Cancer Chemother Pharmacol (1997) 40: 521–525

ORIGINAL ARTICLE

Alfredo R. Sancho · James A. Dowell · Walter Wolf

The effects of anesthesia on the biodistribution of drugs in rats: a carboplatin study

Received: 12 September 1996 / Accepted: 21 February 1997

Abstract *Purpose*: Anesthetics can alter the biodistribution profile of drugs and, consequently, the regional pharmacokinetics of antineoplastic drugs at the tumor site. The effect of coadministered anesthetics on the biodistribution profile of carboplatin was studied in rats. *Methods*: Female Wistar rats were used to compare the effects of ketamine/xylazine, thiopental and pentobarbital on the biodistribution of 30 mg/kg radiolabelled ^{195m}Pt-carboplatin administered intravenously, with conscious rats as the control group. Blood and urine samples were collected between 5 and 120 min. *Results*: The percentage values of the injected dose of platinum per ml (%ID/ml) in plasma at the final time-point were respectively, 0.557%, 0.156%, 0.115% and 0.086%, in pentobarbital-, ketamine/xylazine- and thiopental-injected rats, and in conscious animals. Following the same sequence of groups, the %ID/ml values of platinum in the cumulative urine were 0.001%, 0.619%, 0.184% and 0.118%, respectively. Urine output varied from very little in the pentobarbital group, to several milliliters in the other groups. *Conclusions*: There was an increase of almost 100-fold in total platinum uptake in the kidneys, cerebrum and cerebellum of rats receiving pentobarbital over the uptake in the control rats, whereas the biodistribution profile of the thiopental group had the least variance. These results demonstrate the importance of anesthetic selection in animal pharmacokinetic studies, as it influences the biodistribution and pharmacokinetic profile of the drug being studied.

This work was supported in part by USC Radiopharmacy Program Funds, and by a grant-in-aid from Alkhermes, Inc. We would like to acknowledge the advice of Dr. Stephen N. Steen on effects of anesthesia in living systems, and Ms. Darshana Palekar for the preparation of the ^{195m}Pt-carboplatin.

A.R. Sancho · J.A. Dowell · W. Wolf
Department of Pharmaceutical Sciences University of Southern California, Los Angeles, CA, USA

A.R. Sancho (✉)
Department of Pharmacy, Radiopharmacy Program, 1985 Zonal Ave. #610, Los Angeles CA 90033, USA
Tel. 213–342–1410; Fax 213–342–9804; E-mail: sancho@hsc.usc.edu

Key words Anesthetics · Drug biodistribution · Carboplatin · Pharmacokinetics · ^{195m}Pt · PK-imaging

Introduction

Noninvasive pharmacokinetic imaging studies, such as the ones performed in our laboratory, require that the animal be restrained (e.g. through anesthesia) over varying periods of time. Previous investigations designed to assess the influence of anesthesia on renal function have generally found reductions in water and electrolyte excretion, which in most cases has been associated with a reduction in glomerular filtration rate (GFR) [8, 16]. In preliminary biodistribution studies of carboplatin, we observed significant variability in pharmacokinetic parameters, suggesting that the coadministered anesthetic may have a significant effect on a drug′s biodistribution and terminal pharmacokinetics. The present study was conducted to determine whether different anesthetics would have differential effects on the biodistribution and the renal excretion of antineoplastic drugs of the platinum family, with which renal side effects may be of clinical concern. Carboplatin [*cis*-diammine (1, 1-cyclobutanedicarboxylato)platinum(II)] was selected for this study since it is a very promising and commonly used second-generation platinum compound. This drug is commonly used for ovarian, small-cell lung, testicular, cervical, and head and neck cancers [1, 11, 14, 15, 20].

Carboplatin, when compared with cisplatin [*cis*-dichlorodiammine platinum(II)], has a significantly lower reactivity to nucleophilic DNA sites and therefore requires a proportionately higher dose to obtain a similar antitumor effect [4, 6]. The carboplatin dose of 400 mg/m^2, its maximum tolerated dose (MTD), has fewer negative effects, such as nephrotoxicity, neurotoxicity, ototoxicity and emetogenicity, than those associated with cisplatin at its MTD [12]. With the exception of neurotoxicity, these side effects are reversible up to a dose close of 1000 mg/m^2. Myelosuppression, specifically thrombocytopenia, is the dose-limiting toxicity of carboplatin [3, 5, 9]. Although

carboplatin has a longer ultrafilterable half-life and a higher urine cumulative level than cisplatin, its irreversible protein-bound terminal half-life of 5 to 6 days is similar to that of cisplatin. Carboplatin also exhibits a higher hydrophilicity than cisplatin, which may be a factor in its overall biodistribution and renal elimination. Urinary excretion is the major route of clearance, with clearance of up to 50% of the carboplatin administered within the first 24 h [18]. The free platinum of plasma half-life from carboplatin ranges between 1.5 and 3 h, depending on species.

Owing to the elimination characteristics of carboplatin, it is important to be aware of the consequence of any changes in renal function of the living system to which carboplatin is being administered. A poor or compromised renal function will directly affect the biodistribution and the bioavailability of carboplatin at the tumor site. Moreover, any anesthetic coadministered with carboplatin can affect the drug′s efficacy and toxicity indices becoming important in animal trials in which anesthetics are frequently used.

In this study we compared three injectable anesthetics or combinations that are commonly used in research and in clinical studies. Our choice of anesthetics used was based on availability and published information on their use in rodents. The combination of ketamine/xylazine has been used extensively in studies of rodents in our laboratory, and has worked well as a surgical anesthetic for vein cannulation and other prolonged procedures [2]. We also felt that pentobarbital sodium (Abbott, North Chicago, IL.) and its sulfur analogue, thiopental (Abbott), should be included in this study because of their widespread usage in animal studies.

Materials and methods

Animals

Female Wistar rats (150–200 g), obtained from Simonson (Los Angeles, Calif.), were housed under normal conditions with free access to food and water. They were housed one animal to a cage and maintained in a standard controlled environment (temperature 19–22 °C, 40–60% relative humidity). The animal studies were approved by the USC Vivaria Committee and adhered to the "Principles of Laboratory Animal Care" (Publication No. 85–23, revised 1985). Each group of animals, anesthetized and conscious, consisted of three or four rats.

The conscious rats were briefly restrained in a conventional perspex tube, their tails immersed in warm water (37 °C) to facilitate the location of the lateral tail vein, and injected into the vein with 30 mg/kg of ^{195m}Pt-carboplatin. The conscious rats were then placed in metabolic cages. The study animals were anesthetized just prior to carboplatin administration. The anesthetic dosage varied according to the manufacturer's suggested dose for this particular animal species and the individual animal's weight, as detailed below. Enough anesthetic was given to maintain the rat under sedation for the duration of the study, which lasted for approximately 2 h. Maintenance dosages were administered as needed for each individual animal. All rats from each of the anesthesia groups had a surgically placed cannula (Clay Adams, PE-50, 0.58 mm internal diameter 0.965 mm outside diameter) in their right jugular vein, inserted either the day before or several hours prior to the beginning of the study. The cannula was maintained free of blood clots with heparinized normal saline (250 units of heparin per 1.0 ml of 0.9% sodium chloride), and flushed routinely or as needed. The cannula was used to administer the carboplatin dose, to give maintenance anesthetic dosages as needed, and to withdraw the blood samples at the predetermined time-points.

For urine collection from the anesthetized rats, a catheter (Clay Adams, PE 50) was placed into the bladder through the urethra and secured in place with adhesive surgical tape. The urine emptied into a sealed 15-ml plastic centrifuge tube. For conscious animals, urine was collected from the bottom receptacle of the metabolic cages.

Anesthesia

Thiopental sodium injectable, USP or Pentothal (Abbott), was administered intraperitoneally (i.p.) at a dose of 30 mg/kg, following the recommendations of the drug information insert. The powder form was reconstituted using normal saline (0.9% sodium chloride), to a concentration of 2.0% or 20 mg/ml thus avoiding hemolysis when administered. A ketamine and xylazine mixture at a 1:1 ratio (20 mg/ml xylazine, 100 mg/ml ketamine) was administered at a dose of 0.2 ml/200-g rat. This mixture has demonstrated satisfactory analgesia, anesthesia and central nervous system depression in short as well as in prolonged surgical procedures. Pentobarbital was administered i.p. at a dose of 32.5 mg/kg, following the recommendations of the Abbott Laboratories information pamphlet.

All dosages were kept under lethal levels. All anesthetics were initially administered i.p. after which the maintenance dosages were administered intravenously (i.v.) through the implanted cannulae. The level of analgesia provided by each anesthetic was assessed in terms of the presence or absence of the pedal withdrawal reflex in response to pinching of the skin between the toes of the hind paws, or of the eyelid closure reflex in response to a sharp and brief air blow to the open eyes. The analgesia status was monitored at frequent intervals during the entire duration of the study. Maintenance dosages were given as needed.

The animals used as the control group did not receive any anesthetic. These animals were kept in individual metabolic cages, with the intention of causing the least stress possible. The cages were set up to collect excreted urine over a 2-h period.

Carboplatin

^{195m}Pt was prepared at the University of Missouri Research Reactor (MURR) by thermal neutron irradiation (average flux of 3×10^{14} n/cm^2 for 7 to 14 days) of enriched (96.2%) metallic ^{194}Pt. ^{195m}Pt was produced with a specific activity ranging from 0.4 and 0.7 mCi/mg. ^{195m}Pt was used to produce radiolabelled carboplatin in our laboratory facilities, using a semiautomated system. The microscale synthesis of ^{195m}Pt-carboplatin followed similar steps to that of ^{195m}Pt-cisplatin synthesis, which has been documented in detail [10]. Yield and purity of the synthesized ^{195m}Pt-carboplatin were determined by spectrophotometry. Thin-layer and paper chromatography were also performed to confirm radiochemical purity. This radioactive material was then added to nonradioactive carboplatin to a specific activity of 0.05–0.2 mCi/mg. The administered dose of carboplatin (30 mg/kg) was dissolved in normal saline at a concentration of 1.0 mg/ml. The dose was prepared and administered to each individual rat at the beginning of each study through the lateral tail vein in conscious rats or the right jugular vein cannula in anesthetized rats.

Tissue recovery and processing

All rats were euthanized 2 h after ^{195m}Pt-carboplatin administration. Selected tissues, including cerebrum, cerebellum and kidneys, were surgically extracted from each rat, immediately dried with Kimwipes and weighed. The individual tissue samples were then homogenized in 25 ml grinding medium (75 m*M* NaCl and 24 m*M* EDTA, adjusted to pH 8.0). From each rat, two 1.0-ml samples of each tissue homogenate were placed in separate polyethylene vials for gamma counting. All samples were kept on ice or placed in the refrigerator (5 °C) during processing. Gamma counting was performed with a Beckman Auto-

Table 1 Percent of the injected dose of ^{195m}Pt in selected tissues at the 120-min time-point after administration (% ID/g, ±SD)

	Brain	Cerebellum	Left kidney	Right kidney
Pentobarbital	0.0186 (±1.05 E-5)	0.0094 (±6.78 E-4)	1.1916 (±1.82 E-1)	0.8114 (±7.85 E-2)
Ketamine xylazine	0.0010 (±4.22 E-5)	0.0005 (±5.91 E-5)	0.0877 (±7.58 E-3)	0.1078 (±7.82 E-3)
Thiopental	0.0010 (±8.94 E-5)	0.0003 (±2.13 E-5)	0.0176 (±1.22 E-3)	0.0170 (±1.02 E-3)
Conscious	0.0002 (±1.07 E-5)	0.0003 (±2.79 E-5)	0.0144 (±7.65 E-4)	0.0139 (±7.31 E-4)

Table 2 Percent of the injected dose of ^{195m}Pt in body fluids sampled at the 120-min time-point after administration (% ID/ml, ±SD)

	Urine[a]	Erythrocyte fraction	Plasma fraction	Whole blood
Pentobarbital	0.0011 (±2.28 E-4)	0.2183 (±1.02 E-3)	0.3385 (±2.82 E-3)	0.5568 (±3.86 E-3)
Ketamine/xylazine	0.6191 (±5.49 E-1)	0.0816 (±1.32 E-4)	0.0742 (±2.96 E-4)	0.1558 (±4.28 E-4)
Thiopental	0.1842 (±9.65 E-1)	0.0474 (±2.25 E-4)	0.0678 (±2.70 E-4)	0.1152 (±4.95 E-4)
Conscious	0.1180[b] (±5.60 E-1)	0.0419 (±1.44 E-4)	0.0441 (±1.32 E-4)	0.0860 (±1.38 E-4)

[a] Urine is expressed as the cumulative amount (ID) through 120 min
[b] Conscious rats urinated at will because they had no urinary catheter

mated Gamma Counter (Beckman, Gamma 4000), with the energy window set at the energy peaks of ^{195m}Pt. Duplicate samples were counted and averaged. All counting was performed within 2–3 days after the time of tissue extraction.

Body fluid processing

Whole blood samples of 0.3 ml were withdrawn from each rat through the right jugular vein cannula at the predetermined time-points (5, 15, 30, 60, 90, and 120 min) after administration of the antineoplastic agent using a heparinized 1.0-ml tuberculin syringe. Immediately after, the blood was placed in a 2-ml microcentrifuge tube containing 0.2 ml heparinized saline (250 units of heparin per ml of 0.9% sodium chloride). The blood samples were centrifuged at 5000 rpm for 4 min using a Beckman microfuge to separate the plasma from the sediment, mostly erythrocytes. The plasma was transferred into a polyethylene vial for gamma counting, while the sediment was resuspended in 0.3 ml normal saline and transferred to a separate polyethylene vial for gamma counting. The blood sample from each conscious rat was withdrawn through a heart puncture at the end of the study. The blood samples were immediately processed as described above.

Urine output was monitored and measured at each time-point. The bladder of conscious rats was drained after euthanasia. An aliquot of 10 µl from the accumulated urine was placed in a polyethylene vial and was brought up to a total volume of 1.0 ml using distilled water. These vials were then placed in the Beckman gamma counter to determine the total ^{195m}Pt content.

Platinum determination in tissues and body fluids

Total platinum content was determined in urine, blood and selected tissues by placing the sample vials in a Beckman gamma counter, calibrated for the energy of ^{195m}Pt. Tissues and the final time-point body fluid samples were obtained 2 h after carboplatin administration. Whole blood samples of 0.3 ml were withdrawn and divided into an erythrocyte fraction, and plasma fractions (which included high and low molecular weight proteins and free or unbound fractions). Tissues were surgically excised, dried, weighed and homogenized; then two 1.0-ml aliquots from each tissue homogenate were taken for the measurement of the ^{195m}Pt content. The measurements from each of the two tissue vial samples were averaged. All tissue and body fluid samples were kept refrigerated at 5 °C or on ice until measured. Serial gamma counting of all these samples was done within 2 or 3 days after each study was completed.

Two separate standards, 10^{-3} and 10^{-4} of the injected dose of ^{195m}Pt-carboplatin in 1.0 ml distilled water, were used to correct all counts. The standards were prepared from each individual ^{195m}Pt-carboplatin synthesis, and were kept until the end of each study and processed through the gamma counter with the tissue and body fluid samples of each individual rat, correcting for decay and equipment intrinsic error. Data were expressed as injected dose per gram or milliliter (ID/g or ID/ml), or as percent of injected dose per gram or milliliter (%ID/g or %ID/ml).

Results

Table 1 shows the data from the selected tissues - cerebrum, cerebellum, right and left kidneys - at the 120-min time-point after injection of 30 mg/kg of ^{195m}Pt-carboplatin. These data represent the ^{195m}Pt counts recovered in the various tissues sampled from animals coadministered with pentobarbital, ketamine/xylazine, and thiopental, as well as from the control group.

Of the tissues excised and measured, the kidneys had the highest concentration of platinated species, while the cerebrum had the least. Kidneys from rats anesthetized with pentobarbital had a ^{195m}Pt concentration at least ten times higher than that of rats anesthetized with ketamine/xyla-

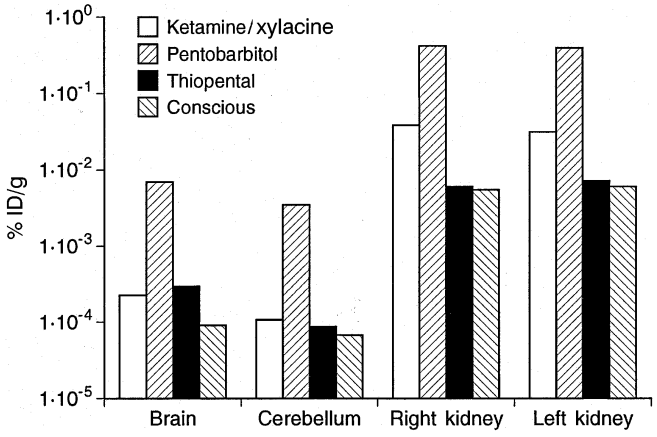

Fig. 1 Biodistribution of radioactively labelled carboplatin at the 120-min time-point in anesthetic-treated and conscious rats

zine, and 100 times higher from that of kidneys from either rats anesthetized with thiopental or conscious rats. The cerebellum data of all the animals demonstrated a similar pattern to that of the kidneys. The cerebrum data exhibited a slight variation from this pattern, but pentobarbital-anesthetized rats remained the group with the highest concentration of ^{195m}Pt, showing levels 10–100 fold higher than those of conscious rats.

Table 2 shows the amounts of ^{195m}Pt recovered in the various blood fractions including whole blood, erythrocyte fraction and plasma fraction. The time-point of 120 min after ^{195m}Pt-carboplatin administration is shown in Table 2 as a comparison index for all rat groups. Overall, the blood data showed higher levels of the tracer when pentobarbital was the anesthetic used. The cumulative amount of ^{195m}Pt in the urine of rats anesthetized with pentobarbital had the lowest concentration of the radioactive tracer compared with the other groups. The data demonstrate an inverse relationship between the recovered ^{195m}Pt in the urine and that in the blood fractions.

Figure 1 shows the accumulation of the radioactively labelled carboplatin in the selected tissues at the 120-min time-point in both the anesthetic-treated rats and the conscious control rats. Standard deviations of each group′s data were all within 20% of the means.

Discussion

The results obtained in this study have provided a comparison of the effect of various anesthetics on the biodistribution of ^{195m}Pt-carboplatin. The data demonstrated that while the cerebrum consistently had the lowest concentration of the ^{195m}Pt tracer, the kidneys were the organ with the highest concentration, regardless of the anesthetic used, as indicated in prior published data [17]. The effect of anesthetics becomes an important factor when deciding dose regimens for drugs such as carboplatin in which their main route of elimination is through the kidneys [18]. The effect of anesthetics, such as those used in this study, on the renal function has been documented in previous studies, through GFR evaluation[19].

In this study the kidneys of rats that were given pentobarbital had up to a 100 fold higher concentration of the tracer to that of the conscious rats, and excreted approximately 1% of the amount of ^{195m}Pt excreted by the conscious rats. These effects were much less for ketamine/xylazine and essentially not observed in the rats anesthetlized with thiopental. One important consequence is that the co-administered anesthetics, by adversely altering the normal pathway of elimination of carboplatin, more than likely increased carboplatin's plasma half-life, drug residence time in plasma and concentration exposure level to the rat's tumor and vital organs. This is evident by the higher levels of the tracer in the plasma and RBC fractions and in the tissues collected from rats given pentobarbital. Although the effect of ketamine/xylazine on renal function is less pronounced than that of pentobarbital, it is still 5 time higher than in conscious rats. The pentobarbital group also shows a higher level of the tracer in plasma and RBC fractions, which would suggest some level of renal dysfunction due to the anaesthetic. Of the anesthetics tested, thiopental appeared not to exhibit any statistically significant difference from conscious animals when measured for its effect on ^{195m}Pt excretion and biodistribution in the selected tissues.

These results clearly suggest that the drug's biodistribution and bioavailability, hence its efficacy and toxicity index, both at the tumors and vital organs, may drastically be affected by a co-administered anaesthetic. The effect of anesthetics on blood hemodynamic parameters, as well as the unique parameters found in tumors that affect drug transport to and into the tumor cells can not be ignored [20]. Consequently, the selection of the anesthetic becomes a critical issue for both invasive as well as non-invasive pharmacokinetic studies, and should be considered carefully prior to the study.

References

1. Bacha DM, Caparros-Sison B, Allen JA, Walker R, Tan CTC (1986) Phase I study of carboplatin (CBDCA) in children with cancer. Cancer Treat Rev 70:865
2. Brammer A, West CD, Allen SL (1993) A comparison of propofol with other injectable anesthetics in a rat model for measuring cardiovascular parameters. Lab Anim 27:250
3. Canetta R, Rozencweig M, Carter SK (1985) Carboplatin: the clinical spectrum to date. Cancer Treat Rev 12(A):125
4. Cleare MJ (1974) Transition metal complexes in cancer chemotherapy. Coordination Chem Rev 12:349
5. Foster BJ, Clagett-Carr K, Leyland-Jones B, Hoth D (1985) Results of NCI-sponsored phase I trials with carboplatin. Cancer Treat Rev 12(A):43
6. Harrap KR, Jones M, Wilkinson CR, Clink HM, Sparrow S, Mitchley BCV, Clarke S, Veasey A (1988) Antitumor, toxic and biochemical properties of cisplatin and eight other platinum complexes. In: Prestayko AW, Crooke ST, Carter SK (eds) Cisplatin: current status and new developments. Academic Press, New York, p 193
7. Jain RK (1994) Barriers to drug delivery in solid tumors. Sci Am 44:58

8. Knight TF, Sansom S, Hawk L, Frankfurt SJ, Weinman EJ (1978) The effects of anesthesia on the excretion of an isotonic saline load in the rat. Pflugers Arch 373:139
9. Newell DR, Siddik ZH, Gumbrell LA, Boxall FE, Gore ME, Smith IE, Calvert AH (1987) Plasma free platinum pharmacokinetics in patients treated with high dose carboplatin. Eur J Cancer Clin Oncol 23(9):1399
10. Palekar D, Anand D, Wolf W (1994) A semiautomated microscale synthesis and quality control of radiolabeled ^{195m}Pt-carboplatin. Pharm Res 11, S-34
11. Peckham M, Horwich A, Brada M, Drury A, Hendry WF (1985) Cis-diammine-1,1-cyclobutane dicarboxylate platinum II (carboplatin) in the treatment of testicular germ cell tumours: a preliminary report. Cancer Treat Rev 12(A):101
12. Siddik ZH, Jones M, Boxall FE, Harrap KR (1988) Comparative distribution and excretion of carboplatin and cisplatin in mice. Cancer Chemother Pharmacol 21:19
13. Smit EF, Willemse PHB, Sleijfer Dth, Uges DRA, Postmus PE, Meijer S, Terheggen PMAB, Mulder NH, de Vries EGE (1991) Continuous infusion carboplatin on a 21-day schedule: A phase I and pharmacokinetic study. J Clin Oncol 9(1):100
14. Smith IE, Evans BD (1985) Carboplatin (JM8) as a single agent and in combination in the treatment of small cell lung cancer. Cancer Treat Rev 12(A):73
15. Sternberg C, Kelsen D, Dukeman M, Leichman L, Heelan R (1985) Carboplatin: a new platinum analog in the treatment of epidermoid carcinoma of the esophagus. Cancer Treat Rev 69:1305
16. Thomsen K, Olesen OV (1981) Effect of anesthesia and surgery on urine flow and electrolyte excretion in different rat strains. Renal Physiol 4:165
17. Tinker N, De Spiegeleer B, Sharma H, Jackson H, McAuliffe C, Reman J (1990) The tissue distribution in rats of [195mPt]carboplatin following intravenous, intraperitoneal and oral administration. Nucl Med Biol 17(4):427
18. Vijgh WJF van der (1991) Clinical pharmacokinetics of carboplatin. Clin Pharmacokinet 21(4):242
19. Walter SJ, Zewde T, Shirley DG (1989) The effect of anesthesia and standard clearance procedures on renal function in the rat. Q J Exp Physiol 74:805
20. Wiltshae E (1985) Ovarian trials at the Royal Marsden Hospital. Cancer Treat Rev 12(A):67