

Clinical Pharmacokinetics of Amifostine and WR1065 in Pediatric Patients with Medulloblastoma

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Abstract

Purpose: We evaluated the pharmacokinetics of amifostine and WR1065 in pediatric patients with newly diagnosed medulloblastoma to assess the influence of patient covariates, including demographics, clinical characteristics, and genetic polymorphisms, on amifostine and WR1065 pharmacokinetic parameters.

Experimental Design: We assessed the pharmacokinetics of amifostine and WR1065 in 33 children who received amifostine (1-minute infusion, 600 mg/m²) just before the start of and 3 hours into a 6-hour cisplatin infusion. Serial blood samples were collected after doses 1 (0 hour) and 2 (3 hours) of course 1. Amifostine and WR1065 were quantitated by high performance liquid chromatography with electrochemical detection. A pharmacokinetic model was simultaneously fit to amifostine and WR1065 plasma or whole blood concentration-versus-time data. The influence of demographic, biochemical, and pharmacogenetic covariates on amifostine and WR1065 disposition was evaluated.

Results: Body surface area was the primary size-based covariate for amifostine pharmacokinetics explaining 53% and 56% of interindividual variability in plasma and whole-blood amifostine clearance, respectively. The population-predicted values for amifostine clearance, volume, and apparent WR1065 clearance from the plasma data were 107 L/h/m², 5.53 L/m², and 30.6 L/h/m². The population-predicted values for amifostine clearance, volume, and apparent WR1065 clearance from whole blood data were 136 L/h/m², 7.23 L/m², and 12.5 L/h/m².

Conclusions: These results support using body surface area for calculating doses of amifostine in children. Similar to data in adults, amifostine and WR1065 are rapidly cleared from plasma and whole blood in children. *Clin Cancer Res*; 16(3); 1049–57. ©2010 AACR.

Medulloblastoma is the most common childhood central nervous system malignancy (1). The use of cisplatin for the treatment of medulloblastoma has improved overall survival and enabled use of lower doses of cranial spinal irradiation (2, 3). However, cisplatin carries with it a host of both short- and long-term toxicities including significant ototoxicity. Approximately 35% of children treated with cisplatin for medulloblastoma have significant ototoxicity (2, 4). Amifostine significantly reduces the risk of severe ototoxicity in patients with average-risk medulloblastoma receiving dose-intense chemotherapy when administered before and during the cisplatin infusion (5).

Amifostine is a prodrug converted to the active thiol metabolite WR1065, which acts as a nonspecific cytoprotectant, reducing the toxicity associated with alkylating agents and radiation therapy by scavenging free radicals (6). The conversion of amifostine to WR1065 is catalyzed by alkaline phosphatase and is pH dependent, occurring more rapidly in alkaline pH. The lower concentration of membrane-bound alkaline phosphatase and lower pH in tumor environments contribute to a relatively low concentration of active chemoprotectant in malignant tissues, providing a relatively selective cytoprotection of normal tissues (7–9). The half-life of amifostine is ~9 minutes, whereas that of the active metabolite, WR1065, is ~15 minutes. Thus, the schedule of amifostine administration is potentially an important factor for optimum efficacy (7, 10, 11).

Despite a promising mechanism of action for the selective protection of normal tissues, investigations of the cytoprotective effects of amifostine have yielded mixed results (5, 12, 13). These conflicting findings may be related to differences in the schedule and dosage of amifostine, inadequate statistical power, and differences in the underlying chemotherapy and patient populations studied. Fouladi and colleagues (14) conducted a phase 1 evaluation of amifostine in combination with

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Translational Relevance

Currently, little is known about the pharmacokinetics of amifostine or its active metabolite WR1065 in children with cancer or about the factors that explain the interpatient variability of these pharmacokinetic parameters. This analysis has defined the disposition of amifostine and WR1065 in children with medulloblastoma. Furthermore, this study investigated the relationship between the expression of drug-metabolizing or transporter genotypes and disposition of amifostine and its primary metabolite. The covariate analysis has identified patient covariates, including body surface area, which are related to the pharmacokinetic parameters and explain the interpatient variability in these parameters.

a 6-hour cisplatin infusion and concluded that the optimum schedule was 600 mg/m² just before and 3 hours into a 6-hour cisplatin infusion. This schedule of administration significantly reduced the ototoxicity associated with the treatment of medulloblastoma (5). To date, investigations of the pharmacokinetic parameters of amifostine and WR1065 in pediatric patients remain limited.

Amifostine and WR1065 are involved in a complex interaction with the glutathione pathway. *In vitro* exposure of cells to WR1065 results in increased precursor uptake and biosynthesis of glutathione (15). Additionally, the elimination of WR1065 may be partially mediated as a glutathione conjugate (16). Significant functional polymorphisms in the glutathione *S*-transferase (GST) pathway have been discovered, with implications for the clearance of multiple drugs including chemotherapeutics (17–20). These polymorphisms include *GSTM1*0*, in which the gene is deleted. Homozygous patients express no M1 protein. *GSTT1* is also polymorphic with homozygotes lacking the GST T1 function. The *GSTP1* polymorphism involves a single nucleotide polymorphism resulting in an amino acid substitution. A resulting steric effect on substrate binding causes substrate-specific functional effects (17, 21). Evaluating functional polymorphisms as covariates for pharmacokinetic parameters may help explain variability in amifostine and WR1065 clearance.

Analytic procedures for measuring amifostine and WR1065 have been developed for both plasma and whole blood. To date, it is not known which sampling matrix provides a better model for relating pharmacokinetics to pharmacodynamics (10, 11, 16). WR1065 is rapidly auto-oxidized, creating a scenario in which sample preparation and procedures for arresting oxidation may have significant implications for the concentrations measured (10, 16, 22). The analytic procedure used in this investigation permitted simultaneous bioanalytic measurements in both blood and plasma (23).

The objectives of this investigation were to: (a) simultaneously assess the pharmacokinetics of amifostine and WR1065 in pediatric patients treated with cisplatin for medulloblastoma; (b) estimate the magnitude of interpatient pharmacokinetic variability along with individual estimates of patient pharmacokinetic parameters; (c) investigate the effects of baseline clinical variables and functional polymorphisms of GST M1, T1, and P1 on the variability of the pharmacokinetic parameter; 4) evaluate parameter estimates derived from whole blood and plasma sampling in relation to pharmacodynamic effects of amifostine; and (d) use the current data set to develop a limited sampling model (LSM) for future investigations of amifostine pharmacokinetics.

Materials and Methods

Patients and treatment. The population pharmacokinetic analysis included amifostine and WR1065 plasma and whole-blood concentration-time data from children that participated in the protocol St. Jude Medulloblastoma-96 (5, 24). Eligibility criteria included patients ages ≥ 3 and ≤ 21 y at the time of diagnosis who had not previously received chemotherapy or irradiation. Prior corticosteroid therapy was allowed. Patients were required to begin treatment within 28 d of definitive surgery. Medulloblastoma risk category was classified according to a modified Chang staging system, as previously described (24). Additional eligibility criteria included normal renal function, normal liver function, normal bone marrow function (hemoglobin of ≥ 10 g/dL, WBC count of $\geq 3,000$ /mL, absolute neutrophil count of $\geq 1,500$ /mL, and platelets of $\geq 100,000$ /mm³), and an Eastern Cooperative Oncology Group performance score of 0 to 3, except in cases of posterior fossa syndrome. Informed consent was obtained from the parent/guardian or patient per the St. Jude Children's Research Hospital Institutional Review Board.

All patients underwent an attempt at maximal surgical resection of the tumor and received treatment with radiation and chemotherapy as previously described (24). After a 6-wk rest period, patients began four cycles of high-dose chemotherapy, each followed by stem cell or bone marrow rescue. The high-dose chemotherapy included 75 mg/m² cisplatin and 1.5 mg/m² vincristine (2 mg max) on day -4, followed by a continuous infusion of 2 g/m² cyclophosphamide with 2-mercaptoethane sulfonic acid on days -3 and -2, hydration on day -1, and infusion of peripheral blood stem cells or bone marrow on day 0, with supportive care and filgrastim in the following days and a subsequent dose of 1.5 mg/m² vincristine (2 mg max) on day +6. Amifostine (600 mg/m²) was administered with each cycle of cisplatin containing chemotherapy as a 1-min i.v. infusion just before the start and 3 h into the 6-h cisplatin infusion.

Blood and plasma sampling and bioanalysis. Samples were obtained preinfusion, 2, 5, 10, 30, 60, 120, and 180 min after both the first and second amifostine dose during course one of cisplatin. Plasma and whole blood concentrations of amifostine and WR1065 were assayed

as previously described (23). In short, for each sample, the concentration of native WR1065 was determined. To measure the concentration of amifostine, a sample was incubated for 4 h at 37°C to convert amifostine to WR1065. After incubation, the sample was assayed to determine the concentration of WR1065. This concentration of WR1065 represented the concentration of amifostine (converted) plus the native WR1065 previously assayed. Subtraction of the native WR1065 concentration from the converted WR1065 determined the concentration of amifostine. The lower limit of quantitation (LLQ) of amifostine and WR1065 was 0.25 µmol/L. The bioanalytic procedure was done for both whole blood and plasma for each sampling time.

Pharmacogenetic analysis. We hypothesized the no or low-activity GST genotypes (*GSTP1**A, *GSTM1* null, and *GSTT1* null) would result in altered systemic clearance of amifostine and WR1065. We used a multiplex PCR technique to amplify both *GSTM1* and *GSTT1* simultaneously in a single PCR as described in detail before (25). In analyses, patients with null genotypes were compared with patients with nonnull genotypes. We determined *GSTP1* rs1695 and rs1138272 polymorphisms by PCR and RFLP methods as described before (25). We analyzed the *GSTP1* results by comparing patients with the *A/*A genotype with the rest of the group, who had at least one variant allele. For quality control, 10% of all of the samples were repeated.

Pharmacokinetic analysis. The pharmacokinetics of amifostine and WR1065 were determined by nonlinear mixed effects modeling through NONMEM (version VI, GloboMax LLC), using the first-order conditional estimation method with INTERACTION through the ADVAN7 subroutine (26). Plasma and whole blood concentration-time data were modeled separately. Xpose (27), Census (28), and the

R-project (version 2.8.1) were used for data management and visualization. Posterior Bayesian individual estimates of amifostine and WR1065 pharmacokinetic parameters were obtained within NONMEM. The pharmacokinetic parameters estimated included amifostine systemic clearance (CL_{am}), volume of distribution (VD), amifostine intercompartmental rate constants k_{12} and k_{21} , apparent clearance of WR1065 (CL_{WR}), and WR1065 intercompartmental rate constants k_{w34} and k_{w43} (Fig. 1). The pharmacokinetic model assumed that the VD of amifostine was equal to the VD of WR1065.

The distribution of parameters was assumed to be log normal. Both interindividual (IIV) and interoccasion (IOV) variability in parameters were modeled as exponential terms. An occasion was defined as one dosing occasion of amifostine. The residual errors for amifostine and WR1065 were each modeled independently using a mixed proportional and additive error model:

$$Cp_{ik} = \hat{C}p_{ik}(1 + \varepsilon_{ik}^{rel}) + \varepsilon_{ik}^{abs}$$

where Cp_{ik} and $\hat{C}p_{ik}$ represent the k^{th} actual and predicted concentrations in individual i , respectively. The error terms ε^{rel} and ε^{abs} are the components of the proportional and additive error, respectively. The proportional error was assumed to have a mean of zero and variance of σ . The components of additive error for amifostine and WR1065 were both fixed to 0.25 in consideration of the LLQ of the assay (0.25 µmol/L). Observed amifostine and WR1065 concentrations below the LLQ were substituted with 0.125 (1/2*LLQ).

Covariates considered in the analysis included body surface area (BSA), age, gender, race, albumin, total bilirubin, GST polymorphisms (M1, T1, and P1), serum creatinine, and calculated glomerular filtration rate (29). A covariate was considered significant in this analysis if the addition of the covariate to the model reduced the objective function value (OFV) at least 3.84 units ($P < 0.05$, based on the χ^2 test for the difference in the -2 log-likelihood between two hierarchical models that differ by 1 degree of freedom).

The relationships between the pharmacokinetic parameters (CL_{am} , VD, and CL_{WR}) and categorical or continuous covariates were described using the following model:

$$\theta = \tilde{\theta} + \sum_{\rho=1}^m \text{covariate}_{\rho} \times \theta_{\rho}$$

where θ was the population estimate, $\tilde{\theta}$ was the population estimate with none of the covariates included, and θ_{ρ} was the effect of covariate ρ on the model. When a categorical variable was considered, the presence of a covariate was coded as covariate $\rho = 1$ and its absence as covariate $\rho = 0$. When sex was evaluated as a covariate, male patients were coded as $\rho = 0$ and females as $\rho = 1$. Patients with incomplete data were censored when covariate analysis included variables for which data were missing.

Bootstrap analysis was used to evaluate robustness of models and calculate SEM estimates and 95% confidence intervals for parameters (30). The nonparametric bootstrap procedure used random sampling from the data used

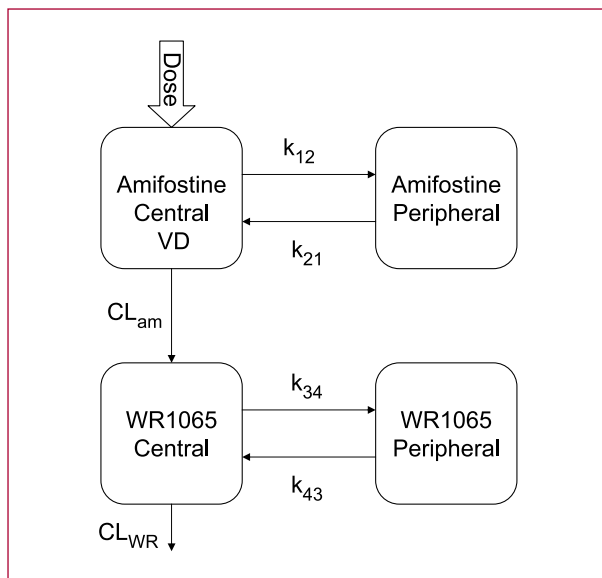


Fig. 1. Amifostine and WR1065 structural model.

for building the base population pharmacokinetic model with replacement to create bootstrap data sets ($n = 500$) with the same sample size as the original. Each new data set was fit to the final model, and population variables were estimated. This procedure was done separately for the plasma and whole blood data sets.

Pharmacodynamic assessment. The primary adverse events of amifostine are hypotension, nausea, and hypocalcemia related to transient hypoparathyroidism (5). We sought to determine whether parameter estimates derived from whole blood or plasma sampling would provide a more robust model in relating pharmacokinetic assessments to the pharmacodynamic effects of amifostine (hypocalcemia and hypoparathyroidism). The decrease from baseline and the duration of decrease in ionized calcium values and parathyroid hormone (PTH) values were used to assess relationships between pharmacokinetic parameters and toxicity with both plasma and whole blood-derived data. Sampling of ionized calcium and PTH was done before and during the 24 h after the amifostine infusion.

Selection of pharmacokinetic LSM. We used the plasma amifostine and WR1065 data to create a LSM for use in future clinical trials of amifostine. The LSM was determined using the estimated population pharmacokinetic parameters in the plasma data. A stepwise process was used to determine an adequate LSM that would estimate individual *post hoc* CL_{am} , VD , and CL_{WR} . The following sampling strategies were evaluated: two samples from one amifostine dose or occasion (5 and 30 min), two samples from each of two occasions (5 and 30 min), three samples from one occasion (5, 30, and 60 min), and three samples from each of two occasions (5, 30, and 60 min).

The following process was used to evaluate limited sampling strategies. First, the concentration-time data were limited to the sampling times of interest. The individual *post hoc* estimates were obtained in NONMEM with each of the limited sampling data sets using the population estimates from the final BSA-normalized plasma model as population priors. This process was repeated for each sampling strategy evaluated. The individual *post hoc* estimates from each limited sampling strategy were then compared with the *post hoc* estimates from the full base model, and the relative bias and accuracy were calculated.

The Wilcoxon signed-rank test was used to compare individual *post hoc* estimates from each of the limited sampling strategies to the *post hoc* estimates from the full base model. All statistical tests used a two-tailed significance threshold of $P < 0.05$ without correction for multiple testing. Statistical analyses were carried out using the JMP software v7.0.1 (SAS Corp.).

Results

Patient population. Of the 134 patients that underwent treatment on the protocol St. Jude Medulloblastoma-96 and received amifostine, 49 consented to participate in the pharmacokinetic studies. One patient enrolled in phar-

macokinetic studies was unable to contribute samples due to hypotension-related to amifostine administration. During the WR1065 assay development, it was determined that because of WR1065 instability, samples assayed >48 hours after collection were not evaluable (23). This restriction created a data set of 33 patients with 649 evaluable whole blood samples and 33 patients with 711 evaluable samples in plasma. Baseline patient characteristics of the patients with pharmacokinetic sampling were comparable with those of the full clinical trial population (24) and are provided in Table 1.

Amifostine and WR1065 disposition. Results from pharmacokinetic studies of amifostine and WR1065 in adult patients showed that a four-compartment model adequately described their disposition (10). We also used a model with a total of four compartments to simultaneously model amifostine (two compartments) and WR1065 (two compartments) with a rate constant describing the conversion of amifostine to WR1065 (Fig. 1).

Using the plasma amifostine and WR1065 concentration-time data, the amifostine population pharmacokinetic

Table 1. Baseline patient characteristics

Patient characteristic <i>n</i> = 33	<i>n</i> (%)
Medulloblastoma risk category	
High	20 (57)
Average	13 (43)
Sex	
Male	23 (70)
Female	10 (30)
Race	
Caucasian	31 (93)
Other	2 (7)
GST genotype (<i>n</i> = 25)	
M1	
Present	9
Null	16
T1	
Present	22
Null	3
P1	
Wt	10
Variant	15
Patient characteristic	Median (range)
Age (y)	7.3 (3.1–20.1)
Weight (kg)	24.6 (13–96.3)
Height (cm)	131 (98–188)
BSA (m ²)	0.93 (0.59–2.24)
Serum creatinine (mg/dL)	0.4 (0.3–0.8)
Albumin (g/dL)	3.5 (3.1–3.9)

Abbreviation: wt, wild-type.

Table 2. Summary of population pharmacokinetic parameters

Parameter	NONMEM estimate	Mean Bootstrap estimate	Bootstrap SEE	Bootstrap 95% CI	NONMEM IIV (%)
Cl_{am}					
Plasma (L/h/m ²)*	107	106	8.41	90.3–123	0.15 (38)
Plasma (L/h)	107	106	11.5	86.3–130	0.31 (56)
Blood (L/h/m ²)*	136	133	10.7	113–155	0.13 (36)
Blood (L/h)	135	132	14.2	105–160	0.3 (55)
VD					
Plasma (L/m ²)*	5.53	5.51	0.55	4.53–6.60	0.12 (34)
Plasma (L)	5.62	5.51	0.72	4.36–7.03	0.32 (56)
Blood (L/m ²)*	7.23	6.97	1.04	4.73–8.71	0.08 (28)
Blood (L)	7.14	6.84	1.2	4.27–8.92	0.23 (48)
CL_{WR}					
Plasma (L/h/m ²)*	30.6	31.3	4.46	22.4–40.4	0.34 (58)
Plasma (L/h)	30.1	30.9	4.26	22.4–39.4	0.34 (58)
Blood (L/h/m ²)*	12.5	13.4	2.87	9.6–20.5	0.17 (42)
Blood (L/h)	12.7	13.7	3.1	9.64–21	0.15 (39)
Population Parameters with sex as a covariate					
CL_{am}					
Plasma (L/h/m ²)*					
Male	99	99	8.7	82.6–117	0.15 (39)
Δ female	24.2	22.7	9.2	3.7–39.4	—
Blood (L/h/m ²)*					
Male	136	133	11.2	112–158	0.13 (36)
Δ female	–0.67	–0.08	12.5	–24.7 to 26.5	—
VD					
Plasma (L/m ²)*	5.41	5.39	0.56	4.4–6.52	0.13 (36)
Blood (L/m ²)*	7.23	6.95	1.1	4.7–8.7	0.08 (28)
CL_{WR}					
Plasma (L/m ²)*	31.2	31.9	4.5	23–40.8	0.33 (57)
Blood (L/m ²)*	12.5	13.4	2.9	9.7–20.9	0.17 (41)

Abbreviations: SEE, SEM of the estimate; 95% CI, percentile bootstrap 95% confidence interval; Δ female, population estimated difference in clearance in female patients.

*Indicates estimate from BSA-normalized model.

parameter estimates included CL_{am}, VD, k_{12} , and k_{21} , and were 107 L/h, 5.62 L, 3.02 h⁻¹, and 4.99 h⁻¹, respectively (Table 2). Population pharmacokinetic parameters of plasma WR1065 included CL_{WR}, k_{34} , and k_{43} , and were 30.1 L/h, 55.3 h⁻¹, and 14.3 h⁻¹, respectively.

The same structural model was used for the whole blood amifostine and WR1065 concentration-time data. The whole blood amifostine pharmacokinetic parameter estimates included CL_{am}, VD, k_{12} , and k_{21} and were 135 L/h, 7.14 L, 4.16 h⁻¹, and 4.13 h⁻¹ (Table 2). Population pharmacokinetic parameters of WR1065 from whole blood data were 12.7 L/h, 25.1 h⁻¹, and 12.9 h⁻¹ for CL_{WR}, k_{34} , and k_{43} , respectively.

The plasma and whole blood estimates of IIV for amifostine clearance were 56% and 55%, respectively. The respective plasma and whole blood estimates of IIV for CL_{WR} in plasma and whole blood were 58% and 39%. The proportional components of residual variability for

amifostine and WR1065 in the plasma model were 0.14 (37%) and 0.19 (44%), respectively. The values for proportional residual variability in the whole blood model for amifostine and WR1065 were nearly identical, 0.13 (36%) and 0.19 (44%), respectively.

We considered BSA and weight as possible size-based covariates. BSA was found as a significant determinant of CL_{am} and VD, explaining 52% and 63% of the IIV, respectively (decrease in OFV, DOFV = –24; $P < 0.001$), in the plasma model (Fig. 2). A similar result was obtained in the whole blood model with BSA explaining 57% and 65% of the IIV for clearance and volume of amifostine, respectively (DOFV = –28; $P < 0.001$). Weight was also found to be a significant determinant of CL_{am} and VD in plasma and whole blood models (DOFV = –22 and –27; $P < 0.001$). However, this change in the objective function was smaller than that observed when comparing the base model to the BSA-normalized data. Moreover, considering

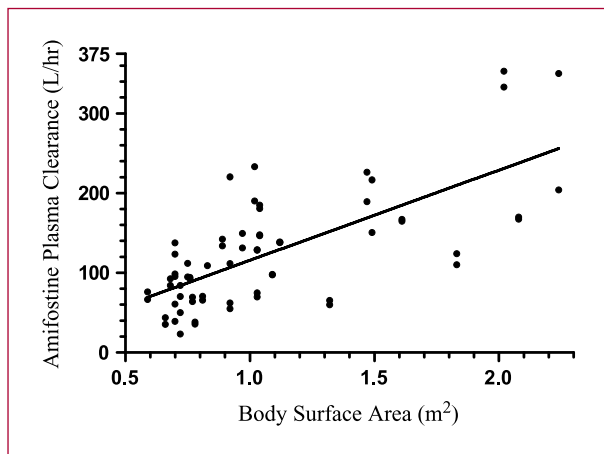


Fig. 2. Plot of amifostine plasma clearance versus BSA.

weight as a covariate explained 46% and 62% of the respective IIV in systemic clearance and volume of amifostine in plasma, which although significant was not as much of an effect as BSA. Thus, BSA normalization was incorporated in the base model and remained in all subsequent analyses of the population model.

The estimates of IOV for CL_{am} were 19% and 27% in the respective plasma and whole blood BSA-normalized models. Inclusion of IOV decreased the OFV by 32 and 71 in the respective plasma and whole blood models. The addition of IOV to the plasma model decreased the estimate of proportional residual error for amifostine from 0.19 (44%) to 0.13 (36%; Table 3). In the BSA-adjusted whole blood model, the addition of IOV decreased the estimate of proportional residual error for amifostine from 0.33 (57%) to 0.19 (44%). The BSA-normalized model with IOV was used to screen each additional covariate in both plasma and whole blood data.

Of the three GST polymorphisms evaluated, the *GST P1* polymorphism provided the most promising results. Addition of the *GST P1* polymorphism to the plasma model (homozygous versus variant) decreased IIV by 2.5%, but the change in objective function did not reach statistical significance (DOFV = -3.1; $P = 0.08$). None of the other GST polymorphisms resulted in significant decreases in measures of variability or improved diagnostic plots when evaluated in relation to amifostine or WR1065 pharmacokinetics in either the plasma or whole blood models.

In the covariate modeling, sex was found as a significant determinant of CL_{am} but not volume or CL_{WR} in the plasma model (DOFV = -7.4; $P = 0.007$). The estimated typical CL_{am} in the plasma model was 99 and 123 L/hr/m² in males and females, respectively (Table 2). The addition of sex to the model in whole blood data did not result in a decrease of the OFV or IIV. In addition, including sex as a covariate in the whole blood-based model did not change the estimate of residual proportional error for amifostine or WR1065 (Table 3).

The model-predicted and individual-predicted concentrations versus observed concentrations of amifostine/WR105 in plasma and whole blood using the final model are represented as scatter plots in Supplementary Figs. S1 and S2. Considering that the model predictions were symmetrically distributed around the line of identity, we conclude that the BSA-normalized model with IOV adequately describes amifostine and WR1065 disposition. The population pharmacokinetic concentration-time curves from the final models as well as observed values for amifostine and WR1065 concentrations are displayed in Fig. 3. The intercompartmental rate parameter estimates (k_{12} , k_{21} , k_{34} , and k_{43}) from the initial and final models are available in Supplementary Table S3.

Pharmacodynamic assessment. We sought to develop a model to investigate the relationships between plasma- and whole blood-derived amifostine and WR1065 pharmacokinetic parameters, and drug effect, namely hypocalcemia and PTH values. A total of 32 patients had baseline and follow-up ionized calcium measurements. Of these patients, 12 (38%) had a decrease in serum-ionized calcium to below 1 mmol/L (reference range, 1.12-1.31 mmol/L). Of these 32 patients, 31 received calcium chloride infusions either to prevent or as a result of hypocalcemia. Visual inspection of the change and duration of change in ionized calcium values did not reveal any relationships between this marker of drug effect and pharmacokinetics of amifostine and WR1065 in plasma or whole blood.

Baseline PTH values were available in 18 patients. All but one of the patients with measurements dropped below the level of detection for the assay (10 pg/mL), and only 7 patients had adequate sampling to determine a time to recovery; all of these patients recovered to normal values within 18 to 24 hours (Supplementary Fig. S4). An additional five patients had sampling out to 19 hours postamifostine, but had not yet recovered to the reference range. Visual inspection of the PTH measurements did not reveal any relationships between this pharmacodynamic marker and plasma or blood pharmacokinetics.

Table 3. Proportional residual error in plasma and whole blood pharmacokinetic models

BSA-normalized models	Amifostine Residual error (%)	WR1065 Residual error (%)
Plasma		
Without IOV	0.19 (44)	0.20 (45)
With IOV	0.13 (36)	0.19 (44)
With sex and IOV	0.19 (44)	0.13 (36)
Whole blood		
Without IOV	0.33 (57)	0.12 (35)
With IOV	0.19 (44)	0.13 (36)
With sex and IOV	0.19 (44)	0.13 (36)

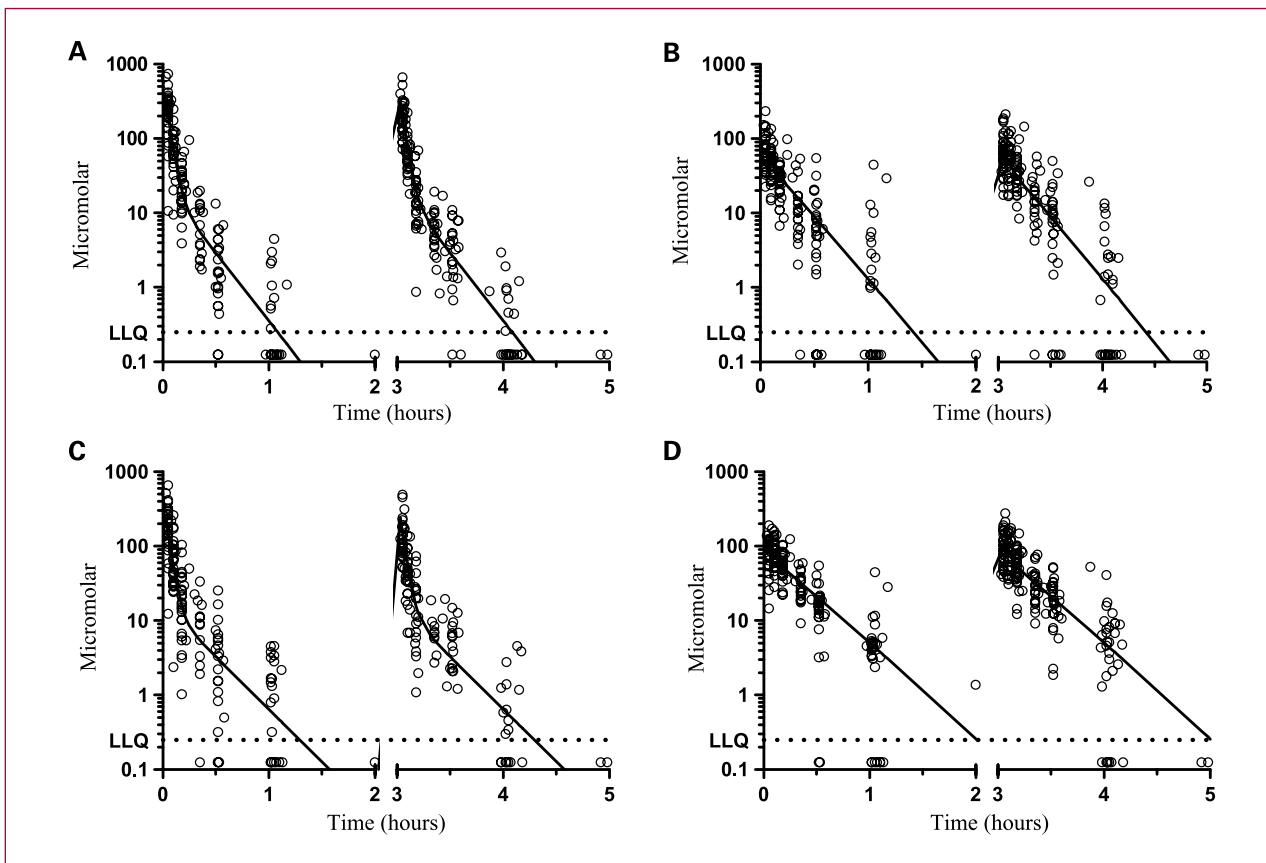


Fig. 3. Population-estimated concentration time curves (solid lines) and observed values (○) for plasma amifostine (A), plasma WR1065 (B), whole blood amifostine (C), and whole blood WR1065 (D). Observed amifostine and WR1065 concentrations below the LLQ were substituted with 0.125 ($1/2 \times \text{LLQ}$).

Limited sampling model. Data from 33 patients were used to create a pharmacokinetic LSM to enhance the ability to conduct studies of amifostine disposition in patient populations. The median biases for CL_{am} and CL_{WR} from the LSM with two samples (5 and 30 minutes) from one amifostine dose (occasion) were 3.6% (SD, 46) and 30.7% (SD, 60), respectively. When a third sample was added (1 hour) to the one-occasion sampling strategy, the bias estimates for CL_{am} decreased to 3% (SD, 52) but increased to 37% (SD, 52) for apparent WR1065 clearance.

When a second occasion was added to the two-sample strategy (total of four samples) the biases for CL_{am} and CL_{WR} were -1.24% (SD, 15) and -9% (SD, 46), respectively. For the strategy that required three samples on each of two occasions, the mean biases for CL_{am} and CL_{WR} were 0.2% (SD, 12.2) and 13% (SD, 24), respectively. Of the models evaluated, the LSM with two samples from two occasions and three samples from two occasions resulted in the lowest calculated biases and were further compared with each other. The estimates of individual pharmacokinetic parameters were not significantly different between these two models for both CL_{am} ($P = 0.8$) and CL_{WR} ($P = 0.9$).

For all of the limited sampling strategies evaluated, the individual parameter estimates for CL_{am} were not statisti-

cally significantly different from those obtained with the full data set. However, the estimates for CL_{WR} from the LSM with two and three samples from one occasion were significantly different when compared with those obtained from the full model ($P < 0.0001$ for both). Similarly, the estimates for CL_{WR} from the LSM with two samples from two occasions and three samples from two occasions were also significantly different from the parameters derived from the full data set with respective P values of 0.005 and 0.001.

Discussion

This is the first population analysis of amifostine and WR1065 pharmacokinetics in a pediatric population. This analysis used a validated bioanalysis technique to measure amifostine and WR1065 in both plasma and whole blood and took advantage of the availability of sampling from both plasma and whole blood to model the data from both analytic matrices (23). The analysis identified a large component of variability between subjects and occasions, indicating large differences in exposure among different patients given the same dosage. BSA was identified as the primary size-based covariate, explaining 52% and

57% of the variability in amifostine clearance in the plasma and whole blood models, respectively.

Gender was identified as a significant predictor of amifostine clearance in the plasma model. Possible explanations for the more rapid decline in plasma amifostine in female patients compared with male patients include the following: potential differences in metabolism; females may have a faster distribution of drug from serum to tissue; or there might be a slower redistribution back to blood from peripheral tissues. This finding was not confirmed when modeled in the whole blood data. Lacking a specific mechanism that explains these results, the differences in clearance between male and female children observed in the plasma data requires further evaluation in future investigations.

WR1065 has several potential pathways for elimination; it may undergo auto-oxidation and may form bonds with mixed disulfides. Among the potential pathways of elimination is conjugation with glutathione (16). We evaluated three functional polymorphisms in the glutathione pathway, none of which significantly explained variability in the pharmacokinetics of amifostine or WR1065. This may be because other pathways are available for the elimination of WR1065, or because with 25 patients with evaluable genotyping, this analysis was unable to elucidate all significant explanatory variables. Addition of *GSTP1* genotype as a covariate for systemic amifostine plasma clearance resulted in a trend, but was not statistically significant. Inclusion of *GSTP1* genotype in future investigations of amifostine and WR1065 pharmacokinetics may clarify the role of glutathione pathway polymorphisms in amifostine distribution.

We sought to evaluate the plasma- and whole blood-derived pharmacokinetic estimates with regard to their relationships with measures of the pharmacodynamic effects of amifostine, specifically, hypocalcemia and hypoparathyroidism. As this dosage of amifostine reliably resulted in hypocalcemia, many patients received calcium in a preventative manner, before amifostine. Administration of calcium can also influence PTH levels, potentially masking pharmacokinetic and pharmacodynamic relationships. Nearly all patients with PTH level measurements dropped below the level of quantitation for the clinical assay used. Thus, the measures of ionized calcium and PTH were insufficient to accurately model the pharmacodynamic effects of amifostine. These data highlight the difficulty of pharmacodynamic modeling in the complex milieu of the clinical environment and illustrate the need for early interim analyses to identify potential problems with study measures.

Overall, other than the WR1065 component, the plasma and whole blood models resulted in similar parameter estimates. Expression of alkaline phosphatase, the enzyme that catalyzes the conversion of amifostine to WR1065, on nucleated blood cells may have contributed to the slightly higher typical value of CL_{am} and the lower typical CL_{WR} in the whole blood (31). A previous investigation of amifostine concentrations in the whole blood, plasma,

and blood elements of five patients reported similar WR1065 concentrations in plasma, whole blood, and blood cells (32, 33), whereas our data suggested higher concentrations of WR1065 in the whole blood compared with plasma. These disparate findings may be related to differences in analytic procedures, such as the methods used to arrest the oxidation of WR1065. Our methods included optimizing perchloric acid concentrations and the immediate immersion of samples into ice to decrease auto-oxidation (23).

The lack of a validation cohort may be considered as a limitation to this investigation. However, the same structural model was used to separately fit data derived from plasma and whole blood sampling. Further investigations would be required to determine whether plasma or whole blood would provide more robust data in relation to drug effect. However, because the majority of estimates were similar in both matrices, either matrix is likely to be suitable for assessing drug distribution and effect.

As expected, the limited sampling strategy with the most sampling time points (total of six samples) resulted in the lowest measures of bias for amifostine clearance. The LSM with two samples (5 and 30 min) drawn from two dosing occasions resulted in a similarly low bias estimate for amifostine clearance and a lower bias estimate for CL_{WR} . In addition, the parameter estimates from the LSM with two samples from two occasions were not significantly different from the LSM with six samples. With 33% fewer samples required, the LSM with two samples from two occasions would be preferable in future pharmacokinetic investigations of amifostine. Although sampling from one occasion would likely provide accurate estimation of systemic amifostine clearance and volume, the estimation of CL_{WR} using one occasion sampling models resulted in a large bias compared with the full sampling model.

In summary, we have developed a population pharmacokinetic model of amifostine and WR1065 in pediatric patients with medulloblastoma. We analyzed for the effects of covariates on amifostine pharmacokinetics using a nonlinear mixed effects approach. In the present analysis, we identified BSA as a covariate that can be used prospectively in a model to predict amifostine clearance. This analysis identified a large component of interpatient variability in clearance of both amifostine and WR1065, which may have significant implications for this clinically important therapy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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