Prospective Evaluation of a Model for Predicting Etoposide Plasma Protein Binding in Cancer Patients

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ABSTRACT

Etoposide protein binding in cancer patients is variable and has been related mathematically to a linear model consisting of serum albumin and total bilirubin [percentage unbound = (1.4 x total bilirubin) - (6.8 x albumin) + 34.4]. In this prospective evaluation of the model, plasma samples were obtained following the administration of etoposide in 31 patients, and the unbound percentage (% unbound) of etoposide in plasma was determined by equilibrium dialysis. The mean measured % unbound was 15.3 ± 11.6 (SD), and the mean model predicted % unbound was 16.7 ± 10.1. The relation between predicted and measured etoposide % unbound was highly correlated (r² = 0.92, P = 0.001). The model was precise but with a slight bias toward overpredicting % unbound (mean prediction error, 1.36%; 95% confidence interval, 0.09 to 2.6%). In patients with abnormal total bilirubin (i.e., >1.5 mg/dl) or with hypalbuminemia (i.e., <3.3 g/dl), the model was both precise and unbiased. These results demonstrate that etoposide % unbound can be predicted using serum albumin and total bilirubin. This model should be useful in prospectively identifying patients at increased risk of experiencing altered pharmacological effects due to altered protein binding of etoposide.

INTRODUCTION

Etoposide is widely used to treat a variety of solid tumors and leukemias in adults and children (1). It is one of the few anticancer drugs that is extensively (>90%) bound to plasma proteins. The unbound fraction of a drug in plasma generally correlates better with pharmacological effects than does the total drug because primarily free drug is available for membrane transport and interaction with receptors or target molecules (2). A model that predicts % unbound, which explained 93% of the interpatient variability in etoposide plasma protein binding, was highly correlated (r = 0.92, P = 0.001). The model was precise but with a slight bias toward overpredicting % unbound (mean prediction error, 1.36%; 95% confidence interval, 0.09 to 2.6%). In patients with abnormal total bilirubin (i.e., >1.5 mg/dl) or with hypalbuminemia (i.e., <3.3 g/dl), the model was both precise and unbiased. These results demonstrate that etoposide % unbound can be predicted using serum albumin and total bilirubin. This model should be useful in prospectively identifying patients at increased risk of experiencing altered pharmacological effects due to altered protein binding of etoposide.

MATERIALS AND METHODS

Eligibility and Treatment Protocol. Patients with histologically confirmed diagnoses of carcinoma of the stomach, pancreas, or hepatobiliary system or of unknown origin who had failed standard treatment or for whom there was no standard therapy were eligible for the clinical study. All patients signed statements of informed consent that met institutional and federal guidelines.

Twenty-four-hour urine creatinine clearance, serum albumin, and total bilirubin were determined in all patients before etoposide therapy began. Patients received etoposide, 100 mg/m² in 250 ml of 0.9% sodium chloride, by a 60-min infusion every other day for up to three doses (on days 1, 3, and 5). In addition, all patients received cisplatin, 70 mg/m², on day 1.

Sample Collection and Drug Analysis. Blood samples were collected in heparinized tubes immediately before the etoposide infusion and serially at 2, 6, 7, 16, 24, and 48 h after the end of the etoposide infusion. Samples were centrifuged and plasma was separated and frozen at −20°C until analysis.

Etoposide Protein Binding Method. The unbound fraction of etoposide in each plasma sample analyzed for total etoposide was determined utilizing an equilibrium dialysis technique with tritiated etoposide (6). Tritiated etoposide was obtained from Moravek Biochemical (Brea, CA) and had a specific activity of 1 Ci/mmol. The radiochemical purity of the radiolabeled etoposide, determined by liquid scintillation counting of fractions after high pressure liquid chromatography (9), was 96%.

Plasma samples were dialyzed against an equal volume of isotonic 0.134 M phosphate buffer at pH 7.4 (Sorensen's buffer) and 37°C for 6 h. The unbound fraction of etoposide was calculated from the ratio of the disintegrations per minute of [³H]etoposide in the buffer to the disintegrations per minute in an aliquot of plasma using an external standardization method for quench correction then expressed as a percentage. The correction for volume shift was made by the method of Huang (10). Correction for radiochemical purity was made by the method of Bjornsson et al. (11).

Mathematical Model. We previously characterized the plasma protein binding of etoposide in a population of 17 patients with cancer (6) and performed a multivariate analysis to assess the relationship between etoposide protein binding and measures of renal and hepatic function. From that analysis we determined that etoposide % unbound was related to total bilirubin and serum albumin (r² = 0.93), as described by the equation

% unbound = (1.4 x total bilirubin) - (6.8 x serum albumin) + 34.4

This equation represents the mathematical model that was prospectively tested in the study.

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The performance of our model was also compared to the performance of an absolute standard or naive predictor (i.e., one that makes the same prediction), as described by Sheiner and Beal (12). The mean of the measured etoposide % unbound for the respective population under analysis was used as the naive predictor for this comparison.

Statistical analysis was performed with the MINITAB statistical package (release 5.1.3) as implemented on a DEC VAX 8600 (13). The *a priori* level of significance was set at $P < 0.05$.

**RESULTS**

Thirty-one adult patients (7 women and 24 men) with cancer were studied. The median age was 61 years, with a range of 37 to 82 years. Twenty-five patients had pancreatic, hepatobiliary, or other upper gastrointestinal tumors; six had tumors of unknown origin. Laboratory values in this patient group are similar to those in the patient population in which our mathematical model was developed. Creatinine clearance measured in 27 patients was 71.9 ± 35.6 ml/min/1.73 m$^2$. The mean ± SD for total bilirubin and albumin was 3.5 ± 5.9 mg/dl (range, 0.3–21.5 mg/dl) and 3.3 ± 0.4 g/dl (range, 2.2–4.0 g/dl), respectively.

The mean measured etoposide % unbound in these 31 patients was 15.3% ± 11.6% (range, 5.3 to 45.8%). The total etoposide concentrations in this group of samples ranged from the lower limits of detection of our assay (~0.25 µg/ml) to 28.5 µg/ml. The actual values of total bilirubin and serum albumin for each patient were used to estimate the percentage of unbound etoposide according to our previous model (see equation in "Materials and Methods"). The mean calculated percentage of unbound etoposide was 16.5% ± 10.0% (range, 7.6 to 46.1%). In Fig. 1, the predicted (calculated) percentage of unbound etoposide is plotted versus the measured unbound percentage; the dashed line represents the line of identity. The slope of the regression line is 0.833, which is not statistically different from the line of identity. The intercept is 0.03, which is not statistically different from 0 ($t$ test; $P > 0.1$).

The performance characteristics of our mathematical model are listed in Table 1. For the entire population the model had a positive bias. The precision of the model as measured by the RMSE was 3.6%, or approximately 24% of the measured unbound percentage. This level of precision is good (i.e., relatively low) when compared to the wide range of measured etoposide % unbound (coefficient of variation, 75%).

The model had a statistically positive bias in 21 patients with normal total bilirubin (i.e., total bilirubin ≤ 1.5 mg/dl); the RMSE (precision) was 3.5% or approximately 38% of the mean unbound percentage. However, when the model was applied to estimating the unbound percentage in ten patients with abnormal total bilirubin (i.e., total bilirubin > 1.5 mg/dl), no statistically significant bias was noted, and the RMSE (precision) was 3.8%, or 14% of the mean unbound percentage for that subgroup.

The entire population was divided into subgroups based on a serum albumin of 3.3 g/dl, and the same analysis was performed. The value for albumin was chosen to divide the population into subgroups with approximately equal numbers of patients in each. The model performed better (i.e., bias and precision) in the subgroup with albumin ≤ 3.3 g/dl than in the subgroup with albumin > 3.3 g/dl (see Table 1).

Summarized in Table 2 are results of the evaluation of the performance of the model relative to that of an absolute standard. The mean percentage of unbound etoposide for each subgroup (see Table 1) served as the absolute standard (i.e., naive predictor). For each subgroup evaluated the model was statistically more precise than was the naive predictor.

**DISCUSSION**

Initial studies from our laboratory reported that etoposide % unbound is approximately 5% and that etoposide plasma protein binding in patients with cancer can be significantly different from that in normal volunteer plasma and is highly variable (6 to 37%) (6). We have also shown that hematologic toxicity correlated with systemic exposure to unbound etoposide. A model to assist clinicians in identifying patients who may have altered etoposide protein binding may reduce the risk of patients.
developing severe hematological toxicity due to increased exposure to unbound etoposide. In this prospective study of a mathematical model to predict the percentage of unbound etoposide, we have shown that this model, which utilizes serum albumin and total bilirubin, can be used to estimate the percentage of unbound etoposide. The model was originally developed by performing a multiple stepwise regression analysis of the percentage of unbound etoposide versus various demographic and biochemical variables in a separate population of patients with cancer (6).

The ME for the mathematical model in all patients (n = 31) was 1.4% (95% confidence interval, 0.09–2.62%), indicating a slight but statistically significant bias toward overestimation of etoposide % unbound. However, no statistically significant bias was observed when the model was evaluated in a subgroup (n = 10) of patients with elevated bilirubin (range, 1.5–21.5 mg/dl) or in a subgroup of patients (n = 16) with hypoalbuminemia (range, 2.2–3.3 g/dl). Thus, in patients with hyperbilirubinemia or hypoalbuminemia, a knowledge of the patient’s total bilirubin and serum albumin will allow one to predict etoposide % unbound without bias.

The role of protein binding on etoposide disposition in patients with liver dysfunction has recently been reported (14). In this study, the mean etoposide systemic clearance was not different in patients with elevated bilirubin; however, the mean clearance of unbound etoposide was significantly lower and the % unbound was significantly greater. This study suggested that patients with hyperbilirubinemia, many of whom had hypoalbuminemia, have a higher exposure to unbound (“active”) etoposide, providing an example of the potential importance of identifying patients at risk of aberrant protein binding.

When the model was evaluated in a subgroup of patients (n = 21) with total bilirubin of less than 1.5 mg/dl (i.e., normal values), the bias was statistically significant, although the percentage prediction error

\[ \text{% PE} = \frac{P - M}{M} \times 100 \]

was greater than 50% in only 6 of 21 patients. Two of the 15 patients with a serum albumin of greater than 3.3 g/dl had a prediction error greater than 50%; both of these patients were also in the group of six patients with normal total bilirubin with prediction errors greater than 50%. The prediction error was greater in these patients because the measured percentage unbound in these six patients with normal total bilirubin was low compared to the percentage unbound calculated by the model. The greatest prediction error in the entire population was in a patient with a bilirubin of 0.3 mg/dl, a serum albumin of 3.5 g/dl, a measured percentage unbound of 5.3%, and a predicted percentage unbound of 11% (prediction error, 107%). In patients with normal total bilirubin and albumin, factors unaccounted for in this model yield variability in etoposide binding, leading to an overestimate of the percentage unbound.

Sheiner and Beal suggested that, when evaluating the predictive performance of a model, one might want to determine if the model performs better than a method that makes the same prediction in all cases (12). The naive predictor used in this approach is typically the average value for the measured values. One then calculates the precision of the model relative to the naive predictor. In our case the model was statistically more precise than was the naive predictor. Thus, use of the mathematical model will provide a more precise estimate of etoposide % unbound than will use of a population average percentage unbound.

As more data become available describing the concentration-effect relationship (i.e., pharmacodynamics) for etoposide, the use of etoposide serum concentrations to adjust etoposide dosage will become more widespread (15, 16). Typically only total etoposide concentrations are available to evaluate pharmacodynamics and adjust doses; however, as with other highly protein-bound drugs such as phenytoin, certain clinical situations (e.g., hyperbilirubinemia, hypoalbuminemia) may alter protein binding and increase the concentration of the free (active) drug. With phenytoin, one may use a nomogram or mathematical equation to adjust the observed total concentration and estimate the concentration of unbound drug (17). The mathematical model described in the present study represents a comparable approach for etoposide, using serum albumin and total bilirubin of a patient to estimate the % unbound and the unbound concentration, given a total etoposide concentration.

Results of the present study suggest that this model performs best in patients with elevated total bilirubin or decreased albumin. Since this is the population of patients most likely to have aberrant plasma protein binding of etoposide, this model will be clinically relevant. The ultimate application of this method of estimating etoposide protein binding will require further study of the pharmacodynamics of etoposide as it relates to total and unbound drug concentrations.

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REFERENCES

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