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The relative nephrotoxicity of cisplatin, *cis*-$[Pt(NH_3)_2(guanosine)_2]^{2+}$, and the hydrolysis product of cisplatin in the rat

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Summary. An examination of the comparative nephrotoxicity in the rat of cisplatin, its hydrolysis product (mostly *cis*-$[Pt(NH_3)_2Cl(H_2O)]^+$ under the conditions applied), and *cis*-$[Pt(NH_3)_2(guanosine)_2]^{2+}$ revealed that these compounds differed significantly in the extent of renal damage they produced following their i.v. injection in Sprague-Dawley rats. The hydrolysis product was found to be the most toxic of the three complexes studied and produced nephrotoxicity at doses lower than those at which cisplatin was nephrotoxic. Under the conditions used, the i.v. administration of *cis*-$[Pt(NH_3)_2(guanosine)_2]^{2+}$ resulted in no observable signs of nephrotoxicity at levels at which an equimolar dose of cisplatin produces clear evidence of renal function impairment and morphological alterations. The nephrotoxicity of these complexes appears to be generally related to the ease with which they undergo nucleophilic substitution reactions. The lack of substantial nephrotoxicity found for *cis*-$[Pt(NH_3)_2(guanosine)_2]^{2+}$ suggests that the products resulting from the action of the DNA repair processes on platinated DNA do not contribute significantly to the nephrotoxicity of cisplatin. Renal platinum levels found following the administration of these compounds correlated with the degree of nephrotoxicity produced by each compound, but no general correlation of nephrotoxicity and renal platinum levels was found. The nephrotoxicity of *cis*-$[Pt(NH_3)_2Cl(H_2O)]^+$ on a molar basis was estimated to be approximately 3 times as great as that of cisplatin itself.

Introduction

The nephrotoxicity that accompanies the administration of cisplatin has been investigated in considerable detail [5, 6, 18]. It is also known that cisplatin undergoes hydrolysis to only a modest extent in the serum, in which the chloride concentration is high, but to a much greater extent in intracellular sites, in which the chloride concentration is much lower [10, 13, 14]. The species formed from cisplatin by hydrolysis are known to be more reactive and more nephrotoxic than the parent compound [3, 17], but quantitative information on their relative nephrotoxicity is limited. The antineoplastic activity of cisplatin is attributed to its preferential reaction with the N-7 atoms on the guanosine bases in DNA; such reactions ultimately form compounds in which both chlorides are replaced by nucleic acid groups [19]. Cisplatin also reacts with a number of intracellular nucleophiles [2, 20], yielding products that are presumably responsible for its adverse effects. The nephrotoxic activity of the products resulting from the repair of platinated DNA has not previously been examined. The purpose of the present study was to compare the nephrotoxicity of cisplatin (*cis*-$[Pt(NH_3)_2Cl_2]$), its initial hydrolysis product (*cis*-$[Pt(NH_3)_2Cl(H_2O)]^+$), and *cis*-$[Pt(NH_3)_2(guanosine)_2]^{2+}$, a complex that exhibit most of the features met in the repair fragments from platinated DNA [11, 15]. Evidence obtained in the course of several clinical studies suggests that renal repair subsequent to severe damage is limited following the use of cisplatin [6, 7]. *cis*-$[Pt(NH_3)_2(guanosine)_2]^{2+}$, which serves as a model for the platinated fragments resulting from DNA repair [11, 15], was included to determine whether compounds of this type have any significant toxic action on renal function in a rat model.

Materials and methods

cis-$[Pt(NH_3)_2Cl_2]$ was purchased from Aesar/Johnson Matthey (Seabrook, N. H.). *cis*-$[Pt(NH_3)_2(guanosine)_2]Cl_2 \cdot 2 \cdot H_2O$ was prepared as previously described [8] (analyses/theory: C, 26.6; N, 18.6; H, 3.99; found: C, 26.01; N, 18.30; H, 3.95). The hydrolysis product of *cis*-$[Pt(NH_3)_2Cl_2]$ was prepared using the procedure of Daley-Yates and McBrien [3], in which a solution containing 1 mg cisplatin/ml was prepared in deionized water and allowed to stand at room temperature for 2 weeks.

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Table 1. Comparative nephrotoxicities of *cis*-$[Pt(NH_3)_2Cl_2]$, *cis*-$[Pt(NH_3)_2(guanosine)_2]^{2+}$, and hydrolyzed cisplatin

Group	△ Body wt.(g)	Kidney wt.(g)	BUN (mg/dl)	Serum creatinine (mg/dl)	Renal Pt (μg/g)	Pathology[a]	C_{Cr}
Untreated controls	17 ± 2**	0.72 ± 0.06**	19.5 ± 1.5**	0.5 ± 0.05**	ND	+0	28.8 ± 3.7**
cis-$[Pt(NH_3)_2Cl_2]$*	−34 ± 7***	0.9 ± 0.04***	128 ± 30***	2.8 ± 1.5***	8.8 ± 0.7	+3 to +4	4.9 ± 2.7***
cis-$[Pt(NH_3)_2(guanosine)_2]Cl_2$:							
22.5 mg/kg	+20 ± 5**	0.74 ± 0.04**	16.3 ± 3.3**	0.6 ± 0.05**	ND	+0	22 ± 7.3**
90.2 mg/kg	11 ± 4**	0.75 ± 0.05**	21 ± 1.9**	0.5 ± 0.05**	8.9 ± 1.2	+0	21.6 ± 2.8**,***
Hydrolyzed cisplatin:							
0.5 mg/kg	14 ± 2**,***	0.67 ± 0.02**	22 ± 2.2**	0.4 ± 0.05**	0.8 ± 0.1**	0	33.4 ± 6.6**
1 mg/kg	−4 ± 4**,***	0.76 ± 0.08**	21.6 ± 3.9**	0.5 ± 0.05**	1.5 ± 0.1**	+2	32.4 ± 4.8**
2 mg/kg	−28 ± 8***	1 ± 0.12***	57 ± 26***	1.8 ± 0.7***	2.7 ± 0.4**	+4	9.1 ± 3.6***
4 mg/kg	−34 ± 7***	1.05 ± 0.09**,***	182 ± 17**,***	6.4 ± 1.8**,***	3.8 ± 0.2**	+4	1.3 ± 0.9**,***

All data represent mean values ± SD. Each group consisted of five animals. Cisplatin was injected i. v. at 7.5 mg/kg; other compounds were given at the doses indicated. After 5 days, the creatinine clearance values (C_{Cr}) were measured, the animals were killed, and tissue samples were taken for analysis and histopathology. ND, Not detectable. C_{Cr} values are expressed in milliliters per hour per 100 g body weight

[a] Evaluated on a scale of 0 to +4; 0, no change from normal control values; +1, minimal renal damage associated with limited areas of focal tubular necrosis and few, if any, proteinic luminal casts; +2, focal areas of tubular necrosis and proteinic material in scattered tubular lumen; +3, moderate to severe renal damage involving tubular necrosis, with epithelial sloughing and proteinic material being present in the tubules; +4, severe, widespread tubular necrosis associated with minimal regenerative change and numerous observable proteinic casts

* Significantly different from the guanosine complex groups for all parameters ($P \leq 0.01$) except for the renal Pt levels in the 90.2 mg/kg group

** Significantly different from animals receiving only cisplatin ($P \leq 0.005$)

*** Significantly different from the control group ($P \leq 0.05$)

Female Sprague-Dawley rats (obtained from Sasco Ind., Omaha, Neb.) were used in all of the experimental studies. The animals were allowed food and water ad libitum, except during creatinine clearance determinations and were housed in an AAALAC-approved animal facility. Platinum compounds were given by tail-vein injection while the animal was under ether anesthesia. Cisplatin was injected at a dose of 7.5 mg/kg or 0.025 mmol/kg; *cis*-$[Pt(NH_3)_2(guanosine)_2]^{2+}$ was given at a dose of 0.025 mmol/kg (22.5 mg/kg) and at a dose that was 4 times greater, 0.1 mmol/kg (90.2 mg/kg); hydrolyzed cisplatin was injected at doses of 0.5 mg/kg (0.0018 mmol/kg), 1 mg/kg (0.035 mmol/kg), 2 mg/kg (0.0071 mmol/kg), and 4 mg/kg (0.0142 mmol/kg).

Creatinine clearance rates were determined by the method of Reznik et al. [16]. At 5 days after administration of the platinum compounds, the animals were given a water load of 5 ml/100 g body weight orally using an 18-gauge feeding needle while the animal was under light ether anesthesia. The animals were placed in metabolic cages and urine was collected over ice for 4 h. Blood urea nitrogen (BUN) and serum creatinine determinations were carried out on blood samples taken from the tail vein just prior to the administration of the water load. BUN and creatinine assays were performed using an IL Multistat III centrifugal analyzer and the appropriate test kit.

Following determinations of creatinine clearance, the animals were sacrificed by cervical dislocation and tissue samples for platinum determination and histopathological examination were immediately removed. Tissue samples for platinum determination were digested in nitric acid, which was subsequently evaporated at 140° C. The residue was dissolved in 40 μl nitric acid under warming, and the sample was brought to volume in distilled water. Platinum analyses were carried out on a Perkin-Elmer 403 atomic absorption spectrometer equipped with an HGA 2100 graphite furnace operated using deuterium background correction under standard operating conditions. Tissues to be evaluated by light microscopy were immediately placed in buffered formalin. The samples were embedded in paraffin and stained with hematoxylin and eosin. Histopathology evaluations were carried out using previously published studies as a guide [4, 9]. The statistical significance of the differences between the groups was evaluated using analysis of variance. All values given are expressed as means ± standard deviations.

Results

The results of all experiments are summarized in Table 1. Untreated control animals displayed normal indices of renal function. The guanosine complex injected at 22.5 mg/kg produced no overt measurable nephrotoxicity as evidenced from the measures that were examined. However, when this complex was given at 90.2 mg/kg, BUN levels were elevated slightly above those in the control group and creatinine clearance values were slightly depressed. The animals in both the untreated and the *cis*-$[Pt(NH_3)_2(guanosine)_2]^{2+}$-treated groups gained weight throughout the experiment, in addition to exhibiting kidneys of normal weight, normal serum creatinine levels, and normal histopathology. Only at the highest dose given did *cis*-$[Pt(NH_3)_2(guanosine)_2]^{2+}$ cause even a modest indication of renal damage. The finding that no platinum was detectable in renal tissues following the administration of 22.5 mg/kg *cis*-$[Pt(NH_3)_2(guanosine)_2]^{2+}$ indicates that the concentration was below 0.5 μg/g tissue.

The animals that had received cisplatin at 7.5 mg/kg exhibited the characteristic toxic signs induced by this compound: weight loss, renal swelling, increases in both BUN and serum creatinine values, increased renal platinum levels, and a significant decrease in creatinine clearance. The administration of hydrolyzed cisplatin produced one or more of these characteristics at all doses at which it was injected. The observable histopathological changes in the kidney that were caused by hydrolyzed cisplatin given at doses of ≥ 1 mg/kg revealed that renal involvement increased with increasing dose.

Renal platinum levels resulting from the administration of *different* platinum compounds were not found to be directly related to the level of nephrotoxicity produced. A renal platinum content of 8.9 ± 1.2 μg/g tissue, which

was found following the injection of 90.2 mg/kg *cis*-$[Pt(NH_3)_2(guanosine)_2]^{2+}$, was accompanied by few obvious signs of renal damage (a very slight) elevation in BUN values and a slight depression of creatinine clearance), whereas the platinum concentration of 8.8 ± 0.7 μg/g tissue that resulted from the administration of 7.5 mg/kg cisplatin was accompanied by severe renal damage as indicated by all of the parameters used to assess toxicity. The presence of 3.8 μg Pt/g tissue after the administration of 4 mg/kg of the hydrolysis product of cisplatin was accompanied by a level of renal damage that was significantly greater than that found for a platinum level of 8.8 ± 0.7 μg/g tissue that resulted from the injection of 7.5 mg/kg cisplatin.

The histopathological changes that occur in the kidneys of animals that have been given varying doses of hydrolyzed cisplatin have not previously been reported in detail. In animals that received 4 mg/kg of the hydrolyzed cisplatin, renal injury observed at 5 days after treatment was characterized by extensive tubular involvement of the inner and outer cortex and the corticomedullary junction. Tubular necrosis associated with areas of complete epithelial desquamation and exposure of the basal lamina was apparent throughout the kidney. Nuclei of injured cells exhibited pyknosis and karyorrhexis. The lumen of most tubules contained desquamated epithelial cells, cellular debris, and widespread proteinic casts. The involvement of almost all of the tubules was noted. The kidneys of animals that received 2 mg/kg of the hydrolyzed cisplatin showed a pattern of injury similar to that found in animals that were given 4 mg/kg, although the renal involvement at the former dose indicated a sparing of tubules within the outer cortex. Severe renal injury at the corticomedullary junction was observed. Widespread tubular necrosis was apparent, with most tubules containing sloughed epithelial cells, cellular debris, and proteinic casts. Some areas revealed exposure of the basement membrane after complete sloughing of the tubular epithelium. Animals that received 1 mg/kg of the hydrolyzed cisplatin exhibited moderate to severe renal damage. Renal involvement was characterized by areas of tubular necrosis at the corticomedullary junction. A loss of tubular epithelium was noted, with desquamated epithelial cells and debris being present in the lumen of the tubules. Collecting tubules contained proteinic casts, although the tubules in the outer cortex were spared. Normal renal morphology was observed at 5 days following drug administration in rats that had been given 0.5 mg/kg hydrolyzed cisplatin. The kidneys were essentially unchanged as compared with those of untreated (normal) controls.

Discussion

The nephrotoxicity found in the present study for cisplatin is very similar to that reported previously, in terms of both the histopathology and the effects on various measures of renal function [4, 9]. The effects of other doses of cisplatin have been published elsewhere [3, 4, 9, 12]. The relatively modest level of nephrotoxicity associated with the administration of high doses of *cis*-$[Pt(NH_3)_2(guanosine)_2]^{2+}$ as compared with the other platinum complexes investigated suggests that analogous compounds resulting from the repair of platinated DNA will not produce species that exacerbate the nephrotoxicity resulting from the administration of the parent compound. Such repair products can be expected to be released slowly as the characteristic cisplatin-DNA lesions are repaired. Since this compound does not readily undergo substitution processes in which the guanosine is removed [8], the renal retention of platinum following its administration may be dependent on the occurrence of ligand substitution processes in which one or more of the ammonia groups bonded to the platinum are replaced by groups such as sulfur atoms that are present in the sulfhydryl groups of proteins. An alternative explanation may involve a process in which the metabolism of the coordinated guanosine may be responsible for the retention of platinum-containing species by the renal tissue. Higher doses of this compound were not given because the levels that would be encountered as metabolic products of cisplatin would be far lower, as this is only one of the many products formed in vivo by the reaction of cisplatin [1, 2, 15, 17, 19].

The lack of any *overall* correlation between renal platinum concentrations and the level of nephrotoxicity is apparent from the data shown in Table 1. The renal platinum level correlated with renal damage at various doses of a single compound, but there was no general correlation between renal platinum concentrations resulting from treatment with different compounds and the observed level of nephrotoxicity. This suggests that the form in which platinum occurs in the kidneys is functionally different following the administration of these different compounds.

The data on hydrolyzed cisplatin provides more detailed evidence that this substance is at least 2–3 times more toxic than is cisplatin itself, depending on the index used to measure the nephrotoxicity. The toxicity of the hydrolyzed species derived from cisplatin, mostly *cis*-$[Pt(NH_3)_2Cl(H_2O)]^+$ under the conditions used in the present study can be expected to depend on the environment in which the hydrolysis occurs. When given by the i.v. route, *cis*-$[Pt(NH_3)_2Cl(H_2O)]^+$ in the serum can be expected to react to a considerable extent with serum proteins and serum amino acids in reactions that compete with those at more sensitive sites, e.g., those involving cellular surface enzymes such as Na^+/K^+ ATPase [1, 21]. When formed from the hydrolysis of cisplatin in intracellular sites, *cis*-$[Pt(NH_3)_2Cl(H_2O)]^+$ reacts in an indiscriminate fashion with many types of intracellular nucleophiles in reactions that can be reversed only slowly, if at all, by intracellular protective nucleophiles such as glutathione. Litterst and other investigators have previously shown that an increase in the chloride concentration, which suppresses the hydrolysis of cisplatin [3, 12] also suppresses its nephrotoxicity. It is possible that the rate at which the hydrolyzed species are taken up by renal tubular cells is greater than that at which these cells take up cisplatin, but this has not yet been examined.

Using the equilibrium constants of Miller and House [13, 14], it can be calculated that when a solution is prepared in distilled water using 1 mg cisplatin/ml and is allowed to sit for 14 days at 25°C, as was done in the

present study, approximately 78% of the platinum occurs as *cis*-$[Pt(NH_3)_2Cl(H_2O)]^+$, with the remainder being cisplatin. Under similar conditions whereby the cisplatin is dissolved in normal saline, the hydrolysis product accounts for only about 6% of the platinum at equilibrium. The thorough kinetic and thermodynamic analysis of this compound previously presented by Miller and House [13, 14] enables the determination of concentrations of the various hydrolysis products obtained from cisplatin if the initial composition and history of the solution are known.

The results obtained in the present study confirm and extend previous investigations demonstrating that the hydrolysis products of cisplatin make a significant contribution to the adverse effects of the parent drug. Furthermore the results suggest that any steps that reduce the levels of hydrolyzed cisplatin in solutions that are given clinically will reduce the ensuing nephrotoxicity to some extent.

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