Metabolism of Docetaxel by Human Cytochromes P450: Interactions with Paclitaxel and Other Antineoplastic Drugs

Isabelle Royer, Bernard Monsarrat, Michelle Sonnier, Michel Wright, and Thierry Cresteil

ABSTRACT

The metabolism of docetaxel by human liver microsomes was investigated in vitro and compared with that of paclitaxel. A main docetaxel metabolite was generated by human liver microsomes in the presence of NADPH: retention time in high pressure liquid chromatography and its ion fragmentation in mass spectrometry were identical to those of the authentic derivative hydroxylated at the butyl group of the C13 side chain. Kinetic measurements and chemical and immunological inhibitions demonstrated that CYP3A was implicated in the hydroxylation of docetaxel: Km (2 μM) and Vmax values of docetaxel for human liver microsomes were comparable to those calculated for the formation of metabolite p-hydroxyphenyl C3'-paclitaxel (M4). Docetaxel hydroxylation correlated only with the CYP3A content of microsomes and with CYP3A-dependent 6β-hydroxylation of testosterone and 16-hydroxylation of dehydroepiandrosterone. The formation of hydroxydocetaxel was strongly reduced by CYP3A inhibitors such as ketoconazole, midazolam, erythromycin, testosterone, orphenadrine, and troaleandomycin, whereas quinidine (CYP2D6), hexobarbital, tolbutamide, and mephenytoin (CYP2C) had no or little effect.

The hydroxylation of docetaxel exhibited a highly positive correlation with the formation of metabolite M4 of paclitaxel (r = 0.929, P < 0.0001, n = 12), but not with its 6α-hydroxylation (r = 0.48, P > 0.15). Docetaxel abolished the hydroxylation of paclitaxel metabolite M4, but was totally inactive on its 6α-hydroxylation. Conversely, paclitaxel reduced significantly the hydroxylation of docetaxel. We examined in vitro the possible interaction among docetaxel, paclitaxel, and drugs which could be associated during chemotherapy. Cisplatin, verapamil, doxorubicin, vinblastine, and vincristine at concentrations usually recommended did not markedly modify taxoid metabolism. Ranitidine and diphenhydramine had no effect, but 100 μM cimetidine partially inhibited the formation of 6α-hydroxypaclitaxel.

Pre-treatment of patients with barbiturates strikingly stimulated docetaxel hydroxylation, whereas no acceleration of docetaxel hydroxylation was noticed in a patient receiving steroids.

INTRODUCTION

Different laboratory approaches including antisense RNA and antibodies have been recently developed for the treatment of human carcinomas. However, for economical and practical reasons, the use of classical chemotherapy will remain the most useful treatment during the next decades. In this respect, new microtubule poisons exhibit a potent and highly promising antiproliferative efficiency. In contrast with Vinca alkaloids, which prevent the assembly of microtubules, the mechanism of action of taxoids primarily involves the stabilization of microtubule assembly, thereby disrupting the cytoskeleton architecture and disturbing the orderly progression through mitosis (1, 2). Taxoids demonstrate a high antimitotic activity on experimental tumors and in animal models (3–7). In clinical trials, taxoids have been shown to be active antineoplastic agents in patients with solid tumors, particularly cisplatin-refractory ovarian cancer and also in breast, lung, head, and neck cancers (8–12).

Two taxoids (Fig. 1) are under current clinical evaluations: paclitaxel (Taxol), mainly extracted from the bark of the Pacific yew (Taxus brevifolia; Ref. 13), and docetaxel (Taxotere), a semisynthetic molecule prepared from 10-deacetylbaccatin III isolated from the leaves of the European yew (Taxus baccata; Refs. 14 and 15). In vivo, paclitaxel was mostly biotransformed at the hepatic level and generated hydroxylated derivatives recovered unconjugated in rat (16) and human bile (17, 18). In humans, metabolites result from the hydroxylation of paclitaxel at position 6 of the taxane ring (M5), at the para position of the phenyl ring at the C3′ on the C13 side chain (M4), and at both positions leading to a dihydroxy derivative (M3; Fig. 1). In vitro studies on the biotransformation of paclitaxel by human liver microsomes confirmed these observations: Cresteil et al. (19) first concluded that CYP2C accounted for the formation of 6α-hydroxypaclitaxel, which is the major metabolite formed in vivo and in vitro, while CYP3A supported the second hydroxylation reaction. In contradiction to these conclusions, Kumar et al. (20) claimed that CYP3A was responsible for the formation of 6α-hydroxypaclitaxel. Recent investigations conducted by Harris et al. (21) and Rahman et al. (22) confirmed the former conclusions and extended the identification of the major isomeric involved in the 6α-hydroxylation of paclitaxel to the CYP2C8.

In contrast to paclitaxel, the biotransformation pathway of docetaxel is similar in animals and humans. The initial metabolite results from the hydroxylation of the methyl of the tert-butyl group at the C13 side chain of docetaxel (Fig. 1), suggesting the participation of cytochrome P450 isoform(s). This metabolite is further oxidized and cyclized (23) in humans as in several animal species studied (24).

In this study, we intended to compare the metabolic pathways involved in paclitaxel and docetaxel biotransformation in the human liver. Preliminary studies tentatively identified the CYP3A as the site of hydroxylation for docetaxel (25). Herein, we investigated the in vitro docetaxel metabolism by human liver microsomes, and we compared docetaxel and paclitaxel metabolic pathways to estimate the relative participation of individual P450 isoforms. The CYP3A subfamily has already been implicated in the biotransformation of several molecules such as verapamil (26), vinblastine (27), and tamoxifen (28). Since clinical protocols have been designed to combine many prescriptions during chemotherapy, in the second part of this study, we were concerned by the impact of drug association on the in vitro metabolism of paclitaxel and docetaxel to predict possible drug interactions modifying the pharmacokinetics of the active compounds and their cytotoxic effects.

MATERIALS AND METHODS

Chemicals. Paclitaxel (Taxol, NSC 125973) was kindly provided by Bristol-Myers Squibb (Wallington, CT) and docetaxel (Taxotere, RP 56976, NSC 628503) by Rhône Poulenc Rorer (Antony, France). All chemicals were from the highest available grade and purchased from Sigma, except pentoxysorufin (Molecular Probes, Eugene, OR). Quinidine was a gift from Dr. P. Dayer, erythromycin was provided by Abbott (Rungis, France), racemic mephenytoin was generously supplied by Sandoz (Basel, Switzerland), mida...
zolam by Roche (Basel, Switzerland), and ketoconazole by Janssen (Beerse, Belgium).

**Tissue Collection.** All protocols were conducted according to the recommendations of the Ethical Committee of the Institut National de la Santé et de la Recherche Médicale. Adult liver samples (n = 12) were obtained from donors for kidney transplantation. Occasionally, one kidney was obtained under the same conditions. Donors had no severe chronic pathology and generally died from a traffic accident. They had no repeated drug consumption. No information was available regarding their smoking and drinking habits. Alternatively, liver samples were collected from three fetuses (22, 26, and 27 weeks, respectively) and eight children ages 12 h to 6 months as previously reported, as well as from five children given barbiturates, benzodiazepines, or ketoconazole, as reported, as well as from five children given barbiturates, benzodiazepines, or steroids for therapeutic purposes (19). Samples were frozen in liquid nitrogen immediately after removal and kept at −80°C.

**Microsome Preparation and Determination of Individual P450 Isoform Content.** Frozen tissues were thawed in ice-cold saline, homogenized, and microsomes were prepared as previously reported (29). Cytochrome P450 was measured according to the procedure of Greim (30), and the protein content of liver microsomes was quantified as detailed elsewhere (19). The individual P450 isoform content of liver microsomes was quantified as detailed elsewhere (19).

**Monoxygenase Activities.** Except when otherwise specified, reactions were performed with 0.3 nmol total P450. Pentoxysresorufin dealkylation was followed by spectrofluorometry according to the method of Burke et al. (32).

**Characterization and Quantitation of Docetaxel Metabolites.** Ethyl acetate extracts evaporated to dryness were dissolved in 200 μl acetonitrile/water (90/10) and added to the incubation mixture at concentrations ranging from 0.1 to 100 μM in the presence of a fixed amount of methanol. Troleandomycin (10 μM) was preincubated for 15 min at 37°C in the presence of NADPH to generate a complex with CYP3A prior to the addition of reagents for docetaxel incubation. In experiments designed to estimate the inhibitory effect of docetaxel on paclitaxel metabolism, docetaxel (from 1 to 100 μM) was incubated with 50 μM paclitaxel. Conversely, paclitaxel concentrations between 10 and 100 μM were added to incubation mixtures containing 25 μM docetaxel. After two extractions with 2.5 ml ethyl acetate, residues were divided in two aliquots and analyzed for the respective content in metabolites according to the separation procedures described below.

Reilly et al. (35), and the biotransformation of paclitaxel was performed as previously reported (19).

**Metabolism of Docetaxel.** The conditions of incubation of docetaxel with liver microsomes were derived from those set for paclitaxel. Microsomal proteins were incubated in 1 ml 100 mM sodium phosphate buffer (pH 7.4), 10 mM MgCl2, 20% glycerol containing 0.1 mM NADP and 1 mM glucose 6-phosphate as an NADPH-generating system. Docetaxel (10 mM in methanol) was added at a final concentration of 25 μM, except when otherwise indicated. Incubations carried out for 30 min at 37°C after the addition of glucose 6-phosphate dehydrogenase were stopped by the addition of 2.5 ml ethyl acetate. The parent drug and its derivative were extracted twice by 2.5 ml ethyl acetate. Then, the extracts were pooled, evaporated to dryness, and analyzed using HPLC.

For kinetic measurements, docetaxel concentrations ranged from 1 to 50 μM. For immuno-inhibitions, liver microsomes were incubated at 23°C for 15 min with increasing amounts of preimmune or anti-CYP3A4 IgG prior to the addition of other reagents (19). Inhibition studies were performed in the presence of 10 μM quinidine, 20 μM orphenadrine, 50 μM DHEA, 160 μM testosterone, and 400 μM erythromycin or 500 μM hexobarbital, tolbutamide, racemic mephentoin, and midazolam. Cisplatin and diphenylhydramine (1 μM-1 mM) and verapamil, cimetidine, and ranitidine (10 μM-10 mM) were dissolved in buffer. Doxorubicin, vinblastine, vincristine, and ketoconazole were dissolved in methanol and added to the incubation mixture at concentrations ranging from 0.1 to 100 μM in the presence of a fixed amount of methanol. Troleandomycin (10 μM) was preincubated for 15 min at 37°C in the presence of NADPH to generate a complex with CYP3A prior to the addition of reagents for docetaxel incubation.

In experiments designed to estimate the inhibitory effect of docetaxel on paclitaxel metabolism, docetaxel (from 1 to 100 μM) was incubated with 50 μM paclitaxel. Conversely, paclitaxel concentrations between 10 and 100 μM were added to incubation mixtures containing 25 μM docetaxel. After two extractions with 2.5 ml ethyl acetate, residues were divided in two aliquots and analyzed for the respective content in metabolites according to the separation procedures described below.

**Characterization and Quantitation of Docetaxel Metabolites.** Ethyl acetate extracts evaporated to dryness were dissolved in 200 μl acetonitrile/water
Table I  Correlations between total cytochrome P450 content, individual P450 isoform content, and related monooxygenase activities in adult human liver microsomes

<table>
<thead>
<tr>
<th>Activity</th>
<th>F6 Doc</th>
<th>M4 P</th>
<th>P450</th>
<th>CYP2C</th>
<th>CYP3A</th>
<th>CYP2E</th>
<th>CYP2D6</th>
<th>CYP1A2</th>
<th>CYP4A1</th>
<th>PROD</th>
<th>Test 6j</th>
<th>Tolb</th>
<th>DM Diaz</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-OH P</td>
<td>0.48</td>
<td>0.93</td>
<td>0.31</td>
<td>0.33</td>
<td>0.87</td>
<td>−0.24</td>
<td>0.79</td>
<td>0.09</td>
<td>0.10</td>
<td>0.34</td>
<td>0.86</td>
<td>0.78</td>
<td>0.64</td>
</tr>
<tr>
<td>M4 P</td>
<td>0.63</td>
<td>0.41</td>
<td>0.72</td>
<td>0.33</td>
<td>0.21</td>
<td>−0.34</td>
<td>0.13</td>
<td>0.17</td>
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<td>0.82</td>
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<td>0.89</td>
</tr>
<tr>
<td>P450</td>
<td>0.73</td>
<td>0.42</td>
<td>0.87</td>
<td>−0.28</td>
<td>0.26</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.38</td>
<td>0.92</td>
<td>0.86</td>
<td>0.68</td>
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<tr>
<td>CYP2C</td>
<td>0.48</td>
<td>0.19</td>
<td>0.03</td>
<td>0.60</td>
<td>0.32</td>
<td>0.19</td>
<td>0.19</td>
<td>0.19</td>
<td>0.19</td>
<td>0.58</td>
<td>0.62</td>
<td>0.62</td>
<td>0.43</td>
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<tr>
<td>CYP3A</td>
<td>0.39</td>
<td>0.34</td>
<td>0.34</td>
<td>0.31</td>
<td>0.05</td>
<td>0.45</td>
<td>0.77</td>
<td>0.23</td>
<td>0.77</td>
<td>0.88</td>
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<tr>
<td>CYP2E1</td>
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<td>0.81</td>
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<td>0.20</td>
<td>0.81</td>
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<td>0.63</td>
<td>0.63</td>
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<tr>
<td>CYP2D6</td>
<td>0.28</td>
<td>−0.49</td>
<td>0.63</td>
<td>0.31</td>
<td>−0.38</td>
<td>0.16</td>
<td>0.01</td>
<td>0.01</td>
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<tr>
<td>CYP1A2</td>
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<td>0.62</td>
<td>−0.13</td>
<td>0.43</td>
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<tr>
<td>CYP4A1</td>
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<td>0.24</td>
<td>0.10</td>
<td>0.33</td>
<td>0.33</td>
<td>0.33</td>
<td>0.33</td>
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<td>0.33</td>
<td>0.33</td>
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<tr>
<td>PROD</td>
<td>0.30</td>
<td>−0.27</td>
<td>0.04</td>
<td>0.51</td>
<td>0.51</td>
<td>0.51</td>
<td>0.51</td>
<td>0.51</td>
<td>0.51</td>
<td>0.51</td>
<td>0.51</td>
<td>0.51</td>
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</tr>
<tr>
<td>Test 6j</td>
<td>0.24</td>
<td>0.61</td>
<td>0.81</td>
<td>0.81</td>
<td>0.81</td>
<td>0.81</td>
<td>0.81</td>
<td>0.81</td>
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<td>0.81</td>
<td>0.81</td>
<td>0.81</td>
<td>0.81</td>
</tr>
<tr>
<td>Tolb</td>
<td>0.63</td>
<td>0.57</td>
<td>0.57</td>
<td>0.57</td>
<td>0.57</td>
<td>0.57</td>
<td>0.57</td>
<td>0.57</td>
<td>0.57</td>
<td>0.57</td>
<td>0.57</td>
<td>0.57</td>
<td>0.57</td>
</tr>
</tbody>
</table>

*Total P450 content was spectrally determined, and the content of each individual isoform was estimated immunochemically. Monooxygenase activities supported by CYP2C (DM Diaz, diazepam demethylation; Tolb, tolbutamide hydroxylation), CYP3A (Test 6j, testosterone 6α-hydroxylation), and pentoxyresorufin dealkylase (PROD) were assayed with human liver microsomes (n = 12), and activities were correlated with monooxygenase activities toward docetaxel (F6 Doc) and paclitaxel (6 OH P, 6α-hydroxylation of paclitaxel; M4P: metabolite M4 of paclitaxel), P450 isoform content (expressed as arbitrary units mg−1 protein) and total P450 content (nmol mg−1 protein)."
Fig. 5. Effect of increasing concentrations of cisplatin, verapamil, or ketoconazole on docetaxel and paclitaxel metabolism by human liver microsomes. Inhibitions were performed with increasing concentrations of chemicals as detailed in "Materials and Methods." Results are given as the mean values of experiments performed in duplicate and expressed as the percentage of activity measured in the absence of chemicals. ▲, hydroxydocetaxel (F6); □, 6-hydroxypaclitaxel (metabolite M5); ○, metabolite M4 of paclitaxel.

Combined evidence from the retention time and the characteristic fragment ions suggest that this compound corresponds to a hydroxylated derivative of docetaxel. In agreement with the data obtained with the pure reference compound, this docetaxel metabolite resulted from a monohydroxylation reaction at the tert-butyl group of the C13 side chain (Fig. 1).

**Pharmacokinetic Determinations.** The formation of the hydroxydocetaxel metabolite by human liver microsomes was relatively low, with a mean value of 2.6 ± 0.6 pmol · min⁻¹ · mg⁻¹, while extreme values ranged from 0 to 7.5 pmol · min⁻¹ · mg⁻¹. This rate was comparable to those observed for the formation of metabolite M4 of paclitaxel (5.3 ± 1.4 pmol · min⁻¹ · mg⁻¹) when incubations were performed with the same microsomal fractions, and was far below the rate of formation of 6α-hydroxydocetaxel (120 ± 30 pmol · min⁻¹ · mg⁻¹). The time dependence of the reaction was observed for up to 30 min with human liver microsomes. Moreover, the formation of metabolites increased with doses of docetaxel (1–50 μM) following a typical monophasic Michaelis-Menten kinetics, with an apparent $K_m$ of 2.1 ± 0.3 μM (calculated from three separate experiments) and an extrapolated $V_{max}$ of 10 pmol · min⁻¹ · mg⁻¹. Thus, a concentration of 25 μM docetaxel was routinely used in additional 30-min incubations.

**Identification of P450 Isoform Involved in Docetaxel Biotransformation.** Several approaches have been used to unequivocally identify the P450 isoform responsible for the biotransformation of docetaxel. First, we compared the rates of hydroxylation of docetaxel and paclitaxel by human liver microsomes (Table 1). A highly positive correlation occurred between the hydroxylation of the lateral chain of docetaxel at C13 and the hydroxylation of the corresponding lateral chain of paclitaxel (metabolite M4) in 12 samples ($P < 0.0001$), whereas no correlation was found with the production of 6α-hydroxypaclitaxel ($r = 0.48; P > 0.15$).

The formation of metabolite M4 of paclitaxel has been assigned to CYP3A (19). Thus, we further compared the rate of hydroxylation of docetaxel with other CYP3A-dependent monooxygenase activities and with the CYP3A content of the microsomal preparations used to assay the hydroxylation of docetaxel. As expected, the docetaxel hydroxylation was correlated with the 6β-hydroxylation of testosterone ($r = 0.864; P < 0.001$) and with the 16-hydroxylation of DHEA ($r = 0.837; P < 0.001$), as well as with the CYP3A content (Table 1). Conversely, no correlation was observed between docetaxel hydroxylation and two CYP2C-dependent monooxygenases, or with the CYP2C content of microsomes. Similarly, no positive relation could be noticed with the microsomal total P450 content or the content in other individual P450 isoforms.

We examined the inhibitory effect of well-defined substrates of monooxygenases. Substrates of CYP3A including erythromycin, testosterone, midazolam, or orphenadrine, and troleandomycin elicited a marked reduction of docetaxel hydroxylation, whereas three substrates of CYP2C have no or little inhibitory effect (Fig. 3). In another set of experiments, DHEA, a substrate of CYP3A, was incubated...
Fig. 7. Effect of increasing concentrations of ranitidine, cimetidine, and diphenylhydramine on docetaxel and paclitaxel metabolism by human liver microsomes. Inhibitions were performed in the presence of increasing concentrations of chemicals as detailed in “Materials and Methods.” Results are given as the mean values of experiments performed in duplicate, and expressed as the percentage of activity measured in the absence of chemicals. A, hydroxydocetaxel (F6); □, 6-hydroxypaclitaxel (metabolite M5); ●, metabolite M4 of paclitaxel.

with liver microsomes in the presence of docetaxel or paclitaxel: 50 μM DHEA substantially reduced the formation of the docetaxel metabolite (by 86%) and the metabolite M4 of paclitaxel (by 78%), but moderately affected the 6α-hydroxylation of paclitaxel (<25%). As expected, 10 μM quinidine had no pronounced inhibitory effect on the biotransformation of either paclitaxel or docetaxel.

Immunoinhibition of docetaxel hydroxylation by anti-CYP3A IgG was performed on two different microsomal preparations. A dose-dependent reduction of docetaxel hydroxylation was observed, leading to a potent 80% inhibition with 5 mg IgG nmol⁻¹ P450, whereas preimmune IgG at the same dose exerted a limited nonspecific reduction of activity (Fig. 4). Altogether these data definitively demonstrated the implication of CYP3 in the hydroxylation of docetaxel.

Drug Interactions. The inhibitory potential of the antifungal ketoconazole of the calcium channel blocker verapamil and of cisplatin on docetaxel and paclitaxel biotransformations is shown in Fig. 5. Cisplatin at doses varying from 1 μM to 1 mM was totally devoid of an inhibitory effect on both molecules. In contrast, low concentrations of ketoconazole specifically inhibited CYP3A-dependent reactions, i.e., docetaxel hydroxylation and formation of the hydroxylated M4 derivative of paclitaxel (IC₅₀ ~ 1 μM), whereas the inhibition of the 6α-hydroxylation of paclitaxel required a 100 times higher concentration. Verapamil exerted a weaker inhibitory effect than ketoconazole. The production of metabolite M4 of paclitaxel was more sensitive to paclitaxel addition (IC₅₀ ~ 100 μM) than the 6-hydroxylation of paclitaxel and the hydroxylation of docetaxel, which required a concentration of 1 mM to be effective. Doxorubicin at doses below 1 μM had a limited in vitro effect on taxoid metabolism (Fig. 6); a IC₅₀ could be calculated around 10 μM whatever the reaction studied. Vinblastine and vincristine caused no inhibition at doses below 10 μM, except vincristine which reduced the 6-hydroxylation of paclitaxel. Finally, three drugs given in association with taxoids to reduce or prevent side effects were tested (Fig. 7): diphenylhydramine and ranitidine exhibited only a marginal effect on the metabolism of paclitaxel and docetaxel. The only effective inhibitor was cimetidine, which partially prevented the formation of metabolite M5 of paclitaxel at doses higher than 100 μM.

Interaction of Docetaxel-Paclitaxel. When docetaxel was incubated with human liver microsomes in the presence of increasing doses of paclitaxel, a reduction of the rate of hydroxylation of docetaxel occurred concomitantly with the rise of paclitaxel metabolism.
Table 2 Docetaxel hydroxylation in patients with drug history or under pathological conditions

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Disease</th>
<th>Treatment and duration</th>
<th>Docetaxel metabolite F6 (pmol · min⁻¹ · mg⁻¹ microsomal protein)</th>
<th>CYP3A (arbitrary units · mg⁻¹ protein)</th>
<th>CYP2C (arbitrary units · mg⁻¹ protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>4 days</td>
<td>Maternal fetal infection</td>
<td>None</td>
<td>Not measurable</td>
<td>7.6*</td>
<td>0.66</td>
</tr>
<tr>
<td>86</td>
<td>6 days</td>
<td>Epilepsia</td>
<td>Phenobarbital, 5 mg/kg/day for 5 days; pentobarbital, 2 mg/kg/day for 3 days</td>
<td>13.3*</td>
<td>2.2</td>
<td>1.4*</td>
</tr>
<tr>
<td>77</td>
<td>3 mo</td>
<td>Epilepsia</td>
<td>Prednisone, 1 mg/kg/day for 1 yr</td>
<td>26.8*</td>
<td>2.2</td>
<td>1.4*</td>
</tr>
<tr>
<td>79</td>
<td>9 yr</td>
<td>Medullar aplasia</td>
<td>Phenobarbital, 5 mg/kg/day; pentobarbital, 2 mg/kg/h; pregnenolone, 2 mg/kg/day for 7 days; diazepam, 13 mg, 1 day</td>
<td>13.3*</td>
<td>0.45</td>
<td>1.08</td>
</tr>
</tbody>
</table>

*M Different from value in control patients within the same group of 9-year-old children are compared to control adults (0.57 for CYP3A and 1.13 for CYP2C).

(Fig. 8a). Conversely, the two pathways involved in the metabolism of paclitaxel were diversely affected by the addition of docetaxel to the incubation mixture: the 6a-hydroxylation of paclitaxel remained (Fig. 8a). Conversely, the two pathways involved in the metabolism activity (e.g., an activity <0.1 pmol · min⁻¹ · mg⁻¹) was detected in abolished by docetaxel (Fig. 8b).

Ontogenesis and Induction of Docetaxel Metabolism. The low level of docetaxel hydroxylation made this determination difficult. No activity (e.g., an activity <0.1 pmol · min⁻¹ · mg⁻¹) was detected in microsomes prepared from fetuses and newborns. However, in children ages 3–12 months, the activity reached a value close to the adult level: 2.1 ± 1.1 (n = 3) versus 2.6 ± 0.6 (n = 12). In fetal and neonatal samples, the amount of reactive material reacting with the polyclonal CYP3A4 antibody was roughly similar to the amount of CYP3A4 detected in adult livers. Some samples have been considered as induced according to their therapeutic history. These samples have already been examined for their ability to metabolize paclitaxel and showed an important increase of the formation of metabolite M4. When incubated with docetaxel, high levels of hydroxylation were observed in microsomes prepared from patients given barbiturates (Table 2). Furthermore, when docetaxel hydroxylation in these induced patients was plotted versus the rate of formation of paclitaxel metabolites, a positive relationship was calculated (r = 0.952; P < 0.001, n = 5) with M4, but not with the 6a-hydroxylated metabolite (r = 0.488; P > 0.25). Microsomes obtained from a patient receiving prednisone for 1 year for the treatment of medullar aplasia did not show an increased docetaxel metabolism.

DISCUSSION

Clinical effectiveness of antineoplastic drugs depends on the balance between the cytotoxicity toward tumor cells and general toxicity. Usually, the therapeutic window is narrow and requires a careful dosage. Thus, it is crucial to accurately estimate the biotransformation of antineoplastic drugs which occurs essentially at the hepatic level and is catalyzed by cytochrome P450-dependent monoxygenases. Paclitaxel has been shown to undergo two pathways: the major leads to its 6-hydroxylation and is catalyzed by CYP2C (19, 22), whereas a minor human metabolite results from the hydroxylation of the lateral chain by CYP3A isofrom(s). These in vitro data have been supported by the recovery of monohydroxylated and dihydroxylated metabolites in bile of patients given paclitaxel.

In this report, we characterize the main metabolite of docetaxel formed by human liver microsomes and identify the P450 isoform involved in the hydroxylation reaction. Additionally, we assess drug interactions comparatively to the P450 isoforms involved in the hydroxylation of paclitaxel. For that purpose we used the human microsomes which have already allowed the characterization of paclitaxel metabolic pathways. Correlation studies, immunological inhibitions, and chemicals inhibitions support the conclusion that, in vitro, the first docetaxel metabolite is formed by CYP3A isofroms. Kinetic measurements are consistent with this statement: the hydroxylation rate on the lateral chain of paclitaxel is similar to that of docetaxel and never exceeds 5–10% of the reaction occurring on the taxane ring of paclitaxel catalyzed by CYP2C8. The lack of hydroxylation on the taxane ring of docetaxel is somewhat surprising and is currently under investigation.

The CYP3A content of microsomes obtained from the liver of patients treated with barbiturates exceeds the mean value measured in controls of the same age. These samples present a markedly higher activity toward docetaxel and confirm the implication of CYP3A in the metabolic process. In the CYP3A subfamily, three major proteins have been characterized. Their expression is age related: CYP3A7 is detected in fetal livers (38, 39), whereas CYP3A4 (HLp or P450 NIF) was the major P450 isoform present in adult livers. Recently, all fetuses and a fraction of the adult population were shown to contain CYP3A5 RNA (40), although the existence of the protein was not demonstrated. Data collected herein from fetal samples demonstrate that CYP3A (CYP3A7 and potentially CYP3A5) has no activity toward docetaxel. Thus, the hydroxylation of docetaxel seems to restrict the catalytic efficiency to CYP3A4. This point will be further examined with recombinant 3A4 and 3A7 proteins expressed in Cos cells, as already done for testosterone and DHEA biotransformations.

These conclusions were further confirmed by drug interactions. We first used several compounds known for their capacity to specifically inhibit CYP3A monoxygenases such as endogenous testosterone and DHEA and exogenous drugs. All reduce the hydroxylation of docetaxel from 50 to 100%. Next, several drugs have been incubated in vitro with docetaxel. Ketoconazole is the more potent inhibitor of docetaxel hydroxylation and paclitaxel minor metabolite M4 hydroxylation exhibiting IC₅₀ below 1 μM. This observation is consistent with the high affinity of ketoconazole for CYP3A-dependent activities exemplified by a Kᵢ toward vinblastine hydroxylation of 0.2 μM (27). Verapamil, a calcium channel blocker, down-regulates the expression of the mdr gene at the transcriptional level and thus could reverse the resistance of proliferative cells to anticancer drugs (41, 42). Doses of verapamil between 15 and 50 μM are required to decrease in vitro the mdr gene expression in leukemic cell lines. This range of concentration is compatible with that which efficiently reduces paclitaxel and docetaxel metabolism, and is in agreement with the Kᵢ of verapamil

*4 Unpublished data.
biotransformation by human microsomes (26). Cisplatin is widely used in chemotherapy in association with taxoids (43); however, in contrast to other anticancer drugs, it was not a substrate for microsomal monooxygenases nor impaired docetaxel and paclitaxel metabolism, even at the highest dose studied. A major side effect of treatments with paclitaxel was associated with the use of Cremophor as vehicle. This compound induces the release of histamine leading to allergic reactions. This effect can be prevented by the coadministration of H₁ and H₂ receptor blockers such as ranitidine, cimetidine, and diphenylhydramine. Apparent Kₘ of cimetidine and ranitidine for human liver microsomes are 0.6 and 5 mM, respectively (44, 45), explaining the higher inhibitory effect of cimetidine on paclitaxel metabolism observed in vitro. However, due to the dose of cimetidine used in therapeutics, drug interaction should remained limited, as shown in the rat model (46).

The biotransformation of Vinca alkaloids and anthraclycines by liver microsomes was mainly supported by CYP3A (27, 47). Since the reported Kₘ of these molecules for CYP3A were in the 10 μM range as well as the Kₘ of docetaxel and paclitaxel, it is not surprising that the extent of inhibition caused by Vinca alkaloids on taxoid metabolism is modest at doses below 10 μM.

These observations are of interest because the different classes of anticancer drugs combined for chemotherapy act on different targets at the cellular level, either on the cell structure through a modification of the microtubule assembly or by intercalation into the DNA molecule; therefore, their biological effect could be additive or synergistic. The only limitation is relative to the drug disposition and depends on the elimination capacity of active compounds by the organism. According to the P450 isofrom involved in the elimination process and to the relative affinity of associated drugs toward that isofrom, an accumulation could overcome the beneficial effect of chemotherapy. Thus, the involvement of distinct P450 isofroms in the hepatic metabolism of paclitaxel and docetaxel might lead to different disposition of these two drugs in human patients.

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REFERENCES

Metabolism of Docetaxel by Human Cytochromes P450: Interactions with Paclitaxel and Other Antineoplastic Drugs

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