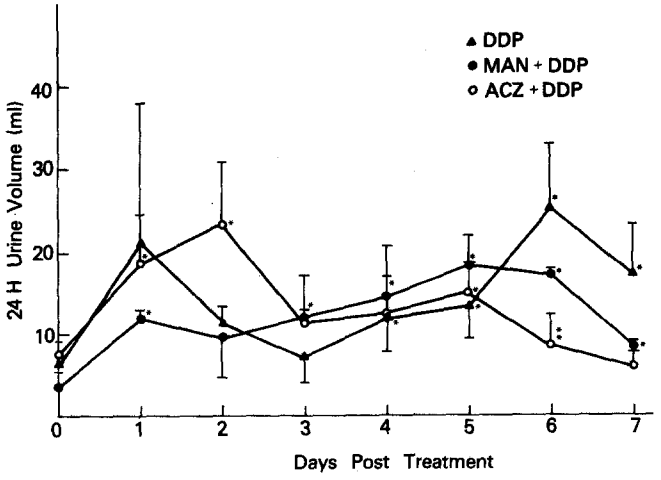

Fig. 3. Effect of ACZ (20 mg/kg IV) or MAN (1.8 g/kg IV) on 24-h urine volumes in rats receiving DDP (5 mg/kg IP). Values are means ± SD. * Values significantly higher than on day 0. ** Values significantly lower than in DDP and MAN groups

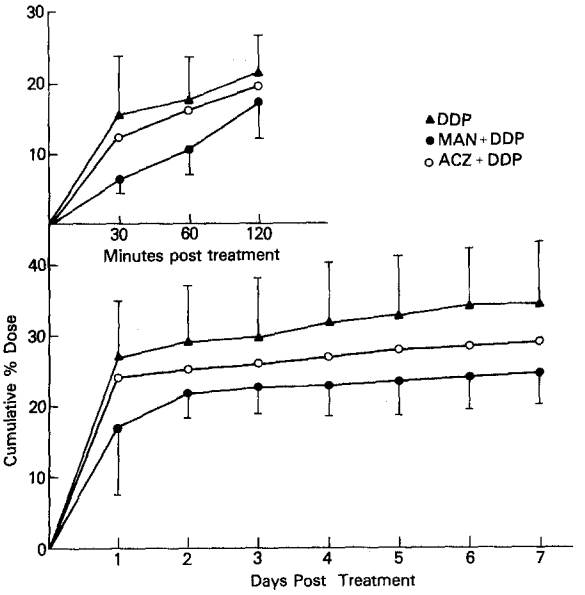

Fig. 4. Cumulative urinary excretion of Pt in rats treated with DDP (5 mg/kg IP) alone or with ACZ (20 mg/kg IV) or MAN (1.8 g/kg IV). Values are means ± SD. Middle bars are left out for clarity

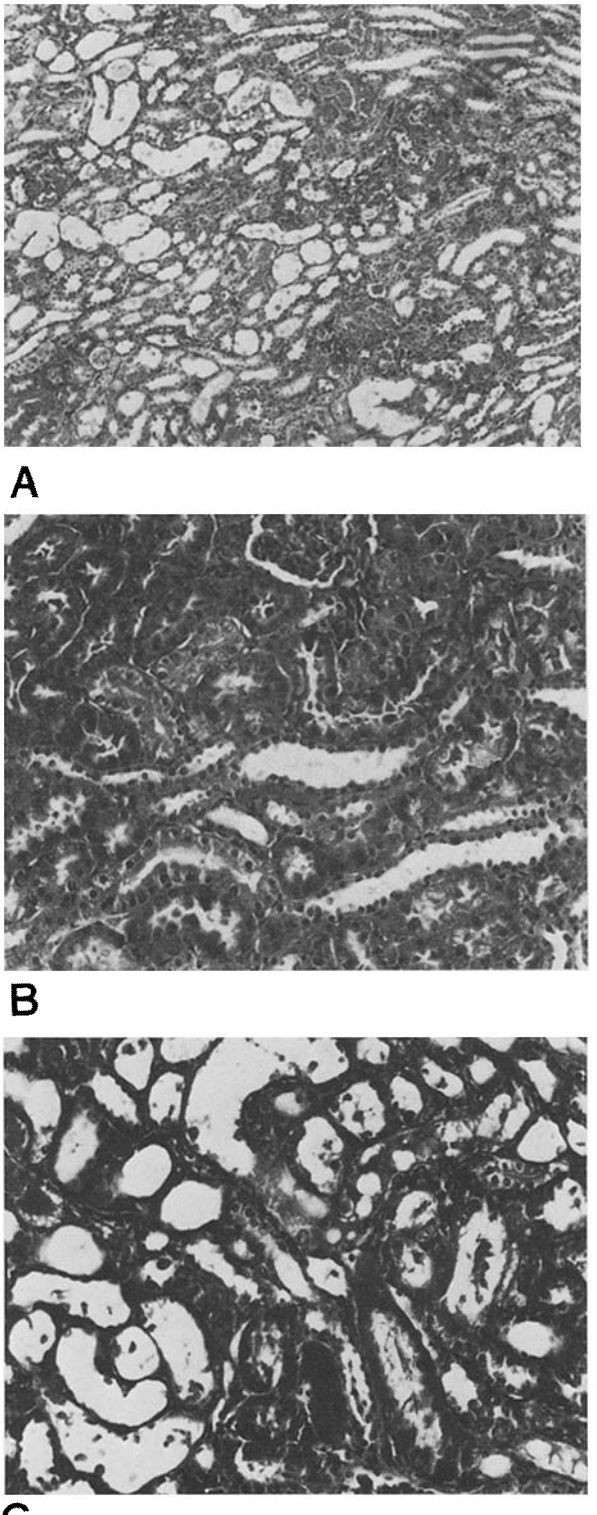

Fig. 5. A Histopathologic changes at the corticomedullary junction (CMJ) in rats 7 days after treatment with DDP, characterized by protein casts, enlarged cells with foamy cytoplasm and large pale nuclei, dilated tubules with flattened epithelium, occasional luminal debris, and aberrant mitotic figures (10×); **B** cisplatin-induced histopathologic changes at the CMJ in ACZ-pretreated animals; the lesion appeared to be resolving, with occasional dilated tubules lined with regular, slightly basophilic, cuboidal cells, and little necrosis or luminal debris (25×); **C** cisplatin-induced histopathologic changes at the CMJ in MAN-pretreated animals. The lesion is similar to that in animals treated with DDP only (25×)

DDP group. In the ACZ group, however, the lesion appeared to be resolving, with occasional dilated tubules lined by regular, slightly basophilic, cuboidal cells, and little necrosis or luminal debris. None of the large foamy cells seen in the DDP group were present. As can be seen in Fig. 5, there was little regeneration in the DDP and MAN groups compared with the ACZ group.

Discussion

Cvitkovic et al. [3] demonstrated that prehydration and mannitol diuresis significantly decreases DDP nephrotoxicity in dogs. A subsequent clinical trial confirmed the utility of the hydration-diuresis regimen in preventing or minimizing nephrotoxicity. The study demonstrated no difference between the two diuretics furosemide and mannitol, however [12]. Various animal studies on the effects of mannitol or furosemide on DDP-induced nephrotoxicity have given conflicting results regarding their effectiveness, depending on which parameters, or time course was investigated [8, 16]. Some studies even demonstrated an enhanced nephrotoxicity when mannitol or furosemide were utilized with DDP [13].

The present study has shown that both MAN and ACZ give rats approximately equal protection against changes in gross indicators of general toxicity (e.g., body weight loss) and renal damage (e.g., BUN elevation). However, ACZ-pretreated animals exhibited less tubular necrosis and a more rapid recovery from the renal damage than animals pretreated with MAN, which had similar renal lesions to animals treated with DDP alone. The discrepancy between renal structure and renal function measurements observed with MAN pretreatment has also been noted in other reports. Thiel et al. [14] found that pretreatment with 0.9% NaCl plus desoxycorticosterone acetate, ACZ, or furosemide protected rats from mercuric chloride impairment of renal function but did not alter the degree of necrosis in the straight portion of the proximal tubule. The improvement of the tubular lesion observed in the present study in the ACZ group but not in the MAN group could be attributed to the lower platinum content of the kidney in the ACZ than in the MAN group. In spite of the reduction of renal platinum content by both diuretics, the percentage of platinum bound to the renal tissue was similar in all treatment groups.

Although there was an apparent but not statistically significant increase in urinary platinum excretion in the ACZ group relative to the MAN group, animals in the ACZ group exhibited higher plasma platinum concentrations.

The mechanism of action of ACZ in protecting against cisplatin nephrotoxicity is still unclear, although several suggestions have been made [11]. In addition to its action as a diuretic, ACZ is an organic acid which may competitively decrease tubular reabsorption of cisplatin. Also, ACZ is a sulfur-containing drug and several sulfur-containing compounds have been shown to be effective in blocking or reducing cisplatin toxicity [1, 7], besides which, a methionine-Pt complex has been identified in the urine following cisplatin administration [4]. Acetazolamide thus may reduce cisplatin nephrotoxicity by a chemical interaction with reactive sites on the cisplatin molecule. This possibility, however, might also be expected to decrease antitumor efficacy and tests are currently under way in our laboratory to explore this possibility. Several possibilites also have been suggested for the renal protection afforded by MAN [15].

These results indicate that caution must be exercised when traditional relatively gross parameters of toxicity are used to indicate potential renal damage. Inspection of BUN and body weight suggested no difference in protection between the ACZ and MAN groups, but histologic evaluation indicated a significant difference in response between the two pretreatments. It has previously been shown that pretreatment with cisplatin produces a decreased renal excretion of subsequent cisplatin doses and a increased toxicity [10]. The present study suggests that ACZ may be more useful than MAN as a diuretic in multiple-treatment protocols with cisplatin because of the milder histological lesion and earlier recovery of normal renal morphology.

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