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Pharmacokinetics of high-dose oral CCNU in bone marrow transplant patients

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Abstract *Purpose*: CCNU (lomustine) and other nitrosourea compounds are rapidly metabolized to alkylating moieties, which are active against various malignancies. In humans, CCNU undergoes biotransformation to the geometric isomers of 4′-hydroxyCCNU. The pharmacokinetics of *trans*- and *cis*-4′-hydroxyCCNU were determined in five patients with Hodgkin's or non-Hodgkin's lymphoma receiving sequential doses of CCNU (15 mg/kg), etoposide (60 mg/kg) and cyclophosphamide (100 mg/kg) prior to autologous bone marrow transplantation. *Methods*: Plasma concentrations of the isomeric forms of the metabolites were determined by high-performance liquid chromatography. Data were analysed using noncompartmental pharmacokinetic methods. *Results*: Formation of the *trans*-isomer predominated over that of the *cis*-isomer, with an average exposure ratio of 1.4 (range 1.0–2.1). Peak plasma concentrations occurred between 1 and 4.1 h postdosing and averaged 1.56 mg/l for the *trans*-isomer and 1.10 mg/l for *cis* isomer. Peak plasma concentrations and systemic exposures varied approximately six- and ninefold, respectively, reflecting significant interindividual variability in alkylating activity after high doses of CCNU. Plasma half-lives of the metabolites were 3.1 h (range 1.1–4.5 h) for the *trans*-isomer and 3.5 h (range 1.3–6.4 h) for the *cis*- isomer, and varied linearly with increasing patient body weight. There was no significant difference in plasma half-lives after high-dose CCNU administration observed in this study and those reported previously after the administration of substantially lower doses of CCNU. *Conclusion.* Despite linearity in the pharmacokinetics of the isomeric forms of 4′-hydroxyCCNU at high doses, large interindividual variability in exposure to the CCNU metabolites was observed. Potential saturation of metabolic pathways to the isomers at these doses may manifest as toxic symptoms since alkylating moieties formed directly from the parent, CCNU, may be associated with greater toxicity than those formed from the isomeric forms of the 4′-hydroxylated metabolite.

Key words Lomustine (CCNU) · Pharmacokinetics · Metabolism

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Introduction

The nitrosourea drugs, including BCNU (carmustine, 1,3-bis(2-chloroethyl)-1-nitrosourea) and CCNU (lomustine, 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea), are used clinically in the chemotherapy of a wide variety of human malignancies [13]. Recent studies have demonstrated high-dose BCNU to be an efficacious adjuvant preparatory therapy for autologous bone marrow transplantation [8, 12]. Because of the propensity for BCNU to produce pulmonary toxicity [6], Chao and coworkers investigated the potential clinical advantage of substituting high-dose CCNU in a preparatory chemotherapeutic regimen for patients with advanced lymphoid malignancies in whom pulmonary function was compromised [3]. As part of the study, the pharmacokinetics following administration of high doses of CCNU were examined, and form the basis for this report.

Unlike BCNU, which undergoes biotransformation by both enzymatic and spontaneous chemical processes to active chemotherapeutic moieties, CCNU undergoes

extensive first-pass hepatic metabolism to the geometric isomeric forms of a monohydroxylated metabolite, which are subsequently bioactivated [19]. Hydroxylation at the 4′-position of the cyclohexyl ring confers both isomers of the metabolite with enhanced alkylating activity and reduced toxic effects relative to CCNU, resulting in an approximately twofold increase in the therapeutic index of each of the metabolites relative to the parent compound [20]. Following high-dose CCNU administration, the possibility exists that saturation of this enzymatic hydroxylation pathway would favor increased systemic availability of unmetabolized parent drug, with an increased incidence of toxic effects. Therefore, the purpose of this investigation was to characterize the disposition of the hydroxylated metabolites after administration of high-dose CCNU. Specifically, the aims of the study were to determine the degree of interpatient variability in exposure to the active metabolites of CCNU, to examine the possibility that there might be saturable metabolism at high doses with an enhanced potential for toxicity and to determine whether nonlinearity in the elimination kinetics of the metabolites occurs at these doses.

Material and methods

Patients

Institutional Review Board approval was obtained at Stanford University Medical Center prior to commencement of the study. Patients with Hodgkin's or non-Hodgkin's lymphoma, who had given informed consent to enroll in an efficacy study of sequential escalating doses of CCNU (6–15 mg/kg) on day −6, etoposide (60 mg/kg) on day −4 and cyclophosphamide (100 mg/kg) on day −2 prior to autologous bone marrow transplantation (day 0), were eligible to participate in this pharmacokinetic study [3]. The cohort assigned to the highest CCNU dose were fasted prior to administration of a single oral dose of 15 mg/kg CCNU as the first part of the preparatory regimen. Renal and hepatic function for all patients were within normal limits.

Sample collection

Blood samples were withdrawn from an indwelling cannula in a peripheral antecubital vein at 0, 1, 2, 3, 4, 5, 6, 12, 18 and 24 h after administration of the CCNU dose. Samples were collected into heparinized tubes and immediately placed on ice. After centrifugation, plasma was transferred to a clean polypropylene tube and stored at −80°C prior to analysis.

HPLC assay

Trans- and *cis*-4′-hydroxyCCNU were kindly donated by Dr C. Temple Jr., Southern Research Institute, Birmingham, Ala., and by Dr T. Mulcahy, University of Wisconsin Medical School, Madison, Wis. CCNU and phenytoin (diphenylhydantoin) were purchased from Sigma Chemical Co. (St Louis, Mo.). CCNU and its hydroxylated metabolites were stored in amber glass vials on desiccant at −80°C. Methanol and dichloromethane (Baker, Phillipsburg, N.J.) were HPLC grade, and all other reagents were reagent grade.

Preparation of standards was performed at the time of patient sampling, and all stock solutions and standards were immediately stored at −80°C to minimize degradation. Final standard concentrations of 4, 2, 1, 0.2, 0.1, 0.05 and 0.01 mg/l were prepared by serial dilutions of a methanolic 10 mg/l stock solution of *trans*- and *cis*-4′-hydroxyCCNU using pooled blank plasma. The internal standard (IS) was a methanolic solution of phenytoin, prepared to a final concentration of 2 mg/l.

Plasma samples were analyzed in duplicate for *trans*- and *cis*-4′-hydroxyCCNU by HPLC. For the first two patients, samples collected at 1 h after CCNU dose administration were also analyzed for CCNU. However, as CCNU was not detectable, as expected from literature data [9], subsequent patient samples were not analyzed for the parent compound. Briefly, 0.5-ml standards and samples were spiked with 100 µl IS and extracted with 2 ml dichloromethane by mixing for 5 min. Samples were centrifuged at 2800 rpm and the dichloromethane fraction was transferred to a clean tube and evaporated to dryness in a Speed-Vac (Savant Instruments, Farmingdale, N.Y.). Dried residues were reconstituted with 120 µl methanol by vortexing for 30 s, and 100 µl samples were injected onto the column for analysis.

An isocratic HPLC assay was employed for determination of *trans*-and *cis*-4′-hydroxyCCNU plasma concentrations. The chromatographic system consisted of an IBM LC/9533 Ternary Gradient solvent delivery system (Danbury, Ct.) equipped with a Waters model 712 WISP autosampler, a Waters Lambda-max 481 variable wavelength ultraviolet detector (Milford, Mass) and Spectra-Physics model 4290 integrator (Fremont, Calif.). Chromatographic separations were effected on an Ultrasphere ODS analytical reversed-phase 4.6 mm (internal diameter) × 25 cm column with 5 µm particle size (Beckman, Fullerton, Calif.) and UV detection was at a wavelength of 254 nm. The mobile phase consisted of 1:1 methanol/phosphate buffer (0.1 *M* KH_2PO_4, pH 7.4) at a flow rate of 1 ml/min. Under these conditions, the relative retention times for *trans*-, *cis*-4′-hydroxyCCNU and IS were 7.7, 8.7 and 13.5 min respectively, with a total run time of 15 min, BCNU and CCNU eluted at 9.5 and 48 min, respectively.

For each of the isomeric forms of the 4′-hydroxylated CCNU metabolite, peak height ratios of drug to IS were plotted and regressed on spiked plasma concentrations. Unknown concentrations of metabolites were determined by interpolation from the regression line thus obtained.

Daily calibration curves were linear ($r^2 > 0.99$) in the range 0.05–4 mg/l for both isomers of 4′-hydroxyCCNU. The limit of detection for the assay was 0.01 mg/l. Recoveries of *trans*-and *cis*-4′-hydroxyCCNU from plasma were 90% and 95%, respectively. Interassay coefficients of variation were 5.8% for the *trans*-isomer and 7.0% for the *cis*-isomer, and intraassay precision was determined to be 5.7% and 4.2%, respectively. Because CCNU, and presumably its metabolites, are known to degrade in aqueous and alcoholic solutions [1], plasma samples and methanolic solutions were tested for stability during storage at −80°C. We found these to be stable without significant loss of potency for periods of up to 7 days. In order to ensure integrity of the analytes in the collected plasma samples, they were assayed immediately, within 7 days of blood draw.

Pharmacokinetic analysis

Plasma concentration versus time data for each of *trans*- and *cis*-4′-hydroxyCCNU were analyzed using noncompartmental methods. The maximum plasma concentration (C_{max}) and time of its occurrence (T_{max}) were determined by visual inspection of the data. The elimination rate constant (k) for each of the CCNU metabolites in plasma was calculated from least squares linear regression of the terminal portion of the log–linear plasma concentration–time curve, and plasma half-life was computed using the equation $t_{1/2} = \ln 2/k$.

Exposure to the active drugs was calculated as the area under the plasma concentration–time curve from zero to infinity (AUC) using the trapezoidal rule, with correction for the area beyond the time of the last measurable concentration (C_{last}) to infinity, as determined by C_{last}/k [4]. The exposure ratio was calculated as the ratio of AUC_{trans} to AUC_{cis}. Under the assumptions that there was rapid and complete absorption of CCNU ensuring that the total dose was supplied to the liver, and that there was complete biotransformation during the hepatic first pass to *trans*- and *cis*-4′-hydroxyCCNU [11], fractional exposure was calculated:

$$fr_{isomer} = AUC_{isomer}/AUC_{total}$$

where fr_{isomer} is the average fractional exposure to the geometric isomer and AUC_{total} is the total hydroxylated CCNU metabolite exposure ($AUC_{trans} + AUC_{cis}$). Precision in the estimates of the pharmacokinetic parameters was expressed using 95% confidence intervals.

Linearity in pharmacokinetics after high-dose CCNU administration was assessed by comparing mean metabolite C_{max} and total exposure values with those obtained in the only other detailed pharmacokinetic analysis of the disposition of CCNU after a single oral dose of 130 mg/m^2 (or approximately one-fifth of the CCNU dose administered in the present high-dose study) [9]. The difference in plasma half-life between the two dose groups was assessed using the Mann-Whitney *U*-test.

Ordinary least squares regression was employed to determine whether a relationship existed between body weight and plasma half-lives for each of the isomeric forms of the 4′-hydroxylated CCNU metabolite. The data obtained in another study in a similar group of patients [9] was included in the analysis in order to assess the validity of the association. The statistical significance of the relationship was tested using the *F* statistic, and the unpaired Student's *t*-test was used to determine whether the slope of the regression line differed significantly from zero. For all statistical analyses, $P < 0.05$ was considered to be significant.

Results

Five male patients, aged 32 to 55 years with body weights ranging from 55 to 104 kg, and who received 15 mg/kg oral dose of CCNU, were included in the study. The plasma concentration–time curves of the isomeric forms of 4′-hydroxyCCNU for two individuals are shown in Fig. 1. For one patient, there was extensive biotransformation to the metabolites, with peak plasma concentrations of 2.2 and 1.6 mg/l of the *trans*- and *cis*- isomers, respectively, occurring at 3.6 h postdosing. In contrast, the other patient presented in the graph demonstrated much lower concentrations of the metabolites of CCNU. For this patient, maximum plasma metabolite concentrations of 0.3 and 0.1 mg/l, respectively, were achieved at 1.2 h after dose administration. Total exposure to the geometric isomeric metabolites of CCNU for this patient was 15% of that for the other patient shown in Fig. 1.

A summary of the pharmacokinetic parameters of the CCNU metabolites in this high-dose study are presented in Table 1. For comparison, the pharmacokinetics determined in the study by Lee et al. [9] after lower doses of CCNU are also tabulated. Plasma concentrations of the *cis*-isomer were found to parallel those of the *trans*-isomer during the absorption phase,

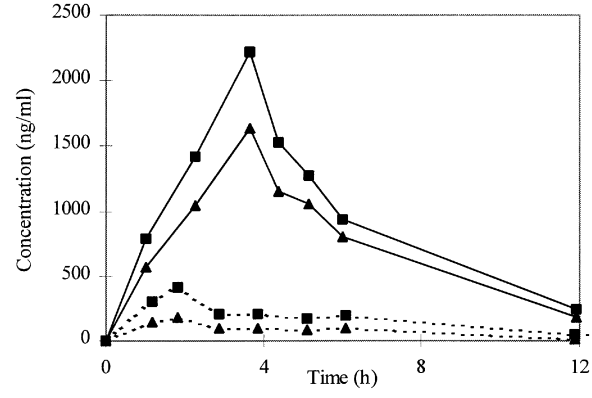

Fig. 1 Plasma concentration time curves of *trans*- (■) and *cis*- (▲) 4′-hydroxyCCNU for two individuals. For one individual, there was extensive metabolism (*solid lines*) while the other demonstrated limited capacity to metabolize CCNU (*dashed lines*)

and simultaneously reached peak concentrations at an average time of 2.5 h (range 1–4.1 h) postdosing. Plasma half-lives of the metabolites were similar, averaging 3.1 h (range 1.1–4.5 h) and 3.5 h (range 1.3–6.4 h) for the *trans*- and *cis*-isomers, respectively.

Mean maximum plasma concentrations of 1.56 mg/l (0.41–2.31 mg/l) and 1.10 mg/l (0.18–1.82 mg/l) were approximately three times those obtained in the study by Lee et al. (Table 1) [9]. Overall exposure, expressed in units of AUC was 14.27 mg.h/l (range 3.29–21.56 mg.h/l) and was about four times that previously reported [9]. Formation of the *trans*-isomer predominated over that of the *cis*-isomer, with an average exposure ratio of 1.4 (range 1.0–2.1). However, we observed large interindividual variability in exposures to each of the isomeric forms of the 4′-hydroxylated CCNU metabolite. For the *trans*-isomer, exposures varied approximately six-fold, while for the *cis*-isomer there was about a ninefold variability.

There was no significant difference between plasma half-lives of the metabolites after high-dose CCNU administration (CCNU dose range 820–1560 mg) and those reported by Lee et al. [9] after substantially lower doses (CCNU dose range of 200–260 mg). An apparent association between the plasma half-lives of the metabolites and patient body weight was assessed by regression analysis (Fig. 2). For the *trans*-metabolite, a statistically significant linear relationship was observed, characterized by the equation $t_{1/2-tr} = 0.06 \times wt - 1.88$ and a correlation coefficient (r^2) of 0.85. Similarly for the *cis*-metabolite, plasma half-lives increased linearly with increasing body weight according to the equation $t_{1/2-cis} = 0.09 \times wt - 3.57$, with a correlation coefficient of 0.90. These relationships were valid for body weights in excess of 50 kg, and therefore likely reflect drug distribution relative to the body composition (fat:lean body mass) of adult patients. A weaker association between body weight and dose-normalized total exposure (AUC) ($r^2 = 0.46$, $P = 0.05$)

Table 1 Summary of pharmacokinetic parameters for *trans*- and *cis*- 4′-hydroxyCCNU (D_{CCNU} administered dose of CCNU, *fr* average fractional exposure, T_{max} time to peak plasma concentration (C_{max}), *AUC* area under the plasma concentration vs time curve extrapolated to infinite time, $t_{1/2}$ plasma half-life.)

Subject	D_{CCNU} (mg)	fr		C_{max} (mg/l)		T_{max} (h)	AUC (mg.h/1)		Total Exposure Exposure ratio (mg.h/1)		$t_{1/2}$(h)	
		trans	*cis*	*trans*	*cis*		*trans*	*cis*			*trans*	*cis*
1	1237.5	0.51	0.49	0.41	0.18	2.0	5.9	5.7	11.5	1.0	3.9	3.0
2	1560	0.68	0.32	0.85	0.66	1.9	2.2	1.1	3.3	2.1	4.5	6.4
3	1125	0.60	0.40	2.31	1.23	1.0	12.3	8.4	20.7	1.5	3.3	3.8
4	975	0.56	0.44	2.22	1.63	3.6	12.2	9.4	21.6	1.3	2.9	2.7
5	825	0.52	0.48	2.02	1.82	4.1	7.4	6.9	14.3	1.1	1.1	1.3
High-dose ($n = 5$)												
Mean ± SD	1145 ±279	0.57 ± 0.07	0.43 ± 0.07	1.56 ± 0.87	1.10 ± 0.68	2.5 ± 1.3	8.0 ± 4.3	6.3 ± 3.2	14.3 ± 7.5	1.4 ± 0.4	3.1 ± 1.3	3.5 ± 1.9
95% CI		0.51–0.63	0.37–0.49	0.80–2.33	0.51–1.70	1.4–3.7	4.2–11.8	3.4–9.1	7.7–20.8	1.0–1.8	2.0–4.3	1.8–5.1
Lee et al. [9] ($n = 4$)												
Mean ± SD	238 ± 26	0.61 ± 0.03	0.39 ± 0.03	0.53 ± 0.03	0.32 ± 0.04	2–4	2.1 ± 0.4	1.3 ± 0.4	3.4 ± 0.8	1.6 ± 0.2	1.8 ± 0.6	1.9 ± 0.7
95%CI		0.58–0.64	0.36–0.42	0.50–0.56	0.28–0.36		1.6–2.5	1.0–1.7	2.6–4.1	1.4–1.8	1.3–2.4	1.2–2.6

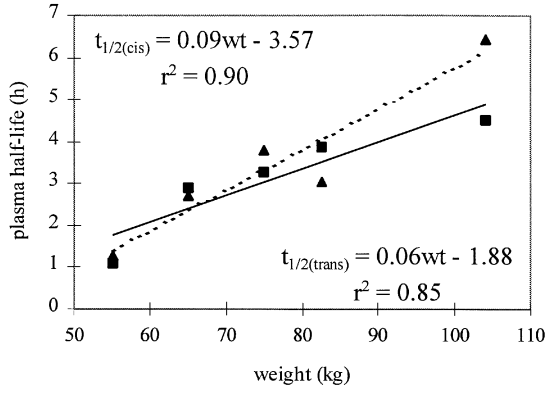

Fig. 2 Plot showing the linear relationship between patient body weight (kg) and plasma half-life (h) for *trans*- (■) and *cis*- (▲) 4′-hydroxyCCNU

was consistent with this observation. When data from the study by Lee et al. [9] were included in the analysis, there was no change in the slope of the regression equation for either metabolite, and significant linear relationships were observed for both the *trans*- ($r^2 = 0.59$) and the *cis*-isomers ($r^2 = 0.66$).

Discussion

Evaluation of the clinical pharmacokinetics of nitrosourea drugs, including CCNU, is complicated by complex metabolic and spontaneous chemical degradation pathways which produce active metabolites and reactive, but unstable, intermediates [11]. While the active moieties have been poorly characterized, the primary cytotoxic mechanism, intracellular alkylation of DNA, has been attributed to the formation of a chloroethyl carbonium ion [11, 17]. In addition, isocyanate moieties are involved in intracellular carbamoylation of proteins and are believed to play a role in producing adverse effects in humans [11, 17].

In early studies in which investigators monitored radioactivity following oral administration of ^{14}C-labeled CCNU to cancer patients, absorption from the gastrointestinal tract was shown to be rapid and complete [18]. However, it was concluded that CCNU undergoes rapid systemic degradation since intact parent drug was undetectable in plasma [18]. It was subsequently shown in in vitro and animal experiments that CCNU undergoes extensive oxidative metabolism mediated by stereoselective cytochrome P450 isozymes, and this pathway predominates over aqueous degradation of CCNU in blood [7, 16]. In a detailed pharmacokinetic analysis of CCNU disposition after an oral dose of 130 mg/m^2 administered to four male cancer patients, Lee et al. determined that CCNU undergoes complete biotransformation on first pass through the liver [9].

In humans, a single biotransformation product is produced by hydroxylation at the 4′-position of the cyclohexyl ring of CCNU [5, 9]. The metabolite exhibits geometric isomerism, and exists in both the *trans*- and *cis*- forms, with the chemically more stable *trans*- configuration predominating in humans. Exposure to the *trans*-isomer averaged 1.4 times that to the *cis*-isomer after high doses of CCNU to bone marrow transplant patients, in close agreement with the findings reported by Lee et al. after substantially lower CCNU doses [9]. The chemotherapeutic activity of CCNU is therefore expected to be mediated through the subsequent degradation of the isomeric forms of the metabolite to active alkylating moieties. In comparison with the parent drug, *trans*- and *cis*-4′-hydroxyCCNU

have demonstrably greater alkylating activity and produce less toxicity in vitro, with consequently increased therapeutic margins [20].

Reported maximum plasma concentrations of both isomeric forms of the metabolite after low-dose CCNU administration, as administered by Lee et al., are associated with minimal variability [9]. In contrast, substantial variability was observed after high-dose CCNU and was approximately twice that observed after low-dose CCNU administration [9]. Mean maximum plasma concentrations and total metabolite exposure were approximately 20–40% lower than expected for the high-dose group. Although the numbers in both this high-dose study and the low-dose study were small, the reduction in metabolite exposure and large interindividual variability are suggestive of significant variability in the extent of metabolism of CCNU between individuals and potentially saturable formation of metabolites, which becomes apparent after high doses of CCNU.

Studies of the effects of a variety of anticancer drugs on specific subfamilies of cytochrome P450 isozymes in human liver microsomes have revealed that CCNU is a competitive inhibitor of both CYP3A and CYP2D6 activity [10, 21]. CYP3A has been characterized as the primary cytochrome P450 subfamily responsible for the biotransformation of cytotoxic drugs which similarly undergo cytochrome P450-mediated ring hydroxylation, including the isomeric alkylating agent cyclophosphamide and the antimitotic vinca alkaloids [2, 10, 15, 21]. Such oxidative metabolic activity is associated with large interindividual variability, ranging from four- to ninefold among individual human liver microsome isolates [2, 21]. Furthermore, biotransformation of drugs by the polymorphic CYP2D6 isozyme is associated with a poor metabolizer trait in 5–10% of Caucasians [14]. The relative importance of these metabolic pathways in the biotransformation of CCNU remain to be fully elucidated; however, it is evident that the potentially large interindividual variability with which they are associated may have significantly contributed to the variability observed in individual metabolite exposures in our study. Furthermore, a reduction in the metabolism of CCNU to its hydroxylated isomeric forms could produce a larger proportion of alkylating degradation products formed from the parent drug. These are associated with a narrower therapeutic margin than those derived from either of the isomeric forms of the metabolite [20], and may manifest clinically as an increased risk of toxicity.

There was no evidence of nonlinear elimination of isomeric metabolites after high-dose CCNU administration, since plasma half-lives of the hydroxylated CCNU metabolites did not differ from those observed after the administration of lower doses in the study by Lee et al. [9]. There was a significant linear correlation between the plasma half-lives of each of the isomeric forms of the metabolite and body weight. This observation is consistent with sequestration by body fat, providing a depot from which the metabolite is slowly released. The slightly longer half-life of the *cis*- compared with the *trans*-isomer may be due to its slightly higher oil–water partition coefficient [20]. While longer half-lives of the isomeric forms of the metabolite in obese patients may be of minimal clinical importance following single-dose administration, it warrants further consideration should repetitive dosing be employed, because some accumulation may occur.

Although there was no evidence for substantial nonlinearities in metabolite formation after high CCNU doses, the interindividual variability in exposure to the CCNU metabolites was much larger than that seen at low doses, and may contribute to toxicity at this dose level. Further investigation of the disposition, efficacy and toxicity of such doses of CCNU are warranted to ensure that optimal dosing strategies are employed to ensure maximal efficacy while minimizing the potential for toxicity.

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