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Inhibition of *cis*-diamminedichloroplatinum (II) – induced renal toxicity in the rat*

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Summary. The hydroxyl-containing dithiocarbamates, sodium (di(hydroxyethl)-dithiocarbamate (NaY) and sodium *N*-methyl,*N*-dithiocarboxy-*D*-glucamine (NaG), appear to possess definite advantages over sodium diethyldithiocarbamate (DDTC) in reducing the *cis*-dichlorodiammineplatinum (Cis-Pt)-induced renal damage in rats given Cis-Pt as an IV bolus of 7.5 mg/kg 1 h before the IP administration of the dithiocarbamate. Renal damage, as estimated by blood urea nitrogen (BUN) values and serum creatinine levels, was less at all times up until sacrifice in animals given NaY or NaG than in those given DDTC.

An even more effective method for suppression of Cis-Pt renal toxicity is to use a combination of procedures. The most efficacious combination involves a 24-h pretreatment with DDTC or NaG plus acetazolamide and normal saline hydration 30 min before administration of Cis-Pt, followed by post-treatment with NaG. With this combination therapy renal function can be almost completely spared. Although DDTC or NaG pretreatment is highly effective when used in conjunction with NaG post-treatment, DDTC or NaG pretreatment alone has no renal sparing effect on renal function or renal platinum accumulation.

In experiments in which antidotes were given 1 h after Cis-Pt and the animals were followed up for 75 days, a chronic interstitial nephritis at 75 days, suggesting a persistent cell-mediated immune response to Cis-Pt-induced renal damage, may be the basis for chronically abnormal renal function resulting from Cis-Pt. Treatment with all three dithiocarbamates, NaY, NaG, and DDTC, reduced the intensity of this cellular reaction and also reduced platinum levels in the kidneys.

Although NaY and NaG are effective heavy metal chelators and renal function is spared and kidney platinum levels are substantially reduced by the dithiocarbamates, no parallel loss of antineoplastic activity by Cis-Pt on the rat Walker carcinoma was observed. Since the dithiocarbamates have no known human toxicity that would disqualify their clinical use, phase 1 clinical trials are indicated.

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Introduction

Nephrotoxicity is one of the primary limitations for the therapeutic administration of *cis*-dichlorodiammineplatinum (II) (Cis-Pt) in human malignancies [6, 8, 12, 22]. The important finding by Borch and Pleasants [2] that sodium diethyldithiocarbamate (DDTC) could partially inhibit this nephrotoxicity has since been confirmed by several investigators [3–5, 11]. Studies with other chelating agents have generally been less successful [7, 17], although sodium thiosulfate (NaT) has been shown to be effective when given IV at the same time Cis-Pt is administered IP [9, 10, 21]. The purpose of the present study was (1) to compare DDTC, sodium thiosulfate (NaT), and two hydroxyl-substituted dithiocarbamates, NaY (Fig. 1a) and NaG (Fig. 1b)

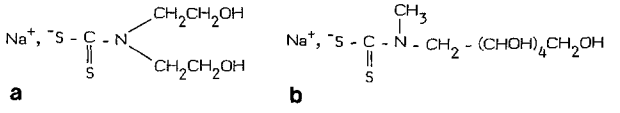

Fig. 1a, b. Structure formulas of NaY *(a)* and NaG *(b)*

for their ability to block the chronic nephrotoxicity of Cis-Pt; (2) to examine the effect on this nephrotoxicity of combining saline hydration and acetazolamide with the dithiocarbamate; and (3) to ascertain whether or not inhibition of nephrotoxicity by chelate therapy affects the anticancer activity of Cis-Pt.

Materials and methods

Cis-Pt (Platinol) was obtained from Bristol Laboratories, Syracuse, NY 13201. DDTC and BUN test kits ≠640 B were from Sigma Chemical Co., St. Louis, Mo 63178. IL BUN test kit ≠3516 and IL creatinine test kit ≠35164 were obtained from Instrumentation Laboratory, Lexington, MA 02173. Acetazolamide (Diamox) was from from Lederle Laboratories Division, Pearl River, NY 10965. Sodium thiosulfate was from Fisher Scientific Co., Fair Lawn, NJ 07410. NaY and NaG were prepared as described previously [18, 19].

Blood urea nitrogen (BUN) and creatinine determinations were performed in male F344 rats (average weight approx. 160 g) obtained from Harlan Industries, Indianapolis, Ind. Tumor response studies were carried out in female Sprague-Dawley rats (average weight approx. 165 g),

also obtained from Harlan Industries. The animals were allowed a 4-day acclimation period after shipment before being used experimentally. Food and tapwater were allowed ad libitum.

In initial experiments for which only the BUN determinations are presented, determinations were performed with Sigma Chemical BUN kit #640 B. In subsequent experiments where both BUN and creatinine values were determined IL urea nitrogen kit #3516 and IL creatinine test kit #35164 were used. These determinations were performed with the aid of IL Multistar III Micro centrifugal analyzer.

Blood samples for all determinations were obtained in each case from the tip of the tail while the animal was under light ether anesthesia. After BUN and creatinine analyses were completed animals were sacrificed by cervical dislocation, after which the kidneys were removed and placed in 10% buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin for histopathological examination.

Platinum levels were determined using flameless atomic absorption spectrophotometry (Perkin Elmer 403 equipped with an HGA 2100 graphite furnace), using standard operating parameters with deuterium arc background correction. Samples were wet ashed in nitric acid, the acid evaporated at 140 °C, and the samples brought to volume using distilled water.

Tumor response studies were performed using the Walker 256 carcinoma (ATCC CCL 38). The induction of tumor growth was achieved by inoculating test animals SC with a cell suspension containing 10^6 tumor cells at two sites in the axillary region for tumor response assessment. The cell suspension was obtained by removing tumors from animals which had been inoculated 8 days earlier, placing the tumors in saline, mincing, and passing the tumor material through a 50 mesh cell sieve. Inoculations were 0.3 ml in volume at each site. After being inoculated, the animals were randomly divided into experimental treatment groups as described in the text. Palpable tumors were noted on day 3 after inoculation, at which time treatment was started. All animals were sacrificed on day 9 after tumor inoculation, and the tumors were then excised, measured and weighed.

All injectate solutions for both BUN and creatinine experiments and for the tumor response experiments were prepared in normal saline immediately before use. Cis-Pt was administered at a dose level of 7.5 mg/kg via tail vein injection. Cis-Pt solutions contained Cis-Pt at a concentration of 2.0 mg/ml. Acetazolamide was administered SC at a level of 20 mg/kg and a concentration of 1 mg/ml [15]. DDTC and NaG were administered IP at the indicated levels, solutions containing these compounds being so constituted that 0.3 ml solution was administered per 160 g body weight. Other treatments (i.e., NaY, NaT) were also administered IP at the indicated levels.

Where appropriate, statistical evaluation of numerical data was carried out using standard analyses of variance procedures.

Results

Individual chelating agents

In these studies experimental protocols similar to those described by Borch and Pleasants [2] were used to provide data comparable to those already present in the literature. BUN and renal platinum concentrations as a function of chelator administration are summarized in Table 1. All the antidotes resulted in a lower BUN value at day 5. Among the antidotes, two (DDTC and NaT) were less effective in reducing the day 5 BUN level. The two dithiocarbamates which contain hydroxy groups (NaY and NaG) were significantly more effective in controlling the rise in BUN concentration and in producing lower BUN values at the end of the experiment. None of the individual antidotes was able to prevent a significant rise in BUN levels or to facilitate a normal BUN value at the end of the 75-day test period. No significant differences were seen in animal weights as a function of treatment. Renal platinum levels at 75 days were reduced by all the dithiocarbamates. NaT, however, had no effect on the renal platinum levels.

Control Cis-Pt group kidneys at 75 days were affected by a chronic interstitial nephritis consisting in cortical mononuclear cell infiltration with relative sparing of the outer one-fifth of the cortex (Fig. 2). Tubular fibrosis was most evident in the areas of greatest mononuclear cell involvement. Scattered clefts were suggestive of past cortical edema, although there was no microscopic evidence of acute interstitial edema. Occasional protein casts were noted in the tubular lumens. With the exception of chronic interstitial nephritis our histopathological findings at 75 days were consistent with the acute effects of Cis-Pt reported by Borch and Pleasants [2], with the majority of damage at the corticomedullary junction involving primarily the proxi-

Table 1. Blood urea nitrogen and renal platinum levels (±SD) of rats receiving 7.5 mg/kg Cis-Pt (IV) followed by antidotes (IP) 1 h later

Group	N	Single antidote dose/kg body wt (mol ratio antidote to Cis-Pt)	BUN (mg/dl) Day						Renal Pt conc at 75 days (ppm/wet wt)
			5	10	15	25	35	75	
Negative control	3		13 ± 0.5						–
Positive control	4		199 ±33	70 ± 7	52 ± 6	40 ±7	–	41 ±2	3.3 ±0.3
DDTC	3	2.92 mmol (117:1)	121 ±29	71 ±23	42 ±12	40 ±6	39 ±2	39 ±1	0.9 ±0.1
NaT	3	6.05 mmol (242:1)	157 ±28	69 ±10	51 ± 3	43 ±3	47 ±2	42 ±6	3.6 ±0.7
NaY	3	2.92 mmol (117:1)	52 ±35	55 ± 9	26 ± 4	25 ±2	25 ±2	24 ±0.5	0.9 ±0.1
NaG	3	2.92 mmol (117:1)	50 ±28	45 ±12	33 ± 3	26 ±6	24 ±2	25 ±1	1.4 ±0.7

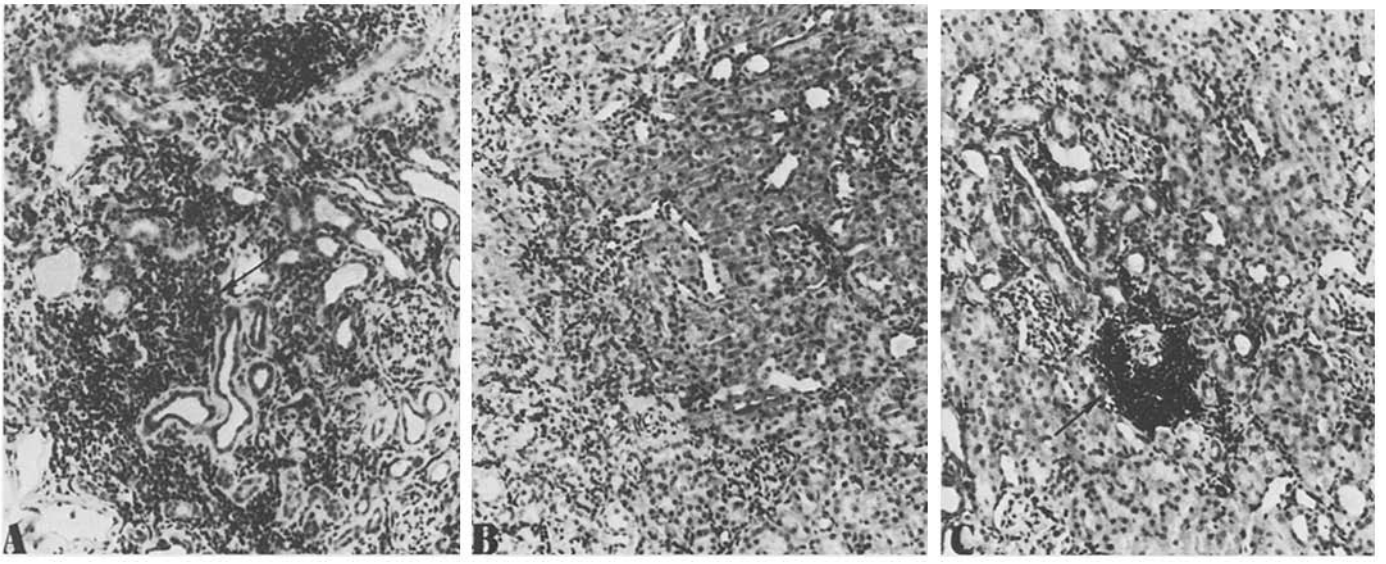

Fig. 2A–C. Effect of Cis-Pt on rat kidney at day 75. **A** No chelator protection (control). Note heavy mononuclear cell infiltrate *(arrows)*, atrophic tubules, and sclerotic interstitium. **B** NaG protection. Mononuclear cell infiltrate is substantially reduced compared with control **(A)**, with no tubular atrophy or interstitial sclerosis. **C** DTTC protection. Focal areas *(arrow)* of mononuclear cell infiltrate, but less tubular damage than in control **(A)**

Table 2. Blood urea nitrogen (±SD) and creatine (±SD) versus renal platinum (±SD) of rats receiving 7.5 mg/kg Cis-Pt IV 24 h after chelator administration

Group no.	Treatment regimen	N	BUN[a] (creatine)[a]			Renal platinum at day 15 (ppm/wet weight) (g)
			Day 5	Day 10	Day 15	
1	DDTC (50 mg/kg IP) at −24 h	5	200±24 (4.8±0.8)	78±8 (1.5±0.8)	56±7 (1.2±0.2)	9.0±0.7
2	NaG (50 mg/kg IP) at −24 h	3	200±37 (5.1±1.7)	90±47 (1.5±0.6)	65±14 (1.2±0.3)	9.8±1.1
3	Cis-Pt only	3	133±37 (2.5±0.8)	57±10 (1.2±0.1	50±12 (1.0±0.04	8.0±0.7

[a] mg/dl

mal tubules in the residua of acute tubular necrosis with nonuniform extensions laterally to the outer cortex and no apparent primary glomerular involvement. All chelator treatment groups were less diffusely involved in mononuclear cell infiltration, with NaG-, DDTC-, and NaY-treated groups faring significantly better than those treated with NaT. The findings in this last group included large tissue clefts, presumably residue of interstitial edema, as well as increased numbers of tubular hyaline casts. The failure of Cis-Pt-treated kidneys to regain full function in these experiments despite adequate time for tubular cell regeneration may be secondary to this apparent cell-mediated immune response.

Combination of procedures

In view of the improved renal protection afforded by NaY and NaG and the report that DDTC and heavy metals may induce metallothionein synthesis in the kidney [1, 20], we tried a variety of combination chemotherapeutic protocols, including administration of chelators prior to Cis-Pt administration. Table 2 demonstrates that administration of DDTC or NaG 24 h before Cis-Pt results in greater renal toxicity than Cis-Pt alone. In contrast, Table 3 demonstrates that the most effective protection against Cis-Pt renal toxicity was provided when pretreatment with DDTC (50 mg/kg) 24 h before Cis-Pt was combined with other measures. These included acetazolamide administered 30 min before Cis-Pt for its diuretic effect [15] and its effect on raising urine pH, which prolongs urinary DDTC half-life [2]. At 1 and 3 h after the administration of Cis-Pt, NaG at 500 mg/kg was given because of its highly effective single dose spraing (Table 1). Remarkably impressive protection is afforded the kidney when this regimen (groups 5 and 6 of Table 3) is followed (i.e., normal BUN and creatinine at days 5, 10, and 15). This regimen, moreover, has no deleterious effect on the antitumor activity of Cis-Pt. Table 4 compares the antitumor effect of Cis-Pt and chelators on the Walker rat carcinoma and renal function of the tumor-bearing animals. Various chelator (DDTC and NaG) combinations are statistically identical with respect to their lack of effect on Cis-Pt-induced reductions in tumor size and weight. Similarly, no differences referable to antidote therapy were observed on histo-

Table 3. Combination chelator effects on inhibition of Cis-Pt-induced renal toxicity and residual platinum renal concentration

Group no.	Treatment regimen[a]	N	BUN[b] (creatine)[b]			Renal platinum at day 15 (ppm/wet weight) (g)
			Day 5	Day 10	Day 15	
1	Control Cis-Pt (7.5 mg/kg)	5	133.4±16.8 (3.10±0.32)	73.6±14.4 (1.16±0.27)	51.6±8.4 (0.9±0.1)	9.2±0.6
2	Pretreat with physiological saline 3 ml at −30 min (saline substituted for antidote vol.)	6	89.3±13.2 (2.4±0.42)	52.4±10.3 (1.1±0.13)	49.3±4.6 (0.96±0.07)	9.4±0.6
3	Pretreat DDTC 50 mg/kg at −1 h Acetazolamide 20 mg/kg at −30 min DDTC 362 mg/kg at +1 h, +3 h	3	83.7±19.0 (1.68±0.23)	47.6± 8.3 (0.87±0.06)	51.5±7.0 (0.8±0.1)	2.7±0.2
4	Pretreat DDTC 50 mg/kg at −1 h Acetazolamide 20 mg/kg at −30 min NaG 500 mg/kg at +1 h, +3 h	6	31.7±4.9 (0.78±0.03)	40.5±3.2 (0.83±0.05)	33.3±3.7 (0.82±0.12)	4.5±1.2
5	Pretreat DDTC 50 mg/kg at −24 h Acetazolamide 20 mg/kg at −30 min NaG 500 mg/kg at +1 h, +3 h	6	23.0±5.6 (0.77±0.06)	25.3±3.8 (0.77±0.04)	25.4±2.6 (0.75±0.02)	2.4±0.2
6	Pretreat DDTC 500 mg/kg at −24 h Acetazolamide 20 mg/kg at −30 min NaG 500 mg/kg at +1 h, +3 h	3	22.2±4.1 (0.80±0.05)	29.2±4.5 (0.80±0.04)	26.8±1.3 (0.78±0.01)	3.5±0.4
7	Pretreat DDTC 500 mg/kg at −24 h Acetazolamide 20 mg/kg at −30 min DDTC 500 mg/kg at +1 h, +3 h	3	29.8±2.9 (0.86±0.04	33.6±1.9 (0.89±0.03)	29.3±0.9 (0.76±0.08)	3.9±0.7

[a] Cis-Pt administered IV at time 0. All antidotes given IP atstated time relative to Cis-Pt
[b] mg/dl

Table 4. Antitumor activity of Cis-Pt as a function of chelator-induced renal sparing

Group no	Treatment regimen[a]	N	Tumor[b]		BUN[b] (mg/dl)	Creatinine[b] (mg/dl)
			Weight (g)	Size (mean diameter)		
1	None	6	4.8±1.2	2.2±0.4	22.0± 2.0	0.82±0.06
2	Cis-Pt (7.5 mg/kg)	6	0.2±0.1	0.7±0.1	173 ±64	3.3 ±2.7
3	DDTC (50 mg/kg) at −24 h Acetazolamide (20 mg/kg) at −30 min DDTC (360 mg/kg) at +1 h, +3 h	6	0.3±0.1	0.8±0.2	65 ±36	1.1 ±0.4
4	NaG (500 mg/kg) at +1 h, +3h	6	0.3±0.2	0.8±0.2	50 ±20	1.0 ±0.1
5	DDTC (50 mg/kg) at −24 h Acetazolamide (20 mg/kg) at −30 min NaG (500 mg/kg) at +1 h, +3 h	6	0.3±0.2	0.8±0.2	28 ± 8	0.8 ±0.1
6	NaG (70 mg/kg) at −24 h Acetazolamide (20 mg/kg) at −30 min NaG (500 mg/kg) at +1 h, +3 h	5	0.2±0.1	0.7±0.2	31 ±12	1.0 ±0.1

[a] Cis-Pt administered IV at time 0 to all groups except group 1
[b] Animals sacrificed at day 9 following tumor inoculation. In the treated animals, the only remaining evidence of the tumor was a focal necrotic area and an inflammatory response

pathological examination. In each case treatment with Cis-Pt resulted in a marked reduction of the growth, giving a focal necrotic area with a mantle of reactive cells surrounding an area of central necrosis and liquefaction. The most effective regimens tested proved to be either DDTC or NaG pretreatment followed by acetazolamide at 30 min before Cis-Pt and then by NaG at 1 and 3 after Cis-Pt administration (Tables 3 and 4, groups 5 and 6 in each). Under the conditions used in this experiment no deleterious effects on Cis-Pt-induced tumor remission were observed with this highly effective method of renal sparing.

Discussion

In comparison with diethyldithiocarbamate (DDTC), the use of dithiocarbamates bearing hydroxy groups (NaY and NaG) results in a less pronounced acute rise in BUN and serum creatinine levels in rats treated with Cis-Pt and

fewer chronic renal toxicity residua as judged by BUN/creatinine and renal histopathology at 75 days. These compounds are also approximately as effective as DDTC in reducing platinum levels in the kidney. Of the drugs tested, NaY and NaG were superior to DDTC in preventing the chronic renal toxic effects of Cis-Pt with the dosages and administration sequences used in this study.

The mechanism of Cis-Pt induced nephrotoxicity is unknown. Levi et al. [13] demonstrated a reduction in free sulfhydryl groups following Cis-Pt, although no direct sulfhydryl Cis-Pt chelation could be demonstrated such as is seen with mercury and the sulfhydryl reduction has not been demonstrated to be a general feature of acute tubular necrosis. Our studies demonstrate that platinum remains chronically bound in the kidneys and that at 75 days the kidneys inner cortex is involved in a mononuclear cell interstitial nephritis. We are unaware of any previous reports of this chronic effect of Cis-Pt. The extent of interstitial nephritis appears to be related to the renal concentration of Cis-Pt in treated versus nontreated animals. Since Cis-Pt nephrotoxicity appears to be related to accumulated dose and since allergically mediated anaphylactic shock has been experienced in sensitive human subjects [16], rapid renal elimination by dithiocarbamate chelators may modify such responses. Moreover, the demonstration by Borch et al. [3] that DDTC administration 2 h after Cis-Pt treatment of an experimental mammary carcinoma in rats did not alter the therapeutic response of the neoplasm to the drug suggests that rapid renal removal of bound Cis-Pt by chelation therapy should be clinically evaluated as a standard regimen in Cis-Pt chemotherapy to reduce cumulative nephrotoxic damage. Our data indicate that the substituted dithiocarbamates NaY and NaG are more effective in reducing renal platinum levels than is DDTC.

The results obtained with the combination of procedures (Table 4) show clearly that this combination is superior to the other individual procedures examined. This is in accord with the notion that the various procedures may operate via different mechanisms in reducing the nephrotoxicity of the Cis-Pt [1, 4, 5, 8, 12, 14, 20–22]. The results obtained with the animals that had been inoculated with the Cis-Pt-sensitive Walker 256 rat carcinoma demonstrate that the steps taken to reduce the nephrotoxicity of Cis-Pt had no apparent effect on its antitumor activity. Although our experiments were not designed to detect escape from Cis-Pt-induced antitumor activity, the residual zone of reactive cells surrounding the central zone of tumor necrosis may be made up of tumor cells capable of providing a means of escape from remission. If this is true, it means that a second course of Cis-Pt is more likely to be a curative procedure. Since Cis-Pt is most commonly administered in humans as single doses with subsequent courses of therapy based on normal renal function, any mechanism which can spare renal function would allow higher cumulative doses of Cis-Pt to be administered, to take advantage of its steep dose-response curve.

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