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Effects of arachidonic acid, prostaglandins, retinol, retinoic acid and cholecalciferol on xenobiotic oxidations catalysed by human cytochrome P450 enzymes

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1. Effects of arachidonic acid, prostaglandins, retinol, retinoic acid and cholecalciferol on xenobiotic oxidations catalysed by 12 recombinant human cytochrome P450 (P450 or CYP) enzymes and by human liver microsomes have been investigated.

2. Arachidonic acid (50 μM) significantly inhibited CYP1A1- and 1A2-dependent 7-ethoxycoumarin *O*-deethylations, CYP2C8-dependent taxol 6α-hydroxylation and CYP2C19-dependent *R*-warfarin 7-hydroxylation. This chemical also inhibited slightly the xenobiotic oxidations catalysed by CYP1B1, 2B6, 2C9, 2D6, 2E1 and 3A4 in recombinant enzyme systems.

3. Retinol, retinoic acid and cholecalciferol were strong inhibitors for xenobiotic oxidations catalysed by recombinant CYP1A1, 2C8 and 2C19.

4. Dixon plots of inhibitions of CYP1A1-, 1A2-, 2C8- and 2C19-dependent xenobiotic oxidations by arachidonic acid, of CYP1A1-, 2B6- and 2C19-dependent activities by retinol, and of CYP1A1- and 2C19-dependent activities by cholecalciferol indicated that these chemicals inhibit P450 activities mainly through a competitive mechanism.

5. In human liver microsomes, arachidonic acid inhibited CYP1A2-dependent theophylline hydroxylation, CYP2C8-dependent taxol 6α-hydroxylation and CYP2C19-dependent omeprazole 5-hydroxylation. Taxol 6α-hydroxylation was also inhibited by retinol and retinoic acid, and omeprazole 5-hydroxylation was inhibited by retinol in human liver microsomes.

6. These results suggest that xenobiotic oxidations by P450 enzymes are affected by endobiotic chemicals and that the endobiotic-xenobiotic interactions as well as drug-drug interactions may be of great importance when understanding the basis for pharmacological and toxicological actions of a number of xenobiotic chemicals.

Introduction

Cytochrome P450 (P450 or CYP) consists of a superfamily of enzymes that catalyse the oxidation of many xenobiotic chemicals such as drugs, toxic chemicals and carcinogens as well as endobiotic chemicals including steroids, fatty acids, prostaglandins and lipid-soluble vitamins (Guengerich and Shimada 1991, Guengerich 1995, Nelson *et al*. 1996). Xenobiotic oxidations have been shown to be catalysed mainly by P450 enzymes belonging to the families of CYP1, 2 and 3 in mammals (Nebert *et al*. 1989) and these P450 enzymes have also been reported as having a role in the metabolism of endobiotic chemicals (Guengerich and Shimada 1991). Endobiotics reported to be catalysed by these P450 forms in man include testosterone and progesterone (6β- and 2β-hydroxylations) by CYP3A4, oestradiol and oestrone (2-hydroxylations) by CYP1A2 and CYP3A4, oestradiol (4-hydroxylation) by CYP1B1, retinol and arachidonic acid (oxidations) by CYP2C8 and 2C9 (Daikh *et al*. 1994, Zeldin *et al*. 1995, 1996b), arachidonic acid (epoxidation) by

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CYP2J (Zeldin *et al*. 1996a, 1997), arachidonic acid (oxidations) by CYP1A2, 2C8, 2C9 and 2E1 (Rifkind *et al*. 1995), and dehydroepiandrosterone 3-sulphate (16α-hydroxylation) by CYP3A7 (Kitada *et al*. 1987).

We have recently shown that CYP2C19 (and also CYP2C9 at slower rates) catalyses progesterone to form 21-, 16α- and 17α-hydroxyprogesterones and testosterone to form androstenedione in human liver microsomes (Yamazaki and Shimada 1997b). The conclusions reached are based on the initial findings that progesterone and other steroid chemicals strongly inhibit the *R*-warfarin 7-hydroxylation by CYP2C19 and *S*-warfarin 7-hydroxylation by CYP2C9 (Yamazaki and Shimada 1997b).

The present study was undertaken to determine if arachidonic acid, prostaglandins, retinol, retinoic acid and cholecalciferol inhibit P450-dependent xenobiotic oxidations in man because these chemicals are oxidized by several forms of human P450 enzymes (Daikh *et al*. 1994, Rifkind *et al*. 1995, Zeldin *et al*. 1995, 1996a, b, 1997). We used recombinant human P450 enzymes including CYP1A1, 1A2, 1B1, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, 3A4 and 4A11 and also human liver microsomes. All of the recombinant P450s were obtained from the baculovirus expression systems that co-express NADPH-P450 reductase (Yamazaki *et al*. 1998). Substrates used for the determination of P450 catalytic activities included theophylline, 7-ethoxycoumarin, coumarin, taxol, *S*- and *R*-warfarin, omeprazole, bufuralol, nifedipine, and lauric acid.

Materials and methods

Chemicals

Arachidonic acid, retinol, retinoic acid, cholecalciferol and lauric acid were purchased from Sigma Chemical Co. (St Louis, MO, USA). Prostaglandin E1, E2, A1, A2, B1 and B2 and 20-hydroxy-prostaglandin E1 and E2 were kindly donated by Ono Pharmaceutical Co. (Osaka, Japan). Other chemicals and reagents used in this study were obtained from sources described previously or were of highest quality commercially available (Shimada *et al*. 1997, Yamazaki and Shimada 1997b).

Enzymes preparations

Recombinant CYP2C9, 2C19, 2D6 and 3A4 expressed in microsomes of *Trichoplusia ni* cells infected with a baculovirus containing human P450 and rabbit NADPH-P450 reductase cDNA inserts were prepared as previously described (Shaw *et al*. 1997, Yamazaki *et al*. 1997). Other recombinant P450 enzymes including CYP1A1, 1A2, 1B1, 2A6, 2B6, 2C8, 2D6 and 2E1 in baculovirus system co-expressing human NADPH-P450 reductase were obtained from Gentest Co. (Woburn, MA, USA); the P450 contents in these latter systems were used as described in the data sheets provided by the manufacturer.

Human liver samples were obtained from organ donors or patients undergoing liver resection as described previously (Shimada *et al*. 1994). Liver microsomes were suspended in 10 mM Tris-Cl buffer (pH 7.4) containing 1.0 mM EDTA and 20% glycerol (v/v) (Guengerich 1994).

Enzyme assays

P450-dependent drug oxidation activities were determined by the HPLC methods as described previously (Shimada *et al*. 1994, 1997, Yamazaki and Shimada 1997a, b, Yamazaki *et al*. 1998). Briefly, standard incubation mixtures (final volume of 0.20 ml) for theophylline 8-hydroxylation by CYP1A2 and bufuralol 1′-hydroxylation by CYP2D6 contained P450 (5 pmol) in 100 mM potassium phosphate buffer (pH 7.4) (25 mM when CYP2D6 was used) containing an NADPH-generating system (0.10 μmol of $NADP^+$, 1 μmol glucose 6-phosphate and 0.05 units glucose 6-phosphate dehydrogenase) and theophylline (100 nmol) or bufuralol (40 nmol). Product formation was determined by HPLC with a 5 μm C_{18} analytical column (4.6×150 mm, Kanto Chemical, Tokyo, Japan). 7-Hydroxylation of *R*- and *S*-warfarin (both final incubation volumes of 0.20 ml) were determined in systems containing CYP2C9 or 2C19 and in some instances CYP1A1 and 1A2 (5 pmol) in 50 mM potassium phosphate buffer (pH 7.4) containing the NADPH-generating system and *R*- or *S*-warfarin (20 nmol). The separation of hydroxylated warfarin metabolites by HPLC was carried out with a 5-μm C_{18} analytical column.

The incubation mixture (final volume of 0.20 ml) for 7-ethoxycoumarin *O*-deethylation by CYP1A1

1A2, 2B6 and 2E1 and for coumarin 7-hydroxylation by CYP2A6 consisted of P450 (5 pmol) in 100 mM potassium phosphate buffer (pH 7.4) containing the NADPH-generating system and 7-ethoxycoumarin (20 nmol) or coumarin (20 nmol). Product formation was determined by HPLC using a 5 μm C_{18} analytical column (unpublished data).

Nifedipine oxidation by CYP3A4, taxol 6α-hydroxylation by CYP2C8 and lauric acid ω-hydroxylation by CYP4A11 were determined as described (Tanaka *et al*. 1990, Dirven *et al*. 1991).

Kinetic parameters for substrate oxidation by recombinant P450 enzymes and human liver microsomes were estimated using a computer program (KaleidaGraph program, Synergy Software, Reading, PA, USA) designed for nonlinear regression analysis.

Other assays

Concentrations of P450 and protein were estimated spectrally by the methods of Omura and Sato (1964) and Lowry *et al*. (1951) respectively.

Results

Effects of several endobiotic chemicals on xenobiotic oxidations by recombinant human P450 enzymes

Effects of 50 μM concentration of each of arachidonic acid, prostaglandin E2, retinol, retinoic acid and cholecalciferol on xenobiotic oxidations catalysed by 12 forms of cDNA-expressed human P450 enzymes were investigated (figure 1). Arachidonic acid significantly inhibited the CYP1A1- and 1A2-dependent 7-ethoxycoumarin *O*-deethylations, 2C8-dependent taxol 6α-hydroxylation and 2C19-dependent *R*-warfarin 7-hydroxylation. This chemical also moderately suppressed the CYP2B6-dependent 7-ethoxycoumarin *O*-deethylation, CYP2C9-dependent *S*-warfarin 7-hydroxylation, CYP2D6-dependent bufuralol 1′-hydroxylation, CYP2E1-dependent 7-ethoxycoumarin *O*-deethylation and CYP3A4-dependent nifedipine oxidation.

Prostaglandin E2 did not strongly inhibit the xenobiotic oxidations catalysed by recombinant P450 enzymes except that it affected slightly the activities of CYP2B6-dependent 7-ethoxycoumarin *O*-deethylation and CYP2C9-dependent *S*-warfarin 7-hydroxylation. We also determined the effects of several prostaglandin derivatives including 20-hydroxyprostaglandin E2, prostaglandin E1 and its 20-hydroxy-derivative, prostaglandins A1 and A2, and prostaglandins B1 and B2 as well as prostaglandin E2 on CYP2C9-dependent *S*-warfarin 7-hydroxylation and found that these derivatives did not inhibit significantly the CYP2C9-catalytic activities (data not shown).

Retinol strongly inhibited xenobiotic oxidations catalysed by CYP1A1, 2B6, 2C8, 2C9 and 2C19. Retinoic acid was also observed to be an inhibitor of CYP1A1-, 2B6-, 2C8- and 2C19-dependent xenobiotic oxidations. Cholecalciferol was a strong inhibitor for taxol 6α-hydroxylation by CYP2C8 and also inhibited the activities catalysed by CYP1A1 and 2C19.

Nature of inhibition of CYP1A1- and 1A2-dependent xenobiotic oxidations by arachidonic acid, retinol and cholecalciferol

Analysis (Dixon plots) of the inhibition of CYP1A1-dependent *R*-warfarin 7-hydroxylation by arachidonic acid, retinol and cholecalciferol, and of CYP1A2-dependent *R*-warfarin 7-hydroxylation by arachidonic acid were carried out in recombinant enzyme systems (figure 2). CYP1A1-dependent *R*-warfarin 7-hydroxylation was competitively inhibited by arachidonic acid, retinol and cholecalciferol having $K_i \sim 10$–20 μM.

CYP1A2-dependent *R*-warfarin 7-hydroxylation was also competitively inhi-

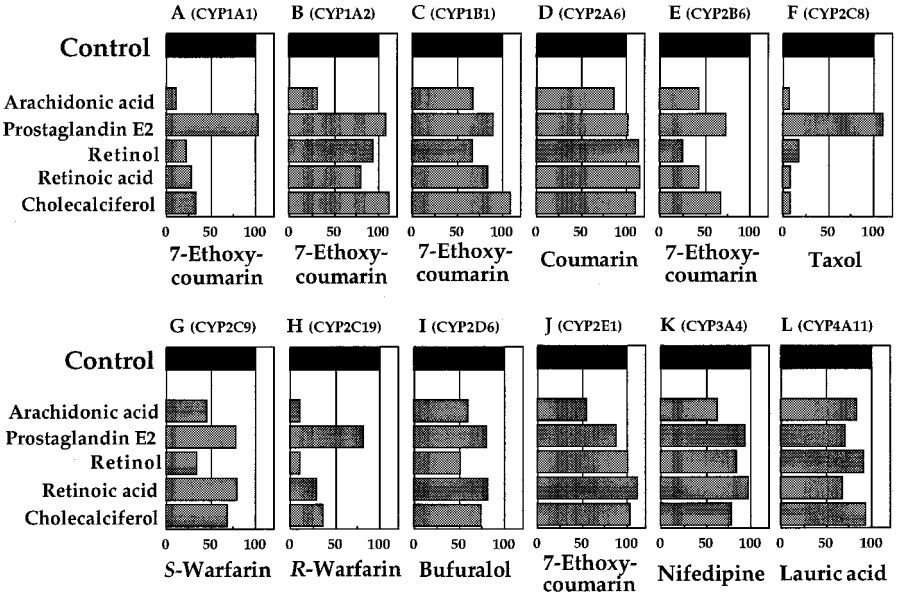

Figure 1. Effects of several chemicals (50 μM) on xenobiotic oxidation activities catalysed by recombinant CYP1A1 (A), 1A2 (B), 1B1 (C), 2A6 (D), 2B6 (E), 2C8 (F), 2C9 (G), 2C19 (H), 2D6 (I), 2E1 (J), 3A4 (K) and 4A11 (L). Activities determined were 7-ethoxycoumarin *O*-deethylation (A–C, E, J), coumarin 7-hydroxylation (D), taxol 6α-hydroxylation (F), *S*- and *R*-warfarin 7-hydroxylation (G and H respectively), bufuralol 1′-hydroxylation (I), nifedipine oxidation (K) and lauric acid ω-hydroxylation (L). Substrate concentrations used were 100 μM in all cases and P450 concentrations used were 1 pmol/200 μl incubation mixtures except that 5 pmol/250 μl incubations were used for CYP1B1, 2C8 and 3A4. Control activities (nmol products formed/min/nmol P450) without chemicals were 29.7 for CYP1A1, 2.1 for CYP1A2, 0.02 for CYP1B1, 6.2 for CYP2A6, 1.2 for CYP2B6, 0.91 for CYP2C8, 0.22 for CYP2C9, 0.06 for CYP2C19, 7.5 for CYP2D6, 2.9 for CYP2E1, 29.4 for CYP3A4 and 35.3 for CYP4A11. Data are the average of duplicate determinations.

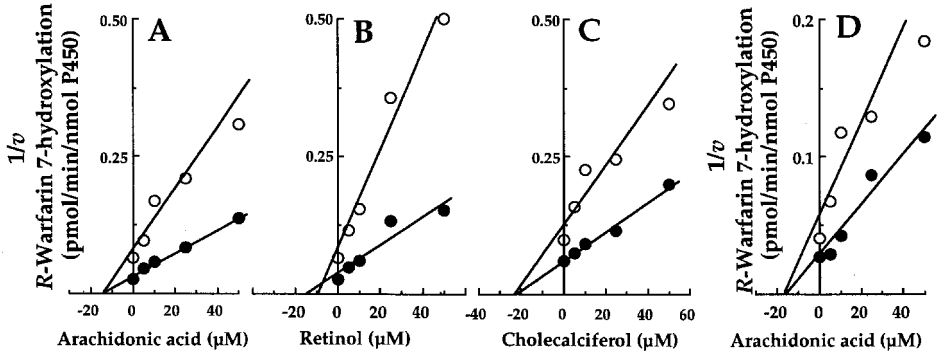

Figure 2. Dixon plots of the effects of arachidonic acid (A), retinol (B) and cholecalciferal (C) on *R*-warfarin 7-hydroxylation catalysed by recombinant CYP1A1 and of the effects of arachidonic acid (D) on *R*-warfarin 7-hydroxylation catalysed by recombinant CYP1A2. *R*-warfarin was added at 20 μM (○) and 100 μM (●) in all cases. Data are the average of duplicate determinations.

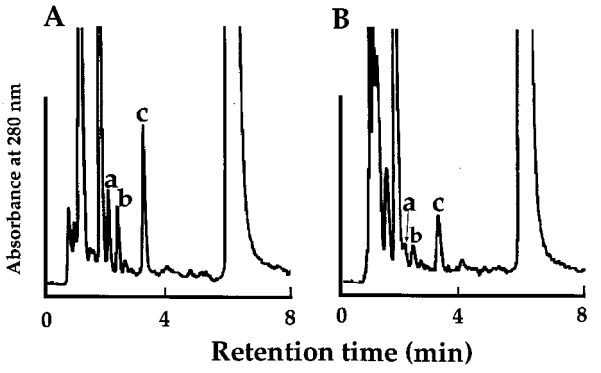

Figure 3. HPLC profiles of the effects of arachidonic acid on theophylline hydroxylation by human liver microsomes (HL-19). Theophylline hydroxylation was determined in the absence (A) or presence (B) of arachidonic acid (at 10 μM). Control activities (pmol/min/nmol P450) in the absence of arachidonic acid were 3.3 for N1-demethylation (a), 4.7 for N3-demethylation (b) and 6.0 for 8-hydroxylation (c). Liver microsomal P450 concentration used was 350 pmol/250 μl and incubations were carried out at 37°C for 30 min. None of the matabolites was formed when the NADPH-generated system was omitted from the incubation mixture.

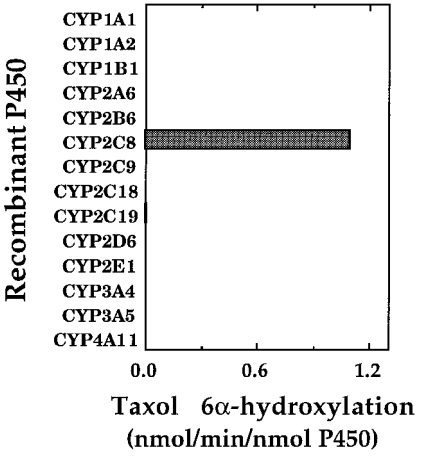

Figure 4. Taxol 6α-hydroxylation activities by recombinant human P450 enzymes. Data are average of duplicate determinations.

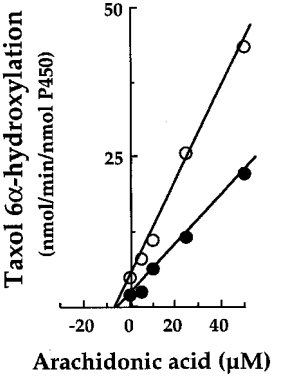

Figure 5. Dixon plots of the effects of arachidonic acid on taxol 6α-hydroxylation catalysed by recombinant CYP2C8. Substrate concentrations used were 20 μM (○) and 100 μM (●). Data are the average of duplicate determinations.

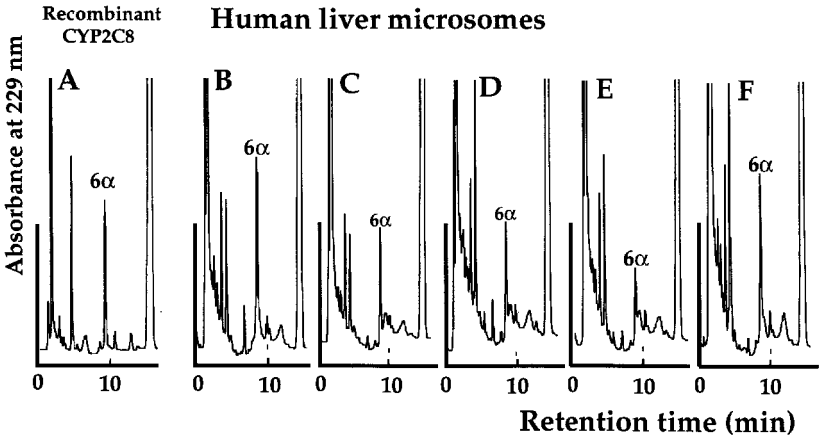

Figure 6. Taxol 6α-hydroxylation by recombinant CYP2C8 (A) and human liver microsomes (HL-19) (B) and the effects of arachidonic acid (C), retinol (D), retinoic acid (E) and cholecalciferol (F) on liver microsomal taxol 6α-hydroxylation activities. These chemicals and substrate were added at 10 μM concentration to the reaction mixture. P450 concentrations were 5 pmol/250 μl for CYP2C8 and 125 pmol/ml for liver microsomes. Control activities for taxol 6α-hydroxylation (nmol/min/nmol P450) in the absence of chemicals were 0.91 for CYP2C8 and 0.034 for liver microsomes. 6α-, 6α-Hydroxytaxol. None of the metabolites was formed when the NADPH-generating system was omitted from the incubation mixture.

bited by arachidonic acid in recombinant enzyme system (figure 2D). To determine if liver microsomal CYP1A2-catalytic activities are affected by arachidonic acid, the effects of this chemical on the hydroxylation of theophylline, one of the marker activities for CYP1A2 (Tjia *et al*. 1996, Shimada *et al*. 1997), were examined (figure 3). Arachidonic acid extensively inhibited the N1- and N3-demethylation and 8-hydroxylation of theophylline in human liver microsomes.

Inhibition of CYP2C8-dependent taxol 6α-hydroxylation by endobiotic chemicals

It has previously been reported that CYP2C8 is the principal enzyme catalysing taxol 6α-hydroxylation in man (Rahman *et al*. 1994). Other studies have, however, shown that CYP3A4/5 and other P450 enzymes as well as CYP2C8 catalyse taxol hydroxylation by human liver microsomes (Cresteil *et al*. 1994, Kumar *et al*. 1994, Sonnichsen *et al*. 1995, Royer *et al*. 1996). Of the 12 forms of recombinant human P450 enzymes examined in this study, only CYP2C8 was found to catalyse taxol 6α-hydroxylation (figure 4). CYP2C19 only hydroxylated taxol at a very low rate and other forms including CYP1A1, 1A2, 1B1, 2A6, 2C9, 2C18, 2D6, 2E1, 3A4, 3A5 and 4A11 did not catalyse taxol hydroxylation at detectable rates.

Inhibition of CYP2C8-dependent taxol 6α-hydroxylation was of a competitive nature and the K_i determined was ~ 7 μM in the recombinant enzyme system (figure 5).

Human liver microsomal taxol 6α-hydroxylation activities were inhibited by arachidonic acid, retinol and retinoic acid (figure 6).

Nature of inhibition of CYP2C9- and CYP2C19-dependent xenobiotic oxidations in recombinant and human liver microsomal P450 systems

R-warfarin 7-hydroxylation by recombinant CYP2C19 was inhibited by arachidonic acid and retinol mainly by a competitive mechanism (figure 7). The K_i determined in these cases were < 10 μM.

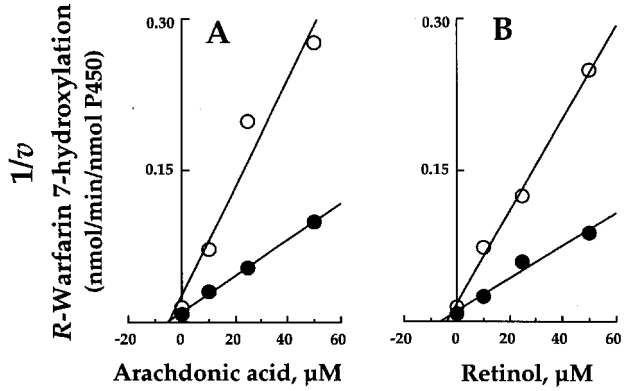

Figure 7. Dixon plots of the effects of arachidonic acid (A) and retinol (B) on *R*-warfarin 7-hydroxylation catalysed by recombinant CYP2C19. Substrate concentrations used were 20 μM (○) and 100 μM (●). Data are the average of duplicate determinations.

Table 1. Effects of arachidonic acid and retinol on *S*-warfarin 7-hydroxylation and omeprazole 5-hydroxylation catalysed by human liver microsomes (HL-19).

		S-warfarin 7-hydroxylation		Omeprazole 5-hydroxylation	
Chemical	μM	(pmol/min/mg protein)	(%)‡	(pmol/min/mg protein)	(%)‡
None	–	2.8± 0.3	(100)	220± 25	(100)
Arachidonic acid	10	2.2± 0.4	(79)	112± 18*	(51)
Retinol	10	2.4± 0.3	(86)	105± 25*	(48)

Values are means ± SD of triplicate determinations. *$p < 0.01$.
‡ Bracket indicates the % of control activity (in the absence of arachidonic acid and retinol).

Two reactions in human liver microsomes, *S*-warfarin 7-hydroxylation and omeprazole 5-hydroxylation, were chosen for the determinations of activities of CYP2C9 and 2C19 respectively (table 1) (Yamazaki and Shimada 1997a, Yamazaki *et al*. 1997). Both arachidonic acid and retinol inhibited significantly the CYP2C19-dependent omeprazole 5-hydroxylation by human liver microsomes. The effects of these chemicals on *S*-warfarin 7-hydroxylation by human liver microsomes were less marked.

Discussion

Drug-drug interactions associated with P450 enzymes have been shown to be one of the important factors in causing serious side effects in man when two or more drugs are dosed to patients simultaneously (Guengerich 1997). At least two mechanisms underlying P450-dependent drug-drug interactions have been suggested (Periti *et al*. 1992, Li *et al*. 1997). One is induction of particular form(s) of P450 by a drug causing an increase in the metabolism of co-administered drug(s) (Li *et al*. 1997). In this case, the pharmacological and toxicological actions of co-administered drugs may be altered, depending on the reactivities of the metabolites that are more inactive or reactive than the parent drugs (Li *et al*. 1997).

The other mechanism, which is suggested to be more important than the induction mechanism in P450-associated drug interactions, is selective inhibition of specific form(s) of P450 by a drug, resulting in decreases in the metabolism of co-

administered drugs (Guengerich 1997). There are at least four important mechanisms underlying P450 inhibition by drugs, namely (1) competitive inhibition, (2) reversible inhibition by nitrogen heterocycles, (3) inhibition through formation of metabolite intermediate complexes and (4) mechanism-based inhibition (Gibaldi 1993, Halpert 1995). Among these, competitive inhibition has been considered as the most common in drug-drug interactions in man (Halpert *et al*. 1994, Halpert 1995). Since some of the P450 enzymes involved in xenobiotic oxidations in mammals have also been reported to catalyse oxidations of endobiotic chemicals such as arachidonic acid, prostaglandins, retinol, retinoic acid and cholecalciferol (Theoharides and Kupfer 1981, Hayashi *et al*. 1988a, b, Gonzalez 1990, Ryan and Levin 1990, Tanaka *et al*. 1990, Guengerich and Shimada 1991, Laethem and Koop 1992, Daikh *et al*. 1994, Rifkind *et al*. 1995, Zeldin *et al*. 1995, 1996a, b, 1997), the possibility exists that these endobiotic chemicals affect drug oxidations by acting as competing substrate for the binding sites of P450 enzymes.

Our current results have shown that arachidonic acid, retinol, retinoic acid and cholecalciferol inhibited the xenobiotic oxidations catalysed by particular forms of P450 based on the results obtained from recombinant human P450 enzymes. Reactions inhibited by these endobiotic chemicals (at 50 μM concentration) in recombinant P450 systems included the CYP1A1-dependent 7-ethoxycoumarin *O*-deethylation by arachidonic acid, retinol, retinoic acid and cholecalciferol, the CYP1A2-dependent 7-ethoxycoumarin *O*-deethylations by arachidonic acid, the CYP2C8-dependent taxol 6α-hydroxylation by arachidonic acid, retinol, retinoic acid and cholecalciferol, and the CYP2C19-dependent *R*-warfarin 7-hydroxylation by arachidonic acid, retinol, retinoic acid and cholecalciferol. Dixon plots of the inhibition of CYP1A1-, 1A2-, 2C8- and 2C19-dependent xenobiotic oxidations by arachidonic acid, of CYP1A1-, 2B6- and 2C19-dependent activities by retinol and of CYP1A1- and 2C19-dependent activities by cholecalciferol support the view that these chemicals inhibit P450 activities mainly through a competitive mechanism. It is also found that arachidonic acid, retinol and cholecalciferol inhibited CYP1A1-dependent *R*-warfarin 7-hydroxylation having $K_i \sim 10$–20 μM. It is interesting in this connection to note our previous findings that *S*-mephenytoin 4′-hydroxylation (by CYP2C19) and *S*-warfarin 7-hydroxylation (by CYP2C9) are competitively inhibited by progesterone with $K_i = 9$ and 17 μM respectively in human liver microsomes (Yamazaki and Shimada 1997b).

Human liver microsomal studies support the view that arachidonic acid inhibited theophylline hydroxylation catalysed by CYP1A2, taxol 6α-hydroxylation catalysed by CYP2C8 and omeprazole 5-hydroxylation catalysed by CYP2C19. We also observed that retinol inhibited CYP2C19-supported omeprazole 5-hydroxylation and arachidonic acid, retinol and retinoic acid inhibited CYP2C8-supported taxol 6α-hydroxylation in human liver microsomes.

It is not known at present whether these endobiotic chemicals really affect the biotransformation of xenobiotic chemicals when the latter chemicals are administered to man *in vivo*. The levels of the endobiotics in the body, particularly in the microsomal fractions of several organs including liver, lung, kidney, intestine and others, may be important in understanding the basis for endobiotic-xenobiotic interactions in man. However, detailed information on the exact levels of these endobiotic chemicals in the microsomal membranes are lacking, whereas the plasma levels of arachidonic acid, prostaglandins, retinol, retinoic acid and cholecalciferol are reported to be in the range 0.1–10 μM (Japanese Biochemical Society 1979).

Since the levels of endobiotic chemicals in the microsomal membranes may be higher than those in the blood, the possibility exists that these endobiotic chemicals affect the biotransformation of drugs by inhibiting P450 catalytic activities.

It has previously been reported that CYP2C8 is the principal enzyme in catalysing taxol 6α-hydroxylation in human liver microsomes (Rahman *et al*. 1994). Other studies have, however, shown that CYP3A4/5 and some other P450 enzymes also catalyse taxol hydroxylation by human liver microsomes (Cresteil *et al*. 1994, Kumar *et al*. 1994, Sonnichsen *et al*. 1995, Royer *et al*. 1996). Of the 12 forms of recombinant human P450 enzymes examined in the current study, CYP2C8 had the highest catalytic activity for taxol 6α-hydroxylation. CYP2C19 had a very low rate of taxol hydroxylation and other forms including CYP1A1, 1A2, 1B1, 2A6, 2C9, 2C18, 2D6, 2E1, 3A4, 3A5 and 4A11 did not catalyse taxol at significant levels. These results support the importance of CYP2C8 in the 6α-hydroxylation of taxol by human liver microsomes, consistent with the results reported recently by Rahman *et al*. (1994).

Prostaglandins were not very good inhibitors for xenobiotic oxidations catalysed by recombinant P450 enzymes, as compared with arachidonic acid, retinol, retinoic acid and cholecalciferol. Since prostaglandin E2 slightly inhibited the activities of CYP2B6-dependent 7-ethoxycoumarin *O*-deethylation and CYP2C9-dependent *S*-warfarin 7-hydroxylation, we also determined the effects of several prostaglandin derivatives (including 20-hydroxyprostaglandin E2, prostaglandin E1 and its 20-hydroxy-derivative, prostaglandins A1 and A2, and prostaglandins B1 and B2) on CYP2C9-dependent *S*-warfarin 7-hydroxylation. The results suggested again that these prostaglandin derivatives were not very good inhibitors of CYP2C9-dependent *S*-warfarin 7-hydroxylation activity. It should, however, be mentioned that prostaglandins are rapidly metabolized by several forms of P450 in microsomal membranes of the liver, lung, kidney and intestine (Vatsis *et al*. 1982, Kaku *et al*. 1984, Miyata *et al*. 1994, Yamamoto *et al*. 1984, Laethem and Koop 1992, Karara *et al*. 1993).

In conclusion, the present results suggest that xenobiotic oxidations by P450 enzymes are affected by endobiotic chemicals and that endobiotic-xenobiotic interactions as well as drug-drug interactions may be of great importance in the understanding of the basis for pharmacological and toxicological actions of a number of xenobiotic chemicals.

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