Time-dependent Pharmacodynamic Models in Cancer Chemotherapy: Population Pharmacodynamic Model for Glutathione Depletion following Modulation by Buthionine Sulfoximine (BSO) in a Phase I Trial of Melphalan and BSO

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Abstract

The development of time-dependent pharmacodynamic models in cancer chemotherapy has been extremely limited. A population approach was used to develop such a model to describe the effect of buthionine sulfoximine (BSO), via its active S-isomer (S-BSO), on glutathione (GSH) depletion in peripheral mononuclear cells. The Phase I trial utilized escalating doses of BSO, from 5 to 17 g/m², as a multiple infusion regimen. The population model consisted of a linear 2-compartment pharmacokinetic model coupled to an indirect response model. The indirect response model consisted of a GSH compartment with input and output rate processes that are modulated as a function of S-BSO and GSH concentrations. The model predicted the observed gradual depletion of GSH, a nadir at approximately 30 h after the last dose of BSO, and a return to baseline GSH levels. On the basis of an IC₅₀ estimate of about 1.6 μM for inhibition of γ-glutamylcysteine synthetase, the target enzyme of BSO, the population model predicted near identical GSH concentration time profiles over the dose range studied. Time-dependent pharmacodynamic models are seen as a powerful means to design dosing regimens and to provide a mathematical platform for mechanistic based models.

Introduction

Pharmacokinetic analyses have been a critical component of Phase I trials of anticancer drugs. Pharmacokinetic parameters are obtained to provide an initial characterization of drug disposition in patients and may also be used prospectively in dose escalation protocols (1, 2). Pharmacodynamic analyses have become a newer addition to the quantitative pharmacological data obtained in Phase I investigations (3–5). These pharmacodynamic analyses have utilized relationships based on single-timed pharmacological or toxicological end points. Most frequently relations are sought between the percentage decrease in an index of myelosuppression at the nadir and the AUC. The Sigmoid Eₘₐₓ model has been commonly used to describe these relationships, and in this context may be generally referred to as a discrete pharmacodynamic model because the temporal nature of the kinetics and action of the drug are not accounted for. It can be appreciated that drug-induced myelosuppression and biochemical effects have unique time dependencies that are amenable to mathematical description. In fact, a time-dependent model to describe the hematologic toxicity of etoposide has been presented recently (6). Such pharmacodynamic models, referred to as time-dependent or temporal pharmacodynamic models, may have a powerful role in characterizing drug action and toxicity, and may further serve as a means to design therapeutic regimens.

BSO² is a diastereoisomer (the S-BSO isomer is the active species) that has been shown to modulate concentrations of the endogenous intracellular tripeptide GSH through inhibition of γ-GCS, the rate-limiting step in the synthesis of GSH (7). GSH is the most abundant intracellular thiol, and in that capacity participates in numerous metabolic and protective functions. GSH is involved in the detoxication of xenobiotics by several mechanisms including oxidative-reductive processes that alter reactive functions; conjugative processes that may neutralize a reactive site and render the xenobiotic more water soluble, and an incompletely characterized influence on DNA repair (8). Anticancer drug resistance is associated with elevated tumor steady-state GSH content and an enhanced capacity to form GSH drug conjugates. Inhibition of GSH-drug conjugate formation through GSH depletion has been proposed as a therapeutic strategy (9).

A Phase I study of the combination of melphalan and BSO has been completed (10). As part of this protocol, two cycles of BSO were given as a multiple dose infusion in escalating doses. In the first cycle BSO was administered alone, whereas in the second cycle melphalan was incorporated into the regimen. Serial plasma S-BSO concentrations and GSH concentrations measured in PMN obtained during the first cycle provided the data necessary to develop a temporal pharmacodynamic model. In this communication, we focus on the derivation of the model and its potential applications.

Materials and Methods

Patient Demographics. Patients eligible for this study had a histological diagnosis of cancer and had exhausted the standard therapeutic options for their disease. They were over 18 years of age, with an Eastern Cooperative Oncology Group performance status of 0–2. They had adequate bone marrow (WBC count, ≥4,000/ml³; granulocyte count, ≥2,000/ml³; platelets, ≥100,000/ml³), liver (bilirubin, ≤1.5 mg/dl; aspartate aminotransferase, ≤5 times upper normal value), and kidney (serum creatinine, <1.5 mg/dl; creatinine clearance, ≥60 ml/min) function. Patients must not have been treated previously with i.v. melphalan, and were required to have recovered from all toxicities of prior treatment. Baseline clinical tests and patient monitoring throughout the study were standard for a Phase I trial and will be reported in greater detail elsewhere (10).

Drug Administrations and Plasma Sampling. BSO was supplied in 10-ml vials containing 100 mg/ml BSO in water for injection, USP, and was reconstituted in 50 ml 0.9% sodium chloride. The BSO regimen was designed to be administered i.v. at 12-h intervals as either a 10- or 30-min constant rate infusion for 6 doses. Actual drug administration times and infusion durations were recorded and used in the development of the model. Doses ranged from 5 to 17 g/m², the 4 patients at the 5 g/m² and 1 patient at 7.5 g/m² BSO dose levels were administered BSO as a 10-min infusion, whereas all other patients received BSO as a 30-min infusion.
The pharmacokinetics of BSO were investigated in a total of 22 patients at 5 dose levels; 5, 7.5, 10.5, 13, and 17 gm/m². Blood samples (5 ml) were drawn into heparinized tubes, centrifuged immediately at 4°C, and plasma was separated. Samples were obtained before treatment and at 5, 10, 15, 30, 45, 60, and 90 min, and 2, 4, 8, and 12 h after the start of the first infusion. Plasma was stored at -70°C until the time of analysis. Drug administration and pharmacokinetic sampling were performed in the Mary S. Schinagl Clinical Pharmacology Unit at Fox Chase Cancer Center.

**Measurement of BSO and GSH.** GSH was assayed in PMN based on a modification of the method of Griffith et al. (11), in which the rate of formation of the GSH conjugate of 5,5’-dithiobis(2-nitrobenzoic acid) was determined spectrophotometrically. Blood samples (5 ml) were drawn into heparinized tubes immediately before and 2, 4, 6, 8, 10, and 12 h after the first dose of BSO; sampling was continued periodically (usually three times daily) during the remainder of BSO treatment, and until 84 h after the last BSO dose. PMN were isolated by Ficoll-Hypaque gradient centrifugation [LSM (density, 1.080), Organon-Bioteknika, Durham, NC], pelleted, and stored dry at -70°C.

For analysis, PMN were thawed and then sonicated in 1 ml cold PBS using an ultrasonicator. Proteins were precipitated by the addition of 12% 5-sulfosalicylic acid (1:3) and pelleted after incubation for 1-4 h at 4°C. The reaction mixture consisted of 0.7 ml of 0.3 mM NADPH, 0.1 ml of 6 mM 5,5'-dithiobis(2-nitrobenzoic acid), 0.1 ml supernatant, 0.1 ml of 0.5 mM potassium phosphate (pH 7.5), and 0.5 unit of GSH reductase. Absorbance was monitored at 412 nm for 3 min at 25°C, and results are expressed as GSH content per mg of cytosolic protein. GSH concentrations were determined by reference to a standard curve that was run with each batch of samples. Protein concentrations were measured by the Bradford assay (Bio-Rad, Richmond, VA).

A recently developed HPLC method was used for the quantitation of the R- and S-BSO isomers (12). Plasma samples were prepared for analysis by addition of internal standard (L-norleucine), followed by ultrafiltration using disposable centrifugal filtration units (molecular weight cutoff, ~10,000; Amicon, Danvers, MA). All samples were derivatized with phenylisothiocyanate. The derivatized amino acids were separated by HPLC on an octadecyl column (Adsortosphere C18, 250 x 4.6 mm, 5-μm particle size) using a mobile phase of 0.14 mM sodium acetate, acetonitrile, triethylamine, and EDTA.

The column effluent was monitored at 254 nm, and quantification was performed using peak areas. The linear range for each diastereoisomer of L-(SR)-BSO was from 2 to 100 μg/ml in plasma.

**Pharmacokinetic and Pharmacodynamic Models.** A population pharmacokinetic model was used to describe the S-BSO plasma concentrations obtained after the first dose in 22 patients. The model consisted of a linear 2-compartment model with a constant coefficient of variation statistical model. The parameters associated with this model were used to simulate plasma S-BSO concentrations according to the actual multiple dose infusion regimen in each patient. The simulated plasma S-BSO concentrations were converted to PMN S-BSO concentrations based on the partition coefficient that was estimated in the model. The PMN S-BSO concentrations are then used in the pharmacodynamic model as follows:

\[
\frac{d}{dt} g_{sh} = k_f g_{sh} - k_g g_{sh}
\]  
(Equation 1)

\[
f_r = 1 - \frac{C_{pmn}}{IC_{50} + C_{pmn}}
\]  
(Equation 2)

\[
f_r = \exp(-t/R)
\]  
(Equation 3)

where g_{sh} = glutathione concentration (μmol/mg protein) in PMN, k_f = synthetic rate constant of g_{sh}, k_g = output rate constant of g_{sh}, C_{pmn} = S-BSO concentration in PMN, IC_{50} = S-BSO concentration that causes 50% inhibition of the maximal g_{sh} synthetic rate, and R = modulation constant for S-BSO output. The output process of GSH includes chemical degradation and its utilization in metabolic and protective functions. f_r is referred to the fractional inhibition of the GSH synthetic rate, and f_r is the fractional stimulation of the output rate of GSH. The value of f_r is an exponential function, is activated at times between 24 and 96 h, and is based on GSH concentrations (see "Discussion"). The final value of R was fixed, before estimation of k_g, IC_{50} and R, based on initial optimization studies.

The population pharmacokinetic/pharmacodynamic model was developed with the NONMEM software program (13). Simulations that utilized the final NONMEM parameter estimates and graphical presentation of the data were completed with the SimuSolv and S-Plus computer programs (14, 15).

The ABEC can be used as a measure of the cumulative drug effect, similar to the AUC, providing a measure of drug exposure. ABEC is the cumulative difference between the baseline PMN GSH concentration and the model-predicted GSH concentrations. ABEC values were used to probe the relationship between dose and number of doses and effect.

**Results**

Table 1 lists the population pharmacokinetic/pharmacodynamic model parameters. The population total clearance (CL_t), 7.12 liters/h, and volume of distribution at steady-state (V_{ss} = V_1 + V_2), 17.8 liters, for S-BSO were similar to values obtained by noncomparative analysis of individual patient data at each dose level (10). In the latter analysis the mean CL_t (± SD) was 8.5 (2.2) liters/h, and the mean V_{ss} was 19.4 liters (9.0).

Fig. 1 illustrates the observed and population model-predicted PMN GSH concentrations. The predicted GSH concentrations were generated at a BSO dose of 10.5 gm/m². On the basis of the low IC_{50} (i.e., 0.354 μg/ml or 1.6 μM), all BSO doses produced analogous GSH concentration-time profiles, and thus the predicted curve in Fig. 1 is representative of the population. Thus, at all BSO dose levels the fractional inhibition term (see f_r, Equation 2) is near zero, consistent with a maximum BSO effect, throughout much of the time course, and returns to a value of 1 in parallel with GSH levels returning to baseline. The time-dependent f_r term was activated at 24 h after initiation of BSO treatment, at a time corresponding to about 50% depletion of baseline GSH concentrations. This exponentially increasing function was based on enhanced utilization of available GSH caused by increased cellular redox stress (see "Discussion"). Modulation of GSH output ceased near the GSH nadir (~96 h), signifying a return to baseline activity. Assessment of the accuracy of the model predictions can be further examined in Fig. 2, where the observed versus predicted GSH concentration plot is shown. It can be seen that the model systematically underpredicts large observed GSH values that occurred at early time points (see Fig. 1). Barring these deviations, differences in observed and predicted GSH concentrations are small and random throughout the remainder of the GSH concentration-time profile.

After BSO treatment, GSH values tend to converge to nadir values at approximately 90 h after initiation of treatment. The ability of the model to predict GSH concentrations at each dose level can be noted in Fig. 3, in which the mean PMN GSH data at each dose level is shown. At the 5 and 7.5 gm/m² BSO dose levels, mean GSH values were generally underpredicted. At the three higher doses, representing 14 of 22 patients, the model predictions are in close agreement with the observed mean GSH data. The mean prediction errors (16), a function of the residuals, were negative at the two lower doses, positive at the three higher doses, and random throughout the remainder of the GSH concentration-time profile.

The population pharmacokinetic/pharmacodynamic model was developed with the NONMEM software program (13). Simulations that utilized the final pharmacodynamic model parameters are shown. The population total clearance (CL_t), 7.12 ± 0.43 (liters/h), and the volume of distribution (V_{ss}) = V_1 + V_2), 17.8 liters, for S-BSO were similar to values obtained by noncomparative analysis of individual patient data at each dose level (10). In the latter analysis the mean CL_t (± SD) was 8.5 (2.2) liters/h, and the mean V_{ss} was 19.4 liters (9.0).

**Table 1. Population model parameters for S-BSO pharmacokinetic-pharmacodynamic model**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value ± SE (unit)</th>
</tr>
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<tbody>
<tr>
<td>Total systemic clearance (CL_t)</td>
<td>7.12 ± 0.43 (liters/h)</td>
</tr>
<tr>
<td>Distributional clearance (CL_d)</td>
<td>21.8 ± 6.66 (liters/h)</td>
</tr>
<tr>
<td>Volume of the central compartment (V_1)</td>
<td>5.55 ± 0.69 (liters)</td>
</tr>
<tr>
<td>Volume of the peripheral compartment (V_2)</td>
<td>12.2 ± 0.95 (liters)</td>
</tr>
<tr>
<td>GSH synthetic rate constant (k_s)</td>
<td>0.0756 ± 0.0019 (μmol/mg-h)</td>
</tr>
<tr>
<td>GSH output rate constant (k_o)</td>
<td>0.0162 ± 0.0016 (h^{-1})</td>
</tr>
<tr>
<td>S-BSO concentration that produces 50% of maximal response (IC_{50})</td>
<td>0.354 ± 0.0511 (μg/ml)</td>
</tr>
<tr>
<td>PMN/plasma partition coefficient (R)</td>
<td>28.6 ± 33.9</td>
</tr>
<tr>
<td>Modulation constant for S-BSO output (Γ)</td>
<td>0.01 (h^{-1})</td>
</tr>
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</table>
ABEC values at all dose levels studied were nearly equivalent, consistent with the predicted GSH data shown (Figs. 1 and 3). Fig. 4 illustrates ABEC values at the 10.5 gm/m² BSO dose level for 1, 3, and 6 doses. An increase in the number of BSO doses increases the ABEC values.

Discussion

The proposed pharmacodynamic model that describes GSH depletion by BSO is a member of a general class of models recently referred to as physiological indirect response models (17). These models can characterize the pharmacodynamics of diverse drug classes that have in common a time lag between peak plasma or tissue concentration and peak effect. The physiological designation for this class of model indicates that the models have the potential to provide a mechanistic account of drug action at the cellular level. However, caution is warranted in consideration of the current model as mechanistic.

The biochemical pathways that detail the synthesis and degradation of GSH are multistep, and for the synthesis of GSH include reactions for the precursor amino acids (glutamine, cysteine, and glycine). BSO, and specifically S-BSO, inhibits γ-GCS that catalyzes the formation of γ-GC from its precursors, glutamine and cysteine. Although the latter is considered the rate-limiting step in GSH production, GSH-transferase catalyzed reaction of γ-GC with glycine is requisite for GSH synthesis. The current model has treated the multistep synthesis of GSH as a single zero-order rate process, due to the lack of information on the time course of the intracellular precursors. Recent HPLC methods have been proposed to measure various components of the GSH pathway (18, 19). Typically, these involve derivatization by fluorophores, and in one case allows simultaneous and specific quantitation of γ-cysteine, γ-GC, and GSH (18). The current non-protein thiol assay, although nonspecific, is readily adaptable to patient samples. Improvements in model specificity could, nonetheless, be obtained by development of a specific assay for GSH in PMN samples. Measurement of one or more amino acid precursors and GSH would enable rate equations specific for individual components of the GSH synthesis pathway to be represented in the model. It should be appreciated that not every species in the reaction pathway be measured because some species can be simulated by the model. The utility of such an endeavor has to be evaluated based on the feasibility of measuring the components of interest in clinical samples against the added information translating into pharmacodynamic models of improved accuracy and less assumptions compared to the current approach. Certainly, some experimentation should be pursued to test the rationale of more specific cellular pharmacodynamic models.

Modulation of the output process for GSH (see Equation 3) is based on the potential of an increased demand or utilization of GSH once it is depleted. The normal protective functions of GSH, such as combining with oxygen radicals, will be compromised in the presence of BSO. The prevalence of oxygen radicals can result in macromolecular damage of normally active detoxification enzymes, placing an even greater demand on the available GSH. Thus, the compromised protective pathways result in an increased demand (stimulation) of GSH utilization, and in terms of the model, its output. Consistent with this we have observed induction of γ-GCS mRNA expression after treatment of HT29 adenocarcinoma cells with hypoxia or with mitomycin C (20). Addition of a modulation process to GSH output (fₙ, Equation 3) was deemed necessary because indirect response models that only contained the inhibition term (fₐ term, Equation 2) resulted in a significantly poorer value for the optimization function than an indirect response model that includes the stimulation term. Further, approximately 100-fold smaller IC₅₀ₐ were obtained with only inhibitory models, IC₅₀ₐ much smaller than the approximate 0.5 μM (0.111 μg/ml) values reported in vitro (21), and in agreement with the model estimate (see Table 1). At present, modulation of the output term has to be viewed functionally rather than strictly mechanistically.

The indirect response model for GSH depletion utilizes a population approach to obtain model parameters in a one-step procedure. Thus, data from all dose levels is fit simultaneously to provide a single set of model parameters. There was a bias in overpredictions at the 2 lower doses, yet both underpredictions and overpredictions are observed at the remaining doses. Overall, the model characterizes the salient features of GSH depletion caused by S-BSO and the expected return to baseline GSH concentrations. Because PMN GSH concentrations were not measured after 144 h, the model-predicted return to baseline, at ~200 hr, will require further verification. A Phase II trial of BSO in combination with melphalan is planned that will measure serial PMN GSH concentrations for up to 300 h after termination of

\footnotesize{\textsuperscript{3}A. Hageboutros, P. Ford, K-S. Yao, and P. J. O'Dwyer. Induction of expression of DT-diaphorase in colon adenocarcinoma cell lines by mitomycin C through the AP-1 and NF-κB elements. Submitted for publication.}

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![Fig. 1. Observed (●) and model-predicted (——) GSH concentrations in PMN in 22 patients receiving between 5- and 17-gm/m² doses of BSO. Each patient received six doses of BSO designed to be administered every 12 h as either a 10- or 30-min continuous infusion. Predicted concentrations were obtained from the population pharmacodynamic model described in "Materials and Methods."](cancerres.aacrjournals.org)
PHARMACODYNAMIC MODEL FOR BSO

BSO treatment. This data will be used to validate and, if necessary, revise the current model. Any revision would occur in the values of the model parameters rather than in the structure of the indirect response model.

The utility of this and other temporal pharmacodynamic models in cancer chemotherapy are multifold. Understanding the time-dependent changes in drug action, such as enzyme inhibition, and drug toxicity, such as myelosuppression, provides critical information needed to design rational chemotherapeutic regimens. The magnitude and timing of drug doses to produce a specific effect, such as degree of enzyme inhibition, and when treatment may be repeated, can be ascertained from time-dependent models. Discrete pharmacodynamic models based on an $E_{max}$ model and utilizing the AUC of the drug are only able to focus on a single response element, such as the nadir of myelosuppression. Two time-dependent pharmacodynamic end points that could be used to design chemotherapeutic regimens are the

Fig. 3. Mean observed (■) and model-predicted (—) GSH concentrations in PMN at each dose level. a, 5 gm/m²; b, 7.5 gm/m²; c, 10.5 gm/m²; d, 13 gm/m²; e, 17 gm/m². Predicted concentrations were simulated from the population pharmacodynamic model parameters based on administration of 6 doses of BSO every 12 h as either a 10- or 30-min continuous infusion.
duration of the response (e.g., time from line to nadir to baseline return) and the ABEC. As shown in Fig. 4, ABEC values are a function of the number of BSO doses, and a more profound and long-lasting depletion of GSH is anticipated with the current regimen than with a multiple dose regimen of for example, three doses. Simulated ABEC values can also be derived for other viable BSO dosing strategies such as continuous infusions. Other time-dependent parameters that can assist in optimizing treatment are the time to nadir and the time of return to baseline. In the BSO-GSH model, the nadir is reached at about 90 h, which suggests the time of treatment with a concomitant cytotoxic agent. During the second cycle of BSO, melphalan was administered at 48 h (time of fifth BSO infusion), which in light of the results may not have been optimal. Return to baseline GSH values occurs at about 200 h, which could suggest a time of retreatment. It must also be noted that these observations have been made in PMN; values in tumor were in general similar to those at nadir points (10), but the kinetics in tumor may differ.

Initial formulation of time-dependent pharmacodynamic models will undoubtedly be confined to Phase I investigations where intensive blood sampling can be accomplished. However, infrequent samples collected in a Phase II trial can be used to update or revise the model developed in Phase I. Individual patient Bayesian parameter estimates that incorporate pertinent covariates can be obtained from a population model derived in Phase I and used in dosage regimen design. Subsequently, model-derived variables such as the ABEC can be used to adjust drug dosage and enable pharmacodynamically guided dosing to be achieved.

In conclusion, a population indirect response pharmacodynamic model has been developed to characterize the time dependence of GSH depletion after treatment with BSO. Further refinements may be realized by application of a specific HPLC method for GSH and precursors, collection of GSH concentrations for longer times, and extension of the model to predict GSH depletions in tumors. The model is representative of time-dependent pharmacodynamic models, a class of models that has not been developed previously for anticancer drugs. These models may have direct impact on pharmacotherapy and provide the basis for mechanistic-based cellular pharmacodynamic models.

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References

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