Cancer Chemother Pharmacol (1995) 35: 254–256

SHORT COMMUNICATION

Kadir Erkmen · Merrill J. Egorin · Leonard M. Reyno
Robert Morgan, Jr. · James H. Doroshow

Effects of storage on the binding of carboplatin to plasma proteins

Received: 11 March 1994 / Accepted: 29 June 1994

Abstract Plasma ultrafiltrates are routinely used in pharmacokinetic studies of carboplatin. Experiments were performed to detect and quantitate artifactual decreases in the platinum concentration of ultrafiltrates prepared from plasma samples stored at −20 °C and −70 °C. Carboplatin was added to anticoagulated, whole human blood to produce a 20 μg/ml concentration. Plasma produced from the blood was stored frozen at either −20 °C or −70 °C. Aliquots from each storage condition were thawed and ultrafiltered once a week for up to 100 days. Platinum concentrations in ultrafiltrates and plasma were determined by flameless atomic absorption spectrometry. There was no loss of ultrafilterable platinum in plasma samples stored at −70 °C, whereas there was a steady decrease in free platinum concentration in ultrafiltrates prepared from plasma samples stored at −20 °C. These results imply that pharmacokinetic studies of carboplatin should use ultrafiltrates prepared immediately or that plasma for such studies should be stored at −70 °C. Storage of carboplatin-containing plasma at −20 °C and subsequent ultrafiltration is not acceptable, because measurement of platinum in such ultrafiltrates will be artifactually low.

Key words Carboplatin · CBDCA · Protein binding

K. Erkmen · M. J. Egorin (✉) L. M. Reyno[1]
Division of Developmental Therapeutics, University of Maryland Cancer Center, 655 W. Baltimore Street, Baltimore, MD 21201, USA
Tel. (+1)410 328 3685; fax (+) 410 328 6559

M. J. Egorin
Division of Hematology-Oncology, Department of Medicine, University of Maryland School of Medicine, Baltimore, MD 21201, USA

R. Morgan, Jr. · J. H. Doroshow
Department of Medical Oncology and Therapeutics Research, City of Hope National Medical Center, Duarte, CA 91010, USA

[1] *Present address:*
Department of Medical Oncology, Hamilton Regional Cancer Centre, 699 Concession Street, Hamilton, Ontario, L8V 5C2, Canada

Introduction

The important platinum-containing agents, cisplatin and carboplatin, have been and continue to be frequent subjects of pharmacokinetic studies. Because both drugs are inactivated after covalent binding to plasma and other proteins, plasma ultrafiltrates are the common matrix in which concentrations of active drug are assessed [3, 6, 8, 9, 11–16, 19–21]. The known high reactivity of cisplatin with plasma proteins has resulted in the practice of immediate plasma ultrafiltration after blood samples are obtained for pharmacokinetic analysis. In contrast, carboplatin is much less reactive with plasma proteins [6, 9, 11, 15, 16, 19–21], and as a result, questions have been raised as to whether carboplatin-containing plasma could be stored frozen until it is ultrafiltered. This latter option has obvious advantages for institutions where manpower and equipment for pharmacokinetic sampling and preparation are limited. In a recent collaborative effort in which plasma samples from patients treated with carboplatin were stored at −20 °C for varying periods of time before being ultrafiltered, we found substantially less platinum in the ultrafiltrates than would be expected. As a result, we undertook a study in which we added carboplatin to whole human blood, prepared plasma from that blood, and stored the plasma at −20 °C or −70 °C to determine whether, and if so at what rate, ultrafilterable platinum is lost due to ex vivo binding during storage.

Materials and methods

Materials

Whole human blood from four separate individuals was collected into heparinized containers. Clinically formulated carboplatin (Paraplatin) was purchased from Bristol–Myers Squibb (Princeton, N.J.).

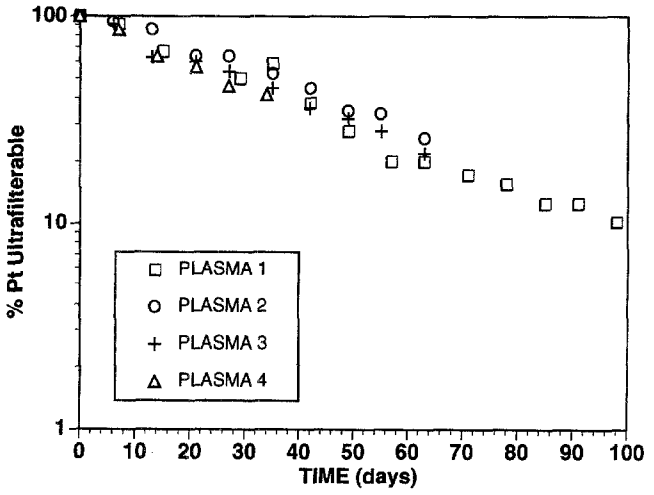

Fig. 1 Concentrations of platinum in ultrafiltrates of four plasmas stored at −20 °C

Methods

Within 30 min after blood was obtained, carboplatin was added to the blood to produce a final drug concentration of 20 μg/ml. Immediately thereafter, the blood was centrifuged at 1000 *g* for 10 min. The resulting plasma was separated into two equal volumes and frozen in 3-ml aliquots at either −20 °C or −70 °C. Thereafter, on a weekly basis, an aliquot of plasma stored at each temperature was thawed at 37 °C until liquid in consistency and immediately ultrafiltered. Ultrafiltrates were prepared by placing a portion of that plasma into Amicon Centrifree micropartition devices (Amicon Division, W.R. Grace, Beverly, Mass.) and centrifuging the devices at 2000 *g* for 20 min at 4 °C in a Sorvall RC-2B centrifuge (DuPont, Wilmington, Del.). Platinum concentration in plasma and ultrafiltrates was assessed with a Perkin–Elmer model 1100 flameless atomic absorption spectrometer (Perkin–Elmer, Norwalk, Conn.) monitoring 265.9 nm. The temperature program used for both plasma and ultrafiltrates was as follows: ramp over 30 s to 90 °C and hold for 60 s; ramp over 10 s to 110 °C and hold for 10 s; ramp over 30 s to 300 °C and hold for 30 s; ramp over 60 s to 1500 °C and hold for 90 s; and atomize at 2700 °C with no ramping. Argon gas flow was 300 ml/min during all heating steps except atomization when it was interrupted. Platinum concentrations were determined by comparison with a standard curve performed on the same day as the assay. When visual assessment of a semilogarithmic plot of ultrafilterable platinum vs storage time for each individual plasma stored at −20 °C indicated first-order decay, the rate of decay of ultrafilterable platinum in stored samples was determined from the formula $A = A_o e^{-kt}$, where A represented the amount of platinum present at any given time, t, and A_o represented the concentration of platinum present at the start of the experiment. Data were fitted using non-linear, least-squares regression as implemented by the computer program ADAPT II [2]. The half-life ($t_{1/2}$) of ultrafilterable platinum in stored samples was calculated from the relationship: $t_{1/2} = 0.693/k$.

Results

When plasma was stored at −70 °C for as long as 100 days, there was no loss of ultrafilterable platinum. In contrast, storage of plasma at −20 °C resulted in a progressive decrease in ultrafilterable platinum as storage time increased (Fig. 1). On visual inspection, this decay in ultrafilterable platinum appeared to be a first-order process. When modelled as such, values of 0.0230, 0.0204, 0.0240, and 0.0274 were calculated for the decay constants of ultrafilterable platinum in the four respective plasma sources studied. These data indicate that storage of plasma containing carboplatin at −20 °C is associated with an average loss of ultrafilterable platinum of approximately 2.4%/day, and imply a half-life of 29.5 ± 4.0 (mean ± SD) days for ultrafilterable platinum when plasma is stored at −20 °C.

Discussion

The platinum-containing antineoplastic agent carboplatin is important because it produces therapeutic responses in a number of tumor types [1, 22] and because its pharmacokinetics have been related to both the therapeutic and the toxic consequences of its administration [4, 5, 10, 17]. As a result, carboplatin remains the subject of a considerable number of ongoing and planned pharmacokinetic studies. In recognition of the potential impact of such pharmacokinetic studies and the need for studying relatively large numbers of patients to assess pharmacokinetic/pharmacodynamic interactions, clinical studies of carboplatin with companion or intrinsic pharmacokinetic components are no longer entirely the province of the relatively limited number of institutions with extensive pharmacokinetic sampling, analytical and modelling resources. In an effort to facilitate pharmacokinetic studies at institutions with more limited personnel and equipment, a number of activities have been initiated. Among these are the development of limited sampling strategies for defining carboplatin exposure [7, 18] and the availability of designated, central analytical facilities for cooperative groups or other multi-institution, clinical trial consortia. Even with the reduced number of plasma samples involved with a limited sampling strategy, the requirement for two centrifugation steps to convert whole blood to plasma and subsequently plasma ultrafiltrate can strain the resources of many clinical centers. A simpler procedure would be to prepare plasma in the clinical setting and store it under appropriate conditions until a later time when: (1) multiple ultrafiltrates could be prepared as a batch; or (2) ultrafiltrate could be produced at the site where flameless atomic absorption spectrometry or other analysis of platinum content would be performed. These considerations were not raised previously in studies of cisplatin because no storage conditions were felt to be capable of preventing that compound's high reactivity from resulting in ex vivo binding to plasma proteins and artifactual reduction in platinum concentration of plasma ultrafiltrate. The less reactive nature of carboplatin has led to the question of whether plasma from patients treated with that drug could be stored frozen until plasma ultrafiltrates could be produced at a later time.

Our data demonstrate that storage of carboplatin-containing plasma at −20 °C is not suitable. Accurate determination of ultrafilterable platinum requires production of

plasma ultrafiltrate immediately after production of plasma or immediately after thawing of plasma stored at −70 °C. Investigators involved in planning or reviewing pharmacokinetic studies of carboplatin should be aware of these limitations.

Acknowledgements This work was supported in part by grant CA33572 from the National Cancer Institute. L.M.R. was the recipient of a Gordon Richards Fellowship from the Canadian Cancer Society, Ontario Division, and a McEachern Fellowship from the Canadian Cancer Society, National Division.

References

1. Bunn PA Jr, Canetta R, Ozols RF, Rozencweig M (eds) (1990) Carboplatin (JM-8). Saunders, Philadelphia
2. D'Argenio DZ, Schumitzky A (1979) A program package for simulation and parameter estimation in pharmacokinetic systems. Comput Methods Programs Biomed 9: 115
3. DeConti RC, Toftness BR, Lange RC, Creasey WA (1973) Clinical and pharmacological studies with *cis*-diamminedichloroplatinum (II). Cancer Res 33: 3310
4. Egorin MJ, Van Echo DA, Olman EA, Whitacre MY, Forrest A (1985) Prospective validation of pharmacologically based dosing scheme for the cisplatin analog, carboplatin. Cancer Res 45: 6502
5. Egorin MJ, Van Echo DA, Tipping SJ, Olman EA, Whitacre MY, Thompson BW, Aisner J (1984) Pharmacokinetics and dosage reduction of carboplatin in patients with impaired renal function. Cancer Res 44: 5432
6. Gaver RC, George AM, Deeb G (1987) In vitro stability, plasma protein binding and blood cell partitioning of ^{14}C-carboplatin. Cancer Chemother Pharmacol 20: 271
7. Ghazal-Aswad S, Newell DR, Calvert AH (1991) A single sample assay for the calculation of the area under the free carboplatin plasma concentration v. time curve. Br J Cancer 63: 7
8. Gonias SL, Pizzo SV (1983) Complexes of serum albumin and *cis*-dichlorodiammineplatinum (II). J Biol Chem 258: 5764
9. Gormley PE, Bull JM, Leroy AF, Cysyk R (1979) Kinetics of *cis*-dichlorodiammineplatinum. Clin Pharmacol Ther 25: 351
10. Jodrell DI, Egorin MJ, Canetta RM, Langenberg P, Goldbloom EP, Burroughs JN, Goodlow JL, Tan S, Wiltshaw E (1992) Relationships between carboplatin exposure and tumor response and toxicity in patients with ovarian cancer. J Clin Oncol 10: 520
11. 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
12. Leroy AF, Thompson WC (1989) Binding kinetics of tetrachloro-1,2-diaminocyclohexaneplatinum (IV) (tetraplatin) and *cis*-diamminedichloroplatinum (II) at 37 degrees C with human plasma proteins and with bovine serum albumin. Does aquation precede protein binding? J Natl Cancer Inst 31: 427
13. Leroy AF, Lutz RJ, Dedrick RL, Litterst CL, Guarino AM (1979) Pharmacokinetic study of *cis*-dichlorodiammineplatinum (II) (DDP) in the beagle dog: Thermodynamic and kinetic behavior of DDP in a biologic milieu. Cancer Treat Rep 63: 59
14. Litterst CL, Gram TE, Dedrick RL, Leroy AF, Guarino AM (1976) Distribution and disposition of platinum following intravenous administration of *cis*-diamminedichloroplatinum (II). Cancer Res 36: 2340
15. Perera A, Jackson H, Sharma HL, McAuliffe CA, Fox BW (1992) A comparative binding of platinum anti-tumour compounds to plasma proteins in the rat (in vivo) and mouse (in vitro). Chem Biol Interact 35: 133
16. Reed E, Kohn KW (1990) Platinum analogues. In: Chabner BA, Collins JM (eds) Cancer chemotherapy: principles and practice. Lippincott, Philadelphia, p 465
17. Reyno LM, Egorin MJ, Canetta RM, Jodrell DI, Swenerton KD, Pater JL, Burroughs JN, Novak MJ, Sridhara R (1993) Impact of cyclophosphamide on relationships between carboplatin exposure and response or toxicity when used in the treatment of advanced ovarian cancer. J Clin Oncol 11: 1156
18. Sorensen BT, Stromgren A, Jakobsen P, Jakobsen A (1993) A limited sampling method for estimation of the carboplatin area under the curve. Cancer Chemother Pharmacol 31: 324
19. Tosetti F, Rocco M, Fulco RA, Chiara S, Bruzzone M, Campora E, Esposito M (1988) Serial determination of platinum, protein content and free sulfhydryl levels in plasma of patients treated with cisplatin or carboplatin. Anticancer Res 3: 381
20. Vijgh WJF van der, Klein I (1986) Protein binding of five platinum compounds. Comparison of two ultrafiltration systems. Cancer Chemother Pharmacol 18: 123
21. Vijgh WJF van der (1991) Clinical pharmacokinetics of carboplatin. Clin Pharmacokinet 21: 242
22. Yarbro JW, Bornstein RS, Mastrangelo MJ (eds) (1992) Carboplatin (JM-8) update: current perspectives and future directions. Semin Oncol, [Suppl 2]: 19 (1)