MODIFICATION OF PACLITAXEL METABOLISM IN A CANCER PATIENT BY INDUCTION OF CYTOCHROME P450 3A4

BERNARD MONSARRAT, ETIENNE CHATELUT, ISABELLE ROYER, PAUL ALVINERIE, JOELLE DUBOIS, ANNIK DEZEUSE, HENRI ROCHE, SUZY CROS, MICHEL WRIGHT, AND PIERRE CANAL

Institut de Pharmacologie et de Biologie Structurale (B.M., I.R., P.A., S.C., M.W.), Centre Claudius Regaud (E.C., A.D., H.R., P.C.), and Institute de Chimie des Substances Naturelles, Centre National de la Recherche Scientifique (J.D.)

(Received June 16, 1997; accepted November 25, 1997)

This paper is available online at http://www.dmd.org

ABSTRACT:

Biliary, plasma, and urinary disposition of paclitaxel and paclitaxel metabolites were determined simultaneously in a patient with percutaneous biliary drain. The complete chemical structures of the major metabolites were established by mass spectrometry and NMR spectroscopy. A nonlinear elimination model was indicated by the fact that the rate of biliary excretion of paclitaxel rose as plasma concentrations fell. Dihydroxypaclitaxel was the predominant biliary metabolite, in contrast to the barely detectable levels in two previous patients. This derivative results from hydroxylation at the C6 position of the taxane ring and at the phenyl C3’-position on the C13 side chain mediated by cytochrome P450 2C8 and 3A4, respectively. In line with this mechanism, the two other main metabolites corresponded to 6α-hydroxypaclitaxel and to the paclitaxel derivative hydroxylated in the para-position on the phenyl ring at the C3’-position of the C13. A high CYP3A4 activity in the patient is consistent with the repeated administration of methylprednisolone for 14 days before paclitaxel treatment, a compound known to induce the CYP3A isoform, and with the increased ratio of 6β-hydroxy cortisol/cortisol in urine, an index of CYP3A activity. These findings emphasize the influence of pretreatment with corticoids on the disposition of paclitaxel.

Paclitaxel has a significant clinical activity against a broad range of tumor types including breast, lung, head and neck, bladder, and platinum-refractory ovarian carcinoma (Rowinsky, 1997). Accurate information on the disposition and metabolism of this drug would help optimize administration. Pharmacokinetic studies performed at the plasma level have demonstrated that its disposition is well characterized by either biexponential or triexponential models, whereas renal excretion of parent compound accounts for less than 14% of the total administered dose (Rowinsky 1995, 1997; Sonnichsen and Relling, 1994; Walle et al., 1995). The primary route of systemic elimination of paclitaxel occurs via hepatic metabolism and biliary excretion, which may account for the marked interpatient variability in systemic clearance (Monsarrat et al., 1993; Sonnichsen et al., 1995; Walle et al., 1995). In a previous study, we characterized the main hepatic metabolites in the bile of a patient; among the five hepatic metabolites detected, 6α-hydroxypaclitaxel was the major metabolite, p-hydroxy-phenyl-C3’-paclitaxel constituted a minor metabolite, and dihydroxytopaclitaxel was barely detectable (Monsarrat et al., 1993). The quantitative predominance of 6α-hydroxypaclitaxel has also been described by Harris et al. (1994a) in the bile of another patient. These metabolites have also been detected in human plasma and feces (Gianni et al., 1995; Royer et al., 1995; Sparreboom et al., 1995; Wright et al., 1995). Two cytochrome P450 isoforms are involved in the biotransformation of paclitaxel by human liver microsomes (Cresteil et al., 1994; Harris et al., 1994b; Kumar et al., 1994; Rahman et al., 1994). The formation of the major metabolite, 6α-hydroxypaclitaxel, is catalyzed by CYP2C8*1 (Cresteil et al., 1994; Rahman et al., 1994), whereas the minor metabolite, p-hydroxy-phenyl-C3’-paclitaxel, is formed by CYP3A4 (Cresteil et al., 1994; Harris et al., 1994b; Kumar et al., 1994). It is assumed that the dihydroxylated metabolite resulted from stepwise hydroxylations at the two previously described sites (fig. 1) (Cresteil et al., 1994; Harris et al., 1994b; Wright et al., 1995). Systemic elimination of paclitaxel has been demonstrated to be saturable in vivo (Huizing et al., 1993; Rowinsky, 1997; Sonnichsen et al., 1994), and neutropenia has been shown to be related to plasma concentration (Beijnen et al., 1994; Gianni et al., 1995; Kearns et al., 1995; Rowinsky, 1997). Marked variability in paclitaxel metabolism may stem from interindividual differences in cytochrome P450 activity and drug-induced interactions (Berg et al., 1995; Cresteil et al., 1994; Harris et al., 1994b; Jamis Dow et al., 1995; Royer et al., 1996; Schlichenmeyer et al., 1995; Sonnichsen et al., 1995).

We report the disposition and metabolism of paclitaxel in a cancer patient with a biliary percutaneous drainage. In contrast to the two previous cases studied, plasma, bile and urine were simultaneously collected during the treatment. The metabolic profile of paclitaxel in bile and plasma was qualitatively and quantitatively different from...
those recorded in the two previous patients (Harris et al., 1994a; Monsarrat et al., 1993).

**Patient, Materials and Methods**

**Patient and Drug Administration.** A 60-year-old female patient had a tubular subtype breast cancer with disseminated metastases on ovary, liver, and in the retroperitoneal space. Paclitaxel (Taxol, Bristol-Myers Squibb) was administered as a 3-hr iv infusion at a dose of 135 mg/m² (243 mg) following premedications with methylprednisolone (200 mg per os), cimetidine (300 mg iv), and dexamethasone (5 mg iv) for prophylaxis of hypersensitivity reactions. In addition, the patient had a subacute bowel obstruction in relation to her metastatic disease and also received methylprednisolone (40 mg per day per os), neostigmine (0.5 mg per day subcutaneously), and tiemonium (20 mg per day im) for 2 weeks before administration of paclitaxel. Plasma y-glutamyl transpeptidase and alkaline phosphatase levels were 11- and 3-fold the upper normal limits, respectively. Total bilirubin, aspartate transaminase, and alanine transaminase levels were within normal limits. The ratio of β-hydroxy corticosterol/cortisol, used as an index of CYP3A activity, was determined by the HPLC method requiring 1 ml of urine (Lykkesfeldt et al., 1994).

**Bile, Urine, and Blood Collection.** The patient gave informed consent for the sampling protocol. Bile was collected through a percutaneous biliary catheter that was originally implanted to drain an obstructive cholangiocarcinoma. Bile was sampled prior to paclitaxel treatment and then continuously during the infusion period (80 ml) and in six fractions following the end of infusion: 0–1 hr (25 ml), 1–3 hr (60 ml), 3–6 hr (75 ml), 6–12 hr (160 ml), 12–24 hr (350 ml), and 24–48 hr (650 ml) after the end of infusion. Fivelimit blood samples were obtained from the no-infused arm, before infusion, at the middle and the end of infusion, and 0.5, 1, 3, 6, 12, 24, and 48 hr after the end of infusion. Blood samples were centrifuged immediately after collection, and the plasma was removed. Urine was collected in four timed-collection periods corresponding to 0–6 hr, 6–15 hr, 15–27 hr, and 27–48 hr after the start of the infusion. Bile, urine, and plasma samples were stored at −20°C until analysis.

**Purification of Biliary Metabolites for Chemical Characterization.** Pooled bile samples, obtained from different timed-collection periods, were extracted and purified according to previously reported method (Monsarrat et al., 1990, 1993). The purity of all metabolites (>96%) was checked with HPLC using a diode array detector before structural identification by mass spectrometry and nuclear magnetic resonance.

**Bile, Plasma, and Urine Sample Preparation for Pharmacokinetic Analysis by HPLC.** Bile samples (1 ml) were spiked with 10 µl of internal standard (docetaxel, 1 mM) and extracted twice by addition of 4 ml of diethyl ether, shaken for 20 sec, and centrifuged at 2000 rpm for 5 min. The ether fraction was evaporated, and the residue was dissolved in 200 µl of 70% methanol. Paclitaxel and paclitaxel derivatives were extracted from plasma and urine by a solid-phase procedure using C2 Bond Elut cartridges (Analytichem, Harbor City, CA) eluted with 3 × 1 ml methanol and 3 × 1 ml 10 mM ammonium acetate, pH 6.0. Before addition of the internal standard (docetaxel, 10 µl at 10 mM) to plasma and urine samples (1 ml), contaminants were washed out successively with 3 × 1 ml 10 mM ammonium acetate and 3 × 1 ml 35% methanol. Paclitaxel and paclitaxel derivatives were then eluted in 2 × 1 ml 80% methanol. After evaporation, the final residues were dissolved in 0.2 ml 80% methanol (recovery of 90–95%). The chromatographic analysis was conducted as previously described (Monsarrat et al., 1993; Royer et al., 1995). Paclitaxel, 6α-hydroxy paclitaxel, p-hydroxyphenyl-C3′-paclitaxel, dihydroxy paclitaxel, and 10-deacetylpaclitaxel were quantified from linear calibration curves obtained with pure reference compounds.

**Identification of Metabolites.** The HPLC–mass spectrometry system (LC/MS) was performed as published (Royer et al. 1995). The nuclear magnetic resonance spectra were recorded in a Brucker 400 MHz spectrometer (Wissembourg, France) after solubilization of the compounds in deuterated methanol/ chloroform (9:5). The absence of glucuronide and sulfate derivatives of paclitaxel in human bile and urine was checked as described (Monsarrat et al., 1990, 1993).

**Drugs and Chemicals.** Paclitaxel, used as reference, was obtained from Bristol-Myers Squibb through the National Cancer Institute. Baccatin III, 10-deacetyl baccatin III, and docetaxel (used an internal standard) were obtained from D. Guénard (CNRS, ICSN, Gif/Yvette, France). 10-deacetylpaclitaxel was prepared as previously reported (Royer et al., 1995). Cortisol was purchased from Sigma, and β-hydroxy corticosterol was obtained from Steraloids (Wilton, NH). All compounds were more than 99% pure by HPLC analysis.

**Pharmacokinetic Analysis.** The plasma concentrations of paclitaxel and paclitaxel metabolites were analyzed using the SIPHER pharmacokinetic program (Simed, Créteil, France) according to 3-compartment and 2-compartment linear models, respectively. The areas under the plasma concentrations vs. time curve (AUC) were obtained using the trapezoidal rule, and the plasma clearance of paclitaxel was calculated by extrapolation to infinity. The overall biliary clearance of paclitaxel was calculated by dividing the cumulative biliary amount of unchanged paclitaxel by the cumulative plasma AUC of paclitaxel 48 hr after the end of infusion. To study the time course of this parameter (e.g. biliary clearance of unchanged paclitaxel) over the 48 hr following administration, the biliary clearance of unchanged paclitaxel was calculated for each period of collection of bile by dividing each rate of biliary excretion by the mean plasma concentration over this interval. The latter value (e.g. mean plasma concentration) was obtained by dividing the trapezoidal

---

**Fig. 1.** Metabolic pathways of paclitaxel biotransformation in human.

**Fig. 2.** HPLC analysis of extracted bile sample from a patient treated with paclitaxel (135 mg/m², administered as a 3-hr iv infusion).

(A) HPLC tracing of bile collected from 6 to 12 hr after the end of the infusion. Peaks representing paclitaxel (P) and predominant biliary metabolites are depicted. P1, deacetyl-baccatin III; P2, baccatine III; P3, dihydroxypaclitaxel; P4, p-hydroxyphenyl-C3′-paclitaxel; P5, 6α-hydroxy paclitaxel; P6, 10-deacetylpaclitaxel; docetaxel was used as internal standard (LS). All other minor peaks (*) showed UV spectra and characteristic fragment ions of paclitaxel derivatives, but their chemical structure remained unidentified. (B) HPLC tracing of bile control during 30 min before the beginning of the infusion.
TABLE 1

<table>
<thead>
<tr>
<th>Compound</th>
<th>Retention Time (min)</th>
<th>Molecular Ion (M' + H)</th>
<th>Characteristic fragment ions (m/z)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(P1) 10-deacetyl baccatin III</td>
<td>5.00</td>
<td>545</td>
<td>527', 509'</td>
</tr>
<tr>
<td>(P2) baccatin III</td>
<td>5.50</td>
<td>587</td>
<td>527', 509'</td>
</tr>
<tr>
<td>(P3) dihydroxypaclitaxel</td>
<td>8.00</td>
<td>886</td>
<td>868', 826', 808', 602', 585', 525', 463', 403', 302', 284'</td>
</tr>
<tr>
<td>(P4) p-hydroxyphenyl C3'-paclitaxel</td>
<td>9.20</td>
<td>870</td>
<td>852', 810', 792', 569', 551', 509', 447', 387', 302', 284'</td>
</tr>
<tr>
<td>(P5) 6a-hydroxypaclitaxel</td>
<td>15.50</td>
<td>870</td>
<td>852', 810', 792', 602', 585', 525', 463', 403', 286', 268'</td>
</tr>
<tr>
<td>(P6) 10-deacetylpaclitaxel</td>
<td>16.40</td>
<td>812</td>
<td>794', 776', 690', 527', 509', 286', 268'</td>
</tr>
</tbody>
</table>

AUC during the time interval by the length of the time interval (Rowland and Tozer, 1989).

Results

Characterization of Paclitaxel Metabolites. The HPLC chromatogram of human bile (fig. 2) revealed, apart from paclitaxel (P), ten peaks absorbing at 235 nm, the maximum absorption wavelength of paclitaxel and its derivatives. Structural identification using HPLC chromatography (table 1), mass spectrometry (table 1), and nuclear magnetic resonance spectroscopy (table 2) showed that all these peaks corresponded to paclitaxel derivatives. Three main paclitaxel metabolites resulted from hydroxylation reactions: 6α-hydroxypaclitaxel (peak P5), p-hydroxyphenyl C3'-paclitaxel (peak P4), and dihydroxypaclitaxel (peak P3). Dihydroxypaclitaxel, resulting from two hydroxylation reactions, was chemically identified by mass spectrometry and, for the first time, by NMR spectroscopy. Using reference compounds of 6α-hydroxy- and 6β-hydroxydocetaxel (P5), 6α-hydroxydocetaxel (P6) p-hydroxyphenyl C3'-docetaxel (peak P4), and 6α-hydroxydocetaxel (peak P3) were identified by HPLC/mass spectrometry (table 1); 10-deacetylpaclitaxel (peak P6) resulting from elimination of the acetyl group at the position 10 of the taxane ring, and 10-deacetylbaccatin III (peak P1) and baccatin III (peak P2) resulting from the removal of the acetyl group at the position 10 of the taxane ring and of the side chain in C13 (fig. 2). β-Glucuro-conjugated and sulfated derivatives were undetectable.

In plasma, among the different absorption peaks (fig. 3 and table 1), unchanged paclitaxel and four paclitaxel derivatives were characteristic by LC/MS coupling: 6α-hydroxypaclitaxel, p-hydroxyphenyl-C3'-paclitaxel, dihydroxypaclitaxel, and 10-deacetylpaclitaxel. In urine, apart from these four metabolites, we also characterized baccatin III and 10-deacetylbaccatin III. No β-glucuron conjugated and sulfated derivatives were detected (data not shown).

Pharmacokinetics of Paclitaxel and Its Metabolites in Plasma, Bile, and Urine. The plasma concentration vs. time profiles of paclitaxel and its four main metabolites are shown in fig. 4 and table 3. Unchanged paclitaxel (P) predominated during the infusion period, but thereafter, dihydroxypaclitaxel was the major species (P3). Paclitaxel plasma clearance and the apparent terminal half-life were 18.8 liters/hr (174 ml/min/m²) and 10.4 hrs, respectively.

From the concentrations of the different paclitaxel metabolites in the successive bile samples, we calculated the time course of their cumulative biliary excretion (fig. 5). During the infusion period (0–3 hr) the 6α-hydroxypaclitaxel (P5) was the major metabolite. Subsequently, dihydroxypaclitaxel (P3) became the main biliary metabolite. The overall amount of dihydroxypaclitaxel recovered in the bile accounted for 16% of administered dose of paclitaxel, whereas the

FIG. 3. HPLC analysis of a plasma sample from a patient treated with paclitaxel (135 mg/m², administered as a 3-hr iv infusion).
6α-hydroxypaclitaxel (P5) accounted for 12.9% (table 3). Paclitaxel (P) and the two other metabolites (P4, P6) represented only 4.8% of the administered dose of paclitaxel. Plotting the biliary clearance of paclitaxel over the different time intervals of collection against mean plasma concentration in this interval showed that clearance increased from 0.12 to 0.79 liters/hr, whereas mean plasma concentrations fell from 2.9 μM to 0.017 μM (fig. 6).

The cumulative urinary excretion of paclitaxel (P), P3, P4, P5, and P6 metabolites in the 48-hr collection period accounted for 3% of the administered dose (table 3). The total cumulative urinary and biliary excretion of paclitaxel and paclitaxel metabolites (P3, P4, P5, P6) 24 and 48 hr after the end of infusion were 29 and 36%, respectively.

**Discussion**

Biliary excretion of paclitaxel and of its metabolites has been previously reported in two patients (Harris et al., 1994; Monsarrat et al., 1993). In both cases, 6α-hydroxypaclitaxel was the main paclitaxel metabolite. Although paclitaxel disposition was determined in the bile of one of these patients, lack of data on the plasma and urinary concentrations of paclitaxel precluded a complete analysis of paclitaxel disposition. In contrast with these previous observations, we quantified paclitaxel and its metabolites in the plasma, bile, and urine of a third patient. We demonstrated that biliary clearance of paclitaxel is saturable. Moreover, this patient exhibited a quantitatively different metabolic pathway due to modified activity of hepatic cytochrome P450.

Paclitaxel plasma clearance and half-life were comparable with those reported in patients on the same treatment schedule (Beijnen et al., 1994; Gianni et al., 1995; Huizing et al., 1995; Rowinsky et al., 1994; Sonnichsen and Relling, 1994; Sonnichsen et al., 1994). Biliary clearance of unchanged paclitaxel increased continuously from 0.12 liters/hr during the infusion to 0.79 liters/hr at the end of the study (fig. 6). A delay for biliary excretion could explain the low initial value but could not account for the progressive increase values. The increase in paclitaxel biliary clearance concomitant with the decrease in the plasma concentrations was thought to reflect a saturable process, either of the hepatocyte influx or secretion of paclitaxel into the biliary canaliculi. Nonlinear elimination of paclitaxel has been ascribed from the more than proportional increase in plasma AUC with increasing dosage (Gianni et al., 1995; Sonnichsen and Relling, 1994; Sonnichsen et al., 1994) or by comparing the AUC values observed after a 3-hr infusion with those observed after a 24-hr infusion (Ohnita et al., 1995). Moreover, the plasma concentrations of paclitaxel were better fitted by a Michaelis-Menten process rather than by a linear elimination process (Gianni et al., 1995; Sonnichsen and Relling, 1994). For the first time, nonlinear elimination was demonstrated by comparing plasma and biliary concentrations determined at the same times. Recently, Sparreboom et al. (1996) have shown in mice that Cremophor EL, a pharmaceutical vehicle of paclitaxel, is a principal determinant in the nonlinear pharmacokinetic

**TABLE 3**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Plasma</th>
<th>Bile (% of the dose)</th>
<th>Urine (% of the dose)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paclitaxel (P)</td>
<td>14.0</td>
<td>0.9</td>
<td>2.7</td>
</tr>
<tr>
<td>Dihydroxy-P (P3)</td>
<td>8.4</td>
<td>16.0</td>
<td>0.13</td>
</tr>
<tr>
<td>p-OH-phenyl-C3'-P (P4)</td>
<td>3.0</td>
<td>2.8</td>
<td>0.02</td>
</tr>
<tr>
<td>6α-OH-P (P5)</td>
<td>2.0</td>
<td>12.9</td>
<td>0.09</td>
</tr>
<tr>
<td>10-Deacetyl-P (P6)</td>
<td>0.2</td>
<td>1.1</td>
<td>0.01</td>
</tr>
</tbody>
</table>

*Fig. 5. Cumulative biliary excretion of paclitaxel and paclitaxel metabolites.*

The cumulative amount excreted in bile is expressed as the percentage of the initial amount of infused paclitaxel. P, paclitaxel; P3, dihydroxypaclitaxel; P4, p-hydroxyphenyl-C3'-paclitaxel; P5, 6α-hydroxypaclitaxel; P6, 10-deacetylpaclitaxel.

*Fig. 6. Biliary clearance of unchanged paclitaxel vs. plasma concentrations of paclitaxel.*
behavior. One may surmise that impairment of biliary secretion of paclitaxel by Cremophor EL is due to reversal of the P-glycoprotein located in the epithelium of liver biliary canaliculi.

The plasma AUC of all the metabolites, except dihydroxy paclitaxel, were low compared with paclitaxel AUC, indicating that these compounds were mainly excreted in bile. As demonstrated previously, biliary elimination constituted the main route of excretion; after administration of 135 mg/m² paclitaxel for 3 hr, 29% of the administered dose was recovered in a 24-hr bile collection period, in line with other published data (Monsarrat et al., 1993; Wright et al., 1995). However, the metabolic profiles were significantly different; whereas the amounts of unmodified paclitaxel (3 and 0.9%, respectively), p-hydroxyphenyl-C3'-paclitaxel (2 and 2.2%, respectively), and 6α-hydroxy paclitaxel (12 and 11%, respectively) were similar, dihydroxy paclitaxel represented 2.5% of the administered dose in the first patient and 14.2% in this patient.

Although few biliary data are available, several in vitro studies have shown that paclitaxel is extensively metabolized in human by liver cytochrome P450 enzymes with marked interindividual variability. All metabolites so far characterized have been found to be less cytotoxic than paclitaxel itself (Harris et al., 1994a; Kumar et al., 1995; Monsarrat et al., 1993; Sparreboom et al., 1995; Wright et al., 1995). In most cases, 6α-hydroxy paclitaxel constituted the major metabolite with p-hydroxyphenyl-C3'-paclitaxel as a minor metabolite (Cresteil et al., 1994; Harris et al., 1994b; Monsarrat et al., 1993; Sonnichsen et al., 1995; Wright et al., 1995). Dihydroxy paclitaxel, an inactive metabolite (Sparreboom et al., 1995), is formed by successive hydroxylation of position 6 of the taxane ring by the P450 cytochrome 2C8 and the para-position of the phenyl at C3. Secondary hydroxylation of position 6 of the taxane ring by the P450 cytochrome 3A4 was low and produced little p-hydroxyphenyl-C3'-paclitaxel. Thus, the presence of large amounts of dihydroxy paclitaxel in the bile and in the plasma may stem from an induction of the P450 cytochrome 3A4, although induction of other cytochromes cannot be ruled out. It has been reported that the urinary excretion of 6β-hydroxy cortisol, the major unconjugated metabolite of endogenous cortisol, constitutes an index of the induction of CYP3A4 in humans (Ged et al., 1989; Lykkefeldt et al., 1994). Quantification of 6β-hydroxy cortisol and cortisol in the urine of this patient demonstrated the presence of 890 ng/ml 6β-hydroxy cortisol and 36 ng/ml cortisol. Thus, the 6β-hydroxy cortisol to cortisol ratio was 5- to 10-fold higher than the normal limit. Alteration of this ratio is indicative of an increase in cytochrome P450 3A4 activity that may affect paclitaxel clearance and pharmacodynamic.

Acknowledgment. We thank Dr. S. Arbuck of the National Cancer Institute for her assistance in obtaining Paclitaxel.

References

A rapid communication in the Journal of Clinical Oncology shows that the production of taxol metabolites is significantly lower in patients with breast cancer who have been treated with tamoxifen compared to those who have not. It is possible that the induction of CYP3A4 by tamoxifen may affect the metabolism of taxol in these patients.


All patients so far characterized have been found to be less cytotoxic than paclitaxel itself (Harris et al., 1994a; Kumar et al., 1995; Monsarrat et al., 1993; Sparreboom et al., 1995; Wright et al., 1995). In most cases, 6α-hydroxy paclitaxel constituted the major metabolite with p-hydroxyphenyl-C3’-paclitaxel as a minor metabolite (Cresteil et al., 1994; Harris et al., 1994b; Monsarrat et al., 1993; Sonnichsen et al., 1995; Wright et al., 1995). Dihydroxy paclitaxel, an inactive metabolite (Sparreboom et al., 1995), is formed by successive hydroxylation of position 6 of the taxane ring by the P450 cytochrome 2C8 and the para-position of the phenyl at C3 of the side chain in C13 by the P450 cytochrome 3A4 (Fig. 1). In most in vivo and in vitro studies, dihydroxy paclitaxel was barely detectable because hydroxylation by the P450 cytochrome 3A4 was low and produced little p-hydroxyphenyl-C3’-paclitaxel. Thus, the presence of large amounts of dihydroxy paclitaxel in the bile and in the plasma may stem from an induction of the P450 cytochrome 3A4, although induction of other cytochromes cannot be ruled out. It has been reported that the urinary excretion of 6β-hydroxy cortisol, the major unconjugated metabolite of endogenous cortisol, constitutes an index of the induction of CYP3A4 in humans (Ged et al., 1989; Lykkefeldt et al., 1994). Quantification of 6β-hydroxy cortisol and cortisol in the urine of this patient demonstrated the presence of 890 ng/ml 6β-hydroxy cortisol and 36 ng/ml cortisol. Thus, the 6β-hydroxy cortisol to cortisol ratio was 5- to 10-fold higher than the normal limit. Alteration of this ratio is indicative of an increase in cytochrome P450 3A4 activity that may affect paclitaxel clearance and pharmacodynamic.