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In Vitro Enhancement of Microsomal Cytochrome P450-Dependent Monooxygenases by Organic Solvents in Rat Liver

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***In vitro* effects of acetone, methanol, and dimethylsulfoxide (DMSO) on liver microsomal cytochrome P450 (P450) content, and P450-dependent arylhydrocarbon hydroxylase (AHH) and 7-ethoxycoumarin *O*-deethylase (ECOD) activities were studied in rats. Acetone at 1% (v/v) enhanced the content of P450, assayed spectrally in 3-methylcholanethrene (MC)- and β-naphthoflavone (BNF)-inducible microsomes by 18 and 7%, respectively. Methanol, up to 5% (v/v) applied, also showed enhancement effects on P450 content in liver microsomes from rats treated with phenobarbital (PB), MC, and BNF, as well as uninduced microsomes with similar but low strength. DMSO, however, did not show such enhancing effects at the ranges of the concentrations applied. AHH and ECOD activities in MC-inducible microsomes were also enhanced by acetone at 1%, which was in proportion to the increase in P450 content by the same concentration. However, the P450 content, and AHH and ECOD activities, were decreased by increasing the concentration of acetone. Methanol at the same concentration with acetone also enhanced ECOD activity but not AHH activity in MC-inducible microsomes. The enhancing effect of acetone on the enzymes was negligible when the microsomes were pretreated with a specific monoclonal antibody of MC-inducible isozyme. The difference in the effects of these solvents on P450 system might be due to their different properties that cause the P450 active site to be exposed *in milieu*.**

Keywords: Acetone, Arylhydrocarbon hydroxylase, Cytochrome P450 isozymes, Dimethyl sulfoxide, 7-Ethoxycoumarin *O*-deethylase.

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Introduction

Cytochrome P450 (P450) is a key enzyme of mixed function oxidase (MFO) systems which catalyzes the oxidation of a number of lipophilic chemicals such as drugs, toxic chemicals including organic solvents (Casazza *et al.*, 1984; Toftgard *et al.*, 1986; Pattern *et al.*, 1986) and carcinogens, as well as endogenous substances including steroids, fatty acids, and prostaglandins (Gonzalez, 1990; Guengerich and Shimada, 1991).

Since most drugs or xenobiotics have hydrophobic properties, they do not readily dissolve in aqueous media; consequently, organic solvents are utilized as vehicles for substrates of P450-dependent monooxygenases (Kontir *et al.*, 1986; Friedman *et al.*, 1988). It is very important to select a suitable solvent which has no side effects for the study of an MFO system. Organic solvents widely used for studies of drug metabolism may influence the microenvironment of membrane lipids as well as membrane-bound enzymes. Some P450 isozymes are induced by solvents in rat liver (Pyykko, 1983; Pyykko *et al.*, 1987; Chang *et al.*, 1997) or destroyed by their direct or indirect actions (Patel *et al.*, 1978; Madyastha *et al.*, 1985).

Since Anders (1968) first demonstrated the enhancement of several P450-dependent monooxygenases by organic solvents, many investigators have studied the effects of organic solvents on these enzymes in microsomes (Powis *et al.*, 1977; Vore and Solivon, 1979; Moldeus and Gergely, 1980) or reconstituted systems (Arinc, 1985). Although their studies were focused on the basis of alteration of the binding site of P450 (Backes and Canady, 1981), electron flow (Miwa *et al.*, 1978), or oxygen consumption of the MFO system (Anders and Gander, 1979), the mechanism underlying the action of the solvents is not yet clearly understood.

Because P450 exists in multiple molecular forms consisting of more than 40 isozymes (Nebert *et al.*, 1991)

and these isozymes are differentially oriented in the endoplasmic reticulum (Vergeres *et al.*, 1989), each isozyme may be affected differentially by the solvents used. Studies of reconstituted systems using purified P450 isozymes may not be valid for evaluating the effects of solvents on their orientations in the biological membrane, and may give wrong information on the characteristics of P450 isozymes.

The present investigation, therefore, was to examine the *in situ* effects of organic solvents used commonly on P450 and P450-dependent AHH and ECOD in rat liver microsomes containing P450 isozymes specifically induced by treatment with P450 inducers.

Materials and Methods

Chemicals Benzo (a) pyrene (B(a)P), β-naphtoflavone (BNF), benzphetamine, sodium dithionite, and NADPH were purchased from Sigma Chemicals Co. (St. Louis, USA). Phenobarbital (PB), ethoxycoumarin, and 3-methylcholanthrene (MC) were purchased from Fluka (Buchs, Switzerland), Aldrich (Milwaukee, USA), and Eastman Kodak Co. (Rochester, USA), respectively. Acetone (99.7% purity), methanol (99.5% purity), and DMSO (99.9% purity) were purchased from Merck Co. (Darmstadt, Germany). Monoclonal antibody, MAb $P450_{1\text{-}7\text{-}1p4}$, specific against P450 isozymes CYP1A1 and CYP1A2 was a kind gift of Dr. Sang-Shin Park (National Cancer Institute, Bethesda, Maryland, USA). Other chemicals used were of the highest grade quality.

P450 induction For the induction of three types of P450 isozymes, male Sprague-Dawley rats (150–160 g) were administered with PB (80 mg/kg), MC (20 mg/kg in corn oil), or BNF (150 mg/kg) by intraperitoneal injections for three consecutive days, respectively, as described elsewhere (Kim *et al.*, 1990). Control rats were maintained *ad libitum* without receiving any chemicals, and the inducer-treated rats were fasted overnight before sacrificing. Microsomes were prepared by differential centrifugation as described previously (Lee and Park, 1989). The content of microsomal P450 was measured as described by Omura and Sato (1964).

Enzyme assays AHH activity was determined by a modified method of Nebert and Gelboin (1968). The reaction mixtures contained 5 μmol magnesium chloride, 0.2 μmol NADPH, and 50 μg microsomal proteins in 1.0 ml of 0.5 M Tris buffer, pH 7.6. The reaction was started by the addition of 80 nmol of B(a)P and incubated for 10 min at 37°C. The reaction was stopped by the addition of 4 ml of 25% acetone in hexane. After vortexing for 10 min, 2.5 ml of the organic phase was transferred into a tube containing 1.0 ml of 1 N NaOH and the concentration of the 3-OH-B(a)P in the organic phase was measured spectrofluorometically (λ_{ex} at 392 nm and λ_{em} at 514 nm). ECOD activity was measured according to the method of Greenlee and Poland (1978). Benzphetamine *N*-demethylase (BPDM) was determined by the method of Thomas *et al.* (1976), briefly described as follows. The reaction mixture contained 0.1 mmol of magnesium chloride, 0.5 μmol of NADPH, 0.5 μmol of NADH, and 300 μg of microsomal proteins in a final volume of 2.0 ml with 0.1 M phosphate buffer, pH 7.6. The reaction was started by the addition of 50 μl of 40 mM of benzphetamine and incubated for 10 min at 37°C. After stopping the reaction by adding both 0.25 ml of 20% zinc sulfate and saturated barium hydroxide, the mixture was centrifuged for 20 min at 3000 rpm. The supernatant was mixed with 0.6 ml of Nash reagent and incubated for 20 min at 60°C to develop the color. Aldehydes formed were determined by measuring the absorbance of the aliquots at 412 nm. Organic solvents (10–100 μl/ml of reaction volume) tested were added and preincubated for 5 min before addition of each substrate.

Measurement of acetone enhancement of P450-dependent AHH and ECOD activities with monoclonal antibody For the evaluation of the enhancement of P450-dependent AHH and ECOD activities in MC or BNF-microsomes by acetone, we assayed the inhibition of these enzyme activities by CYP1A1/1A2-specific monoclonal antibody as previously described (Kim and Park, 1986; Moon *et al.*, 1998).

Statistics All data are presented as the mean ± SD of determinations from at least three experiments. Statistical analysis was performed using the Student's *t*-test on the individual sets of data.

Results

In order to evaluate the effects of organic solvents on MFO systems which are dependent on P450 isozymes, we induced P450 isozymes CYP1A1/1A2 and CYP2B1/2B2 in rat liver *in vivo* by pretreatment with MC, BNF, or PB, respectively. As shown in Table 1, the microsomal content of P450 was increased 2-fold, 1.5-fold, and 1.4-fold by PB, MC, and BNF, respectively. Compared with uninduced microsomes, AHH activity increased 13- and 10-fold in MC- and BNF-microsomes, respectively, but increased slightly (1.3-fold) in PB-microsomes. BPDM activity increased 4-fold in PB-microsomes, but decreased in MC- or BNF-microsomes, which are consistent with the results of other investigations (Guengerich *et al.*, 1982; Ryan *et al.*, 1982). The investigations suggested that uninduced hepatic microsomes contain a small proportion of CYP2B (~1%) and a large proportion of CYP2C11 (43%), and PB treatment induces hepatic CYP2B1/2B2, while CYP2C11 is reduced (Guengerich *et al.*, 1982; Ryan *et al.*, 1982). Since BPDM activity represents CYP2B1 and 2C11 at 100 and 40 on an equimolar basis (Guengerich *et al.*, 1982) and PB treatment increases CYP2B1 and decreases CYP2C11 (Ryan *et al.*, 1982), BPDM activity in PB microsomes is thought primarily to be catalyzed by CYP2B1/2B2. The slight decrease of BPDM activity in MC- or BNF-microsomes is probably accountable from a decreased CYP2C11. These results indicate that an inducer may induce certain isozymes and reduce others. These results support the basic notion that CYP1A1/1A2 is primarily induced by MC and BNF, and CYP2B1/2B2 is primarily induced by PB in rat liver. However, recent studies reported that MC treatment modulates the expression of the CYP2C11 and 3A subfamily in a complex manner

Table 1. The content of cytochrome P450 and activities of AHH and BPDM in liver microsomes of rat treated with inducers.

Microsomes	P450 (nmol/mg protein)	AHA (pmol/mg/min)	BPDM (nmol/mg/min)
Uninduced	1.05 ± 0.04	92 ± 15	8.2 ± 0.3
MC	1.57 ± 0.05*	1304 ± 28**	5.7 ± 0.1
PB	2.09 ± 0.05*	131 ± 15	34.9 ± 1.2**
BNF	1.46 ± 0.04*	947 ± 20**	5.8 ± 0.1

Microsomes were prepared by pooling livers from eight rats. MC, PB, and BNF represent the liver microsomes of rats pretreated with 3-methylcholanthrene, phenobarbital, and β-naphthoflavone, respectively. Values are presented as mean ± SD of at least three determinations, and those marked with asterisks differ significantly from vehicle control values (*$p < 0.001$, **$p < 0.0001$).

involving both suppressive and inductive components (Cooper *et al.*, 1993; Jones *et al.*, 1996), where CYP3A1/3A2 are also inducible by PB (Cooper *et al.*, 1993; Debri *et al.*, 1995). On the basis of our data and the result of these observations, we suggest that the 4-fold increase of BPDM activity in PB-microsomes may probably include the activity by CYP3A1/3A2 as well as by CYP2B1/2B2.

In vitro effects of organic solvents on these MFO systems were evaluated in these microsomes. Figure 1 shows the effects of methanol, acetone, and DMSO on the P450 content in the liver microsomes. The effects of these solvents depended on the source of microsomes as well as on the concentrations of the solvents. The content of P450 in uninduced microsomes was changed slightly by the addition of acetone up to 100 μl (10%, v/v) per ml of reaction mixture, whereas the P450 content in PB-microsomes decreased by 40% by the same concentration of acetone. Acetone at 136 mM enhanced the content of P450 in MC- and BNF-inducible microsomes by 18 and 7%, respectively. Methanol up to 5% (v/v) showed enhancement effects on the P450 content of all four microsomes tested. In contrast, DMSO decreased the P450 content with a similar pattern in all four microsomes, showing only a weak effect at concentrations lower than 5% (v/v).

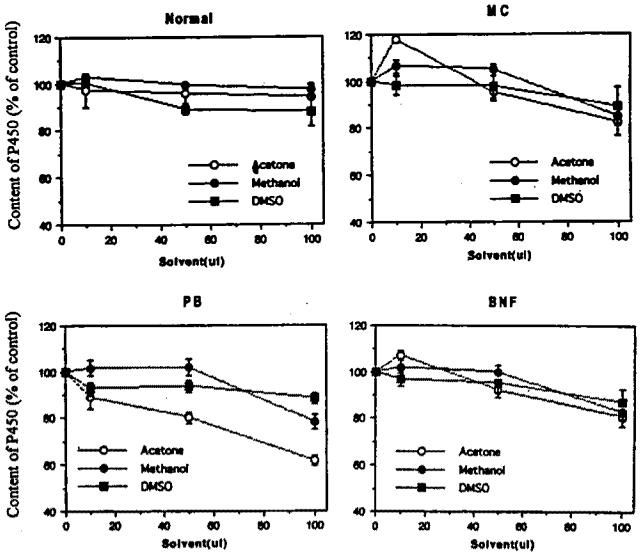

Fig. 1. Effects of acetone, methanol, and DMSO on the content of P450 in rat liver microsomes pretreated with inducers. Microsomes were prepared by pooling livers from eight rats. MC, PB, and BNF represent the liver microsomes of rats pretreated with 3-methylcholanthrene, phenobarbital, and β-naphthoflavone, respectively. Values are presented as mean ± SD of at least three determinations. The P450 concentrations at untreated organic solvents were 1.05, 1.57, 2.09, and 1.46 nmol/mg protein, respectively, for uninduced, MC, PB, and BNF-microsomes.

The effects of methanol, DMSO, and acetone, on P450-dependent AHH and ECOD activities have shown a variety of reaction patterns depending on the P450 isozyme (Fig. 2). In general, methanol and DMSO at increasing concentrations more strongly inhibited AHH activities in uninduced or PB-microsomes than in MC- or BNF-microsomes. Acetone had a lower inhibitory potency against AHH activity in uninduced microsomes than in any induced-P450 isozymes and, within the concentration range used, had the strongest inhibitory potency against AHH activity in PB-microsomes. Surprisingly, acetone had the strongest inhibitory potency against ECOD activity in uninduced microsomes. DMSO had totally different inhibition patterns of AHH and ECOD activities for the P450 isozymes tested. Even though DMSO dramatically inhibited AHH activity in both uninduced and PB-microsomes, it had almost no inhibitory effect on the activities in MC- or BNF-microsomes throughout the whole range of concentrations tested, indicating an isozyme-specific inhibitory potency. DMSO showed a similar, somewhat complicated, inhibition patterns of ECOD activities among the four types of microsomes. In particular, it did not inhibit ECOD activity in PB-microsomes up to a concentration of 20% (v/v). Methanol, within the range of concentrations tested, showed similar inhibitory effects on ECOD activities in all the P450 isozymes, but a little stronger in uninduced and PB-microsomes.

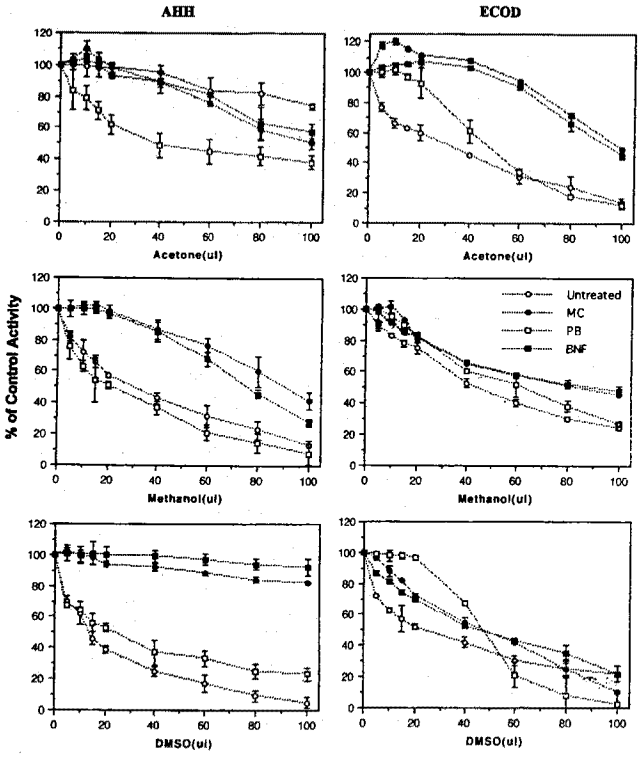

Fig. 2. Effects of acetone, methanol, and DMSO on P450-dependent AHH and ECOD activities in rat liver microsomes pretreated with inducers. Microsomes were prepared by pooling livers from eight rats. MC, PB, and BNF represent the liver microsomes of rats pretreated with 3-methylcholanthrene, phenobarbital, and β-naphthoflavone, respectively. Values are presented as mean ± SD of at least three determinations. The control activity (100%) for AHH was 92, 1304, 131, and 947 pmol/min/mg of protein for uninduced, MC-, PB-, and BNF-microsomes, respectively. The control activity (100%) for ECOD was 2.10, 23.19, 7.44, and 16.64 nmol/min/mg of protein, respectively.

Acetone at concentrations less than 2% enhanced both AHH and ECOD activities in MC- and BNF-microsomes, at 1% showing the highest enhancement of 10 and 20% over those in uninduced microsomes, respectively. However, at the same concentrations, it inhibited those activities in PB-microsomes and did not affect uninduced microsomes. At concentrations higher than 2%, acetone inhibited both enzyme activities in all microsomes. Thus, stimulation of P450-dependent B(a)P and ethoxycoumarin metabolism by acetone depends on its concentration as well as on the P450 isozymes.

Table 2 shows the inhibitory properties of these solvents on AHH and ECOD acitivities more clearly through IC_{50} values. In general, these two monooxygenases in uninduced and PB-microsomes were more strongly inhibited by the solvents than those in MC- or BNF-microsomes. Methanol had stronger inhibitory potency for AHH and ECOD activities in uninduced and PB-microsomes than in MC- and BNF-microsomes, suggesting

Table 2. Concentrations of organic solvents that produce 50% inhibition of AHH and ECOD activities in liver microsomes of rat pretreated with inducers.

	Treatment	IC_{50} (M)		
		Methanol	Acetone	DMSO
AHH	Uninduced	0.74	>1.36	0.14
	MC	2.29	>1.36	>1.01
	PB	0.52	0.63	0.22
	BNF	1.93	>1.36	>1.01
ECOD	Uninduced	1.16	0.48	0.23
	MC	2.22	1.32	0.47
	PB	1.65	0.69	0.49
	BNF	2.34	1.29	0.45

IC_{50}, concentration of solvents which produces 50% inhibition of AHH or ECOD activity. Values are presented as means of at least three determinations from pooled liver microsomes from eight rats.

an isozyme-specific inhibition property. Acetone generally had stronger inhibitory potency against ECOD compared to AHH in all of the isozymes, suggesting a substrate-specific binding property. Interestingly, acetone had low inhibitory potency for AHH in uninduced microsomes, suggesting its weak interaction with CYP2C11. Methanol and DMSO had isozyme-specific inhibitory potency for AHH activity, but less or no isozyme-specific inhibitory potency for ECOD activity. This result suggests that DMSO weakly interacts with CYP1A1/1A2 for the inhibition of AHH activity. In contrast, acetone had the lowest inhibitory potency against AHH activity among the three solvents. DMSO, among the solvents tested, had the strongest inhibitory potency for ECOD activity in MC- or BNF-microsomes.

Table 3 shows the effect of solvents on the relative activities of AHH and ECOD at the same solvent concentration (0.136 M). Acetone did stimulate these enzyme activities in both MC- and BNF-microsomes. Although methanol also showed an enhancement effect on both ECOD and AHH activities in MC- or BNF-microsomes, this effect did not achieve statistical significance. On the other hand, DMSO had no effect on the two enzyme activities in MC- or BNF-microsomes and strongly inhibited AHH activity in uninduced and PB-microsomes and ECOD activity in uninduced microsomes. Acetone also had an inhibitory effect on these enzyme activities in PB-microsomes, but no effect in uninduced microsomes.

In order to investigate whether the enhancements of P450 content and its dependent enzyme activity by acetone resulted from the isozyme specificity, we studied the inhibition of AHH and ECOD by an isozyme-specific MAb in MC- and BNF-microsomes (Fig. 3). MC-MAb

Table 3. *In vitro* effects of organic solvents on AHH and ECOD activities in liver microsomes of rat pretreated with inducers.

Treatment	Control	Methanol	Acetone	DMSO
AHH[a]				
Untreated	92 ± 15 (100)	72 ± 10 (78)	91 ± 8 (99)	56 ± 5 (61)
MC	1,304 ± 28 (100)	1,330 ± 35 (102)	1,434 ± 40 (110)*	1,310 ± 28 (100)
PB	131 ± 15 (100)	108 ± 10 (83)	105 ± 8 (80)	82 ± 5 (53)
BNF	947 ± 20 (100)	956 ± 22 (101)	1,013 ± 25 (107)*	951 ± 20 (100)
ECOD[b]				
Untreated	2.10 ± 0.05 (100)	1.89 ± 0.10 (90)	1.37 ± 0.03 (65)	1.31 ± 0.10 (62)
MC	23.19 ± 2.38 (100)	23.66 ± 2.40 (102)	27.80 ± 3.20 (120)*	20.87 ± 1.92 (90)
PB	7.44 ± 0.76 (100)	6.84 ± 0.56 (92)	7.51 ± 0.73 (101)	7.29 ± 0.88 (98)
BNF	16.64 ± 0.35 (100)	16.72 ± 1.04 (100)	17.48 ± 1.00 (105)*	13.64 ± 1.06 (82)

Microsomes were prepared by pooling livers from eight rats. MC, PB, and BNF represent the liver microsomes of rats pretreated with 3-methylcholanthrene, phenobarbital, and β-naphthoflavone, respectively. Enzyme activities were compared at the same concentration of the solvents used (0.136 M). Values are presented as mean ± SD of at least three determinations, and those marked with asterisks increase significantly from control values ($p < 0.05$).

[a] pmol/mg protein/min.

[b] nmol/mg protein/min.

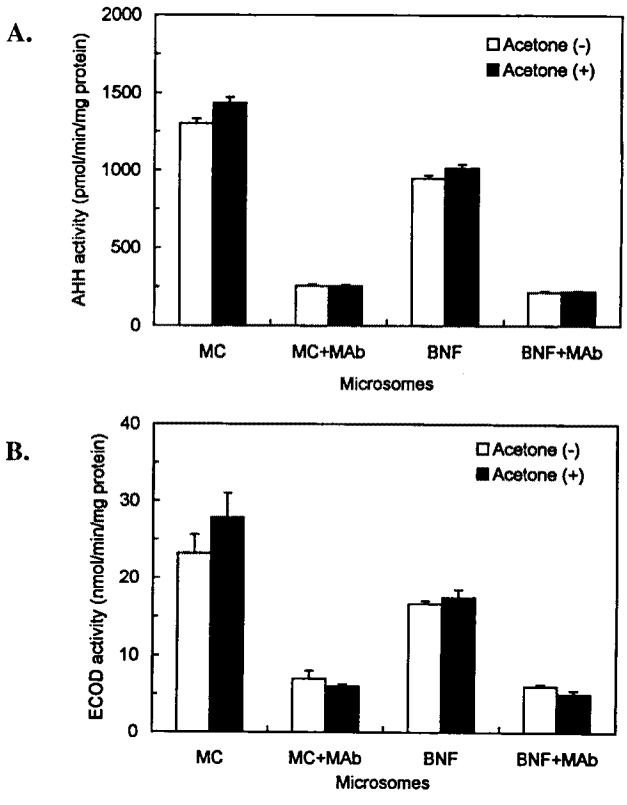

Fig. 3. Inhibition of acetone (at 0.136 M) enhancement in P450-dependent AHH (A) and ECOD (B) activities by pretreatment with the specific monoclonal antibody (MAb $P450_{1\text{-}7\text{-}1p4}$)[#] to MC-inducible isozyme. Microsomes were prepared by pooling livers from eight rats. MC and BNF represent the liver microsomes of rats pretreated with 3-methylcholanthrene and β-naphthoflavone, respectively. Values are presented as mean ± SD of at least three determinations. [#]MAb $P450_{1\text{-}7\text{-}1p4}$; MAb specific to CYP1A1 and 1A2 isozymes.

inhibited AHH activity by 80% in MC-microsomes and by 70% in BNF-microsomes (Fig. 3A), and the inhibition of ECOD also showed a similar pattern (Fig. 3B). However, in both cases, acetone enhancement of the activities was eliminated by pretreatment with their specific MAbs and, in fact, ECOD activity was actually decreased. This difference in the effect of acetone on the two enzymes may result from the overlapping substrate specificity of P450 (Guengerich, 1979). Thus, the disappearance of acetone enhancement by treatment with MAb did not result from other mechanisms such as alteration of electron flow (Miwa *et al.*, 1978) or dissociation of the enzyme-substrate complex (Anders, 1971).

Discussion

The data obtained from our present study indicate the importance of solvent choice as a vehicle for the substrate in the metabolism of the MFO systems. Our results have shown that acetone at a limited concentration (1%, v/v) increases P450 content and this enhancement is closely related to the increase of P450-dependent AHH and ECOD activities in MC- or BNF-microsomes. Solvents used in this study, however, inhibited AHH and ECOD activities at concentrations higher than 2% although some variation occurs depending on the source of microsomes. This inhibition could be due to protein denaturation by the excess organic solvents (Herskovits *et al.*, 1970). Nevertheless, the fact that different effects of the solvents on P450 depends on the source of microsomes is an interesting phenomenon.

It is known that both acetone and methanol are inducers of cytochrome P450 2E1 and are oxidized by the isoform *in vitro* (Sippel *et al.*, 1991; Pankow and Jagielki, 1993;

Chen *et al.*, 1994; Allis *et al.*, 1996). It has been reported that DMSO inhibits P450 2E1 activity (Carriere *et al.*, 1993) and decreases the total cytochrome P450 content in rat hepatocytes by converting it to cytochrome P420 (Lindsay *et al.*, 1991), but DMSO also potentiates the induction of ECOD activity by PB (Schilter *et al.* 1993). Our data suggest that MC- and BNF-inducible P450 isozymes are more resistant to organic solvents than PB-inducible or uninducible isozymes, which was confirmed by our measurements of the activities of P450-dependent monooxygenases and the content of P450.

We evaluated the effects of solvents on the MFO system with respect to the volume of solvent added into the reaction mixture. Generally, 2 to 5% (v/v) solvent concentrations have been used as vehicles for substrates of the MFO system (Kontir *et al.*, 1986). Our data, however, show that the solvents have markedly affected P450 even at concentrations less than 5%.

We also tried to evaluate the effect of solvent polarity on the enzyme because differences in the polarities of the solvents could be a cause of their different effects on P450 content and P450-dependent monooxygenases activities. The polarity of DMSO is the highest among the solvents tested, and acetone is the lowest. The solubility of both B(a)P and 7-ethoxycoumarin in these solvents is the highest in acetone, followed by methanol and DMSO. Our results for these three solvents showed an inverse relationship between polarity and enzyme activities.

Another possibility is that the effect of acetone or methanol on the P450-dependent AHH and ECOD activities might be correlated with their lipophilicities. In the case of alkylbenzenes, it has been reported that they induce hepatic cytochrome P450-dependent ECOD activity in rat and these inducton powers correlate with their molecular weight and lipophilicity (Pyykko, *et al.*, 1987). In contrast, Pyykko *et al.* (1987) reported that rat liver microsomal cytochrome P450, aryl hydrocarbon hydroxylase, and NADPH-cytochrome *c* reductase were only induced by the largest molecules of alkylbenzenes.

Since the solvents in equal volume will have different solvent molarities in the final reaction mixture, the actual molarity of the reactants has to be considered. We evaluated the relationship between enzyme activity and molarity of the solvents at 0.136 M because acetone at this concentration had the highest enhancement of AHH and ECOD activities as well as P450 content, but no correlation between enzyme activity and solvent molarity was observed.

P450-dependent drug metabolism can be also activated by stimulation of its electron transport system. NADPH-P450 reductase, a membrane-bound enzyme consisting of membrane-binding and hydrophilic domains, is the rate-limiting enzyme in the electron transport system (Miwa *et al.*, 1978; Peterson *et al.*, 1989) and can be influenced by some chemicals or solvents. Some agents, including organic solvents, facilitate the transfer of the second electron to P450 (Powis *et al.*, 1977). However, in our study the activity of NADPH-P450 reductase was not affected by the addition of acetone (data not shown), although AHH and ECOD activities were enhanced by this solvent. This indicates that enhancement of these enzyme activities is not due to the stimulation of electron transport system by acetone. However, further study is needed to evaluate whether such effects are P450 isozyme specific.

The P450 isozymes have similar molecular weights and high homologies in amino acids sequence (Guengerich *et al.*, 1982; Lee, 1991; Nebert *et al.*, 1991), but the three-dimensional conformation and orientation in the membrane are known only for a few isoforms (Ozoles, 1989; Vergeres *et al.*, 1989). P450 is anchored to the membrane of endoplasmic reticulum by insertion of series of segments spanning the membrane, which makes the orientation of all of the active site of P450 to the cytosolic side of the endoplasmic reticulum accessible to the interaction with specific antibodies or foreign compounds such as organic solvents, and the other components such as NADPH-P450 reductase and cytochrome b_5 have a specific orientation in the membrane (Nadler and Strobel, 1988; Ozoles, 1989; Vergeres *et al.*, 1989). Therefore, structural changes in the membrane induced by the solvents could cause the enhancement of P450-dependent monooxygenase activity.

Besides their effects on P450 as substrates and inducers, many organic solvents are known to increase the fluidity of artificial and native membranes, which may be particularly important in the content of P450 and P450-dependent monooxygenase activities. This is supported by the studies of Garda and Brenner (1984), who showed that the increase of membrane fluidity with short-chain aliphatic alcohols was paralleled by an increase in NADH-cytochrome *c* reductase activity. Engelke *et al.* (1993) showed that fluidization of the microsomal membrane by organic solvents increased P450 cluster formation.

Since this electron transport system produces reactive oxygen species (ROS) such as O_2^- and H_2O_2 (Morehouse and Dust, 1988; Sakai, *et al.*, 1992), and some organic solvents such as DMSO scavenge these ROS (Klein *et al.*, 1981; Rashba-Step and Cederbaum, 1994) during the oxidation of substrates by the P450 system, we also considered the possibility that free radical scavenging activity of organic solvents may influence their enhancement effects on the P450-dependent AHH or ECOD activity. However, our results indicate that ROS scavenging activity of the organic solvents does not influence the enhancement of AHH and ECOD activities for the following two reasons: First, the enhancing effects of acetone at the concentration of 0.136 M on these enzyme activities also exerted the enhancing effects on the content of P450 which are not resulted from the enhancement by oxygen activation (Fig. 1 and Table 3). Second, organic solvents such as DMSO better reacts with singlet oxygen or hydroxyl radical than O_2^- or H_2O_2 generated by the P450 system. There are also conflicting

reports whether ROS generation in the P450 system is dependent on the P450 isozyme (Sakai, *et al*, 1992) or not (Lambert *et al.*, 1996), which makes our discussion difficult.

On the basis of work from our own and other laboratories, we conclude that the different responses of P450-dependent monooxygenases to different solvents are due to differential effects of the solvents on the three-dimensional conformation of each P450 isozyme in the microsomal membrane. However, it is not clear why this effect is observed only at low concentrations of acetone.

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