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# Increased tissue deposition and decreased excretion of platinum following administration of cisplatin to cisplatin-pretreated animals

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***Summary.*** *Guinea pigs were pretreated IP with cisplatin (10 mg/kg) for various times before IV administration of $^{195m}$Pt-labeled cisplatin. Concentrations of $^{195m}$platinum were greater in tissues of pretreated animals than in those of control animals. Amounts of $^{195m}$platinum in subcellular fractions from pretreated rabbits were similarly greater in pretreated animals. Amounts of radioactivity appeared to be greatest in animals receiving a larger number of pretreatment injections, even though the total amount of cisplatin administered was equal in all groups. BUN was elevated on day 1 after the radioactive dose only in those animals which had been pretreated. Urinary excretion of platinum was significantly less in pretreated than in control animals. It appears that pretreatment with cisplatin damages the kidney severely enough for subsequent doses of cisplatin not to be excreted as efficiently, thus leading to a greater tissue deposition of platinum in pretreated animals.*

## Introduction

Drugs are routinely administered in multiple doses and, particularly in cases of antineoplastic agents, at threshold toxic doses. Traditionally little attention has been devoted to ascertaining the effects of early doses on efficacy, toxicity, and pharmacokinetics of subsequent courses of therapy. Cisplatin is a relatively new and highly promising antineoplastic drug, whose most serious dose-limiting effect is renal toxicity. Recent work designed to investigate the ototoxicity of multiple doses of cisplatin [10] revealed quantitative changes in the distribution of platinum in animals that had received previous doses of cisplatin. The work reported here validates our original observations and presents experimental results to support a likely explanation.

## Methods and materials

*cis*-Dichlorodiammineplatinum-II (cisplatin) labeled with $^{195m}$platinum (141 mCi/mole; 94% radiochemical purity) was purchased from Oak Ridge National Laboratories (Oak Ridge, TN, USA) and non-radioactive cisplatin was obtained from the Drug Development Branch, NCI. Female strain 2 guinea pigs (Murphy Breeding Labs., Plainfield, IN, USA) weighing approximately 300 g were ear-tagged and acclimatized to the animal quarters for 1 week prior to any further treatment or manipulation. All animals were allowed free access to tap water and vitamin C-supplemented guinea pig chow (Ralston Purina, St Louis, MO, USA). Animals were weighed and randomly allocated to one of four treatment groups ($n$ = 3/group). Group 1 received drug vehicle 24 h prior to further treatment. Group 2 received an IP injection of cisplatin (10 mg/kg) 24 h before further treatment. Group 3 received two IP injections (5 mg/kg per injection) spaced at equal 24-h intervals prior to further treatment. Group 4 received three IP injections (3.3 mg/kg/injection) spaced at equal 24-h intervals prior to further treatment. Twenty-four hours after the final pretreatment injection each animal in all four groups received an IV injection (10 mg/kg) of $^{195m}$Pt-labeled cisplatin into the femoral vein after surgical exposure of the vein under Rompun : Ketamin (1 : 4) anesthesia. Following injection of the radioactive drug, the leg incision was closed with wound clips and the animals were returned to their cages. Two hours after radioactive drug administration animals were killed by decapitation and blood was collected into heparinized test tubes. Plasma was separated by gentle centrifugation and urea nitrogen (BUN) was determined using the diacetyl monoxime method. The temporal bones were removed and the perilymphatic spaces perfused with 10% formaldehyde. Cochlear microdissection of organs of Corti, *stria vascularis*, and spiral ligaments was then performed. Spleen, lung, liver, kidneys, and adrenals were removed from the carcass and weighed. All tissues and fluids were added to vials and the radioactivity determined in a Packard Auto-Gamma counter. An aliquot of the treatment solution with a known specific activity was counted at the same time. Samples were counted for 10 min or 900,000 accumulated counts. Statistical comparison of the mean of each of the three pretreatment groups with the mean of the vehicle control group was done using Dunnett's procedure [11] at $P \leq 0.05$.

Next, 10 guinea pigs were acclimatized to stainless steel metabolic cages for 3 days. On day 4, five of the animals were treated IP with 10 mg/kg cisplatin in 0.9% NaCl. Twenty-four hours later all 10 animals were anesthetized and received 10 mg/kg cisplatin IV. Doses were those which had previously been shown to cause BUN elevations and which produced occasional lethality (~ 10%) during a 7-day observation period. Urine was collected from the bladder at 30 and 120 min after treatment by gentle suprapubic pressure and at 6 and 24 h after treatment by means of the metabolic cages. Twenty-four hours after treatment animals were killed with ether, blood was collected from the vena cava, and the plasma was separated. The urinary bladder was emptied and the urine added to that collected in the metabolic cages. Urine and

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**Table 1.** Effect of cisplatin pretreatment on tissue concentrations[a] of radioactivity 2 h after administration of $^{195m}$Pt-labeled cisplatin to guinea pigs

| Tissue | Pretreatment | | | |
|---|---|---|---|---|
| | None | 1 day | 2 days | 3 days |
| Plasma | 2.23 ± 0.14 | 3.30 ± 0.07* | 2.76 ± 0.10 | 2.95 ± 0.41 |
| Kidney | 8.18 ± 0.28 | 13.9 ± 1.4* | 9.68 ± 0.38 | 14.9 ± 1.3* |
| Spleen | 2.18 ± 0.08 | 3.14 ± 0.16* | 3.79 ± 0.46* | 3.94 ± 0.16* |
| Liver | 6.18 ± 0.18 | 11.8 ± 1.7* | 13.7 ± 1.3* | 13.6 ± 0.8* |
| Lung | 2.42 ± 0.01 | 3.00 ± 0.08* | 3.79 ± 0.23* | 4.46 ± 0.23* |
| Adrenal | 1.44 ± 0.03 | 2.30 ± 0.18* | 2.87 ± 0.12* | 2.61 ± 0.10* |
| Cochlea[b] | 12.2 ± 0.2 | 20.1 ± 2.3* | 18.8 ± 2.8* | 25.0 ± 3.6* |

* Significantly different from 'None' ($P \leq 0.05$)
[a] Figures are means ± SD ($n = 3$) and are presented as % dose/g (or % dose/ml) × $10^3$
[b] Total % dose/cochlea determined by combining data from basal membrane and stria vascularis

plasma samples were analyzed for platinum content by flameless atomic absorption spectroscopy [4].

Finally, the distribution of $^{195m}$Pt-labeled cisplatin was examined again in pretreated rabbits to determine whether there were any pretreatment-induced changes in the subcellular distribution of radioactivity. Rabbits were chosen to confirm the guinea pig observations in another species, because of the ease of comparison with previous cisplatin subcellular distribution work, and because lung and kidney from a single animal would provide enough tissue to facilitate the subcellular fractionation procedure. Male New Zealand rabbits (approximately 3 kg) were pretreated for 1, 3, or 5 days (total pretreatment dose = 5 mg/kg) prior to receiving 2 mg/kg $^{195m}$Pt-labeled cisplatin IV into a marginal ear vein. Two hours later animals were killed with ether, and bone, lung, colon, duodenum, skin, kidney, liver, and muscle were removed from the carcass. Whole organs were weighed and the radioactivity in each tissue determined. In addition, erythrocytes were isolated from whole blood and separated into cytosol and membrane fractions by the method of Hanahan and Ekholm [2]. Lung, liver, and kidney were homogenized (1 + 4) in 0.25 *M* sucrose and the following subcellular fractions separated by differential ultracentrifugation: mitochondrial (800 *g* supernatant centrifugated at 8,000 *g* for 10 min), microsomal and cytosolic (8,000 *g* supernatant centrifuged at 105,000 *g* for 60 min). Protein contents of each subcellular fraction and of erythrocyte membrane and cytosol were determined by the method of Lowry et al. [7], using bovine serum albumin as standard.

## Results

The concentration of platinum in various organs from cisplatin-pretreated guinea pigs is shown in Table 1. Concentrations were significantly greater in all organs from pretreated animals. When data were examined relative to plasma concentrations of radioactivity, cochlea and kidney were found to contain the highest concentrations of radioactivity (tissue : plasma = 5.5 and 3.7, respectively). Cisplatin pretreatment increased the tissue : plasma ratio (T : P) for adrenal, spleen, lung, and kidney by approximately 30%, but the T : P for liver and cochlea were increased by 50% and 65%, respectively. In spleen, lung, liver and adrenals, the concentration was greatest in those animals that had received the largest number of pretreatment injections, even though the total amount of drug received during pretreatment was equal for all three groups. The study of whole organ distribution from the rabbit yielded the same results (data not presented) as those shown in Table 1.

**Table 2.** Effect of pretreatment with cisplatin on urinary excretion[a] of platinum following a subsequent dose of cisplatin to guinea pigs

| | Control ($n = 4$) | Pretreated ($n = 5$) |
|---|---|---|
| % Dose excreted | | |
| 0– 30 min | 16.6 ± 5.4 | 16.8 ± 3.6 |
| 30–120 min | 21.6 ± 6.1 | 8.3 ± 3.0* |
| Urine volume (ml) | | |
| 0– 30 min | 3.2 ± 1.2 | 6.0 ± 2.0 |
| 30–120 min | 2.8 ± 0.8 | 2.6 ± 1.0 |
| Plasma level (μg/ml)[b] | 2.36 ± 0.23 | 1.98 ± 0.47 |

* Significantly different from control, $P \leq 0.05$
[a] Means ± SD
[b] 24 h post-treatment

**Table 3.** Recovery of radioactivity[a] from $^{195m}$Pt-cisplatin in subcellular fractions of tissues from rabbits variously pretreated with non-radioactive cisplatin

| Tissue | Fraction | Pretreatment | | | |
|---|---|---|---|---|---|
| | | None | 1 day | 3 days | 5 days |
| Kidney | Cytosal | 904 | 1,260 | 1,368 | 1,736 |
| | Microsomes | 786 | 1,253 | 1,268 | 1,441 |
| | Mitochondria | 740 | 1,362 | 1,552 | 1,811 |
| Liver | Cytosal | 405 | 407 | 554 | 712 |
| | Microsomes | 613 | 552 | 690 | 813 |
| | Mitochondria | 575 | 528 | 770 | 713 |
| Lung | Cytosal | 283 | 159 | 208 | 233 |
| | Microsomes | 329 | 152 | 193 | 265 |
| | Mitochondria | 321 | 162 | 268 | 283 |
| Erythro-cyte | Membrane | 206 | 286 | 302 | 400 |
| | Cytosal | 29 | 29 | 37 | 44 |

[a] Figures give cpm/mg protein from a single rabbit per treatment

Platinum urinary excretion in control and in pretreated guinea pigs is shown in Table 2. During the first 30 min after administration of cisplatin urinary excretion was similar in both groups. Between 30 and 120 min, however, significantly less platinum was excreted by pretreated than by non-pretreated guinea pigs. Urine volume and plasma platinum concentra-

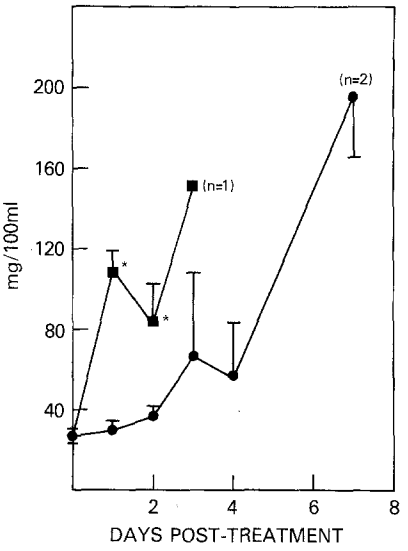


**Fig. 1.** Effect of a single IP pretreatment (10 mg/kg) with cisplatin on cisplatin-induced changes in plasma urea nitrogen (BUN) levels. Figures are means ± SD ($n = 3$). Control value (26.7 ± 2.4 mg/dl) was obtained by pooling data from three vehicle-treated guinea pigs killed 24 h and 3 killed 7 days after dosing. Statistically different values between pretreated (■) and non-pretreated (●) groups are indicated by * for $P \leq 0.05$ (paried $t$-test)

tions were similar for the two groups (Table 2). These results were confirmed by the 2-h urinary recovery of platinum from non-pretreated and cisplatin pretreated rabbits (26.3% vs 19.5% ± 2.1%, respectively).

The effect of pretreatment on the recovery of radioactivity in subcellular fractions of erythrocytes, liver, lung, and kidney from $^{195m}$Pt-labeled cisplatin-treated rabbits is shown in Table 3. These data support the initial observation of increasing tissue concentration following pretreatment observed in both the guinea pig and rabbit. A greater amount of radioactivity was found in all fractions from liver, kidney, and erythrocytes following pretreatment than in fractions from non-pretreated animals. Pretreatment appeared to decrease the amount of radioactivity in fractions of lung. Concentrations of radioactivity in liver and kidney fractions appeared to increase with the number of pretreatment injections.

Figure 1 shows BUN values from pretreated and non-pretreated guinea pigs following treatment with 10 mg/kg cisplatin. In non-pretreated animals 3 days elapsed before a noticeable increase in BUN occurred, and the first death occurred on day 7. In cisplatin-treated animals, BUN values were nearly four times above normal on day 1 after the second dose and two-thirds of the treated animals were dead by day 3 post-treatment.

## Discussion

Because animals in these experiments received non-radioactive pretreatment injections but radioactive drug 2 h before being killed, the uptake of radioactivity is a reflection of how the last dose of drug was distributed rather than the accumulation of several different doses. The reason for the increased concentration of drug after pretreatment with the same drug is unknown but several explanations are possible. First, cisplatin may induce a ubiquitous binding substance that increases in amount after the pretreatment dose and thus accounts for the increased tissue concentration of the radioactive dose in pretreated animals. An obvious candidate for this inducible receptor would be metallothionein (MT), the cysteine-rich metal-binding protein [3] that is known to bind heavy metals [8] and to be inducible by some of them [9]. Previous work from this laboratory, however, has shown that although cisplatin binds to a cadmium-inducible protein, cisplatin itself is not able to induce the synthesis of this substance [5]. Therefore it appears that MT is an unlikely candidate for a platinum-inducible receptor. Also, it would be surprising if the same inducible receptor occurred in approximately equal quantities in such widely different tissues as those studied.

Another possible explanation is based on changes in receptor affinity after interaction with platinum. It is conceivable that platinum-bound receptors might have a greater affinity, but a lower binding constant, for free platinum than is the case with unbound receptors. Much too little is known about the nature of platinum receptors to consider this more than a theoretical possibility.

Another possibility, which has some practical appeal, is that the first dose of cisplatin produced substantial enough damage to the kidney for the excretion of the subsequent dose to be impaired. It is known that cisplatin is very rapidly cleared by the kidney, with greater than 60% of the administered dose appearing in urine as early as 4 h after IV administration to dogs [6]. Hence it might be expected that if the kidney was badly enough damaged by the pretreatment, renal excretion during the 2 h after the radioactive dose would be decreased sufficiently to allow a greater amount of free platinum to be available for additional binding to tissue receptors. The data in Table 2 and Fig. 1 support this argument. In addition, Gonzalez-Vitale et al. [1] have demonstrated that renal function is acutely compromised when aminoglycoside antibiotics are administered to patients after cisplatin dosing, suggesting that cisplatin may in fact influence the toxicity or kinetics of subsequent nephrotoxic drugs. Furthermore, BUN data (Fig. 1) suggest that after the second dose of cisplatin, cisplatin-induced renal toxicity is increased during the 1st day after a second treatment. Thus it is probable that within the 1st h after the radioactive cisplatin dose renal function was decreased, as reflected by the decreased excretion of platinum in the urine.

Thus the increased concentration of platinum in tissues of cisplatin-pretreated animals appears to be due at least in part to a decrease in the renal excretion of platinum, presumably caused by the nephrotoxicity of the initial dose. The use of multiple doses of cisplatin in short times, therefore, warrants continual monitoring of patient renal function.

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