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In vitro stability, plasma protein binding and blood cell partitioning of ^{14}C-carboplatin

Robert C. Gaver, Alice M. George, and George Deeb

Department of Drug Metabolism and Pharmacokinetics, Pharmaceutical Research and Development Division, Bristol-Myers Company, P.O. Box 4755, Syracuse, NY 13221, USA

Summary. Radiochemically pure ^{14}C-labeled carboplatin, *cis*-diammine [1,1-cyclobutane (1-^{14}C) dicarboxylato (2-)-0,0'] platinum (II), was added to fresh human, dog and rat plasma, at concentrations ranging from 1 to 100 μg ^{14}C-carboplatin/ml. After 10 min incubation at ambient temperature, the plasma was ultrafiltered in Amicon Centrifree micropartition units to generate protein-free plasma ultrafiltrate (PU). Total radioactivity was determined by liquid scintillation counting. A mean (±SD) of 102%±2.0%, 99.5%±1.9%, and 99.0%±1.0% of the ^{14}C-carboplatin added to fresh human, dog and rat plasma respectively was recovered in the PU. ^{14}C-carboplatin was incubated at 37° C with fresh plasma (60 μg/ml) and urine (200 μg/ml) from humans and dogs for 120 h, and samples were removed at appropriate times for analysis of carboplatin, 1,1-cyclobutane dicarboxylic acid and cyclobutane carboxylic acid. The latter were separated by HPLC on a C-18 column with a mobile phase of $H_2O/CH_3CN/0.3$ *M* tetrabutylammonium phosphate (880:50:20 v/v/v), and the column eluants at the retention time of each compound were collected and counted for total radioactivity. Carboplatin degraded in each of the matrices with a corresponding release of 1,1-cyclobutane dicarboxylic acid. ^{14}C-carboplatin (50 μg/ml) was incubated at 37° C with fresh human, dog and rat blood and the distribution of radioactivity into the cellular fraction was determined. Radioactivity did not distribute into the blood cells of humans or dogs, but after 5 h, 44% of the radioactivity in rat blood was associated with the cellular fraction. These results show that carboplatin, at physiological concentrations, does not bind instantaneously and reversibly to the plasma proteins of rat, dog or human, and that the molecule slowly degrades in plasma and urine in vitro with the release of 1,1-cyclobutane dicarboxylic acid. The remaining diammine platinum (II) portion of the molecule therefore accounts for the essentially irreversible protein binding of the platinum from carboplatin.

Introduction

Carboplatin, *cis*-diammine [1,1-cyclobutane dicarboxylato (2-)-0,0'] platinum (II) (CBDCA, JM8, NSC 241240, Paraplatin), is a second-generation platinum-containing antitumor agent presently in phase III trials in the United States and Europe. It was selected for clinical evaluation based on experimental antitumor and toxicologic profiles in animal models showing it to be less nephrotoxic and less emetic than cisplatin [3, 8].

Carboplatin has limited stability in human plasma in vitro with a half-life of about 30 h [1, 2]. Degradation also occurs on incubation with human plasma diluted 1:1 with pH 7.4 phosphate buffer [7]. This degradation presumably occurs by displacement of the 1,1-cyclobutane dicarboxylic acid moiety by water molecules with the formation of highly reactive diaquated diammine platinum (II), analogous to the loss of the chlorine atoms from cisplatin but occurring at a slower rate. This compound or the corresponding mono- or dihydroxylated platinum II compound would then react rapidly and essentially irreversibly with the sulfhydryl or amine groups of plasma proteins. In this way, the platinum from carboplatin, but not the parent compound itself, could become bound to plasma proteins.

In vitro studies employing an HPLC assay specific for carboplatin showed that only 10% or less of the parent compound was instantaneously and reversibly bound to human plasma proteins in a manner characteristic of normal drug protein binding [1]. In a similar study, 93% of the carboplatin added to human plasma was recovered in plasma ultrafiltrate; essentially the same percentage of platinum (92%) was recovered in the ultrafiltrate when an aqueous solution of carboplatin was treated identically [11]. These results indicated that little, if any, of the compound was instantaneously and reversibly bound to human plasma proteins.

Carboplatin also appears not to be reversibly bound to the plasma proteins of the dog or rabbit in vitro, but conflicting data have been reported on the binding in rat plasma in vivo. During an 8-h incubation of carboplatin in dog or rabbit plasma, no change in ultrafilterable platinum was noted, indicating that protein binding of platinum, either reversible or irreversible, had not occurred [6]. Immediately after administration of carboplatin (20 mg/kg) to rats, about 20% of the free platinum was reported to be reversibly bound to plasma proteins [10]. This binding remained constant for about 1 h, but free platinum values then decreased due to irreversible binding to plasma proteins. Other investigators, however, reported that 94%–97% of the platinum in rat plasma immediately after administration of carboplatin (20 mg/kg) was present as free or ultrafilterable platinum [5]. These free platinum

Offprint requests to: R. C. Gaver

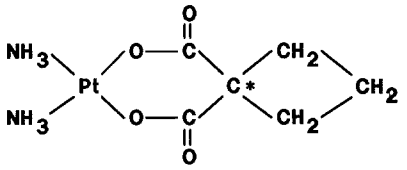

Fig. 1. Structure of carboplatin showing position of ^{14}C (*)

concentrations then decreased and by 2 h accounted for about 70% of the values for total plasma platinum.

The platinum from carboplatin accumulated in rat red blood cells both in vitro (300 μ*M*) and in vivo (80 mg/kg) [9] but no accumulation of platinum was found in the red blood cells of the dog following i.v. administration (12 mg/kg) of carboplatin [12]. No data are available on the possible accumulation in human red blood cells.

Recently, the synthesis of carboplatin, labeled with ^{14}C in the cyclobutane portion of the molecule (Fig. 1), was described [4]. The availability of this material provided us with a rapid and precise means of evaluating the in vitro stability, protein binding and plasma to blood cell partitioning of carboplatin in different animal species. In addition, it enabled us to follow the in vitro release of 1,1-cyclobutane dicarboxylic acid. These studies are the subject of this paper.

Materials and methods

Chemicals and reagents. ^{14}C-labeled carboplatin was synthesized as previously described [4]. After recrystallization, the specific activity was 3.1 μCi/mg or 6882 dpm/μg and the radiochemical purity was 99.8%. The latter was determined by applying the compound to an HPLC system developed for the analysis of carboplatin in urine [1] and determining the amount of radioactivity in column eluants collected at 1-min intervals. The total disintegrations per minute (dpm) in the fractions at the retention time of carboplatin were divided by the total dpm applied to the column and multiplied by 100 to give the percent radiochemical purity. All solutions of ^{14}C-carboplatin were prepared on the day of use. Tetrabutylammonium hydrogen sulfate and 1,1-cyclobutane dicarboxylic acid were from Aldrich Chemical Co., Milwaukee, Wis. Cyclobutane carboxylic acid was from Fluka AG, Chem Fabnik, Marseille, France. Water was prepared by a Milli-Q water purification system (Millipore Corp., Bedford, Mass.). HPLC mobile phase solvents were HPLC grade and all other reagents were ACS grade.

Biological samples. Blood and urine were obtained from normal, healthy male and female volunteers and from adult male beagle dogs and male Sprague-Dawley rats in our departmental colonies. The blood was collected on each experimental day in Vacutainer tubes containing EDTA as the anticoagulant (Becton-Dickinson and Co., Rutherford, NJ). Plasma was generated immediately by centrifugation and was transferred to a separate container. Milliliter quantities of protein-free ultrafiltrates of human plasma or serum were obtained by adding 6 ml plasma or serum to each of several CF50A Amicon Centriflo ultrafiltration cones (Amicon Corp., Danvers, Mass.) and centrifuging at 650 g for 20 min at 3°–7° C. Between 1.2 and 1.4 ml ultrafiltrate was obtained from each 6 ml. Human serum was obtained by collecting blood in tubes with no anticoagulant, allowing the blood to clot for about 0.5 h at 22° C, and then centrifuging it to separate the serum from the cells and the fibrin clot. Dog urine was collected by urinary catheter and rat urine was collected from rats placed in metabolism cages equipped with urine-feces separators. Hematocrit values were determined with an IEC model MB micro-hematocrit centrifuge (Damon/IEC Division, International Equipment Co., Needham Heights, Mass.).

Determination of total radioactivity. Samples of 1.0 ml or less were added to 10 ml Insta-Gel (United Technologies Packard, Downers Grove, Ill.) and mixed. Samples of greater than 1.0 ml, obtained during collection of the HPLC eluants, were mixed with 15 ml Insta-Gel. The samples were counted in a Beckman LS9000 liquid scintillation spectrometer (Beckman Instruments, Inc., Fullerton, Calif.) equipped with an automatic data reduction system. A quench curve of H number vs counting efficiency was generated from a series of quench standards (United Technologies Packard; Beckman Instruments, Inc.). Quench standards were counted with the samples to verify proper operation of the instrument. Samples and standards were counted for 50 min or to a 2% two-sigma error. All results were expressed as dpm after correction for background (50 cpm) and quenching.

HPLC Analysis of carboplatin and degradation products. The amounts of ^{14}C-carboplatin, 1,1-cyclobutane (1-^{14}C) dicarboxylic acid and cyclobutane (1-^{14}C) carboxylic acid in human and dog plasma or plasma ultrafiltrate (PU), dog urine and water were determined by HPLC on a 250 × 4.6 mm IBM octadecyl column (IBM Instrument Co., Danbury, Conn.). The column eluants corresponding to the retention times of the particular compound were collected and counted for total radioactivity. The mobile phase was H_2O/CH_3CN/0.03 *M* tetrabutylammonium phosphate (880:50:20 v/v/v) and was vacuum-degassed prior to use. The tetrabutylammonium phosphate reagent was prepared by dissolving 13.5 g tetrabutylammonium hydrogen sulfate in 127 ml water, adding 5.4 ml 85% phosphoric acid and adjusting the pH to 7.4 with 5 *N* NaOH. Injection volume was 10 μl, flow rate was 2.0 ml/min, chart speed was 12 or 15 cm/h, and the range setting was 0.05 AUFS. Detection was at 229 nm. Under these conditions carboplatin, 1,1-cyclobutane dicarboxylic acid and cyclobutane carboxylic acid eluted at about 2 min, 6–7 min and 8–9 min respectively. The column eluants were collected at 0.5 or 1.0 min intervals for a total of 12 or 12.5 min. Eluants from 12 or 12.5 to 25 min were collected in a 50-ml volumetric flask. After bringing to volume with mobile phase, a 1.0-ml sample was analyzed for radioactivity. The total amount of radioactivity injected onto the column was determined by having the autosampler inject 10-μl samples of the specific matrix directly into a scintillation vial. The amount of radioactivity present as carboplatin, cyclobutane carboxylic acid or 1,1-cyclobutane dicarboxylic acid was the sum of the dpm present in three consecutive 0.5-min fractions beginning at the time the compound started to elute. The total percentage recovery was calculated by summing the dpm present as carboplatin and the two carboxylic acids, dividing this sum by the total dpm applied to the column and multiplying by 100.

The concentrations of carboplatin in human urine were determined by a previously described HPLC procedure with a LiChrosorb diol column (Alltech Associates, Deerfield, Ill.) and $CH_3CN/0.015\%$ H_3PO_4 (89:11 v/v) as the mobile phase [1]. Quantitation was based on the recovery of radioactivity at the elution time of the compounds and not on peak height measurement. In this system, carboplatin eluted at 11.5 min and both 1,1-cyclobutane dicarboxylic acid and cyclobutane carboxylic acid eluted at 3 min. The 48-h and 120-h samples from the urine stability study were also analyzed with the previously described HPLC system to determine the quantities of each of the carboxylic acids.

The HPLC equipment consisted of the following units: WISP, model 710B autosampler; Model 6000A solvent delivery system; Models 441 and 481 LC detectors (Waters Associates, Milford, Mass.); a Model SE120 recorder (BBC-Metrawatt/Goerz, Broomfield, Colo.) and a Model 1200 recorder (Linear Instrument Corp., Reno, Nev.).

In vitro stability at 37° C. A 1.2-ml portion of an aqueous solution of ^{14}C-carboplatin (1.0 mg/ml) was added to 18.8 ml fresh dog or human plasma to give a final concentration of 60 μg ^{14}C-carboplatin/ml plasma. The plasma was placed in a constant temperature water bath at 37° C, and samples (0.25 or 0.50 ml) were removed at the following times for HPLC analysis of carboplatin, 1,1-cyclobutane dicarboxylic acid and cyclobutane carboxylic acid: 0, 2, 6, 24, 48, 72 and 120 h. Additional samples (10 μl) were taken at the same times for determination of radioactivity. Samples of plasma (0.3–0.5 ml) were also added to Centrifree ultrafiltration units and were centrifuged at 200 g for 20 min to generate from 170 to 200 μl PU. The PU was transferred to a microcentrifuge tube, and a sample (10 μl) was removed for determination of radioactivity. The samples for HPLC analysis were immediately frozen on dry ice and stored at −60° C until analyzed.

A 0.3-ml portion of an aqueous solution of carboplatin (10 mg/ml) was added to 15 ml fresh human urine, fresh dog urine or water to give a final concentration of 200 μg ^{14}C-carboplatin/ml urine or water. These solutions were incubated at 37° C and samples (0.5 ml) were removed at the following times for analysis: 0, 2, 6, 24, 48, 72 and 120 h. Samples (10 μl) were also removed for determination of total radioactivity, and additional samples of the water solution were taken at 168 and 240 h. All samples, except those for total radioactivity, were frozen on dry ice and stored at −60° C until analyzed.

Determination of protein binding. A sample of ^{14}C-carboplatin was dissolved in water to give a final concentration of 10.5 mg ^{14}C-carboplatin/ml. Dilutions of this solution were made to give final concentrations of 1.05 and 0.021 mg/ml. A 10-μl sample of each solution was taken for determination of total radioactivity. Appropriate volumes (10–100 μl) of the aqueous standards were dispensed, in triplicate, into 100 × 13 mm tubes, and the total volume of water in each tube was adjusted to 0.10 ml. Samples (2.0 ml) of fresh human plasma or PU were added to each tube and mixed thoroughly. The final concentrations ranged from 1 to 100 μg ^{14}C-carboplatin/ml. Triplicate samples (50 μl) were removed for determination of total radioactivity and three 0.5-ml samples were added to separate Centrifree ultrafiltration units (Amicon Corp., Danvers, Mass.). After standing for 10 min at 22° C, the units were centrifuged at 200 g for 10 min to generate about 60 μl PU. Samples (50 μl) of each PU were analyzed for total radioactivity. Similar samples were prepared on a separate occasion with fresh human serum and serum ultrafiltrate. The same procedures and concentrations were used on separate occasions to prepare ^{14}C-carboplatin in fresh dog and rat plasma, except that the total water and plasma volumes were 0.20 ml and 4.0 ml, respectively, 1.0-ml plasma samples were ultrafiltered and triplicate 100-μl samples were removed for determination of total radioactivity. The percentage of free, non-protein-bound drug was calculated by dividing the mean dpm/ml PU by the mean dpm/ml plasma and multiplying by 100. Subtracting this value from 100 gives the percentage of protein-bound drug.

Determination of plasma cell partitioning. A 0.50-ml sample of an aqueous solution of ^{14}C-carboplatin (1.05 mg/ml) was added to 10.0 ml fresh human, rat or dog blood and mixed gently to give a final concentration of 50 μg ^{14}C-carboplatin/ml. The blood samples were placed in a constant temperature water bath at 37° C and 1.0-ml samples were removed at the following times: 0, 0.25, 0.50, 0.75, 1.0, 3.0 and 5.0 h. The blood samples were centrifuged for 15 min at 200 *g* to generate plasma. The latter was placed in a separate tube and triplicate (50-μl) samples were removed for determination of radioactivity. The hematocrit values for human, dog and rat blood were 0.42, 0.53 and 0.44 respectively.

In a second experiment, the effect of added unlabeled 1,1-cyclobutane dicarboxylic acid on the binding of ^{14}C-carboplatin to rat blood cells was evaluated. A 250-μl sample of an aqueous solution of ^{14}C-carboplatin (1.05 mg/ml) and a 250-μl sample of an aqueous solution of unlabeled 1,1-cyclobutane dicarboxylic acid (0.158 mg/ml) were added to 10 ml fresh rat blood to give final concentrations of 25 μg carboplatin/ml (67 μ*M*) and 3.76 μg/ml of the dicarboxylic acid (26 μ*M*). To a second 10-ml sample of rat blood were added 250 μl of the ^{14}C-carboplatin solution plus 250 μl of water. Both blood samples were incubated at 37° C, and samples were removed at 0, 0.25, 0.50, 0.75, 1, 2 and 3 h and processed as described above. The hematocrit value was 0.39.

The distribution of ^{14}C-carboplatin between the plasma and cells of rat blood was calculated by the following relationship:

$$C = B - P(1\text{-}H), \quad (1)$$

where C = μg drug in the cells in 1 ml blood, B = μg drug in 1 ml blood, P = μg drug in 1 ml plasma and H = hematocrit. The product of P (1-H) is the μg of drug in the plasma portion of 1 ml blood.

Results

In vitro stability

When ^{14}C-carboplatin (60 μg/ml plasma) was incubated with fresh human plasma at 37° C, the radioactivity present as carboplatin decreased with time, with a corresponding increase in the amount of 1,1-cyclobutane (1-^{14}C) dicarboxylic acid (Fig. 2). No significant quantities (> 50 dpm) of cyclobutane (1-^{14}C) carboxylic acid were found. A plot of log carboplatin dpm vs time showed the

loss of carboplatin was first order ($r = -0.999$), with a half-life of 33 h. This value confirmed our previously reported in vitro half-life value of 32 h for a concentration of 25 μg/ml, based on HPLC analysis of unlabeled carboplatin [1]. In addition, essentially all of the radioactivity present as carboplatin and 1,1-cyclobutane dicarboxylic acid was recovered as the respective compounds in the PU. These results indicated that neither compound was reversibly bound to plasma proteins.

Similar results were seen on incubation of ^{14}C-carboplatin (60 μg/ml plasma) in fresh dog plasma. Carboplatin concentrations decreased with a corresponding increase in the concentration of 1,1-cyclobutane (1-^{14}C) dicarboxylic acid. Likewise, no significant quantities (>50 dpm) of the monocarboxylic acid were found, and the decay of carboplatin was first order ($r = -0.999$), with a half-life of 39 h. As with human plasma, the amount of radioactivity recovered as parent compound and as the dicarboxylic acid in the PU was essentially the same as in the plasma. Therefore, carboplatin degraded at a similar rate in both human and dog plasma, and neither parent compound nor 1,1-cyclobutane dicarboxylic acid was reversibly bound to the plasma protein of either species.

Carboplatin also degraded in human and dog urine at 37° C, with the release of the dicarboxylic acid. On incubation of ^{14}C-carboplatin (200 μg/ml) in fresh human urine, the loss of parent compound was first order ($r = -0.998$), with a half-life of 39 h (Fig. 3). Carboplatin was also unstable in dog urine at the same concentration. In contrast to the degradation of carboplatin in human plasma and urine and in dog plasma, the decay in dog urine was not first order, since a plot of the log concentration data vs time was curvilinear. However, only about 50% of the original concentration remained after 23 h at 37° C.

Carboplatin (200 μg/ml) was more stable in water at 37° C than in either plasma or urine (Fig. 4). Although the results were somewhat variable, about 90% of the initial carboplatin concentration was still present after 240 h and only about 2% or less of the radioactivity was present as 1,1-cyclobutane dicarboxylic acid. The increased relative stability of carboplatin in water compared to the biological matrices indicated that the degradation in plasma and urine was promoted by components in these matrices.

Protein binding

The extent of reversible binding of carboplatin to human plasma proteins was determined using Amicon Centrifree micropartition systems. ^{14}C-carboplatin was added to fresh human plasma, collected with EDTA as the anticoagulant, to give concentrations of 1–100 μg carboplatin/ml plasma. A 0.5-ml sample of the plasma was then placed in the Centrifree unit and centrifuged to generate about 60 μl PU. A sample (50 μl) of the latter was counted. ^{14}C-carboplatin was also added to fresh human PU at the same concentration and these samples were processed in the same manner as the plasma. The latter were prepared to determine whether carboplatin was bound to the filter of the Centrifree units. All of the radioactivity added to plasma and PU was recovered in the corresponding ultrafiltrates (Table 1). Therefore, carboplatin at concentrations found in vivo does not undergo the instantaneous and reversible binding to human plasma proteins that is characteristic of normal drug protein binding.

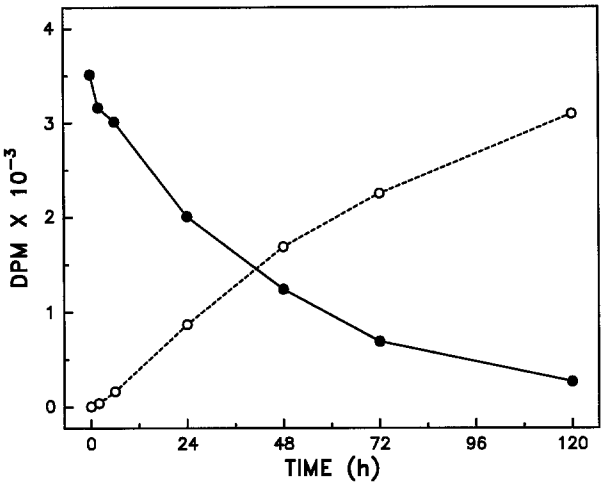

Fig. 2. Radioactivity present as carboplatin (———) and 1,1-cyclobutane dicarboxylic acid (– – – – –) as a function of time on incubation of ^{14}C-carboplatin (60 μg/ml) in fresh human plasma at 37° C

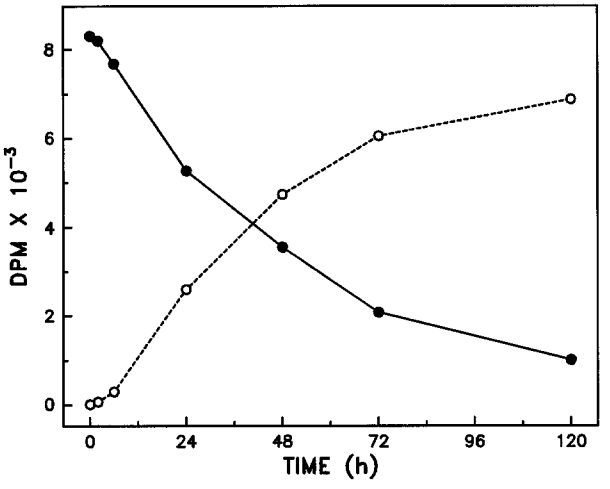

Fig. 3. Radioactivity present as carboplatin (———) and 1,1-cyclobutane dicarboxylic acid (– – – – –) as a function of time on incubation of ^{14}C-carboplatin (200 μg/ml) in human urine at 37° C

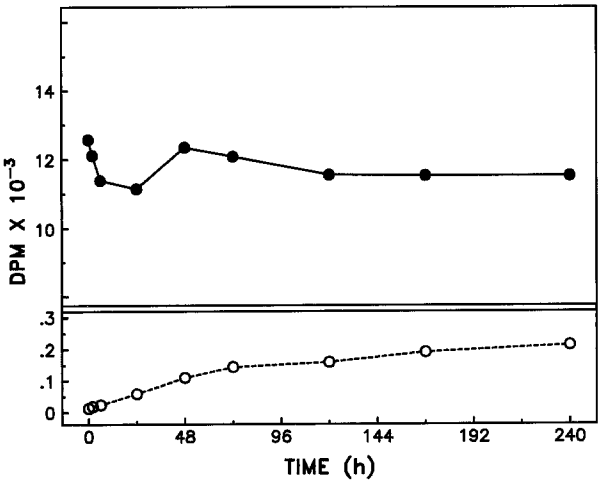

Fig. 4. Radioactivity present as carboplatin (———) and 1,1-cyclobutane dicarboxylic acid (– – – – –) as a function of time on incubation of ^{14}C-carboplatin (200 μg/ml) in water at 37° C

Table 1. ^{14}C-Carboplatin radioactivity in fresh human plasma and serum before and after ultrafiltration in an Amicon Centrifree system

Human plasma (EDTA)			
Carboplatin concentration (μg/ml plasma)	Mean dpm/50 μl ± SD ($n = 3$) Plasma	Plasma ultrafiltrate	Mean percent not protein bound
1	321 ± 10	331 ± 7	103
5	1775 ± 42	1840 ± 13	104
10	3468 ± 111	3488 ± 81	101
25	8422 ± 302	8375 ± 78	99.4
50	16601 ± 490	17102 ± 244	103
100	33442 ± 990	33211 ± 960	99.3
			102 ± 1.97
Human serum			
Carboplatin concentration (μg/ml serum)	Mean dpm/50 μl ± SD ($n = 3$) Serum	Serum ultrafiltrate	Mean percent not protein bound
1	344 ± 10	336 ± 27	97.7
5	1646 ± 60	1640 ± 60	99.6
10	3242 ± 168	3298 ± 62	102
25	8171 ± 257	8473 ± 212	104
50	16915 ± 768	16899 ± 543	99.9
100	32318 ± 1530	33496 ± 670	104
			101 ± 2.56

Table 2. ^{14}C-Carboplatin radioactivity in fresh dog and rat plasma before and after ultrafiltration in an Amicon Centrifree system

Dog plasma			
Carboplatin concentration (μg/ml plasma)	Mean dpm/100 μl ± SD ($n = 3$) Plasma	Plasma ultrafiltrate	Mean percent not protein bound
1	695 ± 25	688 ± 8	99.0
5	3251 ± 66	3302 ± 8	102
10	5236 ± 100	5248 ± 88	100
25	17598 ± 230	17322 ± 104	98.4
50	34330 ± 292	33174 ± 702	96.6
100	63279 ± 1391	64072 ± 2436	101
			99.5 ± 1.93
Rat plasma			
Carboplatin concentration (μg/ml plasma)	Mean dpm/100 μl ± SD ($n = 3$) Plasma	Plasma ultrafiltrate	Mean percent not protein bound
1	668 ± 8	670 ± 20	100
5	3436 ± 27	3360 ± 96	97.8
10	6495 ± 134	6520 ± 140	100
25	17336 ± 168	17106 ± 352	98.7
50	34343 ± 916	34148 ± 258	99.4
100	64663 ± 1654	63348 ± 246	98.1
			99.0 ± 0.949

An identical experiment was performed with fresh human serum instead of plasma to determine if the anticoagulant might have inhibited the binding. As with plasma, all of the radioactivity was recovered in the ultrafiltrate (Table 1). Carboplatin, over a concentration range of 1–100 μg/ml serum, is not reversibly bound to human plasma proteins in the presence or absence of EDTA.

A similar determination of protein binding over the same concentration range was performed with fresh dog and rat plasma, except that 1.0 μl plasma was applied to the Centrifree units and 100 μl PU was counted (Table 2). As with human plasma, all of the radioactivity added to the plasma was recovered in the PU. Carboplatin, therefore, was not reversibly bound to the proteins of rat or dog plasma.

Plasma/cell partitioning

^{14}C-carboplatin (50 μg/ml) was incubated for 5 h at 37° C in fresh whole blood from humans, dogs and rats and samples were removed at various times. Plasma was generated by centrifugation and was counted for total radioactivity. With all three species, the total amount of radioactivity added to the blood was recovered in the plasma at time 0. However, while the amounts of radioactivity in human and dog plasma remained constant with time, the amounts in rat plasma decreased with time. The calculated amounts of radioactivity in the plasma and cells of 1 ml blood (Fig. 5) showed that by 1 and 5 h approximately 30% and 44% of the radioactivity respectively was associated with the cellular components of rat blood.

In a similar experiment, ^{14}C-carboplatin (25 μg/ml, 67 μ*M*) with and without added unlabeled 1,1-cyclobutane dicarboxylic acid (3.76 μg/ml, 26 μ*M*) was incubated in whole rat blood for 3 h to see whether a known degradation product of carboplatin would influence the plasma/cell distribution of radioactivity. At each sampling time, the amounts of radioactivity in the cellular fractions were the same. By 1 h, 29%–32% of the radioactivity in the blood was associated with the cellular fraction. The added dicarboxylic acid did not affect the cellular distribution of radioactivity.

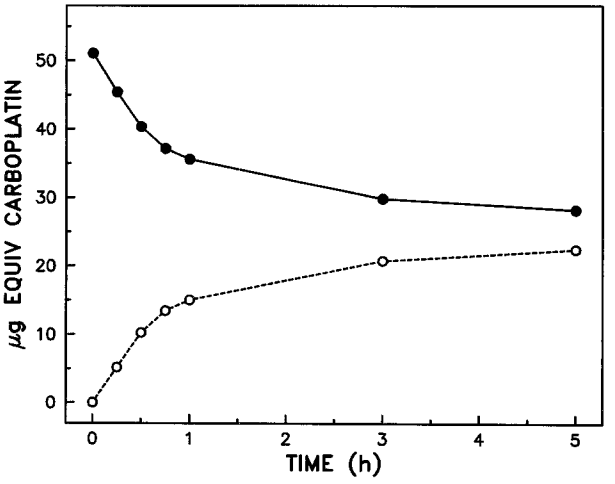

Fig. 5. Partitioning of radioactivity from ^{14}C-carboplatin between the plasma (●) and cells (○) in 1.0 ml rat blood as a function of time at 37° C. Initial concentration was 50 μg ^{14}C-carboplatin/ml blood

Discussion

The results of these studies with ^{14}C-carboplatin demonstrate that the compound is relatively stable in water, but degrades slowly in plasma and urine at 37° C, with the release of 1,1-cyclobutane dicarboxylic acid. Similar degradation would be expected to occur in vivo, but because of the relatively short plasma half-life of 1.5 h in humans [2], compared to the in vitro half-life of about 30 h, it would not be expected to have a significant effect on the plasma pharmacokinetics of the compound. However, as pointed out previously [1, 2], this instability in human plasma and urine could dramatically decrease the amounts of carboplatin found on analysis of samples of these matrices if they are not stored frozen and rapidly analyzed. This would lead to decreased values for the area under the plasma concentration vs time curve (AUC) and the percentage of the dose excreted in the urine and to erroneously high values for total body and renal clearances. Likewise, assay values for free, ultrafilterable platinum will be reduced and will not be a valid measurement of carboplatin if plasma ultrafiltrates are not generated as rapidly as possible after the blood samples are obtained. Therefore, proper sample handling is critical for obtaining valid pharmacokinetic data for carboplatin.

Our in vitro protein binding studies demonstrate that carboplatin, over the range of concentrations found in vivo, does not undergo the instantaneous and reversible binding to plasma proteins that is characteristic of normal drug-protein binding. This confirmed and extended our previous results with unlabeled compound showing that only 10% or less of the compound was protein bound. None of the parent compound, carboplatin, was instantaneously bound in vitro to the plasma proteins of human, dog or rat plasma based on a technique generally employed for evaluating drug-protein binding.

Although parent compound does not bind to plasma proteins, the platinum from carboplatin does become tightly bound to plasma proteins. This binding could occur simultaneously with the displacement of the 1,1-cyclobutane dicarboxylic acid by nucleophiles such as amine or sulfhydryl groups on proteins, or it could occur following displacement by water molecules, analogous to the displacement of chlorine atoms from cisplatin with formation of the highly reactive mono- or diaquated diammine platinum (II) compounds. If the latter occurred, one would expect to see the same platinated proteins after a dose of cisplatin as after a dose of carboplatin, since they would both proceed through the same reactive intermediate. The only difference would be the faster rate of aquation with cisplatin than with carboplatin. In either case, the essentially irreversible binding of platinum to plasma proteins would remove the highly reactive platinum (II) products that are probably responsible for the biological activity of both carboplatin and cisplatin.

Our results confirm and extend previous reports showing that carboplatin distributes to the cellular fraction of rat blood [9] but not to the cells of dog blood [12] following i.v. administration, and show that the compound does not distribute to the cellular fraction of human blood in vitro. The rat, therefore, differs from man and the dog in this aspect of the disposition of carboplatin.

Conclusions

These studies show that while the platinum from carboplatin can become bound to the plasma proteins of all species, the parent molecule, carboplatin, does not become instantaneously and reversibly bound to the plasma proteins of the dog, rat or human. In rats, but not in dogs or humans, carboplatin distributes into the cellular fraction of the blood. Finally, carboplatin degrades slowly in human and dog plasma and urine with the release of 1,1-cyclobutane dicarboxylic acid, and care must be exercised in the handling and storage of samples if valid pharmacokinetic data are to be obtained.

References

1. Gaver RC, Deeb G (1986) High-performance liquid chromatographic procedures for the analysis of carboplatin in human plasma and urine. Cancer Chemother Pharmacol 16: 201–206
2. Harland SJ, Newell DR, Siddik ZH, Chadwick R, Calvert AH, Harrap KR (1984) Pharmacokinetics of *cis*-diammine-1,1-cyclobutane dicarboxylate platinum (II) in patients with normal and impaired renal function. Cancer Res 44: 1693–1697
3. Harrap KR (1985) Preclinical studies identifying carboplatin as a viable cisplatin alternative. Cancer Treat Rev 12 (Suppl A): 21–33
4. Haynes VJ, Swigor JE, Pittman KA (1987) Synthesis of *cis*-diammine [1,1-cyclobutane-[1-^{14}C]-dicarboxylato (2-)-0,0] platinum (II). J. Label Comp Radiopharmaceut 24: 535–539
5. Laznickova A, Laznicek M, Kvetina J, Drobnik J (1986) Pharmacokinetics and plasma protein binding of two platinum cytostatics CHIP and CBDCA in rats. Cancer Chemother Pharmacol 17: 133–136
6. Litterst CL (1984) Plasma pharmacokinetics, urinary excretion, and tissue distribution of platinum following i.v. administration of cyclobutane dicarboxylato platinum-II and *cis*-platinum to rabbits. In: Hacker MP, Double EB, Krakoff IM (eds) Platinum coordination complexes in cancer chemotherapy. Martinus Nijhoff, Boston, pp 71–81
7. Momburg R, Bourdeaux M, Sarrazin M, Roux F, Briand C (1985) In vitro plasma binding of some second generation antitumor platinum complexes. Eur J Drug Metab and Pharmacokinet 10: 77–83
8. Rose WC, Schurig JE (1985) Preclinical antitumor and toxicologic profile of carboplatin. Cancer Treat Rev 12 (Suppl A): 1–19
9. Siddik ZH, Newell DR, Boxall FE, McGhee KG, Harrap KR (1983) Uptake and retention of *cis*-platin and *cis*-diammine-1,1-cyclobutane dicarboxylate platinum II (CBDCA, JM8) in rat erythrocytes. Br J Cancer 48 (1): 132
10. Siddik ZH, Newell D.R. et al. (1984) Biliary excretion, renal handling and red cell uptake of cisplatin and CBDCA in animals. In: Hacker MP, Double EB, Krakoff IM (eds) Platinum coordination complexes in cancer chemotherapy. Martinus Nijhoff, Boston, pp 90–102
11. Van der Vijgh WJF, Klein I (1986) Protein binding of five platinum compounds: comparison of two ultrafiltration systems. Cancer Chemother Pharmacol 18: 129–132
12. Van der Vijgh WJF, Lelieveld P et al. (1984) Pharmacokinetics of five platinum compounds in dogs. In: Hacker MP, Double EB, Krakoff IM (eds) Platinum coordination complexes in cancer chemotherapy. Martinus Nijhoff, Boston, p 140

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