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ORIGINAL ARTICLE

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Cyclophosphamide, cisplatin, and carmustine: pharmacokinetics of carmustine following multiple alkylating-agent interactions

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Abstract Cyclophosphamide, cisplatin, and carmustine (CPA/cDDP/BCNU) constitute a combination alkylating-agent regimen commonly used with autologous marrow support. Its therapeutic effectiveness is accompanied by sporadic life-threatening and fatal toxicities, the most common of which is acute lung injury. We have previously shown that variation in the BCNU AUC can be correlated to the risk of pulmonary injury in patients receiving CPA/cDDP/BCNU. In an attempt to understand further the role of interpatient variation in drug pharmacokinetics (PK) with respect to pharmacodynamic outcomes, we evaluated the effect of pretreatment with CPA, cDDP, or both on BCNU PK in male Sprague-Dawley rats. The drug-administration pattern was designed to mimic that of the CPA/cDDP/BCNU regimen in patients. Each pretreatment increased both the absolute value of and the variation in BCNU AUC relative to the control values. These findings are consistent with an important rate-limiting elimination pathway for BCNU in rats and may explain the wide interpatient variability of BCNU AUC and the sporadic pulmonary toxicity seen in patients receiving CPA/cDDP/BCNU.

Key words Carmustine · Pharmacokinetics · Alkylating agents

Introduction

Combinations of alkylating agents (AA) are being used with increasing frequency to treat patients with advanced or high-risk malignancy. This is particularly true when they are employed at high doses with autologous bone marrow support (ABMS) [1]. Advantages of this strategy include the frequent nonoverlapping nonmyelosuppressive toxicities seen with these agents [2] and the frequent lack of cross-resistance seen in experimental tumor systems [3].

In contrast to the extensive studies that have evaluated pharmacokinetic (PK) interactions of antimetabolites, PK interactions of AA have been the subject of much more limited study. As these combination regimens are used more frequently, an understanding of their drug interactions becomes more important. This is especially true because high-dose AA with ABMS can produce sporadic but fatal visceral organ injury [1]. Understanding the factors that might lead to these toxicities could improve the therapeutic benefit of these regimens.

The cyclophosphamide, cisplatin, and carmustine regimen (CPA/cDDP/BCNU) is among the most widely used of these combinations and is presently being employed in a National Cancer Institute high-priority intergroup trial to treat women with high-risk stage II breast cancer [4]. In this report, we evaluate the effect of pretreatment with CPA, cDDP, or both drugs on BCNU PK in male Sprague-Dawley rats. The drugs were given in doses and schedules intended to mimic the pattern and doses of drug delivery used in the clinical setting.

A stimulus for this study is an attempt to correlate PK observations in patients with rodent PK measurements. The design of preclinical experiments investigating antineoplastic-drug PK such that the latter mimic those observed in humans may be the optimal way to study dose and schedule effects that are predictive of human results [5]. The CPA/cDDP/BCNU regimen as given to patients is shown in Fig. 1.

Materials and methods

Male Sprague-Dawley rats weighing approximately 150 g were supplied by Sasco, Inc. (Omaha, Neb.). They were housed in the University of Colorado Health Sciences Center animal facility and were supplied with food and water ad libitum. The animals were maintained in the facility for 5 weeks prior to the study. The average body weight (mean ± SD) prior to the study was 305 ± 22 g. CPA and cDDP were purchased from Sigma Chemical Company (St. Louis, Mo.), and BCNU was a gift of Bristol-Myers Squibb, Oncology Division.

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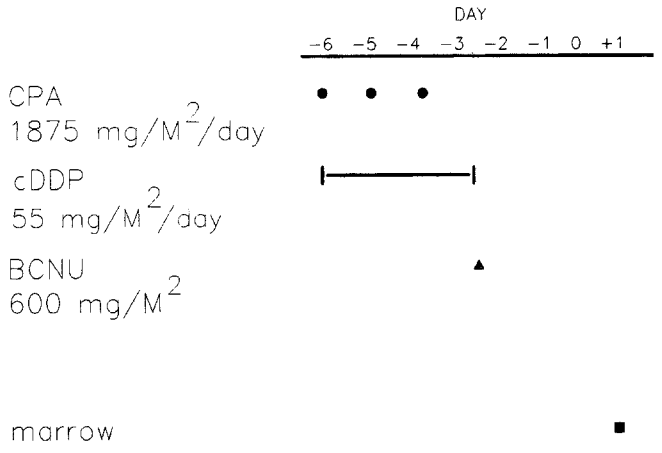

Fig. 1 The cyclophosphamide/cisplatin/carmustine (CPA/cDDP/BCNU) regimen as given to patients. CPA is given as three consecutive daily 1-h infusions; cDDP, as a 72-h continuous infusion; and BCNU, as a 2-h continuous infusion immediately following the administration of cDDP. CPA and cDDP infusions are begun between 9:00 and 12:00 each day

A polyethylene jugular venous catheter was surgically placed 1 day prior to initiation of the treatments. The rats were anesthetized with 6 mg/kg ketamine HCl (Aveco Co., Fort Dodge, Iowa) and 3 mg/kg xylazine (Haver, Shawnee, Kan.), both being given intramuscularly. Venous catheter blood return was verified and the catheter was flushed with 0.3 ml of a normal saline solution containing 100 units heparin and 1 mg chloramphenicol sodium succinate/ml. The exterior portion of the intravenous catheter was clamped and coiled in a protective cranial catheter capsule until use.

Treatments

Beginning 1 day after catheter placement, the rats were given three daily intraperitoneal injections of 0.6 ml physiologic saline/100 g body weight either alone (control) or containing cDDP (1.4 mg/kg), CPA (200 mg/kg), or both agents. CPA and cDDP were admixed under conditions verified to ensure compatibility [6]. All injections were given between 9:00 and 12:00. In contrast to the CPA/cDDP/BCNU regimen for human use, where the cDDP is given as a 72-h continuous infusion, we gave the cDDP to the rats as three daily bolus injections for purposes of convenience.

BCNU (12 mg/kg) was formulated in 10% ethanol solution immediately prior to administration and given through the venous catheter on the 4th day. Whole-blood samples for BCNU PK measurement were collected by cardiac puncture following 20 s of CO_2 anesthesia. No more than 2 ml of whole blood was removed from each rat, and the animals were euthanized with 100 mg/kg intravenous pentobarbital following phlebotomy. Thus, a minimum of three rats were required to complete each BCNU disapearance curve as described below. The doses of CPA/cDDP/BCNU were selected so as to approximate the AUC values obtained in patients treated with CPA/cDDP/BCNU.

Whole-blood samples (1 ml) were collected at 5, 10, 15, 30, 45, and 90 min after BCNU infusion by the methods described above. Sufficient numbers of animals were treated that four to eight blood samples were obtained at each time point for each experimental cohort, with the exception of the 30-min time point for the placebo-treated rats and the 45-min time point for the CPA+cDDP-treated rats, where only three samples were obtained.

Analysis

Pooled whole blood was spiked with >99% pure BCNU over a concentration range of 0.05–20.0 μg/ml to generate a reference standard curve. The standard curve samples and rat whole-blood samples were extracted and analyzed in identical fashion. Heparinized, air-evacuated 3-ml tubes were injected with 2 ml of high-performance liquid chromatography (HPLC)-grade ethyl acetate (Burdick & Jackson, Muskegon, Mich.) containing 20 mg diphenylhydantoin/l as an internal standard. Standard and rat whole-blood samples were extracted by injecting 1 ml of the blood samples into ethyl acetate-containing tubes, which were immediately vortexed for 20 s. The tubes were then centrifuged at 2000 *g* for 5–10 min. The organic layer was removed and evaporated to dryness under a nitrogen stream. Residues were reconstituted with 300 μl of HPLC-grade methanol (Burdick & Jackson) and analyzed by HPLC at a UV-detector wavelength of 237 nm.

The analysis was performed using a Waters Associates HPLC system consisting of model 510 pumps, an 8-mm × 10-cm RCM (radial compression module) fitted with a Nova Pak C-18 radial compression cartridge and a Waters Guard Pak C-18 guard column, a Wisp 712 autosampler, and a model 490 multichannel UV detector. Samples were eluted isocratically at room temperature using a 55% methanol:45% water mobile phase at a flow rate of 1.6 ml/min. The retention times for BCNU and diphenylhydatoin were 4.9 and 6.1 min, respectively. The ratio of the absorption peak area of BCNU to that of the internal standard was compared with the known concentration to generate a standard curve and analyze rat samples. The calibration curve was linear at concentrations ranging from 0 to 20.0 μg/ml, with the correlation coefficient never being less than 0.99. The sensitivity and reproducibility of the assay have been described previously, as has an analysis for interfering substances [7]. No detector response was seen in the absence of BCNU in either rat or human blood extracts.

BCNU blood-elimination curves

Composite BCNU blood-elimination curves were constructed by averaging the BCNU assay results of multiple blood samples. These samples were obtained from different rats at identical times (specified above) following BCNU treatment. Each BCNU blood-assay set that was averaged was obtained from rats that had received identical pretreatment.

The mean assay values for each time point and pretreatment were used as an initial data set to derive a best-fit BCNU elimination curve as described below. Standard deviations for that curve were derived from the PCNONLIN program (see below).

Statistical methods

The BCNU mean-concentration and sample-time data obtained from each cohort of rats were used for calculation of PK parameters. PCNONLIN software (version 3.0, Statistical Consultants Inc., Lexington, Ky.) and an 80836-based microcomputer with a mathematical coprocessor were used to perform the analysis. A one-compartment model (model 1) with i. v. bolus and first-order output was used after inspection of the data confirmed a good fit.

Bartlett's test was used to detect differences in variance between the treatment groups [8]. Differences between area under the curve (AUC) measurements for the various groups were determined by comparing all unweighted assay results for all time points in each data set using the generalized Wilcoxon method [9].

Results

Whole-blood BCNU concentration and time data obtained for the various rat cohorts are shown in Fig. 2. Inspection of the data demonstrated that they were adequately modeled using a one-compartment scheme, consistent with previous reports [10]. The modeled whole-blood elimination curve (+1 SD) generated for rats treated with BCNU alone is shown in Fig. 2 A. The BCNU elimination curves generated

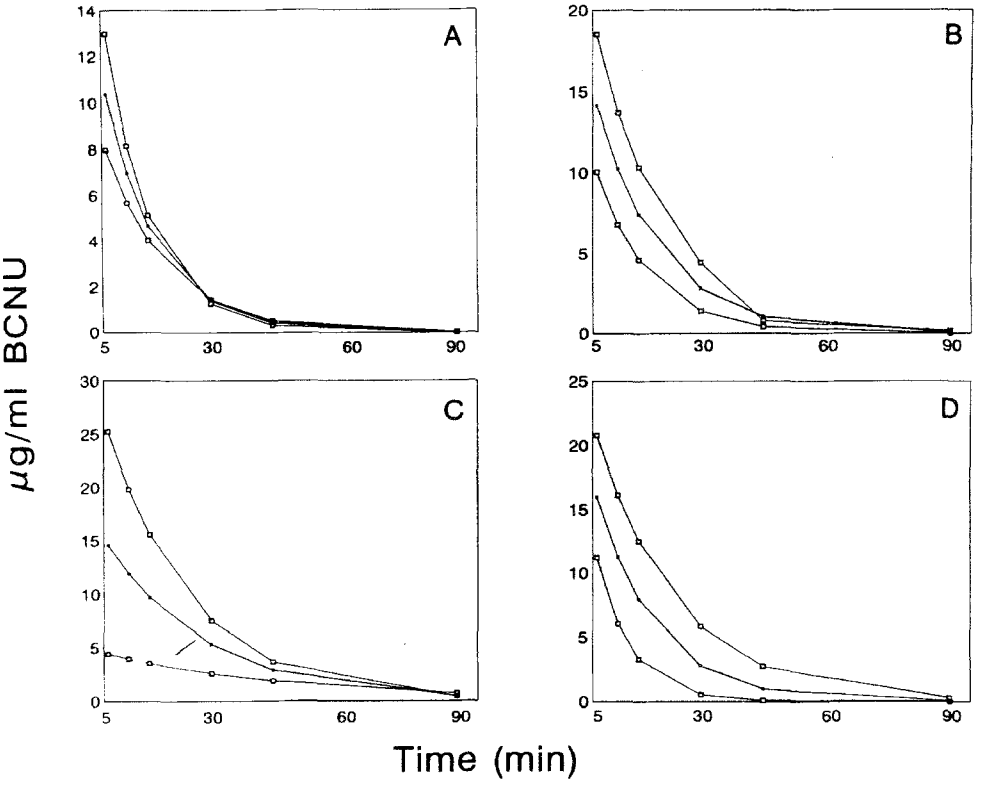

Fig. 2 Whole-blood elimination profiles obtained for BCNU in male Sprague-Dawley rats that were pretreated with either **A** physiologic saline alone, **B** CPA, **C** cDDP, or **D** CPA+cDDP. Intervals corresponding to 1 SD about both curves are shown as *lines with square symbols*

for the various pretreatments are shown in Fig. 2B–D. The four graphs contrast the standard deviations observed for each group, emphasizing the increasing variability of BCNU concentration when any pretreatment is compared with control values.

Derived PK parameters obtained for rats treated with BCNU alone are shown in the first line (*Control*) of Table 1. The variation in BCNU elimination observed between animals was quite small as shown by the coefficient of variation found for AUC and the elimination half-life ($t_{1/2}$ elim.). This contrasts with the much larger coefficients of variation found for the AUC following any of the pretreatments.

Derived BCNU PK parameters obtained for rats pretreated daily for 3 consecutive days with CPA, cDDP, or both drugs are shown in lines 2, 3, and 4, respectively of Table 1. Comparison of the AUC obtained for any of the pretreated animals demonstrated that all pretreatments tended to increase the BCNU AUC. Whereas animals pretreated with CPA+cDDP showed the highest C_{max} values, animals treated with cDDP alone demonstrated the most prolonged $t_{1/2}$ elim and the highest AUC. The BCNU AUC values produced following any pretreatment were significantly higher than the control values ($P < 0.04$, Wilcoxon test). Figure 2 emphasizes the large increase in BCNU AUC variability that was associated with any of the pretreatments ($P < 0.02$ versus control, Bartlett's test).

Table 1 BCNU PK parameters for rats treated with BCNU alone or pretreated with CPA, cDDP, or both agents

Group	AUC[2] (μg min ml^{-1})	$t_{1/2\ elim}$ (min)	C_{max} (μg/ml)
Control	193 (15)	9 (13)	16 (32)
CPA	304 (41)	11 (14)	19 (24)
cDDP	441 (51)	17 (17)	17 (82)
CPA+cDDP	325 (63)	10 (37)	23 (18)
Human[b]	775 (100)	31 (77)	5.5 (84)

[a] Data are presented as mean values (coefficient of variation expressed in percent)

[b] Human data are based on 76 patients receiving CPA/cDDP/BCNU

Discussion

Phase I studies of high-dose BCNU in humans demonstrated that although doses in excess of 2000 mg/m^2 could be given, the practical maximal tolerated dose was approximately 1200 mg/m^2. The dose-limiting toxicities included pulmonary, cardiac, and central nervous system injury [11].

BCNU was subsequently selected by Peters et al. [12] for use in a combination AA regimen with ABMS designed to treat solid tumors. As shown in Fig. 1, the BCNU is given following three daily 1-h infusions of CPA and a 72-h continuous infusion of cDDP. The doses shown were the maximally tolerated doses in a phase I multidrug escalation trial. The limiting toxicities in that trial were hepatic and pulmonary injury that were sporadic in frequency.

As supportive-care mechanisms were improved and the CPA/cDDP/BCNU regimen was investigated further, it became clear that pulmonary injury was the most common toxicity of this regimen and could prove fatal if not treated

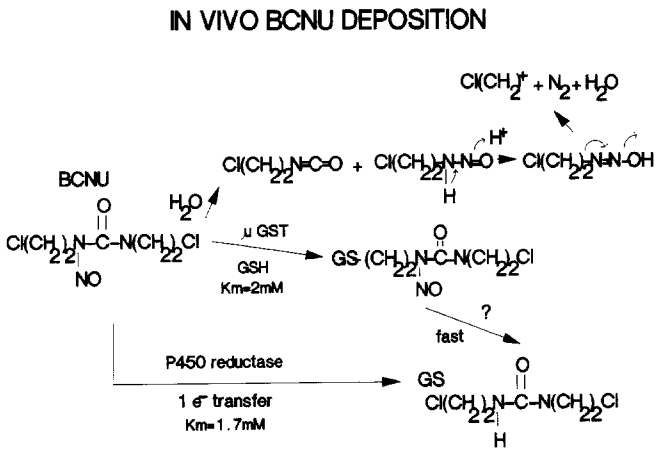

Fig. 3 The three proposed BCNU elimination pathways in rodents are shown, including classic hydrolysis (*top*), glutathione (*GSH*) conjugation followed by denitrosation (*middle*), and microsomal reductase-mediated denitrosation followed by GSH conjugation (*bottom*). The latter two pathways presumably would produce mercapturic acid derivatives (uncharacterized) subject to urinary excretion

rapidly with high-dose steroids. Since BCNU was known to cause frequent pulmonary drug toxicity, even when given at lower conventional doses [13], we investigated whether variation in whole-blood BCNU AUC values between patients receiving CPA/cDDP/BCNU might correlate with the risk for pulmonary injury. In fact, a BCNU AUC value of >600 μg min ml^{-1} was associated with a striking increase in the risk for pulmonary drug toxicity [7].

A surprising finding in that study and additional investigations was a more than 20-fold variation in BCNU AUC between patients. This compared with a 3- to 4-fold variation in CPA and cDDP AUC values in the same patients [14]. The classically described activation and elimination pathway for BCNU was hydrolysis [15, 16], which should not be subject to such wide interpatient variability. Other investigators have suggested that metabolic elimination of BCNU may be important. Levin et al. [17] demonstrated a loss of BCNU therapeutic activity against an intracerebral rat tumor following phenobarbital pretreatment. This therapeutic alteration was correlated with a faster elimination of BCNU [17]. This observation suggested that microsomal metabolism might be important for elimination of BCNU in rats. Other investigators have reported that both microsomal denitrosation [18–20] of BCNU (or its chemical congener lomustine, CCNU) and glutathione conjugation of BCNU followed by denitrosation [21, 22] occur in rats (middle and lower pathways, Fig. 3) and result in deactivation of BCNU. The PK relevance of these pathways (without microsomal induction) as compared with hydrolysis was unclear, and human data were not available.

The doses and schedule used for CPA, cDDP, and BCNU administration to rats in the present study were designed to mimic the PK of the CPA/cDDP/BCNU regimen as given to humans. For reasons of convenience, cDDP was given as three daily injections instead of as a continuous 72-h infusion. The lower AUC value observed for rats was compared with humans is primarily related to the faster $t_{1/2}$ elim value (10 versus 30 min) seen in rats.

The present study demonstrates that pretreatment of rats with CPA, cDDP, or both agents can either slow BCNU elimination, decrease the apparent volume of distribution (resulting in an increased C_{max}), or both, leading to an increase in the BCNU AUC. Additionally, these pretreatments (particularly cDDP) substantially increase the variability in BCNU blood concentration observed between animals, resulting in an increase in the composite-curve AUC variability derived from animals given identical doses of drug.

These findings are consistent with the hypothesis that metabolic elimination of BCNU in the rat is pharmacologically important, particularly since renal or pulmonary excretion of intact BCNU is minimal [23]. Both cDDP [24, 25] and CPA [26, 27] are known to affect P-450 activity and intracellular glutathione concentration in rodents, either or both of which may be relevant and rate-limiting for BCNU elimination. In particular, LeBlanc et al. [24] have shown that cisplatin alters P-450-dependent steroid metabolism in rats through what may be a sex-dependent mechanism. The present studies employed only male rats to avoid such variability. Whether sex-dependent changes in BCNU elimination occur in either rodents or humans is at present unknown. The present study provides no evidence to support a particular mechanism of BCNU elimination or drug-drug interaction between the three components of this regimen.

The three known elimination pathways for BCNU in rodents are summarized in Fig. 3. It is not known whether the metabolic pathways (middle and lower) occur in humans, but it is striking that a greater than 10-fold variation in BCNU AUC is seen in both rats and humans when treatment with CPA and cDDP precede BCNU administration. The metabolic pathways result in BCNU deactivation, whereas hydrolysis produces reactive intermediates necessary for cytotoxicity. Thus, if pretreatment with CPA or cDDP increases the BCNU AUC by decreasing metabolic deactivation, increased BCNU hydrolysis and cytotoxicity might be expected to result. Evaluation of the existence and importance of these two elimination pathways in humans will be the subject of future research.

Although it is possible that inhibition of a rate-limiting metabolic or transport function in a nonlinear fashion could explain the observed increase in BCNU C_{max}, other mechanisms may be responsible for this difference.

The doubling of the BCNU AUC with CPA and cDDP pretreatment in the rat may explain the difficulty in increasing the dose of BCNU above 600 mg/m^2 in the CPA/cDDP/BCNU regimen in humans. This dose may produce a BCNU AUC equivalent to that resulting from the 1200 mg/m^2 single-agent dose that was recommended following the phase I BCNU trial. Unfortunately, PK evaluation was not carried out in that trial. It is interesting to speculate what effect changing the delivery schedule for BCNU relative to CPA and cDDP in this and other regimens might have on the toxic and therapeutic outcome in high-dose chemo-

therapy studies in humans. As shown in Table 1, the BCNU AUC variability seen in humans relative to the absolute AUC is equal to or greater than that observed in rats.

In conclusion, pretreatment of rats with CPA, cDDP, or both agents produces large increases in both the absolute mean value of and the variation in the BCNU AUC. These observations suport a pharmacologically important role for a rate-limiting process (perhaps microsomal metabolism or glutathione conjugation) in BCNU elimination. If these results can be extrapolated to humans, they may also help explain the variability and extent of pulmonary toxicity seen with the CPA/cDDP/BCNU regimen. Further studies of the mechanisms of BCNU elimination are warranted.

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References

1. Armitage JO, Antman KH (1992) High-dose cancer therapy. Pharmacology, hematopoietins, stem cells. Williams and Wilkins, Baltimore
2. Peters WP, Eder JP, Henner WD, Bast RC Jr, Schnipper L, Frei E III (1985) Novel toxicities associated with high-dose combination alkylating agents and autologous bone marrow support. In: Dicke KA, Spitzer G, Zander AR (eds) Autologous bone marrow transplantation. Proceedings of the first international symposium. University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, Texas
3. Skipper HE (1979) Combination therapy: some concepts and results. Cancer Res 17: 215–257
4. Cancer and Leukemia Group B (1990) Protocol 9082, Southwest Oncology Group trial 9114, a randomized comparative study of high dose cyclophosphamide, cisplatin, and BCNU (CPA/cDDP/BCNU) and autologous bone marrow support versus standard dose CPA/cDDP/BCNU as consolidation to adjuvant CAF for patients with operable stage II or stage III breast cancer involving >10 axillary lymph nodes, phase III. NCI
5. Collins JM, Zaharko DS, Dedrick RL, Chabner BA (1986) Potential roles for preclinical pharmacology in phase I clinical trials. Cancer Treat Rep 70: 73–80
6. Cohen MH, Johnston-Early A, Hood MA, et al. (1985) Drug precipitation within i. v. tubing: a potential hazard of chemotherapy administration. Cancer Treat Rep 69: 1325–1326
7. Jones RB, Matthes S, Shpall EJ, Stemmer S, Bearman SI (1993) Acute lung injury following treatment with high-dose cyclophosphamide, cisplatin, and carmustine: pharmacodynamic evaluation of carmustine. J Natl Cancer Inst 85: 640–647
8. Bartlett MS (1937) Some examples of statistical research in agriculture and applied biology. J R Stat Soc 4: 137–170
9. Cox DR, Oakes D (1984) Analysis of survival data. Chapman and Hall 1984, NY
10. Henner WD, Peters WP, Eder JP, et al. (1986) Pharmacokinetics and immediate effects of high-dose carmustine in man. Cancer Treat Rep 70: 877–883
11. Phillips GL, Fay JW, Herzig GP, et al. (1983) A phase I–II study; intensive BCNU (1,3-bis(2-chloroethyl)-1-nitrosourea, NSC 409962) and autologous bone marrow transplantation for refractory cancer. Cancer 52: 1792–1802
12. Peters WP, Eder JP, Henner WS, Schryber S, Wilmore D, Finberg R, Shoenfeld D, Bast R, Gargone B, Antman K, Anderson K, Kruskall MS, Schnipper L, Frei E (1986) High dose combination alkylating agents with autologous bone marrow support: a phase I trial. J Clin Oncol 4: 646–654
13. Aronin PA, Mahaley MS, Rudnick SA, Dudka L, Donohue JF, Selker RG (1980) Prediction of BCNU pulmonary toxicity in patients with malignant gliomas. An assessment of risk factors. N Engl J Med 303: 183–188
14. Jones RB, Matthes S, Dufton C, Bearman SI, Stemmer SM, Myers S, Shpall EJ (1993) Pharmacokinetic/pharmacodynamic interactions of intensive cyclophosphamide, cisplatin, and BCNU in patients with breast cancer. Breast Cancer Res Treat 26: S11–S17
15. Chattergi UC, Greene RF, Gallelli JF (1978) Mechanism of hydrolysis of halogenated nitrosoureas. J Pharm Sci 67: 1527–1532
16. Colvin M, Brundrett RB, Cowens JW, et al (1976) A chemical basis for the antitumor activity of chloroethylnitrosoureas. Biochem Pharmacol 25: 695
17. Levin VA, Stearns J, Byrd A, et al (1979) The effect of phenobarbital pretreatment on the antitumor activity of 1,2-bis(2-chloroethyl)-1-nitrosourea (BCNU), 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU), and 1-(2-chloroethyl)-3-(2,6-dioxo)-3-piperidyl-1-nitrosourea on the plasma pharmacokinetics and biotransformation of BCNU. J Pharmacol Exp Ther 208: 1
18. Hill DL, Kirk MC, Struck RF (1975) Microsomal metabolism of nitrosoureas. Cancer Res 35: 296
19. Potter DW, Reed DJ (1983) Involvement of FMN and phenobarbital cytochrome P-450 in stimulating a one-electron reductive denitrosation of 1-(2-chloroethyl)-3-(cyclohexyl)-1-nitrosourea catalyzed by NADPH-cytochrome P-450 reductase. J Biol Chem 258: 6906–6911
20. Hill DL (1976) *N,N'*-Bis(2-chloroethyl)-*N*-nitrosourea (BCNU), a substrate for glutathione (GSH) S-transferase (abstract). Proc Am Soc Clin Res 17: 52
21. Smith MT, Evans CG, Doane-Selzer P, Castro VM, Tahir MK, Mannervik B (1989) Denitrosation of 1,3-bis(2-chloroethyl)-1-nitrosourea by class mu glutathione transferases and its role in cellular resistance in rat brain tumor cells. Cancer Res 49: 5954–5958
22. Talcott RE, Levin VA (1983) Glutathione-dependent denitrosation of *N,N'*-bis(2-chloroethyl)-*N*-nitrosourea (BCNU): nitrite release catalyzed by mouse liver cytosol in vitro. Drug Metab Dispos 11: 175–176
23. DeVita VT, Denham C, Davidson JD, et al (1967) The physiological disposition of the carcinostatic 1,3-bis-(2-chloroethyl)-1-nitrosourea (BCNU) in man and animals. Clin Pharmacol Ther 8: 566–577
24. LeBlanc GA, Sundseth SS, Weber GF, Waxman DJ (1992) Platinum anticancer drugs modulate P-450 mRNA levels and differentially alter hepatic drug and steroid hormone metabolism in male and female rats. Cancer Res 52: 540–547
25. Andrews PA, Schiefer MA, Murphy MP, et al (1988) Enhanced potentiation of cisplatin cytotoxicity in human ovarian carcinoma cells by prolonged glutathione depletion. Chem Biol Interact 65: 51–58
26. Gurtoo HL, Marinello AJ, Berrigan MJ, Bansal SK, Paul B, Pavelic ZP, Struck RF (1983) Effect of thiols on toxicity and carcinostatic activity of cyclophosphamide. Semin Oncol 10: 35–45
27. LeBlanc GA, Waxman DJ (1990) Mechanisms of cyclophosphamide action on hepatic P-450 expression. Cancer Res 50: 5720–5726