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Cytochrome P450 3A-Mediated Human Liver Microsomal Taxol 6α-Hydroxylation[1]

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ABSTRACT

The antitumor drug taxol was metabolized to one major (6α-hydroxytaxol) and two minor metabolites by human liver microsomes. A 10-fold interindividual variability with a V_{max} of 1.16 ± 0.85 nmol/hr/mg of microsomal protein and a K_m of 18.0 ± 12.2 μM was observed for taxol 6α-hydroxylation (mean ± S.D.; n = 6). The NADPH-dependency and the inhibitory effect of carbon monoxide and piperonyl butoxide on taxol metabolism indicated the involvement of cytochrome P450 (CYP) monooxygenases. Chemical inhibition studies pointed to the CYP 3A subfamily as being responsible for taxol 6α-hydroxylation. However, although some CYP 3A substrates were inhibitory (midazolam, 17α-ethinyl estradiol, quercetin, verapamil and testosterone), others were not (troleandomycin, erythromycin and cyclosporin A). The inhibition was found to be competitive with low K_i values for midazolam (10.5 μM) and 17α-ethinyl estradiol (4.5 μM). Taxol 6α-hydroxylation correlated well with the metabolism of 17α-ethinyl estradiol (r = 0.874; $P < .05$) and midazolam (r = 0.954; $P < .01$) in the same livers. Rabbit anti-rat CYP 3A1 antibodies, which cross-react with human CYP 3A isoforms, were inhibitory of taxol 6α-hydroxylation. Although the evidence from these experiments supported the CYP 3A mediation of taxol 6α-hydroxylation, the lack of effect of some inhibitors combined with the inability of a human CYP 3A4 transfected cell line to metabolize taxol point to a CYP 3A isoform other than 3A4. The findings in this study could prove clinically useful for the prediction of potential drug interactions, both inhibitory and inductive of taxol metabolism.

Taxol (Paclitaxel), an antitumor drug obtained from the Pacific yew tree (*Taxus brevifolia*), has been demonstrated to be active against drug-refractory ovarian and breast cancers as well as malignant melanoma (Rowinsky *et al.*, 1990) with dose-limiting neurological side effects (Lipton *et al.*, 1989). It appears to act by interfering with the tubulin-microtubule system (Horwitz, 1992). The biological fate of taxol is not well understood. In phase I clinical trials, less than 10% of the dose was excreted unchanged in urine (Wiernik *et al.*, 1987), whereas another 20% was detected in bile (Wright *et al.*, 1992). The remaining 70% may likely be metabolites appearing in the bile and feces. We have recently isolated and characterized the major metabolite of taxol, 6α-hydroxytaxol (fig. 1) (Kumar *et al.*, 1994), in human liver microsomes, a metabolite distinctly different from the two known metabolites in the rat (fig. 1) (Monsarrat *et al.*, 1990; Walle *et al.*, 1993). 6α-Hydroxytaxol has also been proposed as the major *in vivo* metabolite of taxol in humans (Wright *et al.*, 1992). Like other anticancer agents, taxol has a narrow therapeutic window. Any factor that alters its disposition is likely to have an effect on the therapeutic/toxicological outcome of taxol therapy. Inhibition or induction of its metabolism or potential genetic polymorphism in the drug-metabolizing enzymes involved could be of clinical significance. In this study we have demonstrated the predominant role of CYP 3A in the human liver microsomal metabolism of taxol to its major metabolite, 6α-hydroxytaxol.

Materials and Methods

Materials. Human livers were obtained from the Liver Tissue Procurement and Distribution System (University of Minnesota, Minneapolis, MN). The cause of death was either cerebral hemorrhage or head trauma. The livers were flash frozen and stored at −80°C. Taxol (Calbiochem, San Diego, CA); [^{3}H]taxol (specific activity, 17–23 Ci/mmol; radiochemical purity, 99.9%; Moravek Biochemicals, Brea, CA); 17α-ethinyl estradiol, tolbutamide, benzphetamine, phenacetin, troleandomycin, testosterone, quercetin and NADP (Sigma Chemical Co., St. Louis, MO); *p*-nitrophenol and aniline (Aldrich Chemical Co., Milwaukee, WI); sparteine, coumarin and erythromycin (Fluka Chemical Corp., Ronkonkoma, NY); cyclosporine A (Sandoz Ltd., Basel, Switzerland); verapamil (Knoll Pharmaceuticals, Whippany, NJ); midazolam (Hoffmann-La Roche, Nutley, NJ); and 6β- and 11β-hydroxytestosterone (Steraloids Inc., Wilton, NH) were used in the study. Other chemicals used were of reagent grade. AHH-1 TK+/- human B-lymphoblastoid cells transfected with *l*-histidinol-resistant vector (cHol, control cells) or vector with human CYP 3A4 cDNA (h3A4 v3) were obtained from Gentest Corporation (Woburn, MA). Rabbit poly-

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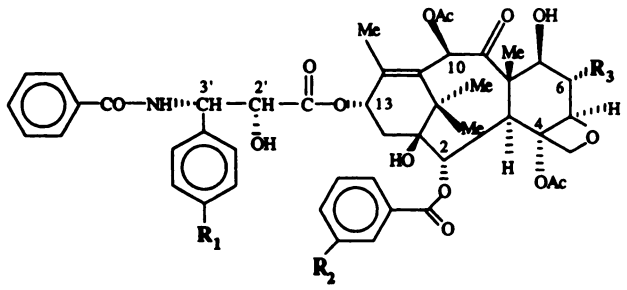
ABBREVIATIONS: HPLC, high-performance liquid chromatography; CYP, cytochrome P450.



	R_1	R_2	R_3
Taxol	H	H	H
6α-hydroxytaxol	H	H	OH
RM1	OH	H	H
RM2	H	OH	H

Fig. 1. Structures of taxol, 6α-hydroxytaxol and two known rat metabolites (RM1 and RM2). All *meta/para*-aromatic protons as well as the 2-, 10- and 3′-protons were tritium labeled in the [^{3}H]taxol used.

clonal anti-rat CYP 3A1 antiserum and preimmune serum were obtained from Human Biologics Inc. (Phoenix, AZ) (Parkinson and Gemzik, 1991).

Incubation. Human liver microsomes were prepared by differential centrifugation (Jurima *et al.*, 1985) and were stored at −80°C until use. Protein concentration was determined according to Lowry *et al.*, (1951) using bovine serum albumin as the standard. Preliminary experiments established the optimal conditions of incubation with respect to time of incubation and protein concentration. The taxol metabolism was found to be linear between 0.2 and 2.0 mg of microsomal protein per ml and up to 40 min. The incubation system consisted of 1 mg/ml of microsomal protein in 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer (0.05 M, pH 7.4) and an NADPH-generating system (2 mM $MgCl_2$, 0.4 mM NADP, 4 mM glucose 6-phosphate and 0.2 U of glucose 6-phosphate dehydrogenase; Jurima *et al.*, 1985). The reaction was started by the addition of taxol stock solution in dimethylsulfoxide containing [^{3}H]taxol (0.5 μCi/ml of incubate). The final concentration of dimethylsulfoxide was 0.2% v/v; 0.3% with inhibitors present. This concentration has no effect on CYP-mediated metabolism (Steiner and Walle, 1992). The incubation was conducted at 37°C for 20 min in a shaking water bath. The reaction was stopped by rapid cooling in ice, followed by extraction of taxol and its metabolites into five volumes of ethyl acetate (extraction efficiency > 90%). The ethyl acetate layer was separated and evaporated under nitrogen. The residue was reconstituted in HPLC mobile phase for further analysis.

HPLC analysis. The HPLC system consisted of a Waters model 510 pump (Bedford MA), a Waters U6K injector, a reversed-phase Curosil G 6 μm (250 × 3.2 mm) column (Phenomenex, Torrance, CA) with a guard column, a Waters model 440 UV detector with a 229 nm filter, a Kipp & Zonen chart recorder (Curtin Matheson Scientific, Houston, TX) and a RediFrac fraction collector (Pharmacia, Piscataway, NJ). The mobile phase consisted of 35% acetonitrile in water. The flow rate was 0.6 ml/min. The retention times for taxol and 6α-hydroxytaxol were 60 and 37 min, respectively. The metabolite quantitation was done by liquid scintillation spectrometry of the HPLC eluate corresponding to the metabolite.

Kinetics. The kinetic characterization of taxol 6α-hydroxylation in six individual human liver microsomal preparations was done as described above using 5 to 67 μM taxol (six concentrations). The apparent V_{max} and K_m values were calculated from the Lineweaver-Burk plots. The highest activity was found with liver UM 208. Hence the inhibition experiments were done with this liver.

Chemical inhibition. The incubation system and conditions were as described above. The final concentrations of taxol and the inhibitor were 10 and 50 μM, respectively. With erythromycin, cyclosporin A and troleandomycin, experiments were also conducted at an inhibitor concentration of 100 μM. The inhibition experiments with midazolam and 17α-ethinyl estradiol were done with a range of concentrations (0–40 μM) to generate Dixon plots. The inhibitor was added to the incubation system immediately before the addition of taxol. With carbon monoxide, the gas was bubbled through the incubate for 30 sec immediately before the start of incubation and the tube was capped tightly. In the case of troleandomycin, the inhibitor, microsomes and NADPH-generating system were preincubated at 37°C for 20 min before the addition of taxol. An appropriate control experiment without troleandomycin was done in parallel.

Correlation studies. The metabolism of taxol (30 μm), midazolam (60 μM) and 17α-ethinyl estradiol (50 μM) was studied under identical conditions in the six individual human liver microsomal preparations. Previously published methods were used for the assay of 2-hydroxy-17α-ethinyl estradiol (Guengerich, 1988) and 1′- and 4-hydroxymidazolam (Kronbach *et al.*, 1989).

Immunoinhibition. A mixture of 20 μg of microsomal protein and varying amounts of preimmune or 3A antiserum was kept at 23°C for 20 min. Then taxol (final concentration, 1 μM) spiked with [^{3}H]taxol and an NADPH-generating system were added and incubated at 37°C for 20 min. The extraction and analysis were as described above.

Taxol metabolism in a CYP 3A4 cell line. The control (cHol) and 3A4 transfected cell line (h3A4 v3) were propagated in Roswell Park Memorial Institute Medium 1640 without histidine and supplemented with horse serum to 9% v/v and 2 mM *l*-histidinol. The testosterone 6β-hydroxylase activity was used as a positive control. For the assay, 20 × 10^6 cells were lysed in 0.5 ml of water. Then 0.5 ml of 0.1 M phosphate buffer (pH 7.4) containing an NADPH-generating system and either taxol (2 μM with 0.25 μCi of [^{3}H]taxol) or testosterone (400 μM) was added to the lysed cells. For inhibition studies, a final concentration of 50 μM of inhibitor was used. The incubation was done at 37°C for 1 hr. The extraction and analysis of taxol and its metabolites were done as above. 11β-Hydroxytestosterone was used as an internal standard for testosterone analysis. Testosterone and its metabolites were extracted into five volumes of methylene chloride, which was evaporated and the residue was dissolved in HPLC mobile phase for further analysis. A Nucleosil C_{18} column (Phenomenex) with UV detection at 254 nm was used for HPLC analysis. The mobile phase consisted of 63% v/v methanol-water at a flow rate of 0.9 ml/min. The quantitation of 6β-hydroxytestosterone was done by comparing HPLC peak heights to those of an authentic standard.

Statistics. All values are expressed as the means ± S.D. Two-tailed Student's *t*-test for paired data was performed to calculate P values; $P < .05$ was considered significant.

Results

Kinetics of 6α-hydroxytaxol formation. Taxol was metabolized by human liver microsomes to one major (6α-hydroxytaxol; Kumar *et al.*, 1994) and two minor metabolites, the latter two not yet identified. The metabolic profile was qualitatively the same in all six livers investigated. Due to the low rates of formation of the minor metabolites, clear data could not be obtained for these metabolites. The formation of 6α-hydroxytaxol exhibited monophasic Michaelis-Menten kinetics. The mean apparent V_{max} value was 1.16 ± 0.85 nmol/hr/mg of microsomal protein and the K_m value was 18.0 ± 12.2 μM (table 1). The *in vitro* intrinsic clearance ($Cl_{int} = V_{max}/K_m$) (Wilkinson, 1987) was calculated to be 0.089 ± 0.075 ml/hr/mg of microsomal protein. A very large, 10-fold, variability in the V_{max} and Cl_{int} values in the six liver preparations was observed. Some of this variability may be sex-dependent. Thus, the V_{max} values, and in particular the Cl_{int} values, were as much as 6 times higher in the livers obtained from the female than the male donors.

Chemical inhibition. Taxol 6α-hydroxylation was found to be NADPH-dependent. Both carbon monoxide (which binds to the active site of CYP) and piperonyl butoxide (a nonspecific

TABLE 1
Taxol 6α-hydroxylation by human liver microsomes

Liver	Age	Sex	V_{max}	K_m	Cl_{int} (V_{max}/K_m)
	yr		nmol/hr/mg protein	μM	ml/hr/mg protein
UM 592	10	M	0.78	22.1	0.035
UM 505	23	M	0.87	18.8	0.046
UM 256	51	M	0.88	39.7	0.022
UM 208	16	F	2.67	12.5	0.214
UM 312	22	F	1.55	8.4	0.185
UM 350	25	M	0.22	6.6	0.033
Mean			1.16	18.0	0.089
S.D.			0.85	12.2	0.075

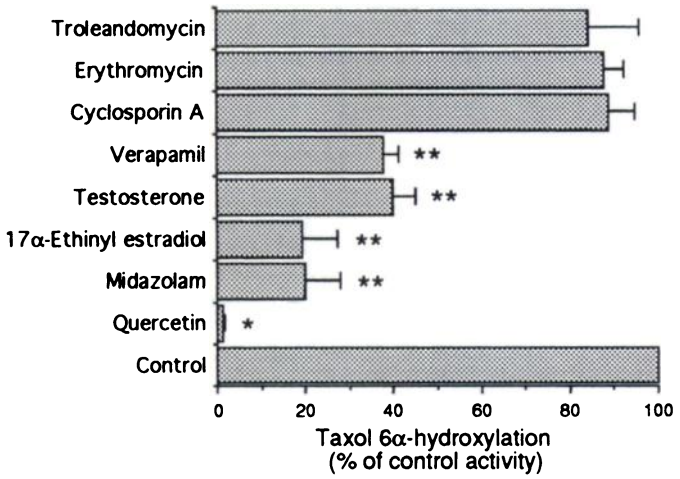


Fig. 2. Inhibition of taxol (10 μM) metabolism by CYP 3A substrates (50 μM). Mean values ± S.D. of three determinations are shown. *P < .001; **P < .005. Control activity was 1.98 nmol/hr/mg of microsomal protein.

inhibitor of CYP) were found to inhibit 94 and 74% of taxol 6α-hydroxylation, respectively, indicating the involvement of CYP in taxol 6α-hydroxylation. The potential inhibition of taxol 6α-hydroxylation by substrates selective for the isoforms of CYP most capable of mediating drug metabolism (Wrighton and Stevens, 1992) was tested in liver UM 208 with a taxol concentration of 10 μM. Whereas 17α-ethinyl estradiol (50 μM), a substrate for CYP 3A (Guengerich, 1988) was found to be inhibitory (81% inhibition of control activity), other CYP substrates (also at 50 μM) were not inhibitory [phenacetin, 1A2, 4% (Gonzalez, 1992); coumarin, 2A6, 0% (Gonzalez, 1992); benzphetamine, 2B, 6% (Riley *et al.*, 1993); tolbutamide, 2C, 0% (Gonzalez, 1992); sparteine, 2D6, 7% (Gonzalez, 1992); aniline, 2E, 2% (Nakura *et al.*, 1992); and *p*-nitrophenol, 2E, 21% (Riley *et al.*, 1993)]. The susceptibility of taxol 6α-hydroxylation to inhibition by 17α-ethinyl estradiol (50 μM) was tested in all six livers. The inhibition was found to be 72.5 ± 7.6% (P < .001). These observations pointed to the possibility of CYP 3A subfamily involvement in taxol 6α-hydroxylation. To gain further support for this notion, the inhibition of taxol 6α-hydroxylation was determined with several known substrates of CYP 3A (fig. 2). In addition to 17α-ethinyl estradiol (Guengerich, 1988), midazolam (Kronbach *et al.*, 1989), verapamil (Kroemer *et al.*, 1992), testosterone (Gonzalez, 1992) and quercetin (Miniscalco *et al.*, 1992) were found to be inhibitory, whereas other CYP 3A substrates, *viz.* erythromycin (Watkins *et al.*, 1989), cyclosporin A (Kronbach *et al.*, 1988) and troleandomycin (Pessayre *et al.*, 1983) were not inhibitory even at a higher concentration (100 μM).

The nature of inhibition was studied using midazolam and 17α-ethinyl estradiol in various concentrations. The inhibition was found to be competitive in both cases, as clearly depicted by the Dixon plots (fig. 3). The K_i values for midazolam and 17α-ethinyl estradiol were 10.5 and 4.5 μM, respectively.

Correlation studies. The taxol 6α-hydroxylation in six liver preparations was correlated with the metabolism of midazolam to its 1′- and 4-hydroxy metabolites and 17α-ethinyl estradiol to its 2-hydroxy metabolite (fig. 4). The experiments were done in parallel under identical conditions so as to minimize experimental variability. The metabolism of taxol and 17α-ethinyl estradiol correlated well with a linear regression coefficient (r) of 0.874 (P < .05) and a Spearman rank correlation coefficient (r_s) of 0.886 (P < .05). The metabolism of taxol and midazolam correlated well with an r of 0.954 (P < .01). The Spearman rank correlation coefficient (r_s) had a value of 0.829 which did not quite reach significance (P < .1). The activity levels of midazolam 1′- and 4-hydroxylation (Kronbach *et al.*, 1989) and 17α-ethinyl estradiol 2-hydroxylation (Purba *et al.*, 1987) obtained in this study compared well with previously reported values.

Immunoinhibition. The anti-rat 3A1 rabbit antiserum inhibited 6α-hydroxytaxol formation in a dose-dependent fashion (fig. 5). This inhibition was significant compared to preimmune serum (fig. 5) at antibody concentrations ≥ 4 mg of immunoglobulin G/mg of microsomal protein. The maximum inhibition achieved was 65%.

Taxol metabolism in a CYP 3A4 cell line. The 3A4 cell line (h3A4 v3) exhibited a 10-fold higher testosterone 6β-hydroxylase activity (5 pmol/min/10^6 cells) compared to the control cell line (0.5 pmol/min/10^6 cells). More than 80% of the testosterone 6β-hydroxylase activity in the 3A4 cell line was inhibited by midazolam (50 μM), whereas the residual testosterone 6β-hydroxylase activity in the control cell line was not inhibited by midazolam. Both control and 3A4 cell lines exhibited very low taxol 6α-hydroxylation (0.005 ± 0.001 pmol/min/10^6 control cells and 0.007 ± 0.002 pmol/min/10^6 3A4 cells; $n = 3$; not significantly different), which is approximately 1/50th of human liver microsomal taxol 6α-hydroxylation activity based on milligram of protein. Furthermore, neither midazolam (50 μM) nor 17α-ethinyl estradiol (50 μm) inhibited taxol 6α-hydroxylation activity in control or 3A4 cell lines.

Discussion

The metabolism of taxol in human liver microsomes is both regiospecific and stereospecific, leading to the formation of 6α-hydroxytaxol (Kumar *et al.*, 1994). The monophasic Michaelis-Menten kinetics observed indicated the involvement of a single enzyme system. The NADPH-dependency of the metabolism and the inhibitory effect of carbon monoxide and piperonyl butoxide implicated the CYP group of monooxygenases in taxol 6α-hydroxylation. Chemical inhibition studies were used to identify initially the CYP isoform(s) responsible for taxol 6α-hydroxylation. None to very weak inhibition by all CYP substrates except 17α-ethinyl estradiol pointed to the involvement of the CYP 3A subfamily. When taxol 6α-hydroxylation was examined in the presence of other CYP 3A substrates, variable results were obtained. Although some substrates were inhibitory, others were not. Verapamil, testosterone, midazolam and in particular quercetin were potent inhibitors. However, troleandomycin, erythromycin and cyclosporine A, although con-

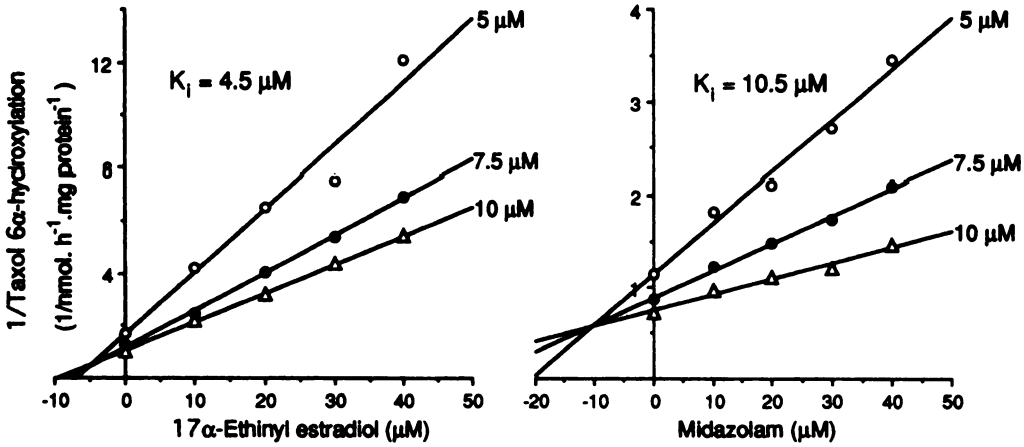


Fig. 3. Dixon plots for the inhibition of taxol metabolism by 17α-ethinyl estradiol (left) and midazolam (right). Taxol concentrations of 5 μM (○), 7.5 μM (●) and 10 μM (△) were used.

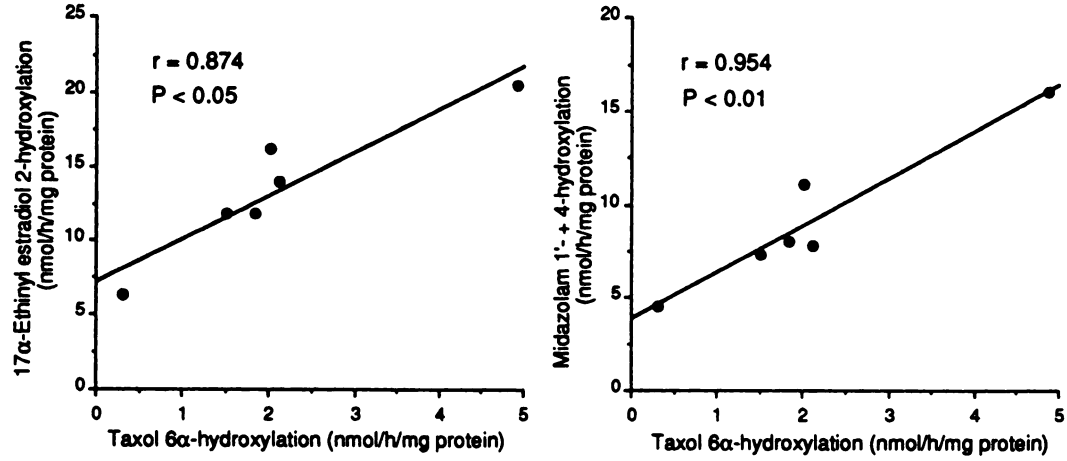


Fig. 4. Correlation of taxol (30 μM) metabolism with the metabolism of 17α-ethinyl estradiol (50 μM) (left) and midazolam (60 μM) (right).

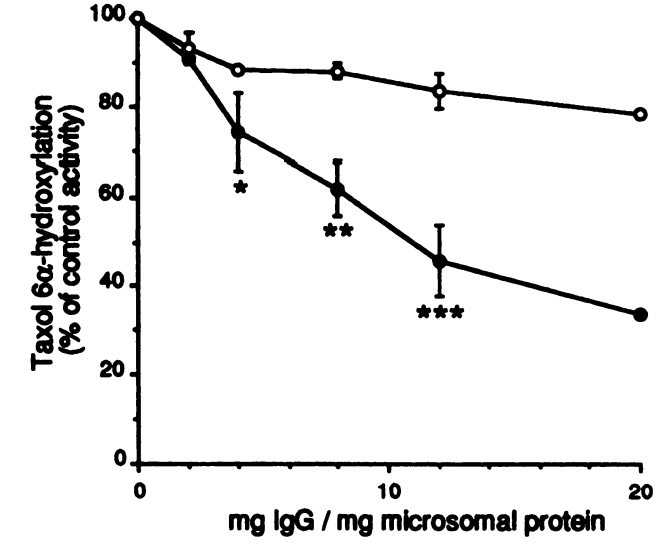


Fig. 5. Inhibition of taxol (1 μM) metabolism by rabbit anti-rat 3A1 antiserum (closed circles) and rabbit preimmune serum (open circles). Mean values ± S.D. of three determinations are shown. *P < .05; **P < .02; ***P < .01. Control activity was 0.84 nmol/hr/mg of microsomal protein. IgG, immunoglobulin G.

sidered typical CYP 3A substrates (Murray and Reidy, 1990), were not inhibitory of taxol 6α-hydroxylation even at rather high concentrations (100 μM). Troleandomycin has been shown previously to be a weak inhibitor of the metabolism of several other CYP 3A substrates such as dapsone (Fleming *et al.*, 1992), terfenadine (Yun *et al.*, 1993) and 4,4'-methylene-bis[2-chloroaniline] (Yun *et al.*, 1992), and cyclosporin A was a rather poor inhibitor of another 3A substrate, vindesine (Zhou *et al.*, 1993). In *in vivo* studies the erythromycin N-demethylase activity (a marker for CYP 3A expression) was found not to correlate with the *in vivo* metabolism of alfentanil (Krivoruk *et al.*, 1993) and cortisol (Hunt *et al.*, 1992), both CYP 3A substrates. It is therefore not unexpected that some of the "known" CYP 3A substrates were not inhibitory of taxol metabolism. The inhibition by midazolam and 17α-ethinyl estradiol was found to be competitive. The K_i values for both of these substrates were low with the potential for clinical interaction with taxol.

The *in vitro* metabolic correlations between taxol hydroxylation and 17α-ethinyl estradiol or midazolam hydroxylation obtained in this study compare favorably with the correlations reported for other CYP 3A substrates (CQA 206–291 *vs.* midazolam, Ball *et al.*, 1992; terfenadine *vs.* nifedepine, Yun *et al.*, 1993; and dapsone *vs.* nifedipine, Fleming *et al.*, 1992). These positive correlations offer further support for the CYP 3A mediation of taxol 6α-hydroxylation.

A more definitive support for CYP 3A mediation of taxol metabolism was obtained with the use of anti-rat CYP 3A1 rabbit polyclonal antibodies which cross-react with human CYP 3A isoforms. These antibodies have been reported to be inhibitory of CYP 3A-mediated metabolism such as tamoxifen N-demethylation (Mani *et al.*, 1993) and testosterone 6β-hydroxylation (Eberhart *et al.*, 1991). The concentration-depend-

ent inhibition of taxol 6α-hydroxylation further confirmed the CYP 3A mediation.

The genetically engineered human CYP 3A4 cell line as expected was found to be active in the 6β-hydroxylation of testosterone, the prototype 3A4 substrate. Only trace amounts of 6α-hydroxytaxol were, however, formed and the activity of the 3A4 cell line did not differ from that of the control cell line. Even the testosterone 6β-hydroxylation was only 4% of the reported human microsomal testosterone 6β-hydroxylation activity (Mani *et al.*, 1993). The low taxol 6α-hydroxylase activity in the 3A4 cell line could therefore be due either to the low expression of CYP 3A4 or more likely to a negligible contribution of this 3A isoform to the 6α-hydroxylation of taxol.

From the chemical inhibition, metabolic correlation and immunoinhibition studies, it can be concluded that taxol 6α-hydroxylation is catalyzed predominantly by the CYP 3A subfamily of monooxygenases. The 3A subfamily enzymes are the most predominant group of CYP enzymes in the human liver (Gonzalez, 1992) with at least four known isoforms [3A3, 3A4, 3A5 and 3A7 (fetal)]. From the present data, it is not possible to pinpoint the contribution of the individual isoforms of CYP 3A. One of the CYP 3A isoforms, 3A5, has been shown to have somewhat different though overlapping substrate specificity from that of CYP 3A4 (Aoyama *et al.*, 1989; Wrighton *et al.*, 1990). With midazolam, livers expressing a polymorphic CYP 3A isoform (possibly 3A5) along with the predominant 3A4 have been reported to have higher activity than livers expressing 3A4 alone (Kronbach *et al.*, 1989). The role of each isoform of the CYP 3A subfamily in taxol 6α-hydroxylation thus needs to be explored further.

As already pointed out (fig. 1), taxol metabolism in the rat produces two main metabolites (Monsarrat *et al.*, 1990, Walle *et al.*, 1993), which are distinctly different from the major human metabolite. This could imply the involvement of distinctly different CYP isoforms. However, the fact that midazolam and verapamil inhibit taxol metabolism in both rats (Walle *et al.*, 1993) and humans, with midazolam somewhat more potent than verapamil, indicates that all three taxol metabolites are derived from the CYP 3A subfamily.

The taxol 6α-hydroxylation by human liver microsomes showed large interindividual variation. A 10-fold variability between the highest and lowest metabolic rates was observed in the six livers studied. This is similar to the interindividual variation in taxol pharmacokinetics observed in cancer patients (Rowinsky *et al.*, 1990; Brown *et al.*, 1991; Wiernik *et al.*, 1987). Comparable interindividual variation has also been seen in the human liver microsomal metabolism of several other CYP 3A substrates such as midazolam (Kronbach *et al.*, 1989), terfenadine (Yun *et al.*, 1993), vindesine (Zhou *et al.*, 1993) and zonisamide (Nakasa *et al.*, 1993). Some of this variability may be due to genetic factors; *e.g.*, one of the CYP 3A isoforms, 3A5, is polymorphically expressed (in about 30% of individuals) (Wrighton *et al.*, 1990). Our preliminary observations suggest that there may also be sex differences in the expression of some members of this subfamily in humans as in the rat (Gonzalez *et al.*, 1986). There is great potential for the alteration of taxol disposition by coadministered drugs or food products, which are either CYP 3A inducers (glucocorticoids such as dexamethasone, phenobarbital or rifampicin) or competing CYP 3A substrates (*e.g.*, verapamil, midazolam, 17α-ethinyl estradiol or quercetin) and possibly other drugs not yet investigated (*e.g.*, gestodene, tamoxifen, nifedepine, vindesine, quinidine or zonisamide). Of these drugs, verapamil, to minimize drug resistance produced by *P*-glycoprotein, dexamethasone, as a premedication in taxol therapy to prevent hypersensitivity reactions (Weiss *et al.*, 1990) and probably quercetin, a flavonoid ingredient of grapefruit juice (Miniscalco *et al.*, 1992), are already used together with taxol.

In conclusion, we have shown that the CYP 3A subfamily of enzymes catalyze the biotransformation of taxol to its major metabolite 6α-hydroxytaxol, although the specific isoform involved has yet to be determined. Many coadministered drugs or food products are likely to modulate the activity or expression of these enzymes and are therefore likely to alter the disposition and actions of taxol. The *in vitro* approach presented here should be useful for further studies of potential drug and other interactions with taxol.

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