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AN UNUSUAL PROFILE OF MUSK XYLENE-INDUCED DRUG-METABOLIZING ENZYMES IN RAT LIVER

NOBUHISA IWATA,* KEN-ICHIRO MINEGISHI, KAZUHIRO SUZUKI, YASUO OHNO,†
TAKASHI IGARASHI,‡ TETSUO SATOH‡ and ATSUSHI TAKAHASHI

Division of Xenobiotic Metabolism and Disposition and †Division of Pharmacology, National Institute of Hygienic Sciences, Setagaya, Tokyo 158, Japan; and ‡Laboratory of Biochemical Pharmacology and Biotoxicology, Faculty of Pharmaceutical Sciences, Chiba University, Chiba 263, Japan

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Abstract—We have demonstrated previously that musk xylene, a non-mutagenic carcinogen, is a novel and specific inducer of CYP1A2 in rats (Iwata *et al.*, *Biochem Biophys Res Commun* **184**: 149–153, 1992). In the present study, the effects of musk xylene (50, 100 or 200 mg/kg body weight, i.p., for 5 consecutive days) on both Phase I and Phase II metabolizing enzymes in rat liver were investigated further and more completely. Among the mixed-function oxidases monitored, 7-ethoxycoumarin deethylase and 7-pentoxyresorufin depentylase activities were increased at all dose levels from 1.6- to 1.7-fold and 2.6- to 3.1-fold, respectively. Benzo[*a*]pyrene hydroxylase activity was increased significantly at only the 200 mg/kg dose level of musk xylene (1.5-fold). Regarding Phase II enzymes, activities of both cytosolic DT-diaphorase and glutathione *S*-transferase (GST) were increased up to 2.0- to 2.4-fold by musk xylene in a dose-dependent manner. Western blot analysis revealed that the changes in these activities were caused by increases in the amounts of DT-diaphorase and GST Ya subunit. Microsomal UDP-glucuronyltransferase (UDPGT) activity assayed with *p*-nitrophenol as substrate was increased 1.6- to 2.0-fold. These results show that musk xylene induces both Phase I cytochrome P450 mixed-function oxidase (CYP1A2 specific) and Phase II metabolizing enzyme systems (DT-diaphorase, GST Ya subunit and UDPGT) in rat liver.

Synthetic nitro musks, such as musk xylene (MX)§, musk ambrette, musk tibetene and musk ketone, have been widely used as fragrance ingredients in soaps, detergents, lotions and foods. These compounds have been detected in the aquatic or marine environment as pollutants [1, 2]. Recently, musk ambrette was found to cause neurotoxic effects in rats [3] and to be mutagenic in *Salmonella typhimurium* TA100 in the presence of S9 [4]. MX was reported to be a potent hepatocarcinogen when administered chronically to B6C3F$_1$ mice of both sexes [5]. However, in contrast to musk ambrette, MX appears to be a non-genotoxic carcinogen in that it was not found to have activity in the Ames test [4]. Therefore, there has been considerable interest in defining its mechanism of action more clearly and in assessing its potential risk to humans.

There are many different classes of non-mutagenic or non-genotoxic carcinogens, ranging from the potent tumor promoters 12-*O*-tetradecanoyl-phorbol-13-acetate and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) that act through specific receptors, to compounds that alter growth control, such as phenobarbital [6, 7]. Some of them, e.g. polychlorinated biphenyls, polychlorinated dioxins, hypolipidemic drugs (peroxisome proliferators), antioxidants, safroles and barbiturates, induce a variety of metabolizing enzymes, such as cytochrome P450, glutathione *S*-transferase (GST), UDP-glucuronyltransferase (UDPGT) and DT-diaphorase [6–27].

These changes in turn can influence the pharmacological actions or toxicity of a variety of compounds including the inducers themselves [8]. Some inducers of drug-metabolizing enzymes, such as barbiturates or polycyclic aromatic hydrocarbons (PAHs), have tumor-promoting activities or increase the toxic effects of drugs in animals. On the other hand, they may sometimes protect animals from the adverse effects of foreign compounds by stimulating the metabolism of them to nontoxic metabolites [8]. Since xenobiotics are, in general, metabolized at first by the mixed-function oxidase system (Phase I) to compounds that subsequently undergo conjugation by GST, *N*-acetyltransferase (NAT), UDPGT or other enzymes (Phase II) to facilitate excretion of the compounds from the body, induction by xenobiotics of both Phase I and Phase II metabolizing enzymes should be investigated as a first step in characterizing the mechanism of action of a foreign compound and its risk for humans.

In a previous study [28], we reported that MX causes a remarkable induction of CYP1A2 and cytochrome b_5, and a small induction of cytochrome CYP1A1. These observations were based on

* Corresponding author: Dr. Nobuhisa Iwata, Division of Xenobiotic Metabolism and Disposition, National Institute of Hygienic Sciences, 18-1 Kamiyoga 1-chome, Setagaya-ku, Tokyo 158, Japan. Tel. 81-3-3700-1141; FAX 81-3-3707-6950.

§ Abbreviations: MX, musk xylene; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; GST, glutathione *S*-transferase; UDPGT, UDP-glucuronyltransferase; PAH, polycyclic aromatic hydrocarbon; NAT, *N*-acetyltransferase; CDNB, 1-chloro-2,4-dinitrobenzene; DCNB, 1,2-dichloro-4-nitrobenzene; PVDF, polyvinylidene fluoride; 3MC, 3-methylcholanthrene; and BHA, 2(3)-*tert*-butylhydroxyanisole.

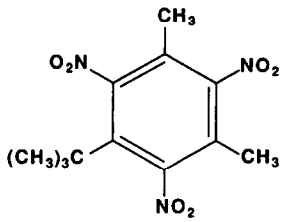

Fig. 1. Chemical structure of musk xylene.

spectrophotometric and immunochemical measurements using anti-cytochrome P450 antibodies for these specific cytochromes. In the present study, we further investigated the effects of MX treatment on various hepatic Phase I and Phase II drug-metabolizing enzymes in livers of rats treated with MX using kinetic and immunochemical methods. Specifically, we determined the enzyme activities for cytochrome P450, NADPH-cytochrome P450 reductase, NADH-cytochrome b_5 reductase, GST, DT-diaphorase, UDPGT and NAT and protein contents of GST Ya subunit, GST Yc subunit, DT-diaphorase and NADPH-cytochrome P450 reductase for a more comprehensive assessment of the changes produced by MX.

MATERIALS AND METHODS

Materials. MX (2,4,6-trinitro-5-*tert*-butyl-xylene; CAS No. 81-15-2; Fig. 1) was obtained from the Takasago Perfume Co. Ltd., Tokyo, Japan. Cytochrome *c*, glucose-6-phosphate, glucose-6-phosphate dehydrogenase, UDP-glucuronic acid and 7-pentoxyresorufin were purchased from the Sigma Chemical Co., St. Louis, MO, U.S.A. Benzo[*a*]pyrene, 7-ethoxycoumarin, 7-hydroxycoumarin and 5,5-dimethyl-1-hydroxymethylhydantoin were from the Aldrich Chemical Co., Milwaukee, WI, U.S.A. Benzphetamine hydrochloride was from the Upjohn Co., Kalamazoo, MI, U.S.A. Pyridine nucleotides were products of the Oriental Yeast Co. Ltd., Osaka, Japan. Other chemicals of reagent grade were obtained from Wako Pure Chemical Industries Ltd., Osaka, Japan. Antibodies against rat liver GST Ya and GST Yc subunits were prepared as described previously [29]. Anti-rat liver DT-diaphorase antibody was prepared according to the procedure of Yoshimura *et al.* [9]. Anti-NADPH-cytochrome *c* reductase antibody was prepared according to the procedure of Noshiro and Omura [30].

Animals. Male Wistar rats (7 weeks old) were obtained from the Japan SLC Co., Shizuoka, Japan. Animals were fed a laboratory chow and water *ad lib.* and housed in plastic cages at constant temperature (24 ± 2°) and humidity (55 ± 20%) under a 12-hr light/dark cycle (light: 7:00 a.m. to 7:00 p.m.). MX was suspended in corn oil (<200 mg/2.5 mL) by sonication. Rats were injected intraperitoneally with either 50, 100 or 200 mg MX/kg body weight for 5 consecutive days and food was withheld for 24 hr prior to killing. Control animals received the vehicle only.

Enzyme preparation. Rats were killed by decapitation. The livers were quickly removed, weighed and perfused with ice-cold 0.154 M KCl, and then homogenized with 4 vol. (w/v) of 10 mM Tris–HCl (pH 7.4) containing 0.154 N KCl, 0.1 mM EDTA, 1.0 mM dithiothreitol and 0.01 μM phenylmethylsulfonyl fluoride in a Potter–Elvehjem homogenizer. The 20% homogenates (w/v) were centrifuged at 9,000 *g* for 20 min and the supernatants were further centrifuged at 105,000 *g* for 1 hr. The resultant clear supernatants were used as the cytosolic fraction (17–23 mg/mL). Microsomes (105,000 *g* pellet) were washed once and resuspended in the same buffer (16–26 mg/mL). All preparations were stored at −80° until used.

Enzyme assays. NADPH-cytochrome *c* reductase activity was measured at 25° by monitoring the reduction of 50 μM cytochrome *c* in the presence of 100 μM NADPH [31]. NADH-cytrochrome b_5 reductase was measured as NADH-ferricyanide reductase using 1 mM potassium ferricyanide and 1 mM NDAH [32]. Mixed-function oxidase activities were measured using either 7-ethoxycoumarin (0.5 mM), 7-pentoxyresorufin (10 μM), aniline (1 mM) or benzo[*a*]pyrene (0.16 mM) as substrates according to the methods described previously [10, 33–35]. Demethylase activities towards aminopyrine (1 mM), erythromycin (1 mM) and benzphetamine (1 mM) were determined by formaldehyde concentrations measured according to the method of Nash [36]. UDPGT activity was determined with *p*-nitrophenol as substrate following the procedure of Isselbacher *et al.* [37]. Cytosolic DT-diaphorase activity was determined by monitoring the reduction of 50 μM cytochrome *c* in the presence of 40 μM menadione [9], and estimated from the decrease in the rate of reduction caused by 40 μM dicoumarol, a specific inhibitor of DT-diaphorase [38]. GST activities were determined with 1-chloro-2,4-dinitrobenzene (CDNB) and 1,2-dichloro-4-nitrobenzene (DCNB) as substrates [39]. NAT activity was determined with *p*-aminobenzoic acid as a substrate according to Andres *et al.* [40]. Protein concentrations were determined by the method of Lowry *et al.* [41] using bovine serum albumin as a standard.

Immunochemical quantitation. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis was performed as described by Laemmli [42] using 7.5 or 12.5% polyacrylamide gel [28]. Microsomal or cytosolic proteins were transferred electrophoretically to polyvinylidene fluoride (PVDF) membranes (Atto Co., Tokyo, Japan) and probed with the respective antibodies. The membranes were immunostained using the Vectastain® avidin–biotin–peroxidase complex kit (Vector Lab., Burlingame Inc., CA), and scanned by a densitometer (CS-9000 model, Shimadzu Co., Kyoto, Japan). The peak area of each sample was calculated, and was linear with respect to the amount of protein loaded. The contents of DT-diaphorase, GSTs and NADPH-cytochrome *c* reductase were expressed as arbitrary densitometric units/mg protein.

Statistical analysis. All data were expressed as means ± SEM. Comparisons of the means were performed by one-way analysis of variance followed

Table 1. Effects of musk xylene on body weight, and on liver microsomal and cytosolic protein contents

	Control vehicle	Musk xylene (mg/kg)		
		50	100	200
Final body weight (g)	144 ± 2.4 (100%)*	149 ± 2.7 (103%)	139 ± 1.3 (97%)	143 ± 2.4 (99%)
Liver weight to body weight ratio (%)	3.48 ± 0.058 (100%)	3.59 ± 0.175 (103%)	3.82 ± 0.036† (110%)	4.14 ± 0.051‡ (119%)
Microsomal protein (mg/g liver)	21.4 ± 1.19 (100%)	21.7 ± 0.33 (101%)	25.6 ± 1.11† (120%)	28.0 ± 1.36‡ (131%)
Cytosolic protein (mg/g liver)	76.5 ± 2.06 (100%)	75.0 ± 0.73 (98%)	74.3 ± 3.42 (97%)	77.0 ± 0.95 (101%)

Young male Wistar rats were treated, i.p., for 5 consecutive days with either vehicle, or 50, 100 or 200 mg musk xylene/kg body weight, and were killed on the following day. Each value is the mean ± SEM of four individual rats.
* Numbers in parentheses indicate the percentages of the control value.
† Significantly different from control ($P < 0.05$).
‡ Significantly different from control ($P < 0.01$).

by Dunnett's multiple range test for significance; $P < 0.05$ was considered significant.

RESULTS

Rats treated with MX, as well as control rats, remained healthy in appearance and normal in behavior during the study. Body weights showed no significant difference from the control on the final day in any of the MX-treated groups. The ratios of liver weight to body weight were elevated in rats given MX at 100 or 200 mg/kg (Table 1). The liver microsomal protein content of control rats was 21.4 mg/g liver and the value was increased by MX administration. No change in the content of cytosolic protein (mg/g liver) was observed.

In a previous study [28], we demonstrated that treatment of rats with MX increases the levels of the total cytochrome P450 and cytochrome b_5 by 1.4- and 1.5-fold, respectively. Several mixed-function oxidase activities in hepatic microsomes are shown in Table 2. Activities of 7-ethoxycoumarin deethylase and 7-pentoxyresorufin dependentylase were increased by MX at all three doses, although these increases were not dose dependent. Aniline hydroxylase and benzphetamine demethylase activities were increased

Table 2. Effects of musk xylene on mixed-function oxidase and related enzyme activities of liver microsomes in rats

	Control vehicle	Musk xylene (mg/kg)		
		50	100	200
NADPH-cytochrome *c* reductase (μmol/mg protein/min)	0.472 ± 0.011 (100%)*	0.527 ± 0.020 (112%)	0.562 ± 0.029 (119%)	0.636 ± 0.053† (135%)
NADH-ferricyanide reductase (μmol/mg protein/min)	9.247 ± 0.272 (100%)	9.299 ± 0.185 (101%)	9.321 ± 0.553 (101%)	8.849 ± 0.358 (96%)
Aniline hydroxylase (nmol/mg protein/min)	1.075 ± 0.027 (100%)	1.220 ± 0.027‡ (113%)	1.090 ± 0.067 (101%)	0.975 ± 0.040 (91%)
Aminopyrine demethylase (nmol/mg protein/min)	3.864 ± 0.162 (100%)	4.212 ± 0.154 (109%)	3.531 ± 0.270 (91%)	3.358 ± 0.098 (98%)
Benzo[*a*]pyrene hydroxylase (pmol/mg protein/min)	260.4 ± 9.6 (100%)	299.6 ± 26.7 (115%)	310.8 ± 15.1 (119%)	387.6 ± 23.7† (149%)
Benzphetamine demethylase (nmol/mg protein/min)	2.997 ± 0.101 (100%)	3.556 ± 0.166‡ (119%)	2.914 ± 0.172 (97%)	2.917 ± 0.194 (97%)
Erythromycin demethylase (nmol/mg protein/min)	3.380 ± 0.129 (100%)	3.216 ± 0.193 (95%)	2.936 ± 0.066 (87%)	3.076 ± 0.147 (91%)
7-Ethoxycoumarin deethylase (nmol/mg protein/min)	2.452 ± 0.075 (100%)	4.000 ± 0.051† (163%)	4.129 ± 0.163† (168%)	4.244 ± 0.232† (173%)
7-Pentoxyresorufin dependentylase (pmol/mg protein/min)	2.289 ± 0.379 (100%)	7.073 ± 1.113† (309%)	5.941 ± 0.914‡ (259%)	5.862 ± 1.197‡ (256%)

Experimental conditions were the same as those given in Table 1. Each value is the mean ± SEM of four individual rats.
* Numbers in parentheses indicate the percentages of the control value.
† Significantly different from control ($P < 0.01$).
‡ Significantly different from control ($P < 0.05$).

Table 3. Effects of musk xylene on hepatic Phase II drug-metabolizing enzyme activities in rats

	Cofactor	Control vehicle	Musk xylene (mg/kg) 50	100	200
Glutathione *S*-transferase					
CDNB (μmol/mg protein/min)		1.289 ± 0.038 (100%)*	1.684 ± 0.042† (131%)	1.816 ± 0.101† (141%)	2.426 ± 0.114† (188%)
DCNB (nmol/mg protein/min)		93.32 ± 2.12 (100%)	113.3 ± 1.02† (121%)	116.0 ± 4.40† (124%)	187.9 ± 6.85† (200%)
DT-Diaphorase (μmol/mg protein/min)	NADPH	0.355 ± 0.032 (100%)	0.556 ± 0.028† (157%)	0.686 ± 0.040† (193%)	0.797 ± 0.044† (225%)
	NADH	0.199 ± 0.015 (100%)	0.315 ± 0.023† (158%)	0.406 ± 0.017† (204%)	0.474 ± 0.020† (238%)
UDP-Glucuronyltransferase (nmol/mg protein/min)		12.66 ± 0.81 (100%)	21.67 ± 0.50† (171%)	19.97 ± 0.63† (158%)	25.54 ± 1.17† (202%)
N-Acetyltransferase (pmol/mg protein/min)		354.0 ± 28.2 (100%)	328.6 ± 3.9 (93%)	328.9 ± 26.3 (93%)	329.3 ± 13.2 (93%)

Experimental conditions were the same as those given in Table 1. Each value is the mean ± SEM of four individual rats.

* Numbers in parentheses indicate the percentages of the control value.

† Significantly different from control ($P < 0.01$).

slightly, but significantly, only in the 50 mg/kg group (1.1- and 1.2-fold, respectively). The activities of benzo[*a*]pyrene hydroxylase and NADPH-cytochrome *c* reductase were increased significantly by 1.5- and 1.4-fold, respectively, relative to the control values at the 200 mg/kg dose level. In contrast, other microsomal mixed-function oxidase and NADH-ferricyanide reductase activities were not altered statistically even at the highest dose of MX (200 mg/kg).

The effects of MX on Phase II drug-metabolizing enzyme activities are summarized in Table 3. DT-diaphorase activities with NADPH or NADH as the cofactor were increased significantly at the 50 mg/kg level and the increases reached 2.3-fold over control values in rats given MX at 200 mg/kg. GST activities towards CDNB and DCNB were also increased significantly in a dose-dependent manner. UDPGT activity was increased potently by MX at 50 mg/kg or more (1.7- to 2.0-fold). NAT activity towards *p*-aminobenzoic acid as a substrate was not affected by MX treatment.

To examine the effects of MX treatment on the contents of the various enzymes, quantitative Western blot analyses were performed, and the results are summarized in Table 4. The patterns of a representative Western blot are shown in Fig. 2. A significant increase (2-fold) in the content of immunoreactive GST Ya in cytosol was observed in rats given MX at either the 100 or 200 mg/kg level, but no significant increase was found in GST Yc. The content of immunoreactive DT-diaphorase was increased significantly in response to MX at all dose levels in a dose-dependent manner. The changes in the contents of these enzymes correlated well with those in the enzyme activities. A significant increase in the content of immunoreactive NADPH-cytochrome *c* reductase was not observed by MX treatment, in contrast to the significant increase found in the enzyme activity.

DISCUSSION

We have demonstrated previously using spectrophotometric and immunochemical techniques that MX treatment of rats results in dramatic induction of both the CYP1A subfamily and cytochrome b_5, and that this induction was selective for CYP1A2 at a low dose (50 mg/MX/kg) [28]. With respect to the induction of the cytochrome P450s in the 1A subfamily, a dose–response relationship was observed for the increase in CYP1A1 content of rat liver microsomes by MX after 5 days at doses of 50, 100 and 200 mg/kg body weight per day, but not for CYP1A2, for which isozymes the maximal increase was observed with the 50 mg/kg dose. These changes are different from those caused by the usual 3-methylcholanthrene (3MC)-type inducers, which increase CYP1A1 more than CYP1A2 [21]. In the present study, we found that benzo[*a*]pyrene hydrdoxylase activity, which is relatively specific to CYP1A1, was increased slightly, but significantly, by MX only at 200 mg/kg (1.5-fold). In contrast, 7-ethoxycoumarin deethylase activity was increased significantly (1.6- to 1.7-fold) by MX at all dose levels tested, precluding demonstration of a dose-dependency (Table 2) within the range of MX doses tested. The dose–response of CYP1A2 induction by MX was shifted to the left (that is, toward lower dose levels), compared to that of CYP1A1. These results suggest that MX may induce CYP1A2, at least in part, through some mechanism(s) other than the *Ah* receptor-mediated mechanism observed in the induction of CYP1A1 by 3MC [22–25]. In regard to the other activities surveyed here, increased levels of either erythromycin demethylase activity or benzphetamine, aminopyrine demethylase and 7-pentoxyresorufin depentylase activities of rat liver microsomes would be indicative of induction of CYP3A [43] or CYP2B [26, 33]. 7-Pentoxyresorufin is the most specific substrate for CYP2B1 [23, 26, 33].

Table 4. Effects of musk xylene on hepatic drug-metabolizing enzyme contents in rats

	Control vehicle	Musk xylene (mg/kg)		
		50	100	200
GST Ya subunit	100.0 ± 7.0	129.3 ± 8.5	194.9 ± 34.6*	209.6 ± 25.4†
GST Yc subunit	100.0 ± 10.3	100.6 ± 11.6	123.3 ± 14.2	116.0 ± 8.4
DT-Diaphorase	100.0 ± 4.9	163.7 ± 25.1†	225.3 ± 5.2†	309.2 ± 4.0†
NADPH-cytochrome *c* reductase	100.0 ± 5.8	113.1 ± 6.4	114.1 ± 8.2	110.0 ± 14.3

Experimental conditions were the same as those given in Table 1. Values represent arbitrary densitometric units/mg protein and are the means ± SEM of four rats.
* Significantly different from control ($P < 0.05$).
† Significantly different from control ($P < 0.01$).

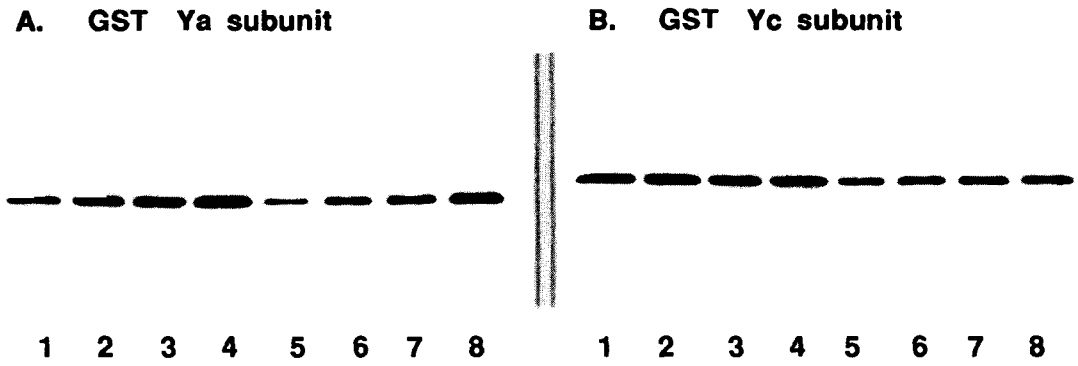

Fig. 2. Western blot analyses of liver cytosol from control (lanes 1 and 5), 50 mg/kg (2 and 6), 100 mg/kg (3 and 7) and 200 mg/kg (4 and 8) MX-treated rats. Cytosolic proteins (lanes 1–4, 2.0 μg; lanes 5–8, 1.0 μg) were electrophoresed on 12.5% sodium dodecyl sulfate–polyacrylamide gels, transferred to PVDF membranes, and probed with antibodies prepared against rat liver GST Ya (A) and GST Yc (B).

MX did not affect erythromycin demethylase activity, and significantly increased 7-pentoxyresorufin depentylase activity, the relative increase of which was very small compared to that observed with phenobarbital treatment (100-fold) [33]. Aniline hydroxylase activity is catalyzed by CYP1A2 and CYP2E1 [27]. MX failed to increase aniline hydroxylase activity at the 100 and 200 mg/kg dose levels (Table 2), although MX induces CYP1A2 activity strongly [28]. There is a possibility that CYP2E1 may be decreased by MX treatment at the highest doses used here. Therefore, MX should not be categorized as a phenobarbital-like, a pregnenolone-16α-carbonitrile-like or an ethanol-like inducer, which are typical inducers of CYP2B, 3A and 2E, respectively [8, 23, 24, 26, 27, 33, 43]; MX appears to be a specific inducer of CYP1A2.

GST, UDPGT and DT-diaphorase have been shown to be induced by various xenobiotics, such as 3MC, TCDD and *tert*-butylhydroxyanisole (BHA) [9, 11–20, 44, 45]. Talalay and coworkers [16, 17] have classified the Phase II drug-metabolizing enzyme inducers into two types: bifunctional inducers (PAHs, TCDD, azo dyes and β-naphthoflavone) that elevate both Phase I enzymes such as CYP1A1 and Phase II enzymes such as UDPGT, GST and DT-diaphorase, and monofunctional inducers [electrophilic compounds such as phenolic antioxidant (BHA), thiocarbamates, 1,2-dithiol-3-thiones and isothiocyanates] that act primarily on Phase II enzymes. As shown in Table 3, MX treatment increased both rat liver DT-diaphorase and GST activities to the same extent. Western blot analysis revealed that the increases in the activities were brought out by alterations in the amounts of these enzyme proteins (Table 4 and Fig. 2). In addition, the activity of UDPGT was increased by MX treatment (Table 3). In comparing these and the preceding results with the characteristics of other known inducers, we can point out the following: (1) MX preferentially induces CYP1A2 as opposed to CYP1A1, which is different from the case of 3MC, and (2) the inductive profile for monooxygenase (Phase I enzymes) activity by MX is different from that of BHA, a monofunctional inducer, which has been reported to decrease benzo[*a*]pyrene hydroxylase activity (0.7-fold) and increase aniline hydroxylase activity (2.7-fold) [19]. Thus, MX shows a different inductive profile of Phase I enzymes from either 3MC or BHA, and is unusual in selectively and strongly inducing the Phase I enzyme CYP1A2 and Phase II enzymes, such as GST, UDPGT and DT-diaphorase. Because of these features, MX should be classified as a new type of bifunctional

inducer with some exceptional characteristics. Further work is needed to elucidate the mechanism of induction of these enzymes by MX.

Recently, we reported that at least eight metabolites can be formed from MX *in vivo* [46]. The main metabolic pathway for MX disposition was reduction of the nitro group at the 2-position to an amino group, followed by acetylation of the resulting amino group [46]. The methyl groups of 2-amino-MX are oxidized further to yield 2-amino-3-hydroxymethyl-MX and 2-amino-5-*tert*-hydroxybutyl-MX. The active form of BHA for induction of Phase II enzymes is *tert*-butylhydroquinone, a major metabolite of BHA, although CYP1A1 induction by TCDD occurs via direct binding of TCDD itself to the *Ah* receptor, thereby enhancing gene transcription [22, 23]. MX itself and its metabolites, in our investigations thus far, were neither electrophilic compounds nor PAHs. MX is not structurally similar to any other monofunctional or bifunctional inducer proposed by Talalay and coworkers [16, 17]. Further studies are required to identify the active metabolites for induction.

Treatment of rats with MX has been shown to induce an increased level of total microsomal cytochrome P450 [28]. In the present study, it was confirmed that the major increase in cytochrome P450 content is associated with the CYP1A2 form and it was also shown that MX can induce several drug-conjugating enzymes. These findings suggest that MX treatment may alter the expressions and/or activities of several drug-metabolizing enzymes responsible for MX metabolism, e.g. the specific form(s) of cytochrome P450, DT-diaphorase, also known as a nitroreductase [47], and the conjugating enzymes.

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