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FUNCTIONAL PROTEIN-PROTEIN INTERACTION OF DRUG METABOLIZING ENZYMES

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1. ABSTRACT

Cytochrome P450 (P450, CYP), a major class of enzymes involved in Phase I drug metabolism, is expressed in the cellular endoplasmic reticulum together with other enzymes, such as microsomal epoxide hydrolase (mEH) and UDP-glucuronosyltransferase (UGT). In many cases, the metabolite produced by P450 is sequentially metabolized by other enzymes to increase its water solubility. It would be reasonable to assume that the metabolite produced by P450 is directly transferred to the other enzymes participating in its subsequent metabolism via protein–protein interactions for rapid metabolism. However, these steps have been considered to take place individually. Previously, we suggested that CYP1A1 specifically associates with mEH, UGTs and NADPH-P450 reductase. This observation strongly supports the view that there is functional cooperation between P450 and mEH/UGT to facilitate multistep drug metabolism. In recent years, accumulating evidence suggests the interaction between drug metabolizing enzymes and a change in enzymatic function by this interaction. In this review, we summarize the interaction between drug metabolizing enzymes and discuss its impact on their function.

2. INTRODUCTION

Drug metabolizing enzymes play important roles in the detoxication and excretion of many endogenous or exogenous compounds. These enzymes are categorized as either phase I or phase II enzymes on the basis of their function. Cytochrome P450 (P450 or CYP) plays key roles in phase I reaction (1, 2) although other enzymes, such as epoxide hydrolase (3, 4) or carboxylesterase (5), are also important. Phase II enzymes involve conjugating enzymes such as UDP-glucuronosyltrasferase (UGT) (6, 7), *N*-acetyltransferase (8, 9) and glutathione-*S*-transferase (GST) (10). Drug metabolism can be explained as steps in which a polar moiety is introduced into the compounds rendering them more suitable for excretion. These steps are also involved in the formation of active metabolites from proximal carcinogens. Thus, enzymes involved in drug metabolism are one of the major targets of cancer research. To date, it has been considered that these enzymes play their roles individually. However, if these enzymes interact with each other and cooperate to modulate the partner enzyme(s), this would represent a unique mechanism for the regulation of the function of drug metabolizing enzymes.

In many cases of drug metabolism, the metabolite produced by P450 is sequentially metabolized by other enzymes to increase its water solubility. It would be reasonable to assume that the metabolite produced by P450 is directly transferred to the other enzymes participating in the subsequent metabolism via protein–protein interactions for rapid metabolism. Interactions between P450 enzymes and between UGT enzymes have been demonstrated. However, thus far, the information reported for the interaction between different sorts of enzymes is very limited. Previously, we suggested that CYP1A1 specifically associates with mEH, UGTs and NADPH-P450 reductase (fp2) (11). This observation strongly supports the view that there is functional cooperation between P450 and mEH/UGT to facilitate multistep drug metabolism (Figure 1). In this review, we deal with

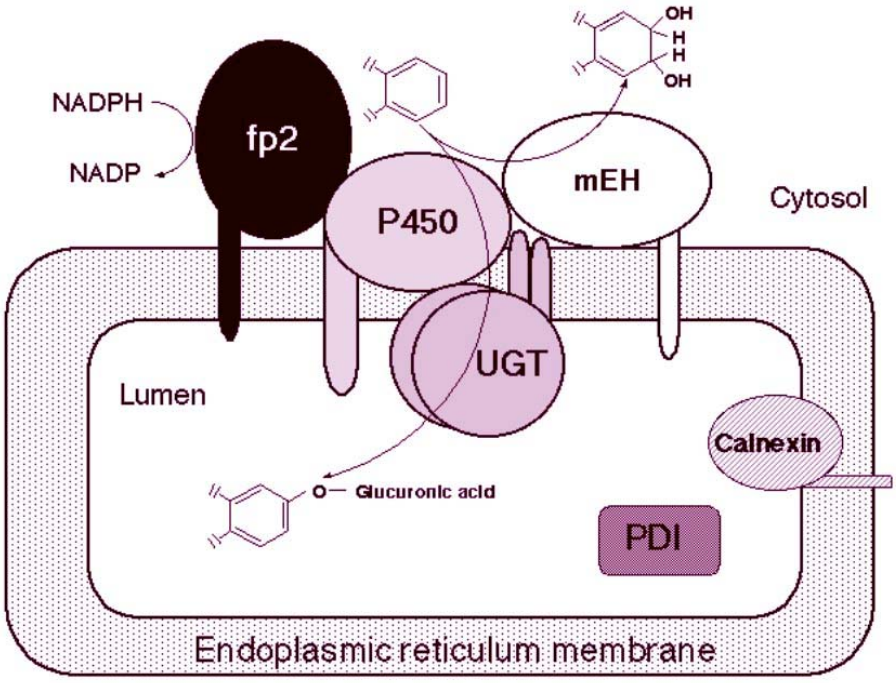

Figure 1. Putative model of microsomal drug metabolizing enzyme complex.

the interaction between drug metabolizing enzymes and its impact on their function.

3. INTERACTION BETWEEN CYTOCHROME P450 ISOFORMS

P450 is a membrane bound enzyme which is localized in the endoplasmic reticulum (ER). The topology of P450 is of the P450-type in which the N-terminal is anchored to the ER-membrane and the major part of the enzyme faces the cytosol (12, 13). However, accumulating evidence suggests that some domains of the P450 molecule are integrated into the ER membrane to permit full catalytic activity (14). P450 needs fp2 and NADPH as an electron donor in order to carry out drug oxidation. One of the two electrons needed for the P450 reaction can be supplied by cytochrome b5 (b5) (15, 16). Thus, it is well known that P450 interacts with fp2 and b5. To our knowledge, the first mention of an interaction among P450s was the report by Alston *et al.* in 1991 (17). In that study, they treated hepatic microsomal proteins from 3-methylcholanthrene (MC)-treated rats with a reversible cross-linking-reagent, sulfosuccinimidyl(4-azidophenyldithio)propionate, and detected a cross-linked complex of CYP1A1 and CYP3A. In addition, bovine adrenocortical mitochondrial P450s, P450scc and P450 11beta (CYP11A1 and CYP11B), interact with each other to modulate the steroidogenic activity (18, 19). Further, an oligomer of CYP2B4 (20) and a hexamer of CYP1A2 (21) have been reported. There is also a series of reports that P450 isoforms can influence the function of other isoforms when combined in a reconstituted system (Table 1). When CYP3A4 was reconstituted with CYP1A1 or CYP1A2, the CYP3A4-catalyzed testosterone 6beta-hydroxlation activity was enhanced (22). On the other hand, competition between CYP2E1 and CYP2A6 for fp2 in the microsomal membrane has been observed (23). In addition, CYP2B4 and CYP1A2 can form an affinity complex (24). When combined in reconstituted systems, formation of a CYP2B4-CYP1A2 complex with a high affinity to fp2 was observed (25). The ternary complex of CYP2B4-CYP1A2-reductase has been shown to be functionally active. Interestingly, X-ray crystallography and gel-filtration investigations have shown that CYP2C8 forms a dimer in solution (26). Peripheral fatty acid binding may assist this dimerization. Although further studies are necessary to understand how the interaction among P450s contributes to drug metabolism *in vivo*, the above research suggest that P450 function is dynamically regulated by the co-existing isoforms. However, more studies using greater numbers of P450 isoforms are needed to generalize this concept. In addition, the question whether the ratio of P450 isoforms in the complex affects their function also needs to be clarified.

4. INTERACTION BETWEEN UDP-GLUCURONOSYLTRANSFERASE ISOFORMS

Glucuronidation is well known as a major detoxification pathway for both exogenous and endogenous compounds. This metabolism is catalyzed by UGTs, members of a superfamily of glycosyltransferases that are found in the ER membrane (6). The UGT gene families are divided into two groups, UGT1 and UGT2, on the basis of evolutionary divergence. The UGT can be referred to as UDP-glucosyltransferase, but we referred to this enzyme as UDP-glucuronosyltransferase in this review because many papers and reviews use the latter terminology. The N-terminal region of UGT is now believed to be an important determinant of substrate specificity (Ref. 6). UGT is a type-

Table 1. Summary of the interaction between drug metabolizing enzymes

Drug Metab. Enzymes		Phenomena observed	Reference
CYP3A4	CYP1A1 CYP1A2	Testosterone 6beta-hydroxylation was stimulated	22
CYP2A6	CYP2E1	Competitive interactions for NADPH-cytochrome P450 reductase	23
CYP2B4	CYP1A2	Affinity complex	24
CYP2B4-1A2	NADPH-cytochrome P450 reductase	Affinity complex	25
CYP2C8	CYP2C8	Homodimer formation	26
CYP1A2	CYP1A2	Hexametric structure	21
UGT1A6/7 (tentative)	UGT (isoform not identified)	Dimer and tetramer formation	38
UGT1A1	UGT1A1 (truncated mutant)	Oligomerization, activity abolished	42
UGT1As	UGT2B1	Hetero-dimerization	40
UGT2B1	UGT2B1	Homo-dimerization	41
UGT1A1	UGT1A1	Homo-dimerization	43
UGT1A9	UGT1A9	Homo-dimerization	45
UGT2B21	UGT2B22	Hetero-oligomerization: simultaneous expression enhances glucuronidation of chloramphenicol & the 6-hydroxyl group of morphine	56, 46
P450 MC	mEH		64
P450 PB	mEH		64
CYP1A1	mEH, UGTs	Trapped on affinity chromatography	11
CYP2C	mEH	Enhanced mEH activity	65
CYP3A4	UGT2B7	Alters regioselectivity of morphine glucuronidation	[a]
CYP1A1	UGT1A7 (tentative)	Substrate supplier	67

[a] Takeda *et al*, Unpublished data

I membrane protein which is anchored to the ER membrane via a C-terminal region having cytosolic tail of approximately 15-20 residues (27). UGT2B1 mutant, which lacks this cytosolic tail, still exhibits activity even although it is much lower than that of the wild-type (28). The double lysine motif, KKXX or KXKXX in the cytosolic tail, which is known as an ER-retention signal (29) is found in all UGTs. The active site of UGT is believed to be located on the luminal side, and this is assumed to be the reason that UGT exhibits its "latency" (27, 30). Since UGT2B1 mutant that lacks the C-terminal trans-membrane region and the cytosolic tail is still resident in the ER, it is assumed that there could be ER-binding site(s) for UGT2B1 other than the C-terminal region (28). In support of this, the internal sequence contributing to the integration into the ER has also been found in UGT1A6 (31). However, the catalytically active soluble truncation mutant of UGT1A9 that lacks the C-terminal trans-membrane region and cytosolic tail can be expressed (32). There is a signature sequence of the UDP-glucosyltransferase superfamily (6). The substrate specificities of UGT isoforms have been characterized so far by single isoform-expression systems in most studies.

4.1. Oligomerization of UGT isoforms

An early study showed the inseparable nature of testosterone UGT (UGT2B3 and/or UGT2B6) and androsterone UGT (UGT2B2) (33). Although successful separation of these UGTs was achieved by another group, the high affinity of both UGTs was suggested (34). Matusi and Nagai purified testosterone UGT from LA-Wistar rats which have a defect in androsterone UGT (UGT2B2) (33). As the homology between UGT2B2 and UGT2B3/6 is quite high (Ref. 6), it is conceivable that these UGTs form a hetero-oligomer. The purification studies mentioned above support this possibility. However, as we have seen, testosterone UGT and androsterone UGT can be isolated and characterized individually (34, 35). It is, therefore, possible that these UGTs can form a homo-oligomer as well as a hetero-oligomer. Hochman *et al.* reported the purification of a UGT (GT_{2P}) from pig liver on the basis of *p*-nitrophenol glucuronidation activity and obtained a preparation exhibiting three bands around 58 kDa with an intensity ratio of 1:2:1 on SDS-PAGE (36). This data suggests that UGT can form a tetrameric structure. This idea was supported by radiation-inactivation analysis (37, 38). Furthermore, Gschaidmeier and Bock suggested from the radiation inactivation of microsomal UGTs that monoglucuronidation of phenols is catalyzed by a dimeric form of UGT while diglucuronidation is catalyzed by a tetramer (38). Matern *et al.* reported oligomeric UGT forms using gel filtration (39). More reliable evidence for a UGT hetero-oligomer has been obtained by cross-linking the UGT1A subfamily with UGT2B1 (40). In this approach, a protein-protein interaction between UGT1As and UGT2B1 was demonstrated by the use of a UGT isoform-selective antibody. However, the functional significance of UGT2B1 and UGT1As oligomer is largely unknown. UGT2B1 also forms a homo-oligomer (41). The importance of the N-terminus domain for dimerization has been shown by preparing chimeric-proteins of UGT2B1 and ecdysteroid glucosyltransferase (41). This is consistent with the observation that when Leu4 of the mature form of wild-type UGT2B1 is mutated to Arg, the activity is abolished (41). Interestingly, when two kinds of inactive UGT2B1 mutant were expressed together in COS cells, the activity was restored (41). This observation again suggests the importance of hetero-oligomerization of UGTs as far as their function is concerned. Another example examining the relationship between a UGT oligomer and its function has been reported for UGT1As. Crigler-Najjar syndrome type II (CNII) shows dominant-negative inheritance for bilirubin glucuronidation. When the truncated mutant form of UGT1A1 in CNII patients was simultaneously expressed with the wild-type enzyme in COS cells, the activity was reported to be approximately 6% that of the wild type (42). This can be explained if the mixed oligomer of wild-type UGT1A1 and the truncated mutant is inactive while the oligomer of wild type UGT1A1 is active. This dominant-negative effect

corresponds closely to the CNII phenotype. Further evidence of the homo-oligomerization of UGT1A1 has been demonstrated by the two-hybrid system (43). When allelic variant UGT1A6*2 was expressed homozygously in HEK293 cells, the UGT1A6*2 allozyme exhibited almost two-fold greater activity than that of wild-type UGT1A6*1 (44). On the other hand, simultaneous expression of UGT1A6*1 and UGT1A6*2 was shown to produce low enzyme activity (44). This observation suggests that the UGT1A6*1/*2 oligomer has a lower activity than UGT1A6*1 or UGT1A6*2 homo-oligomers. In addition, homo-oligomerization of UGT1A9 was demonstrated by the epitope-tagging method (45). These studies suggest that human UGT1A1, UGT1A9 and rat UGT2B1 form homo-oligomers, while rat UGT1As and UGT2B1 form hetero-oligomers.

4.2. Alteration of UGT function by hetero-oligomer formation: study using morphine glucuronidation as the probe

It remains to be clarified whether UGT oligomerizations are functionally relevant, although a change in steroid-UGT activity by co-expression of two catalytically inactive forms of rat liver UGT2B1 has been reported (41). Our previous study suggested that the hetero-oligomer of guinea pig UGT2B21 and UGT2B22 exhibits a regio-selectivity towards morphine glucuronidation that differs from either homo-oligomer consisting of the respective UGTs (46). This was the first example suggesting that UGT hetero-oligomer formation results in a substrate specificity different from that of the corresponding homo-oligomer.

UGT isoforms involved in morphine-3-glucuronide (M-3-G) formation have been purified from experimental animals (47-51). However, the UGT isoform involved in the production of the active metabolite, morphine-6-glucuronide (M-6-G) (52), has not yet been purified. On the basis of the substrate specificity of expressed cloned UGT isoforms, human UGT2B7 and monkey UGT2B9 have been shown to catalyze the glucuronidation of morphine at the 6-hydroxyl group as well as the 3-hydroxyl group (53, 54). However, the guinea pig shows a higher rate of M-6-G formation compared with humans (55).

We have attempted to purify UGT isoforms in guinea pigs involved in M-6-G formation using chromatographic separations involving an omega-(beta-carboxypropionylamino)octyl Sepharose 4B column (50), chromatofocusing, and a UDP-hexanolamine Sepharose 4B column. This has given us an active preparation consisting of two UGT isoforms, UGT55K and UGT59K, which were difficult to separate (Yoshisue *et al*, unpublished data). The approximate ratio on the basis of band intensities on SDS-PAGE stained with Coomassie Blue, was UGT55K : UGT59K (3:1). We have cloned UGT2B21 and UGT2B22 cDNAs, encoding UGT55K and UGT59K, respectively. The analysis of the catalytic activity of these UGTs demonstrated that extensive M-6-G formation was observed only when UGT2B21 and UGT2B22 were expressed simultaneously in COS-7 cells (46). When UGT2B21 and UGT2B22 were expressed in COS cells at a ratio of approximately 3:1, the M-3-G/M-6-G formation was similar to that obtained under *in vivo* and *in vitro* conditions (55). UGT2B21 expressed in COS cells was capable of glucuronidating the 3-hydroxyl group of morphine, 4-hydroxybiphenyl, borneol, testosterone, androsterone, estriol, and chloramphenicol (46, 56). However, UGT2B22 does not exhibit any significant activity toward these substrates. When UGT2B21 and UGT2B22 were expressed simultaneously, the chloramphenicol glucuronidation was enhanced 4.5-fold whereas the activity towards other substrates was little affected except for the 6-hydroxyl group of morphine. These results suggest that simultaneous expression of UGT2B21 and UGT2B22 enhances UGT2B21-catalyzed chloramphenicol glucuronidation as well as morphine-6-glucuronidation. Thus, hetero-oligomer formation of UGT2B21 and UGT2B22 may act by fine-tuning the catalytic glucuronidation of chloramphenicol and the 6-hydroxyl group of morphine. As UGT2B21 and UGT2B22 have a very similar primary sequence, it is conceivable that these UGTs form hetero-oligomers and so such hetero-oligomer-derived alterations in substrate specificity seem to be important for a better understanding of the function of UGT isoforms. Although we have tested a limited range of substrates, no detectable activity of UGT2B22 was observed toward typical UGT substrates under our assay conditions. Like UGT2B22, there have been reports of orphan UGT isoforms whose substrates have not yet been identified, such as human UGT2B10 (57) and UGT2B11 (58).

Little attention has been paid to the active hetero-oligomers of human UGT isoforms. Thus, the physiological functions of UGT isoforms have not been fully elucidated since we focus on a single UGT isoform to clarify its function. Further investigations are necessary to investigate the impact of active UGT hetero-oligomers on polymorphisms in drug sensitivity and post-sequence pharmacogenomics.

5. INTERACTION BETWEEN P450 AND MICROSOMAL EPOXIDE HYDROLASE

Hepatic mEH is expressed in the ER membrane, where it is involved in the metabolism of many xenobiotics, such as polycyclic aromatic hydrocarbon carcinogens, in concert with other proteins, such as members of the P450 superfamily (3, 4). Topological studies have shown that mEH is integrated into the membrane with a multiple transmembrane domain and is expressed in the ER with type I (Ccyt/Nexo) and type II (Cexo/Ncyt) topological orientation. EH that is expressed in the plasma membrane with type II topology has also been reported (59, 60). Thus, the topology of mEH in ER is similar to that of P450.

As we suggested previously, CYP1A1 specifically associates with mEH (11), and this observation strongly supports the view that there is functional cooperation between P450 and mEH. A few early reports have demonstrated the activation of mEH function by P450 (61-64). However, the specificity of P450s and mEH substrates as far as this interaction is concerned remains largely unknown. Our recent study suggests that mEH function is modified by P450 in a P450 isoform-specific fashion (65).

The dissociation equilibrium constants (*K*d) for the affinity of P450 - mEH binding have been estimated by a resonant mirror technique using an optical biosensor cell covalently bound to rat mEH (66). While comparable *K*d values were obtained for CYP1A1 and 2B1, these were greater by one order of magnitude than those for the CYP2C11, suggesting that the latter P450 has a higher affinity for mEH than the former two. To clarify the influences of P450 enzymes on the catalytic activity of mEH, the hydrolyzing activity for styrene oxide and benzo(*a*)pyrene-7,8-oxide was analyzed in the presence or absence of P450s. Styrene oxide hydrolysis was activated by all P450s including the CYP1A, 2B, 2C, and 3A subfamilies. In agreement with the association affinity determined by the resonant mirror technique, CYP2C11 appeared to have enhanced activity for styrene oxide hydrolysis. On the other hand, benzo(*a*)pyrene-7,8-oxide hydrolysis was enhanced by only CYP2C11 while CYP1A1 and CYP2B1 had no effect. These results suggest that 1) many P450 enzymes associate nonspecifically with mEH; 2) particular form(s) of P450 plays a greater role in the association/activation of mEH; and 3) the P450-mediated activation of mEH depends upon the substrate of mEH.

6. INTERACTION BETWEEN P450 AND UGT

6.1. Protein-protein interaction between P450 and UGT

The protein-protein interaction between P450 and UGT was detected by affinity chromatography using CYP1A1-conjugated Sepharose 4B columns. In this approach, sodium cholate-solubilized hepatic microsomes from phenobarbital-treated rats were applied to the columns and the material eluted with buffer containing NaCl was analyzed by immunoblotting. It was found that some forms of UGT as well as fp2 and mEH were efficiently trapped by the CYP1A1 column (11). Protein disulfide isomerase (PDI) and calnexin, non-drug-metabolizing enzymes expressed in the endoplasmic reticulum, were unable to associate with the CYP1A1 column. These results suggest that CYP1A1 interacts with mEH and UGTs to facilitate a series of multistep drug metabolic conversions. Since the membrane topology of CYP1A1, mEH and fp2 are similar, their interaction would not be unexpected. However, UGT has an opposite topology from the above enzymes on the ER-membrane. Therefore, it may not be reasonable to hypothesize that P450 can associate with UGT. However, the above data from the affinity chromatography raise the possibility of a P450-UGT interaction. If the interaction between P450 and UGT takes place in the membrane, alpha-helices of their membrane anchoring regions may contribute to this. Otherwise, particular region(s) of P450 distinct from the anchoring domain of the N-terminal would associate with the membrane to link with UGTs.

6.2. P450 as a substrate supplier

It is conceivable that the P450 plays a role as a substrate supplier to the UGT as well as a function modulator for the UGT. The former possibility is supported by a recent study which examined the effects of P450 ligands and permeabilization of microsomes on 3-hydroxybenzo(*a*)pyrene [3-OH-B(*a*)P] glucuronidation mediated by rat hepatic microsomes (67). While the UGT activity with non-permeabilized microsomes from MC-treated rats was markedly reduced by alpha-naphthoflavone (alpha-NF), this inhibitor had hardly any effect when permeabilized microsomes were used. Kinetic analysis indicated that the inhibitory effect of alpha-NF is competitive. If alpha-NF inhibits UGT directly, the inhibitory effect should be increased by increasing the membrane permeability. This is because, due to so-called "latency", the lipid bilayer interferes with the access of inhibitor/substrate to UGT located within the ER. However, the experimental results reported by above study did not support this. In fact, the inhibitory effect of alpha-NF was greatly reduced when used with detergent-permeabilized microsomes. This observation does not support the possibility that inhibition is caused by the direct action of alpha-NF with UGT. It is likely that alpha-NF inhibition of 3-OH-B(*a*)P UGT is due to the effect on P450 and P450/UGT association is necessary for this. These results suggest that a UGT isoform(s) involved in 3-OH-B(*a*)P glucuronidation is interfered with a CYP1A inhibitor via a mechanism dependent on the intact nature of the microsomal membranes. It is likely that P450 functions as a substrate supplier for some isoforms of UGT via possible interactions between UGT and P450.

Ikushiro *et al.* have reported very recently that simultaneous expression of rat CYP1A1 and UGT1A6 in yeast does not alter the UGT1A6-catalyzed reaction (68). Glucuronidation of 3-OH-B(*a*)P in rats is catalyzed by several UGT isoforms, including MC-inducible UGT1A7 (69-71). Taking these and our own data into consideration, functional interactions between P450 and UGT probably occur in an isoform-specific manner.

6.3. Alteration of UGT function by interaction of P450 and UGT

Regarding the hypothesis of functional co-operation between P450 and UGT, our current work was undertaken to see whether morphine glucuronidation by UGT2B7 is affected by P450 isoforms CYP3A4, CYP1A2, and CYP2C9, the most abundant P450s expressed in human liver (72). We studied the modulation of UGT2B7-catalyzed-morphine glucuronidation by P450 (Takeda *et al*, unpublished data). The effects of CYP isoforms on the kinetic parameters of UGT2B7-catalyzed glucuronidation of the morphine 3-hydroxyl group were examined using the microsomes of COS cells which simultaneously expressed UGT2B7 and either CYP3A4, 1A2 or 2C9. While co-expression of CYP3A4 with UGT2B7 had little effect on *Vmax*, the *Km* was increased by about 9.8-fold in comparison with the UGT2B7 single expression system. The other P450 isoforms (CYP1A2 and CYP2C9) had no, or only a minor, effect on the *Km* and *Vmax* values. The *Km* of CYP3A4-UGT2B7 microsomes was similar to that of human hepatic microsomes (73, 74). Immunoprecipitation of UGT from solubilized human liver microsomes with anti-UGT antibody (48) resulted in co-precipitation of CYP3A4 with UGT2B7. The protein-protein interaction between CYP3A4 and UGT2B7 was further supported by the overlay assay using GST-tagged CYP3A4: i.e., UGT2B7 blotted on the blotting membrane was identified by detecting an overlay probe, GST-CYP3A4 fusion protein, using anti-GST antibody.

Requirement of the integration of CYP3A4 into ER membrane for modifying UGT function has been suggested from the following evidence. While the addition of CYP3A4 to untreated COS microsomes expressing UGT2B7 had no, or only a minor, effect on morphine glucuronidation, the formation of M-3-G by detergent-treated microsomes was reduced by CYP3A4. On the other hand, the formation of M-6-G was enhanced by exogenous CYP3A4, suggesting that the effect of this P450 on UGT2B7 function is quite different as far as the two reactions are concerned. These results strongly suggest that 1) the glucuronidation activity of UGT2B7 towards morphine is specifically modulated by interaction with CYP3A4 in microsomal membranes; and 2) CYP3A4 alters UGT2B7 regioselectivity so that the ratio of morphine activation/detoxication is increased.

7. PERSPECTIVE

The mechanism underlying the change in UGT function by hetero-oligomerization with UGT or P450 will not be fully understood until more data from molecular biological and membrane biological analyses are obtained. To date, altered function of UGT by hetero-oligomerization has been reported by only a few workers. Furthermore, the functional interaction between P450 and UGT is a new concept. Hence, UGT or CYP isoform selectivity on forms of hetero-oligomerization needs to be investigated. However, there is the possibility that UGT affects P450 function. The possibility that P450s interact with drug metabolizing enzymes other than UGT or mEH is also of great interest. The interaction of drug metabolizing enzymes may be one of the major mechanisms explaining inter-individual differences in drug sensitivity which cannot be understood by single nucleotide polymorphisms. To achieve high quality pharmaceutical care for patients suffering from physical and mental pain, the inter-individual differences due to the interaction of drug metabolizing enzymes should be studied in more detail.

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Abbreviations: PDI, protein disulfide isomerase; fp2, NADPH-cytochrome P450 reductase; P450, CYP, Cytochrome P450; mEH, microsomal epoxide hydrolase; UGT, UDP-glucuronosyltransferase; b5, cytochrome b5; ER, endoplasmic reticulum; CNII, Crigler-Najjar syndrome type II; M-3-G, morphine-3-glucuronide; M-6-G, morphine-6-glucuronide; *K*d, dissociation equilibrium constants; alpha-NF, alpha-naphthoflavone; MC, 3-methylcholanthrene; 3-OH-B(*a*)P, 3-hydroxybenzo(*a*)pyrene

Key Words: Cytochrome P450 (P450, CYP); UDP-glucuronosyltransferase (UGT); Microsomal Epoxide Hydrolase (mEH); Protein-Protein Interaction; morphine; benzo(a)pyrene; Affinity Chromatography, Review

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