

# Selective Biotransformation of Taxol to 6 $\alpha$ -Hydroxytaxol by Human Cytochrome P450 2C8<sup>1</sup>

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## Abstract

The principal taxol biotransformation reaction of humans and of human hepatic *in vitro* preparations is 6 $\alpha$ -hydroxylation of the taxane ring, but a separate, minor hydroxylation pathway (metabolite B formation) also exists. Taxol metabolism was studied using membrane fractions from Hep G<sub>2</sub> cells infected with recombinant vaccinia viruses that contain complementary DNAs encoding several human cytochrome P450 enzymes. Only P450 2C8 formed detectable 6 $\alpha$ -hydroxytaxol. Metabolite B formation was catalyzed by complementary DNA-expressed 3A3 and 3A4, but not by 3A5. Each P450 3A preparation catalyzed felodipine oxidation. The apparent  $K_m$  and  $V_{max}$  values for taxol 6 $\alpha$ -hydroxylation were  $5.4 \pm 1.0 \mu\text{M}$  and  $30 \pm 1.5 \text{ nmol/min/nmol P450}$ , respectively, for complementary DNA-expressed P450 2C8; the values were  $4.0 \pm 1.0 \mu\text{M}$  and  $0.87 \pm 0.06 \text{ nmol/min/mg protein}$ , respectively, for human hepatic microsomes. The inhibition of 6 $\alpha$ -hydroxytaxol formation by quercetin was competitive with an apparent  $K_i$  of 1.3 or 1.1  $\mu\text{M}$  with 2C8 or hepatic microsomes, respectively; retinoic acid was inhibitory, showing an apparent  $K_i$  of 27  $\mu\text{M}$  with hepatic microsomes; inhibition by tolbutamide was complex, weak, and unlikely to be clinically relevant. The correlation between hepatic 2C8 protein content and 6 $\alpha$ -hydroxytaxol formation was high ( $r^2 = 0.82$ ), while the correlation with 2C9 content was low ( $r^2 = 0.38$ ).

These data show that human biotransformation routes of taxol result from catalysis by specific enzymes of two P450 families and that taxol 6 $\alpha$ -hydroxylation is a useful indicator of P450 2C8 activity in human hepatic microsomes.

## Introduction

Taxol is indicated currently for the treatment of refractory ovarian and breast cancer. In contrast to *Vinca* alkaloids, taxol increases polymerization of microtubules, stabilizes them once polymerized, and inhibits depolymerization. This results in the mitotic arrest of cells in the G<sub>2</sub> or M phases. The major pathway of taxol metabolism in humans and in human-derived *in vitro* systems is the regiospecific hydroxylation at position six of the taxane ring, with the 6-hydroxyl group *trans* to the 7-hydroxy group, yielding 6 $\alpha$ -hydroxytaxol<sup>3</sup> (1-3). This reaction appears to detoxify taxol (1) and, hence, removes

pharmacological activity. A second minor pathway for taxol metabolism results in hydroxylation at Ph2 of the C-13 side chain (metabolite B<sup>3</sup>; Refs. 1 and 2); metabolite B formation is catalyzed by the human cytochrome P450 3A subfamily, and the reaction is catalyzed by P450 3A4 (4, 5) but not by P450 3A5 (4). Cresteil *et al.* (5) assigned P450 2C enzymes in 6 $\alpha$ -hydroxytaxol formation based on a correlation between taxol 6 $\alpha$ -hydroxylase activity and total immunodetectable P450 2C ( $r^2 = 0.52$ ) determined in hepatic microsomes prepared from 11 individuals. A minimum of four enzymes constitute the human P450 2C subfamily (6), and these enzymes have been shown to have markedly different substrate selectivities (7-9). Because of the complexity of the P450 2C subfamily we have investigated taxol metabolism using cDNA-expressed human cytochrome P450 enzymes and human liver microsomes. Evidence reported herein shows that (a) P450 2C8 efficiently catalyzes taxol 6 $\alpha$ -hydroxylation and (b) this enzyme is the principal, if not the only, catalyst of this reaction in humans.

## Materials and Methods

**Reagents.** Taxol (lot FB10110) and baccatin III were obtained from the Developmental Therapeutics Program (National Cancer Institute, Bethesda, MD) and were 99% pure by HPLC. 6 $\alpha$ -Hydroxytaxol was isolated from a human bile sample and was pure by HPLC, by extinction coefficient, and by nuclear magnetic resonance (1). Felodipine and dehydrofelodipine were gifts from Astra Hässle (Mölnal, Sweden). Hydroxytolbutamide was a gift from Allan Rettie (Department of Medicinal Chemistry, University of Washington, Seattle, WA). Quercetin was purchased from Sigma Chemical Co. (lot 118F0619). Inhibitory chemicals and the constituents of the NADPH-generating system were purchased from Sigma. NADPH was purchased from Boehringer Mannheim (Indianapolis, IN).

**Human Liver Microsomes.** Human liver samples, medically unsuitable for liver transplantation, were acquired under the auspices of the Washington Regional Transplant Consortium (Washington, DC), and microsomes were prepared from these samples as described previously (4). Microsomal preparations from human liver 9 were suspended in 0.1 M phosphate buffer containing 1 mM EDTA and 50  $\mu\text{M}$  MgCl<sub>2</sub> (pH 7.4; reaction buffer) to give a final protein concentration of 0.2 mg protein/ml (0.18 nmol total P450/ml). Inhibitors (quercetin, TAO, and retinoic acid) were dissolved in ethanol and added to each microsomal suspension (10  $\mu\text{l/ml}$ ) while on ice. Tolbutamide was dissolved in reaction buffer. Reactions (4.95 ml) were warmed in a shaking water bath for 3 min at 37°C in the presence of an NADPH-generating system (final concentrations: 1 mM NADP<sup>+</sup>, 1 unit/ml of glucose-6-phosphate dehydrogenase, 10 mM glucose-6-phosphate), 50  $\mu\text{l}$  of 0.2-20 mM taxol in ethanol was added at 0 min, and the reaction was incubated for 15 min.

**Microsomes Containing cDNA-expressed P450s.** Vaccinia virus expression vectors were constructed and viral stocks were prepared as described previously (10). Hep G<sub>2</sub> cells were infected with a recombinant vaccinia virus containing human P450 cDNAs, and the cells were harvested 24 h later. Control Hep G<sub>2</sub> cells were infected with wild-type vaccinia virus. The cells were sonicated and centrifuged at 500,000  $\times$  g for 12 min. Pellets were suspended in 50 mM potassium phosphate buffer, pH 7.4, and the P450 content was measured by CO difference spectra (11). The amount of expressed P450 used in each incubation reported in Table 1 was (enzyme, pmol): 1A2, 66; 3A3, 100; 3A4, 103; 3A5, 113; 2B6, 95; 2C8, 51; 2C9, 69; and 2C9R144C,

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<sup>1</sup> The United States Adopted Name Council has assigned the name "paclitaxel" to taxol and TAXOL.

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<sup>3</sup> The abbreviations used are: 6 $\alpha$ -hydroxytaxol, benzenepropanoic acid:  $\beta$ -(benzoylamino)- $\alpha$ -hydroxy-, 6,12b-bis(acetyloxy)-12-(benzoyloxy)-2a,3,4,4a,5,6,9,10,11,12,12a,12b-dodecahydro-3,4,11-trihydroxy-4a,8,13,13-tetramethyl-5-oxo-7,11-methano-1H-cyclodeca(3,4)benz(1,2-b)oxet-9-yl ester, [2aR-[2 $\alpha$ ,3 $\alpha$ ,4 $\beta$ ,4a $\beta$ ,6 $\beta$ ,9 $\alpha$ ( $\alpha$ R\*, $\beta$ S\*),11 $\alpha$ ,12 $\alpha$ ,12a $\alpha$ ,12b $\alpha$ ]]; metabolite B, benzenepropanoic acid:  $\beta$ -(benzoylamino)- $\alpha$ ,4-dihydroxy-, 6,12b-bis(acetyloxy)-12-(benzoyloxy)-2a,3,4,4a,5,6,9,10,11,12,12a,12b-dodecahydro-4,11-dihydroxy-4a,8,13,13-tetramethyl-4-oxo-7,11-methano-1H-cyclodeca(3,4)benz(1,2-b)oxet-9-yl ester, [2aR-[2 $\alpha$ ,4 $\beta$ ,4a $\beta$ ,6 $\beta$ ,9 $\alpha$ ( $\alpha$ R\*, $\beta$ S\*),11 $\alpha$ ,12 $\alpha$ ,12a $\alpha$ ,12b $\alpha$ ]]; cDNA, complementary DNA; HPLC, high performance liquid chromatography; SIMS, secondary-ion mass spectrometry; TAO, troleandomycin; TK<sup>-</sup>, TK<sup>-</sup> 143 human embryoblast.

Table 1 Formation of taxol metabolites by cDNA-expressed human P450 enzymes

Microsomes were prepared from cells expressing specific P450s and were suspended in 50 mM potassium phosphate buffer, pH 7.4. Taxol (final concentration, 200  $\mu\text{M}$ ) was added, and the reaction mixtures were preincubated at 37°C for 3 min in a shaking water bath. Reactions were initiated by adding NADPH (final concentration, 100  $\mu\text{M}$ ) and were terminated 30 min later.

cDNA-expressed enzyme <sup>a</sup>	Catalytic activity (nmol/min/nmol P450)	
	6 $\alpha$ -Hydroxytaxol	Metabolite B
Control cells	ND <sup>b</sup>	ND
1A2	ND	ND
3A3	ND	0.26
3A4	ND	0.34
3A5	ND	ND
2B6	ND	ND
2C8	10.4	ND
2C9	ND	ND
2C9R144C	ND	ND

<sup>a</sup> Amount of P450 ranged from 51 to 113 pmol/incubation. Control activities for P450 3A enzymes were determined with felodipine (P450 form, nmol product/min/nmol P450): 3A3, 3.5; 3A4, 3.5; 3A5, 2.7.

<sup>b</sup> ND, not detected; limit of detection was 0.1 nmol.

100. Subsequent experiments with 2C8 used 10 pmol P450/incubation. Taxol (2–200  $\mu\text{M}$ ) and inhibitors were added to microsomal suspensions (4.5 ml/reaction), and the reaction mixtures were warmed for 3 min at 37°C in a shaking water bath. Reactions were initiated by adding 500  $\mu\text{l}$  of 1 mM NADPH and were terminated 30 min later.

**Sample Preparation, Chromatography, and Metabolite Structural Analysis.** All reactions were terminated by placing the tubes on ice and by adding 5.0 ml of 2.0  $\mu\text{M}$  baccatin III (internal standard) dissolved in acetonitrile to each tube. Tubes were vortexed and centrifuged, and the supernatant was evaporated to dryness with a Speed-Vac apparatus. Because of chromatographic coelution of inhibitors with taxol metabolites, two sample preparation methods were used: (a) when quercetin or tolbutamide was included in reaction mixtures, the residue from solvent evaporation was dissolved in 200  $\mu\text{l}$  of 1:1 acetonitrile:water (v/v), 5 ml chloroform was added, the organic layer was washed twice with 10 ml of 0.02 N NaOH and twice with 10 ml of water, and the chloroform was evaporated with a stream of air; control reactions for these experiments were prepared similarly; (b) in all other cases, the residue that resulted from solvent evaporation was dissolved in 200  $\mu\text{l}$  of 1:1 acetonitrile:water, and 2 ml of acetonitrile was added; tubes were vortexed and centrifuged, and the supernatant was evaporated to dryness with a Speed-Vac apparatus. All samples were dissolved in 200  $\mu\text{l}$  of 1:1 acetonitrile:water prior to HPLC analysis. Taxol metabolite HPLC analyses used a 4.6 x 100 mm Hewlett Packard ODS Hypersil column and gradient conditions of (time in minutes:percentage of water; remaining composition was acetonitrile) 0:90, 5:90, 30:35, 40:35, 45:90, and 50:90 (gradient A); the assays were conducted and were validated as described previously (4).

A sample of the P450 2C8-catalyzed reaction product was prepared for structural analysis by batch incubation; 308 pmol of P450 2C8 and NADPH were combined in a volume of 6.0 ml of reaction buffer, and this solution was incubated with 200  $\mu\text{M}$  taxol for 1 h at 37°C. Protein and buffer salts were precipitated with 5.0 ml acetonitrile. The supernatant was centrifuged, decanted, and evaporated to dryness, and the product fraction from HPLC analysis (see above) of the residue was collected. The following HPLC protocols were used to test for chromatographic coelution of this product with an authentic standard of 6 $\alpha$ -hydroxytaxol: (a) 4.6 x 100 mm Hewlett Packard ODS Hypersil with gradient A; (b) 4.6 x 250 mm Vydac 201HS C-18 with gradient A; (c) 4.6 x 250 mm Vydac 201HS C-18 with a gradient (time in minutes:percentage of water; remaining composition was acetonitrile) of 0:80, 50:30, 55:80, and 70:80. Each of these HPLC systems has been described in detail (4). The 2C8 reaction product (1 nmol in methanol) was combined with 0, 1, or 3 nmol of 6 $\alpha$ -hydroxytaxol in methanol; the solvent was evaporated, the residue was dissolved in 1:1 acetonitrile:water, and the samples were analyzed using each HPLC method. The 2C8 reaction product was analyzed by positive- and negative-ion SIMS, and taxol was analyzed in parallel experiments (25 nmol of each, dissolved in 200  $\mu\text{l}$  methanol). Initial experiments assessed total ion spectra, and subsequent B/E linked-scan experiments with the 2C8-derived preparation or with taxol studied fragment ions of  $m/z$  868 or of 852, respectively. The conditions that follow were used: instrument, JEOL SX102; matrix, 1:1 dithiothreitol:dithioerythritol (v/v); Xe gun voltage, 6 keV.

**Assessment of Reaction Kinetics.** Inhibition kinetics were analyzed using the methods and programs of Cleland (12).  $K_m$  and  $V_{max}$  values for human liver microsomes and expressed enzymes were determined by least-squares fit to a hyperbola. Inhibition constants were determined from the best fit to competitive, noncompetitive, and uncompetitive equations.

**Protein Electrophoresis and Western Immunoblotting.** Protein electrophoresis and Western immunoblotting were conducted following standard protocols and used rabbit anti-P450 IgG produced against rat P450 2C7 (13). This antibody is known to cross-react with human P450 2C8 and human P450 2C9 (13). For Western blot standards, TK<sup>-</sup> cells were infected with cDNAs encoding P450 2C8 and P450 2C9 for 48 h. Total cell lysate protein (containing 5 pmol P450 2C8 or 4 pmol P450 2C9) from TK<sup>-</sup> cells or microsomal protein prepared from nine human livers (63  $\mu\text{g}$ ) were loaded in each lane. Western blots were analyzed and integrated by densitometric scanning using a Molecular Dynamics computing densitometer and ImageQuant software. Integrated areas were determined after manually setting the baseline. Variation in the extent of antigen staining on separate western blots was corrected for by comparing the integrated areas of identical samples analyzed on each blot. Comigration of 2C8 from TK<sup>-</sup> cells and the second fastest migrating antigen band of human microsomes was established by mixing these samples.

## Results

**Analysis of the P450 2C8 Taxane Product.** HPLC and SIMS analyses indicate that the product of taxol incubation with expressed human P450 2C8 is 6 $\alpha$ -hydroxytaxol. The product was inseparable from authentic 6 $\alpha$ -hydroxytaxol when studied with three HPLC protocols, and the relationship between the peak integral and the amount of taxane added was linear in each case (data not shown). Positive-ion SIMS showed molecular ions of  $m/z$  854 or of 870 for taxol or the 2C8 product, respectively. Negative-ion SIMS showed ions at  $m/z$  852 and 525 for taxol and ions at  $m/z$  868 and 541 for the 2C8 product; these ions represent molecular ions and taxane-ring fragments of these chemicals (1, 2, 14). B/E linked-scan mass spectra were obtained for taxol (positive ion:  $m/z$  794, 569, 509, 286, and 268; negative ion:  $m/z$  792, 525, and 284) and for the 2C8 product (positive ion:  $m/z$  810, 585, 525, 286, and 268; negative ion:  $m/z$  808, 541, and 284); assignments for each of these fragments have been given for taxol (14).

**Form Selectivity and Reaction Kinetics.** cDNA-expressed human cytochrome P450 2C8 catalyzed the biotransformation of taxol to 6 $\alpha$ -hydroxytaxol (Table 1); neither P450 2C9, its variant P450 2C9R144C, nor any other expressed P450 enzyme studied produced detectable 6 $\alpha$ -hydroxytaxol (Table 1). Tolbutamide methylhydroxylation was used as a positive control for each P450 2C preparation, and these enzymes did catalyze the reaction (data not shown). The formation of metabolite B (2, 4) was catalyzed by P450 3A3 and P450 3A4, but not by P450 3A5 (Table 1). Felodipine oxidation was used as a positive control for each P450 3A preparation; P450 3A3, 3A4, and 3A5 were active (Table 1). The kinetic parameters for the formation of 6 $\alpha$ -hydroxytaxol catalyzed by cDNA-expressed P450 2C8 or by human liver microsomes (HL9) were determined, and the apparent  $K_m$  and  $V_{max}$  values were 5.4  $\mu\text{M}$  and 30 nmol/min/nmol P450 with expressed P450 2C8, and 4.0  $\mu\text{M}$  and 0.87 nmol/min/mg protein, respectively, with human hepatic microsomes (Table 2).

**Characteristics of Inhibition.** Quercetin was shown previously to be a strong inhibitor of taxol 6 $\alpha$ -hydroxylase activity (3, 4), and was shown not to affect P450 3A4-mediated metabolite B formation (4). The data in Table 2 shows that quercetin is a competitive inhibitor of 6 $\alpha$ -hydroxytaxol formation catalyzed by cDNA-expressed 2C8 and human hepatic microsomes, with  $K_i$  values of 1.3 and 1.1  $\mu\text{M}$ , respectively. Retinoic acid, another P450 2C8 substrate (15), inhibited taxol 6 $\alpha$ -hydroxylase activity with an apparent  $K_i$  value of 27  $\mu\text{M}$  when studied with human liver microsomes (Table 2). The inhibition of 6 $\alpha$ -hydroxytaxol formation by tolbutamide was weak and showed

Table 2. Kinetic constants for taxol 6 $\alpha$ -hydroxylation<sup>a</sup>

Enzyme source	Inhibitor	Inhibitor type <sup>b</sup>	$K_m$ ( $\mu$ M)	$K_{is}$ ( $\mu$ M) <sup>c</sup>	$K_{ii}$ ( $\mu$ M) <sup>c</sup>	$V_{max}$
P450 2C8 <sup>d</sup>	None		5.4 $\pm$ 1.0 <sup>e</sup>			30.0 $\pm$ 1.5
Microsomes <sup>f</sup>	None		4.0 $\pm$ 1.0			0.87 $\pm$ 0.06
P450 2C8	Quercetin	Competitive	9.1 $\pm$ 1.7	1.29 $\pm$ 0.19		21.0 $\pm$ 1.5
Microsomes	Quercetin	Competitive	9.7 $\pm$ 1.7	1.14 $\pm$ 0.17		1.09 $\pm$ 0.07
Microsomes	Retinoic acid	Competitive	14.4 $\pm$ 2.5	27.0 $\pm$ 3.7		0.86 $\pm$ 0.06
Microsomes	Tolbutamide	Mixed	5.8 $\pm$ 0.7	2370 $\pm$ 490	4020 $\pm$ 500	1.09 $\pm$ 0.04

<sup>a</sup> Kinetic constants and inhibition type were determined using the methods and programs of Cleland (12).

<sup>b</sup> Inhibition type was determined by the best fit to competitive, noncompetitive, and uncompetitive models.

<sup>c</sup>  $K_{is}$ , inhibition constant on the slope (competitive);  $K_{ii}$ , inhibition constant on the intercept (noncompetitive).

<sup>d</sup> cDNA-expressed P450;  $V_{max}$  units are nmol/min/nmol P450.

<sup>e</sup> Mean  $\pm$  SD.

<sup>f</sup> Human liver microsomes prepared from HL9 (4);  $V_{max}$  units are nmol/min/mg protein.

both competitive and noncompetitive components (Table 2). In addition, 35  $\mu$ M taxol inhibited tolbutamide metabolism by 75% when studied with expressed P450 2C8 (300  $\mu$ M tolbutamide).

Because TAO was a strong inhibitor of human taxol biotransformation but was selective for P450 3A4-mediated metabolite B formation (4), the effect of TAO on the 2C8-mediated reaction was tested. TAO did not inhibit taxol 6 $\alpha$ -hydroxylation when added at 2, 50, or 200  $\mu$ M and when the taxol concentration was 10  $\mu$ M; this finding with expressed 2C8 is consonant with the inhibitory effect of TAO of taxol metabolism by human hepatic microsomes (4, 5).

**Correlation Analyses.** Western immunoblot analysis was performed using a rabbit antibody preparation raised against rat P450 2C7, and this preparation recognizes human P450 2C8 and P450 2C9 proteins (13). Bands at  $M_r$  51,000 and 55,000 were found in each of the nine liver microsomal preparations (Fig. 1), and these bands were assigned as P450 2C8 and P450 2C9, respectively, based on these observations: (a) similar molecular weights for P450 2C8 and P450 2C9 purified from human liver have been reported (16); (b) expressed 2C8 or 2C9 gave bands that comigrated with the hepatic proteins (Fig. 1); and (c) no band separation occurred when 2C8 was mixed with human hepatic microsomes. The relative staining of 2C8 or 2C9 bands among liver samples from 9 individuals (4) was assessed by densitometry. Fig. 1 shows that the correlation between P450 2C8 content and *in vitro* synthesis of 6 $\alpha$ -hydroxytaxol was high ( $r^2 = 0.82$ ), whereas the relationship between P450 2C9 content and 6 $\alpha$ -hydroxytaxol formation was poor ( $r^2 = 0.38$ ).

## Discussion

The results of the experiments described here indicate that P450 2C8 is the predominant enzyme of those examined responsible for formation of the principal human metabolite and detoxication product of taxol (1), 6 $\alpha$ -hydroxytaxol. This conclusion and a supporting data set (4) are at variance with another report on human 6 $\alpha$ -hydroxytaxol formation (3). The conclusion of 2C8-selective catalysis is derived from the lines of evidence that follow: (a) of several cDNA-expressed P450 enzymes tested, only P450 2C8 showed a capacity for taxol 6 $\alpha$ -hydroxylation; (b) cDNA-expressed 2C8 was an efficient catalyst of the reaction, as demonstrated by a turnover number of 30 nmol/min/nmol P450; (c) taxol 6 $\alpha$ -hydroxylase activity correlated well ( $r^2 = 0.82$ ) with hepatic P450 2C8 content, but did not correlate ( $r^2 = 0.38$ ) with the hepatic content of P450 2C9; (d) the apparent  $K_m$  value obtained for taxol 6 $\alpha$ -hydroxylation by expressed P450 2C8 (5.4  $\pm$  1.0  $\mu$ M) was similar to that found with a human liver microsomal preparation (4.0  $\pm$  1.0  $\mu$ M); and (e) the inhibition kinetics for quercetin were virtually identical for expressed P450 2C8 and for hepatic microsomes with apparent  $K_i$  values of 1.3 and 1.1  $\mu$ M, respectively.

Metabolite B formation was catalyzed by P450 3A3 and by P450 3A4 with comparable rates, but P450 3A5 appeared incapable of

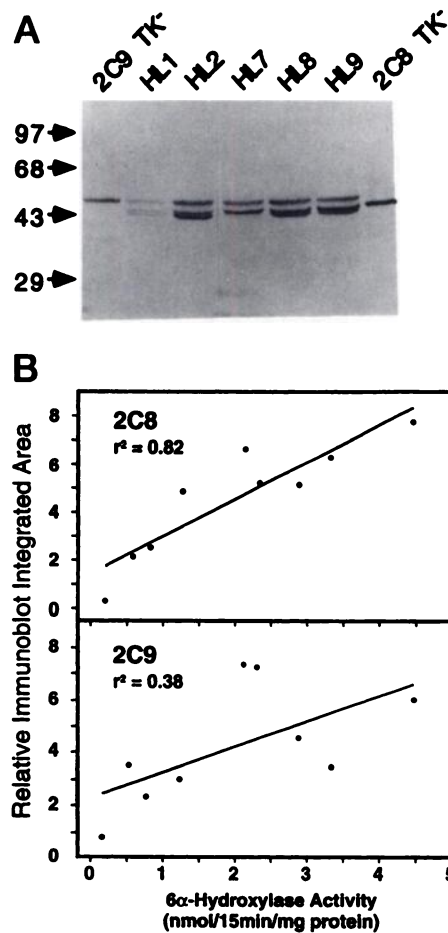


Fig. 1. Western immunoblot analysis of expressed P450 2C8, P450 2C9, or human hepatic microsomes using rabbit anti-rat P450 2C7 IgG (A), and correlation between individual P450 enzyme content and taxol 6 $\alpha$ -hydroxylase activity in 9 human hepatic microsomes (B). Total cell lysate protein (5 pmol P450 2C8 or 4 pmol P450 2C9) from vaccinia-infected TK<sup>-</sup> cells or 63  $\mu$ g microsomal protein from 9 human livers (HL1-HL9) were analyzed. Western blots were analyzed by densitometric scanning, and peak areas were determined by integration. The correlation between integrated areas and taxol 6 $\alpha$ -hydroxylase activities was determined by linear regression.

catalyzing taxol biotransformation (Table 1). These data are another example of frank substrate selectivity differences between P450 3A4 and the polymorphic P450 3A5 (6).

Quercetin [and other flavonoids (kaempferol, naringenin); Ref. 4] is shown here to inhibit a P450 2C8 marker activity; hence, flavonoids can be inhibitory toward P450 1A (17), P450 3A (18), and P450 2C subfamily catalysis. Mephenytoin, tolbutamide, hexobarbital, and sulfaphenazole did not affect the formation of 6 $\alpha$ -hydroxytaxol by human hepatic microsomes (4), however, although they are common inhibitors of enzymes in the P450 2C subfamily. These inhibition data

are consistent with the extant literature and support the assignment of 2C8 in taxol 6 $\alpha$ -hydroxylation because: (a) mephenytoin is a selective substrate for P450 2C19, and 2C8 apparently does not catalyze mephenytoin biotransformation (7); (b) the  $K_m$  for tolbutamide hydroxylation by expressed 2C8 preparations is 450–650  $\mu$ M (9, 19), and there is general agreement that P450 2C9 predominates in this reaction (7, 9); (c) tolbutamide is inhibitory toward *bona fide* 2C9-catalyzed reactions (19, 20), in contrast to tolbutamide effects on taxol 6 $\alpha$ -hydroxylation; (d) hexobarbital is a substrate for 2C9, 2C10, and possibly for 2C19 (6); and (e) sulfaphenazole selectively inhibits 2C9 (9).

There exists only a limited knowledge of 2C8 substrates or inhibitors given to humans (6), but some inhibitors of taxol 6 $\alpha$ -hydroxylation are potential chemotherapeutic agents. Quercetin inhibits the growth of several human tumor-derived cell lines (including leukemia, Ehrlich's ascites tumors, squamous cell carcinoma of head and neck origin, gastric and colon carcinoma, the estrogen receptor-positive breast carcinoma MCF-7; Ref. 21), and quercetin is being assessed in Phase I clinical trials. All-*trans* retinoic acid is a promising experimental agent for acute promyelocytic leukemia. Retinoic acid and retinol are substrates of 2C8 (15), and retinoic acid does inhibit taxol 6 $\alpha$ -hydroxylation (this work). If retinoic acid or quercetin are coadministered with taxol, then these data could be useful in assessing the potential for a pharmacokinetic interaction. Moreover, because of its higher affinity for 2C8 compared to other known substrates (9, 15, 19) and because of the relatively high turnover number of 6 $\alpha$ -hydroxylation, taxol may serve as a useful *in vitro* probe of human P450 2C8 activity.

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