The Effect of an Individual’s Cytochrome CYP3A4 Activity on Docetaxel Clearance

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ABSTRACT

Docetaxel is a chemotherapeutic agent effective in the treatment of various solid tumors. Patients given a standard dose of docetaxel exhibit wide interpatient variation in clearance (CL) and toxic effects. Docetaxel undergoes metabolism by cytochrome CYP3A4. Thus, interpatient variability in CYP3A4 activity may account in part for differences in toxicity and CL. Twenty-one heavily pretreated patients with metastatic sarcomas received docetaxel (100 mg/m²). Hepatic CYP3A4 activity in each patient was measured by the [14C-n-methyl]erythromycin breath test (ERMBT). Blood samples were taken at selected times over the next 24 h for pharmacokinetic analysis. Phenotypic expression of hepatic CYP3A4 activity measured by the ERMBT varied over 20-fold (administered 14C exhaled in 1 h: mean, 2.53%; range, 0.25–5.35%), which is similar to a normal control population. CL of docetaxel varied nearly 6-fold (mean, 21.0 liters/h/m²; range, 5.4–29.1 liters/h/m²). The ERMBT was the best predictor of CL when compared with serum alanine aminotransferase, albumin, alkaline phosphatase, or serum α-1-acidic glycoprotein. The natural log of ERMBT accounted for 67% of the interpatient variation in CL. Multivariate analysis showed that the natural log of ERMBT and albumin together accounted for 72% of the interpatient variation in CL. The greatest toxicity was seen in patients with the lowest ERMBT. Hepatic CYP3A4 activity is the strongest predictor of docetaxel CL and accounts for the majority of interpatient differences in CL. Patients with low CYP3A4 activity are at risk for having decreased CL and may thus experience increased toxicity from docetaxel. Those with high activity may be receiving a suboptimal dose. By measuring CYP3A4 activity, the ERMBT may be clinically useful in tailoring doses of CYP3A4 substrates, such as docetaxel, in certain individuals.

INTRODUCTION

Patients and healthy individuals alike display significant differences in terms of efficacy and adverse effects after exposure to many drugs. An identical dosage of a drug can result in widely different concentrations of the therapeutically active compound or metabolites (1). The presence of genetic variability in the activity of enzymes involved in drug metabolism could lead to clinically important variations in efficacy or adverse effects for some drugs.

The cytochrome P450 system is a family of heme-containing enzymes responsible for the metabolism of nonendogenous substances, such as drug molecules and toxins (2). Specific isoenzymes are designated by the prefix “CYP,” and to date, over 30 isoenzymes have been purified, cloned, sequenced, and characterized in humans. CYP3A4 is found in the liver and small bowel. The CYP3A4 enzyme displays large interpatient differences in both content and catalytic activity in humans (3). These differences exist even in the absence of medications known to induce or inhibit the enzyme and are thus likely to reflect genetic variability (3).

The ERMBT specifically measures the in vivo activity of hepatic CYP3A4 (3, 4) and is therefore ideally suited for drugs given i.v., such as docetaxel. This test is based on the fact that CYP3A4 is the major enzyme responsible for erythromycin metabolism, liberating a carbon atom that is exhaled as carbon dioxide. After injecting a trace dose of radiolabeled erythromycin, the rate of radiolabeled carbon dioxide exhaled is measured. This test has been validated as a specific measure of CYP3A4 activity (3).

In vitro studies suggest that CYP3A4 is the major enzyme involved in docetaxel metabolism (5). Docetaxel is excreted in the feces with less than 8% excreted unchanged (6). It is highly bound to plasma proteins including AAG. Pharmacokinetic data show significant variation in CL between patients (7). Decrease in total body CL is associated with increased frequency and severity of side effects (8). Mutivariate analysis demonstrates that interpatient variability of CL is predicted by body surface area, AAG plasma level, and elevated hepatic enzymes; however, these parameters do not account for total variability (7). Because docetaxel binds chiefly to AAG, high levels of this protein presumably limit the free fraction of docetaxel available for CL in the liver. Due to lower CL of docetaxel in patients with elevated transaminases, it is currently recommended in the United States package insert that patients with an ALT > 1.5X

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3 The abbreviations used are: ERMBT, erythromycin breath test; AAG, α-1-acidic glycoprotein; ULN, upper limit of normal; ALT, alanine aminotransferase; ln ERMBT, natural log of ERMBT; GCRC, General Clinical Research Center; CL, clearance.
the ULN concomitant with alkaline phosphatase > 2.5 × ULN or bilirubin > ULN do not receive this drug. The European Summary of Product Characteristics alternatively recommends a dosage reduction of 25% in patients with concomitant elevations of ALT and alkaline phosphatase.

Previous studies have shown modest activity for docetaxel in patients with advanced sarcomas (response rates of 17% and 18%), thus we decided to try this agent in our sarcoma population (9, 10). We hypothesized that variability in CYP3A4 activity accounts for interpatient differences in docetaxel CL and toxicity. Thus, the ERMBT was used to measure CYP3A4 activity in sarcoma patients receiving docetaxel (Fig. 1).

**PATIENTS AND METHODS**

**Study Population.** Twenty-one patients with measurable, metastatic sarcomas of bone or soft tissue origin were entered into our trial. Eighteen patients had soft tissue sarcomas, whereas three had bone sarcomas. Twelve patients were men, and nine were women (age range, 19–72 years). Thirteen of the patients with soft tissue sarcomas had received extensive prior chemotherapy, whereas five patients with gastrointestinal stromal sarcomas had not received extensive prior chemotherapy. Two of the three patients with bone sarcomas had also received extensive prior chemotherapy. All patients gave written informed consent, and the study was approved by the Protocol Review Committee of the Cancer Center and the Review Committee of the GCRC, as well as the University of Michigan Institutional Review Board. All patients had normal hematological values and liver chemistries within the limits recommended for the use of docetaxel at the time of admission (normal total bilirubin, alkaline phosphatase < 5 × the ULN, and serum transaminase levels < 1.5 × the ULN). Concurrent use of drugs known to alter CYP3A4 activity such as imidazole antifungal agents (itraconazole), macrolide antibiotics (erythromycin), antiseizure medications (phenobarbital and phenytoin), and rifampin were specifically avoided. Other exclusion criteria were concurrent radiotherapy, active infection, pregnancy or lactation, and allergy to erythromycin.

**Study Schema and Procedures.** Three ERMBTs were administered to each patient at the GCRC. The test was done on three occasions in case either the dexamethasone premedication or the chemotherapy introduced variability. The first study served as a baseline and was done before admission for docetaxel (range, 1–19 days before admission). The second study was done 24 h after dexamethasone premedication was initiated and 1 h before the infusion of docetaxel. The third study was done 3 h after the infusion of docetaxel had been completed. A total of 3 μCi (<0.1 mmol) of [14C-N-methyl]erythromycin obtained from Metabolic Solutions, Inc. (Nashua, NH) was given i.v. Exhaled carbon dioxide was trapped in a solution of hyamine hydroxide, ethanol, and a blue indicator before and 20 min after the injection. The percentage of 14C exhaled/h = 49.496 × (percentage of 14C exhaled/min at 20 min − baseline counts) (11).

Docetaxel was administered at 100 mg/m² i.v. over 1 h. Each patient received the following standard premedications: (a) dexamethasone (8 mg, p.o.) every 12 h starting the day before infusion and continuing for 5 days; and (b) granisetron (2 mg, p.o.) 30 min before the infusion. Blood was drawn for pharmacokinetic analysis over 24 h with specimens at optimal sampling times: just before the end of infusion and at 0.25, 0.75, 3.00, 6.50, and 24.00 h after the end of infusion (12). Case report forms documented actual sampling times as well as the actual beginning and end times of the infusion. Docetaxel was assayed from frozen plasma samples using reverse-phase high-performance liquid chromatography (13). All samples were assayed at the same time. A Bayesian criterion was used to calculate the docetaxel plasma concentration area under the curve based on measured drug levels and population pharmacokinetic parameters using NONMEM software (12, 14). The area under the curve was computed as dose/CL. CL was directly estimated by fitting the model (12, 14). Patients had baseline liver chemistries and serum AAG levels drawn. One patient did not have a baseline serum ALT recorded, and the two patients had insufficient sample to determine the AAG level. Baseline and weekly hematological counts were checked. Granulocyte-colony stimulating factor was not given during this first cycle.

Subsequent cycles of chemotherapy were given every 3 weeks if the patient was judged by us to be deriving clinical benefit. The pharmacokinetic data were obtained only during the initial cycle. Treatment was stopped if the disease progressed, if intolerable toxicities developed, or at the patient’s request. Radiological evaluation of patients was done at the discretion of the treating physician to document clinical response or progression.

**Statistical Analysis.** Although the distribution of the ERMBT in a large sample of normal controls is positively skewed, the distribution in this small sample is approximately normal. The paired t test was used to compare the breath test values obtained at the three time points. Pearson’s correlation coefficient (r) further describes the strength of the linear relationship and is presented along with the P. Nonparametric analogues to these test procedures were conducted for confirmation but are not reported.

CL, as determined by pharmacokinetic modeling, is approximately normal. The association between CL and patient characteristics was evaluated using simple and multiple least-squares regression models. Variables considered as potential predictors of CL in this model included ERMBT, AAG, albumin, ALT, and alkaline phosphatase. A natural log transformation was applied if indicated by univariate residual plots. For instance, the relationship between the ERMBT and CL was not linear over the entire range of values, particularly at the lowest ERMBT values, and the low ERMBT values had a better fit when in ERMBT was used. The best model for predicting CL was determined from the set of predictors using a step-wise procedure based on the largest F
Table 1  Variability in breath tests and docetaxel clearance

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERMBT #1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.41</td>
<td>1.08</td>
<td>0.125–4.86</td>
</tr>
<tr>
<td>ERMBT #2</td>
<td>2.53</td>
<td>1.17</td>
<td>0.41–5.35</td>
</tr>
<tr>
<td>ERMBT #3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.25</td>
<td>0.93</td>
<td>0.53–4.37</td>
</tr>
<tr>
<td>Docetaxel CL&lt;sup&gt;c&lt;/sup&gt;</td>
<td>20.42</td>
<td>6.30</td>
<td>5.4–29.1</td>
</tr>
</tbody>
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<sup>a</sup> ERMBT = percentage of 14C exhaled/h.
<sup>b</sup> Significantly lower than ERMBT #2 (P = 0.0047).
<sup>c</sup> Docetaxel CL is given in liters/h/m².

The interpatient variability in ERMBTs and docetaxel CL is evident in the range of values obtained, as shown in Table 1. The ERMBT varied >20-fold, largely because a few patients had very low breath test results. The CL of docetaxel varied >5-fold among these patients. These ERMBT data exhibit similar variability to that seen in 107 normal controls studied previously (ERMBT mean = 2.56; SD, 1.06; range, 0.77–6.15). There was excellent correlation between ERMBT results on all three occasions (r = 0.81–0.92, P < 0.0001). Thus, there is no evidence for a significant effect of dexamethasone premedication on the ERMBT. There is a tendency for the test value to fall modestly after docetaxel administration (ERMBT #3 versus ERMBT #2, P = 0.0047), likely reflecting competition between erythromycin and docetaxel for metabolism by CYP3A4. The ERMBT #2 was used in subsequent analyses.

Fig. 2 demonstrates the relationship between CL and the phenotypic measurement of CYP3A4. As expected, those patients with reduced CL had the lowest ERMBT results. As mentioned in the statistical section, the low ERMBT values had a better fit when ln ERMBT was used, with r² = 0.67, P = 0.0001. Even if the ERMBT was not log-transformed, the relationship between CL and the ERMBT remained strong (r² = 0.49, P = 0.001). Albumin was also a good predictor of CL (r² = 0.46, P = 0.001). Alkaline phosphatase was not as strong a predictor of CL. One patient with osteosarcoma had an exceedingly high alkaline phosphatase (5769) from bone rather than liver origin and was therefore included in the study. When this outlier was removed from the analysis, alkaline phosphatase was a weak predictor of CL (r² = 0.36). The relationships between CL and ALT or AAG were also weak (r² = 0.23 and 0.12, respectively), but in the anticipated inverse direction. Thus, the ERMBT proved to be the best single predictor of docetaxel CL.

Fig. 2  Relationship between CL and phenotypic measurement of CYP3A4.

Both the CL of docetaxel and CYP3A4 activity, as measured by the ERMBT, had marked interpatient variation in this study. All patients were required to have good hepatic function as described previously, and with the exception of dexamethasone, no patient received known potent inhibitors or inducers of CYP3A4. Thus, it appears that the variation seen could reflect genetic differences in the metabolism of docetaxel.

Figure 2 and Table 1

The most significant independent variable examined to predict docetaxel CL in this study was hepatic CYP3A4 activity as measured by ERMBT. Previously, the best predictors of docetaxel pharmacokinetics that had been identified were abnormal liver chemistries and serum AAG level. Albumin was also a significant predictor of CL, but its effect was of low magnitude compared with that of AAG (9). In this study, AAG and the ERMBT results do not appear to be correlated, and the ERMBT is a superior predictor of CL. In fact, the ERMBT and albumin are the strongest predictors of CL, and together they account for 72% of the interpatient variability in CL. Why is the AAG a poorer predictor in this study? The pharmacokinetic data and AAG levels in this study were deter-
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The AAG reflects the free fraction of drug available for CL in the liver, whereas CYP3A4 activity reflects the individual’s inherent ability to metabolize the drug. Although this is a small study, the fact that CYP3A4 activity was a better predictor is an important new finding and will hopefully improve our understanding of the pharmacokinetics of docetaxel. Larger studies are under way and will help clarify the importance of AAG and CYP3A4 activity in relationship to CL.

Although toxicities (namely, grade 3 or 4 neutropenia) were seen in virtually all patients at this dose level, the observation that an ERMBT result of <1 in this study was associated with the severest toxicities is interesting. Although marked interpatient heterogeneity in the expression of CYP3A4 genes is known to exist, the clinical implications of having a “high” or “low” CYP3A4 activity were not necessarily known. In this study “low” CYP3A4 activity (ERMBT < 1) appears to be associated with excess toxicity. It implies that consideration should be given to modifying the dose for patients with such a low ERMBT. Similarly, one would wonder whether patients with “high” CYP3A4 activity are at risk of getting subtherapeutic doses of CYP3A4 substrates such as docetaxel. This hypothesis should be studied further.

In this study, single agent docetaxel was given, but docetaxel is now commonly included in combination regimens. Because the additional drugs are often myelotoxic but not CYP3A4-mediated, the ERMBT may be helpful in sorting out the specific effect docetaxel makes with regard to the toxicity of the regimen.

Logistically, the ERMBT is easy to administer and poses a minimal risk to patients. It proved to be highly reproducible. It was not significantly affected by dexamethasone premedication, and this observation is consistent with prior data (15) that showed no substantial effect of dexamethasone premedication on docetaxel CL. The test can therefore be done at a convenient time, days or weeks ahead of the planned administration of docetaxel.

In conclusion, CYP3A4 activity was found to be the most significant independent variable for predicting the CL of docetaxel. The ERMBT, perhaps together with serum albumin or AAG, may be clinically useful in individualizing the dosage of docetaxel and possibly other drugs that are CYP3A4 substrates, including several new antineoplastic agents. It may also be helpful in individualizing the dose of docetaxel in patients with markedly elevated hepatic enzymes who are currently excluded from treatment with docetaxel.

We plan to initiate a study using tailored dosing of docetaxel based on CYP3A4 activity. We postulate that by tailoring the dose, variability in patient drug exposure could be diminished. Hopefully, modern pharmacogenetics can be put into practical use in the clinic and allow us to treat patients with better precision and less toxicity.

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