Distribution and Disposition of Platinum following Intravenous Administration of cis-Diamminedichloroplatinum(II) (NSC 119875) to Dogs

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SUMMARY

cis-Diamminedichloroplatinum(II) is an antineoplastic drug that is undergoing a renewed clinical interest as a drug for use in combination regimens. In order to increase the understanding of the pharmacokinetics of this drug, the plasma clearance and organ distribution of platinum were followed in female beagle dogs treated with a single i.v. dose of cis-diamminedichloroplatinum(II). Plasma levels of platinum were determined by flameless atomic absorption spectrometry and showed a distinctly biphasic clearance pattern with a rapid-phase half-time of considerably less than 1 hr and a slow-phase half-time of nearly 5 days. During the first 4 hr after treatment, plasma levels fell by 90% while 60 to 70% of the applied dose was recovered in the urine. Sixteen tissues plus plasma, bile, and urine were routinely analyzed for platinum content. An easily measurable plasma concentration of platinum was still detectable 12 days after treatment, with no significant change in plasma concentration between Days 4 and 12. Initial concentrations of platinum were highest in organs of excretion, gonads, spleen, and adrenals but remained significantly elevated only in kidney, liver, ovary, and uterus, where a tissue:plasma ratio of 3 to 4 was maintained for as long as 6 days posttreatment.

INTRODUCTION

Relatively recent and highly interesting additions to the list of drugs currently eliciting clinical interest in the treatment of human neoplasms are salts or complexes of various heavy metals. Perhaps the prototype of this drug class is the series containing the transition element platinum. The most widely studied and extensively utilized drug in this class is DDP. Originaly shown by Rosenberg et al. (14) to be antibacterial against Escherichia coli, DDP was later demonstrated to be active against several animal tumors (15). Clinically, DDP has activity as a single agent in a narrow spectrum of tumors, many of which are gonadal in origin (3). In addition, DDP has shown promise in combination with other agents against several other human solid tumors (7). Numerous data have accumulated on the biochemical effects of DDP (6) and on the metabolism of DDP and of its analogs (17, 19). Manifestations of toxicity in both humans and animals have been well documented (16, 18). Little work, however, has been published on the uptake, distribution, and excretion of DDP in either man or animals. Contributions to the disposition of DDP have dealt mainly with limited numbers of animals or patients and have failed to establish basic pharmacokinetic patterns. Because of the clinical interest in DDP and the limited available data on the distribution of this compound, we felt that it was of value to undertake a series of studies to define some basic dispositional parameters of DDP in animals. This communication is concerned with the tissue distribution and plasma decay following single-dose administration of DDP to dogs over a time course ranging from 5 min to 12 days postinjection.

MATERIALS AND METHODS

Animal Treatment. Adult female beagle dogs (8 to 10 kg) were anesthetized with i.v. (cephalic) pentobarbital sodium (30 mg/kg). A catheter was inserted into the external jugular vein for the collection of blood samples and a human pediatric urinary catheter was passed through the urethra into the urinary bladder for the collection of urine. DDP was administered i.v. (1 mg/kg in 0.85% NaCl solution) through an indwelling catheter in the femoral vein. Beginning at 5 min postinjection, both blood and urine were sampled for analysis. Catheters were allowed to remain in place for no longer than 4 hr, at which time either the dogs were sacrificed by exsanguination or the catheters were removed, wounds closed with sutures, and the dogs were returned to their cages. Food was withheld for 16 hr postoperatively. For study times longer than 4 hr, blood was sampled from the cephalic vein and urine was recovered by rinsing cage pans with distilled water. For the removal of tissues, dogs were sacrificed at 10 min, 60 min, 4 hr, 24 hr, and 4 days postinjection. Dogs at the 2 longest time points were killed by electrocution; those at early times (i.e., while still under anesthesia) were killed by exsanguination. In addition to these routine time points, selected tissues were sampled at times as long as 12 days posttreatment. Tissues routinely analyzed were: lung, liver, kidney, uterus, ovary, skeletal muscle, skin, adrenals, spleen, fat, pancreas, heart, stom-
ach, jejunum, and ascending colon. At longer time points only kidney, liver, lung, skin, muscle, ovary, plasma, and urine were collected. Bile samples were routinely taken from the gallbladder at the time of sacrifice. In addition, in those dogs studied for longer than 24 hr after dosing, blood samples were analyzed for urea nitrogen and creatinine to evaluate renal function during the posttreatment period. Tissues were removed from 1 dog at each time point, but 3 to 6 plasma samples and 2 to 3 urine samples were collected from different dogs at each time.

**In Vitro Plasma Binding.** Binding of DDP to plasma proteins was studied by adding DDP (1 or 5 μg/ml final concentration) to heparinized plasma from control dogs. This plasma was then incubated in the dark at 37° for various times. Binding was determined as described previously (4) by placing triplicate aliquots of incubated plasma into ultrafiltration membranes (Amicon 2100, CF-50) which were centrifuged for 20 min at 1000 rpm in a refrigerated (0-4°) International Model PR-2 centrifuge. The amount of platinum recovered in the ultrafiltrate was taken as the unbound fraction. Preliminary experiments showed that at zero time 95 to 100% of the platinum from a 0.85% NaCl solution of DDP could be recovered in the ultrafiltrate.

**Platinum Analysis.** Details of the platinum assay have been described elsewhere (12). Briefly, tissue samples were digested using nitric and perchloric acids, and after evaporation to dryness the residue was then solubilized in dilute hydrochloric acid. A small aliquot of the HCl solution was then analyzed for platinum on a Perkin-Elmer Model 303 atomic absorption spectrometer equipped with a heated graphite atomizer on which the temperature control was modified to produce gradual increases in temperature. Plasma and urine were assayed without prior digestion. Accuracy of the platinum determination was verified by neutron activation analysis. The recovery of platinum following the addition of known amounts of DDP to tissue and plasma prior to digestion was 85 to 100% depending on the tissue analyzed. This method is for total platinum metal; no attempts were made to determine the biological or chemical state of the platinum.

**RESULTS**

The plasma decay curve and cumulative platinum recovery in urine are presented in Chart 1. Lines were fitted to points by inspection, and the plasma half-times (t1/2) were estimated from those lines by extrapolation to t = 0. It can be seen that the initial fall in plasma concentration is extremely rapid, with an apparent t1/2 of considerably less than 1 hr. After 4 hr, however, the rate of decrease becomes considerably slower, with significant amounts of platinum still detectable in plasma 12 days after injection. The apparent t1/2 for this slow phase of plasma decay is 4 to 5 days. Urinary levels of platinum rise rapidly with 50 to 60% of the administered dose recovered in urine within the 1st 4 hr after treatment (Chart 1). Urinary data are presented for the 1st 4 hr only because this was the longest time studied in which dogs were catheterized and quantitative recovery of urine was possible. Although feces were not analyzed, only small amounts of platinum were detectable in the bile.

Data in Table 1 show the organ distribution of total platinum at 6 time points after treatment. Highest levels are found in the kidney, which correlates well with high urinary levels at early times following treatment. Within the 1st day after treatment, plasma values decline by approximately 90% while many tissues maintain nearly constant levels of the element.

Chart 2 shows the tissue:plasma ratio of platinum for several tissues during the 1st week posttreatment. Although the absolute values are low relative to those on the 1st day after dosing, the concentration of platinum was significantly higher in several tissues than was found in plasma at corresponding times. In several instances renal cortex and medulla were analyzed separately; in each instance it was found that the concentration of platinum was initially slightly higher in the medulla but that, by the end of the 1st day, concentrations had become equal and then became greater in the cortex. This latter distribution was maintained as long as 12 days after dosing.

The apparent in vitro binding to proteins of platinum derived from the incubation of DDP with dog plasma is shown in Chart 3. In other preliminary experiments we incubated 0.1 mm bovine serum albumin with DDP, 1 or 5 μg/ml, at 37° for 48 hr and found a progressive decrease in the platinum recovered in the ultrafiltrate; this suggested a change in binding characteristics of platinum to albumin similar to that seen with whole plasma (Chart 3). As is the case with
Table 1
Organ distribution of platinum following i.v. administration of DDP, 1 mg/kg, to dogs

<table>
<thead>
<tr>
<th>Organ</th>
<th>Min</th>
<th>60</th>
<th>1 Hr</th>
<th>24</th>
<th>4 Days</th>
<th>12 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td>6.65</td>
<td>1.95</td>
<td>1.87</td>
<td>2.15</td>
<td>0.75</td>
<td>0.55</td>
</tr>
<tr>
<td>Plasma</td>
<td>2.64</td>
<td>0.71</td>
<td>0.45</td>
<td>0.27</td>
<td>0.18</td>
<td>0.15</td>
</tr>
<tr>
<td>Ovary</td>
<td>1.81</td>
<td>0.64</td>
<td>1.04</td>
<td>0.78</td>
<td>0.73</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>1.50</td>
<td>1.39</td>
<td>1.34</td>
<td>1.17</td>
<td>0.35</td>
<td>0.32</td>
</tr>
<tr>
<td>Uterus</td>
<td>1.36</td>
<td>0.70</td>
<td>1.18</td>
<td>1.53</td>
<td>0.92</td>
<td></td>
</tr>
<tr>
<td>Skin</td>
<td>1.25</td>
<td>0.16</td>
<td>0.78</td>
<td>0.80</td>
<td>0.66</td>
<td>0.14-0.24</td>
</tr>
<tr>
<td>Stomach</td>
<td>1.20</td>
<td>0.68</td>
<td>0.80</td>
<td>0.78</td>
<td></td>
<td>0.31</td>
</tr>
<tr>
<td>Adrenal</td>
<td>1.06</td>
<td>0.34</td>
<td>0.93</td>
<td>1.08</td>
<td>0.26</td>
<td></td>
</tr>
<tr>
<td>Colon</td>
<td>0.70</td>
<td>0.40</td>
<td>0.24</td>
<td>0.25</td>
<td>0.07</td>
<td>0.04</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.35</td>
<td>0.80</td>
<td>0.47</td>
<td>0.66</td>
<td>0.42</td>
<td></td>
</tr>
<tr>
<td>Spleen</td>
<td>0.26</td>
<td>0.15</td>
<td>0.52</td>
<td>0.43</td>
<td>0.33</td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>&lt;0.06</td>
<td>&lt;0.06</td>
<td>&lt;0.06</td>
<td>&lt;0.06</td>
<td>&lt;0.06</td>
<td></td>
</tr>
<tr>
<td>Fat</td>
<td>0.23</td>
<td>&lt;0.04</td>
<td>&lt;0.04</td>
<td>&lt;0.04</td>
<td>&lt;0.04</td>
<td></td>
</tr>
</tbody>
</table>

DISCUSSION

Results presented here give some insight into the pharmacokinetic behavior of platinum following single-dose i.v. administration of DDP to dogs. Although it has been reported by some investigators that various platinum-containing drugs have a long biological half-life (1, 19), other investigators have reported a rapid clearance and excretion following intravascular administration (10, 17). The explanation for this apparent contradiction was hinted at by Hill, who found in patients treated with DDP a fast and a slow phase of platinum loss from plasma depending on the time after administration that the follow-up study was conducted (7). The present study examines both early and late times posttreatment and demonstrates a clearly biphasic clearance pattern. Very high plasma levels occurring shortly after administration are quickly cleared within the 1st 4 hr. The final phase of clearance is protracted, however, and elimination of platinum from blood is not complete even 12 days after a single dose of the drug.

In an attempt to explain the prolonged 2nd phase of DDP clearance, we examined the apparent binding of platinum to blood plasma in vitro by ultrafiltration. As can be seen in Chart 3, there was a progressive increase in the fraction of platinum retained by the membrane during the 48-hr incubation period. This result supports a suggested change in the DDP molecule (20) with a concomitant alteration in

\[ \text{Data points are the means of 3 analytical replicates and are expressed as the percentage of the incubated dose recovered in the ultrafiltrate.} \]

The plasma decay curve (Chart 1), the break in this biphasic curve occurs at about 6 hr.

Our concern for the toxicity of DDP to treated dogs was generated by the well-known renal toxicity of this drug (5, 11), and we wanted to assure that the distribution and plasma decay of platinum were not affected by compromised renal function. Blood urea nitrogen and creatinine levels in plasma from treated dogs were unchanged for periods of up to 12 days posttreatment.
protein binding characteristics and thus the filterability. The relation between this possible change in the form of platinum with the subsequent change in filtration characteristics may help to explain the striking change in plasma platinum clearance, although it is recognized that additional explanations may also be valid. One alternative hypothesis is that there are different binding mechanisms operating in vivo. Large doses of platinum may be bound with a high rate constant but low affinity, while at lower concentrations of platinum the binding characteristics may favor a low rate constant and high-affinity mechanism.

Although we have no extensive evidence to suggest which blood component may be altered or affected by DDP, preliminary experiments showed that there was little detectable platinum in the RBC fraction after separation from plasma and washing with NaCl solution. This is in agreement with data of Howle et al. (8) who showed no association of the dipyridine analog of DDP with erythrocyte membranes. Our preliminary results of platinum binding to bovine serum albumin (see "Results"), however, are in contrast to data of both Howle et al. (8) and Gale (2), who showed no apparent binding to albumin when the protein was dialyzed for 4 hr against a solution containing 10^{-1} to 10^{-5} M concentrations of either the dipyridine or methylamine analogs of DDP, respectively. In one of these studies (2), the concentration of protein in the dialysis bath was only 0.5 mg/ml, which is 70 times less than the concentration of albumin normally in plasma. In another experiment (8), binding studies were conducted at room temperature. It is possible that the binding characteristics of the 2 analogs differ from DDP, that the concentration of protein used by others was too low, or that any rate of change of filterability is temperature dependent and not observed at short times.

The organ distribution of platinum at various times after administration of the drug is in qualitative agreement with that shown not only for DDP but also for DDP analogs, and in several species including man (1, 2, 7, 10, 17, 19). The localization of platinum in organs of excretion (kidney, liver, and lung) is a finding reported almost universally for several platinum-containing drugs in several species (2, 10, 17). This localization also has been reported to be protracted, at least in kidney and liver, and these results are confirmed by the present study (Table 1). In addition the distribution of platinum shown in Table 1 is consistent with the well-established renal toxicity seen in man and animals, as evidenced here by the high renal content of platinum even 12 days after treatment. Of additional note is the high concentration of protein used by others was too low, or that any rate of change of filterability is temperature dependent and not observed at short times.

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