응용약물학회지, The Journal of Applied Pharmacology 6, 351-357(1998)

Comparison between TCDD and 3MC Action on *CYP1A1* Expression and EROD Activity in the Isolated Perfused Male Rat Liver

Mee R. AHN and Yhun Y. SHEEN*

College of Pharmacy, Ewha Womans University

(Received November 15, 1998; accepted December 15, 1998)

Abstracts – In order to understand the mechanism of the regulation of *CYP1A1* gene expression and ethoxyresorufin deethylase (EROD) activity in *ex vivo* system, we have studied the action of TCDD and 3MC in the isolated perfused male rat liver. CYP1A1 mRNA level and EROD activity were measured in rat liver that was isolated and perfused with various chemicals such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), 3-methylcholanthrene (3MC), 17β-estradiol (E_2), morin. TCDD or 3MC alone perfusion into male rat liver resulted in increase of CYP1A1 mRNA level and the magnitude of stimulation was one and half times higher with TCDD treatment than 3MC treatment. However E_2 perfusion into male rat liver showed slight stimulation of CYP1A1 mRNA level. When 10^{-8} M E_2 was perfused concomitantly with either 10^{-9} M TCDD or 10^{-9} M 3MC, stimulated CYP1A1 mRNA by either TCDD or 3MC was inhibited. Morin was examined for its effects on CYP1A1 mRNA level and result was similar to that was observed with estrogen except that morin alone did not change the level of CYP1A1 mRNA. EROD activity was also stimulated with either TCDD or 3MC perfusion, and the magnitude of EROD stiumlation was similar to that of CYP1A1 mRNA stimulation in response to TCDD or 3MC perfusion. This data is different from the data that we have obtained with female rat liver. Concomitant perfusion either E_2 or morin with TCDD or 3MC inhibited 3MC perfusion or TCDD perfusion stimulated EROD activity. These data confirm the hypothesis that TCDD and 3MC might act through the same mechanism of action on the regulation of *CYP1A1* gene expression in male rat liver.

Keywords□ TCDD, 3MC, EROD, CYP1A1, Morin

The cytochrome P450-dependent monooxygenase system catalyzes oxidative metabolism of a wide variety of substrates including endogenous as well as exogenous compounds. As a preliminary detoxification step, many compounds are first converted to polar metabolites by cytochrome P450, which facilitates their elimination. However, some compounds may also be inadvertently bioactivated by cytochrome P450 to reactive intermediates that produced adverse biological effects (Amdur *et al.*, 1996; Yang *et al.*, 1978). For example carcinogenic polycyclic aromatic hydrocarbons (PAH) such as benzo(a) pyrene (B[a]P) undergo metabolic activation by cytochrome P450 and expoxide hydrolase to chemically reactive ultimate carcinogen diol expoxides (Chou *et al.*, 1986). The *CYP1* family, which consists of at least three enzymes, *CYP1A1, CYP1A2* and *CYP1B1* has been shown to be important in the metabolism of several xenobiotics such as PAH and heterocyclic amines, and expression of these enzymes is inducible by PAHs such as TCDD. TCDD induction of CYP1 transcription is mediated by the cytosolic arylhydrocarbon receptor (AhR), which is known as a ligand-activated transcription factor. Activation of AhR involves ligand binding, dissociation of heat-shock protein-90, nuclear translocation, and dimerization with the AhR nuclear translocator protein (Arnt) followed by binding to DRE (for XRE) enhancer elements in the 5-noncoding region of the responsive gene (Carrier *et al.*, Riddick *et al.*, 1994; Denison *et al.*, 1988). The mechanism of action of this compound is to activate the arylhydrocarbon receptor (AhR) to a form that binds to specific gene regulatory sequence elements, called xenobiotic responsive elements (XREs), through heterodimerization with Arnt (Ko *et al.*, 1996; Mason *et al.*, 1994; Dolwick *et al.*, 1993). AhR and Arnt have a similar overall structure and belong to the basic helix-loop-helix class of transcription factors (Poland and Gloven, 1974; Pendurthi *et al.*, 1993). Upon binding XREs, the AhR-Arnt complex activates transcription of adjacent structural genes which encodes enzymes that are involved in the oxidative metabolism of these compounds (Miksicek, 1995; Sousa and

* To whom correspondence should be addressed.

Marletta, 1995). Thus far, we assumed that arylhydrocarbons such as 3MC, and TCDD would affect drug metabolism via identical mechanism of action. However, very little study have been reported to confirm if it is in deed true that 3MC and TCDD act in an identical mechanism of action on the regulation of *CYP1A1* gene expression. Therefore we have undertaken study to examine how 3MC and TCDD would affect *CYP1A1* gene expression and EROD activity. After livers from male Sprague Dawely rats were isolated and perfused with TCDD or 3MC or E_2 or morin, the level of CYP1A1 mRNA was measured via RT-PCR and EROD activity was determined. Our finding showed that the levels of CYP1A1 mRNA and EROD activity in male liver were increased with either 3MC or TCDD treatment. The magnitude of TCDD stimulation of CYP1A1 mRNA was one and half times higher than that of 3MC stimulation however the magnitude of TCDD stimulation of EROD was similar to that of 3MC. Unlike the data that we have obtained with female rat liver, these data confirm the hypothesis that TCDD and 3MC might act through the same mechanism of action on the regulation of *CYP1A1* gene expression in male rat liver.

MATERIALS AND METHODS

Materials

3MC was purchased from Aldrich Chemical Co (Milwaukee, WI, USA). Quercetin, morin, dimethylsulfoxide (DMSO), 17β-estradiol, β-nicotinamide adenine dinucleotide phosphate reduced form (NADPH), bovine serum albumin (BSA), ethoxyresorufin, resorufin, potassium phosphate, sodium phosphate, sodium bicarbonate, sodium chloride, potassium chloride, glucose, calcium chloride, magnesium sulfate, phenol, mercaptoethanol, and chloroform were obtained from Sigma Chemical Co (St. Louis, MO, USA) and diethylpyrocarbonate, guanidinium thiocyanate, dATP, dCTP, dGTP, dTTP, moloney murine leukemia virus reverse transcriptase, dithiothreitol were from GIBCO (Gaithersburg, MD, USA). Oligo d(T)15, Taq DNA polymerase, RNasin were purchased from Promega (Madison, WI, USA). TCDD was a gift from Dr. Chae K. at NIEHS, NIH, USA.

Isolated Liver Perfusion

Male Sprague-Dawley rats were anesthesized by ketamine chloride (100 mg/kg, *i.p.*). The portal vein of rat was cannulated by polyethylen catheter (18 guage) and isolated into perfusion chamber. The isolated liver was perfused with Krebs-Henseleit Bicarbonate buffer (KHB, pH 7.4) saturated with air ($95\%O_2$, $5\%CO_2$). The isolated liver was perfused with KHB for the first 10 minutes to maintain a stable physiological condition before the perfusion with various chemicals for 4 hrs.

Preperation of Microsomes

Liver tissue was homogenized in phosphate buffer (pH 7.6) using polytron. Microsomal fraction was prepared by differential centrifugation and resuspended in pyrophosphate buffer (pH 7.6) and stored at -70°C until use. Total protein was measured using the bicinchonic acid (BCA) method (Pierce, Rockville, IL USA) using BSA as a standard.

Measurement of Ethoxyresorufin O-deethylase Activity

Liver microsomes were prepared as previously reported (Fujino *et al.*, 1984). Microsomes were incubated with 5 μM ethoxyresorufin for 1 minute at 37℃ and 250 μM NADPH, enzymatic activity was monitored via change in fluorescence based on time change. After the 15 minutes, measurement of enzymatic activity was calculated from the area under the peak (excitation 530 nm, emission 579 nm).

Total RNA Isolation

Total RNA was isolated using method by Chomczynski and Sacchi (1987). Liver tissue was homogenized with denaturing solution and extrated with phenol and chloroform. The RNA pellet was reconstituted in diethylpyrocarbonate treated water.

Reverse Transcription and Polymerase Chain Reaction (RTPCR)

For conversion of total RNA to cDNA, a reaction mixture was prepared containing reverse transcriptase (RT) buffer, 10 mM dithiothreitol, 0.05 mM dNTPs, oligo d(T) 15 primer, 20U RNasin, 200U MLV-RT and 3 μg of total RNA. The reaction was incubated for 1 hr at 37° followed by inactivation of the enzyme at 95° for 10 min. For the PCR amplification of cDNA, a reaction mixture was prepared containing RT mix, polymerase reaction buffer, 2.5 mM $MgCl_2$, 0.125 mM dNTP, 1U *Taq* DNA polymerase, and 3pmol each of the forward and reverse primers. *CYP1A1* forward primer; 5'CCATGACCAGGAACTATGGG3' *CYP1A1* reverse primer; 5'TCTGGTGAGCATCCAGGACA3': β-*Actin* forward primer; 5'CCTCTA TGCCAACACAGT3' β-*Actin* reverse primer; 5'AGCCACCAATCCACACAG3' PCR products were analyzed on in 2% agarose gels (PCR product sizes;

CYP1A1, 341bps; β-*Actin*, 153bps) and quantified by a Gel Doc 1000video imaging analysis system.

RESULTS and DISCUSSION

Change of CYP1A1 mRNA level in male rat liver with 3MC or TCDD treatment

Expression of *CYP1A1* gene in response to either 3MC or TCDD was examined by measuring CYP1A1 mRNA level via RTPCR from the isolated perfused rat liver system in *ex vivo*. As shown in Fig. 1, 1nM 3MC perfusion into male rat liver resulted in 130% of CYP1A 1 mRNA level when that of 0.01% DMSO perfused liver was set at 100%. In case of 1nM TCDD perfusion into male rat liver have increased in CYP1A1 mRNA level over control male rat liver by 200% (Fig. 1). This data suggested the potency of TCDD might be different from that of 3MC in terms of *CYP1A1* gene expression in male rat liver, although they might share the same mechanism of action. Based on reports which showed six times stronger effect with TCDD than with 3MC on *CYP1A1* gene expression in female rat liver (Ahn and Sheen, 1998), it is possible to think that gender difference might exist in regualtion of *CYP1A1* gene expression.

Change of EROD activity in male rat liver with 3MC or TCDD treatment

The EROD activity was determined in the 1 nM 3MC or 1 nM TCDD perfused male rat liver microsome to be 2.42 pmole/min/mg protein and 2.65 pmole/min/mg protein, respectively. 0.01% DMSO perfused male rat liver microsome gave 1.55 pmole/min/mg protein EROD activity (Fig. 2). These changes in EROD activity in response to 1 nM 3MC or 1 nM TCDD seems to agree with changes in CYP1A1 mRNA level (Fig. 1). 3MC is known to stimulate both CYP1A1 mRNA and CYP1A2 mRNA, whereas TCDD stimulate only CYP1A1 mRNA (Gonzales, 1988). Since EROD activity represented both *CYP1A1* and *CYP1A2*, EROD activity alone could not be a best indicator to monitor *CYP1A1* gene expression. Also it is shown that the basal activities of EROD between male and female liver are different, female liver appears to have three times more EROD activity than male liver does (Ahn and Sheen, 1998). The high level of basal EROD activity might mask the effect of TCDD so that fold stimulation by TCDD in female rat liver.

Effects of estrogen and morin on TCDD stimulated CYP1A1 mRNA level

In male rat liver, 1 nM TCDD perfusion stimulated CYP1A1 mRNA by two fold over that of control male

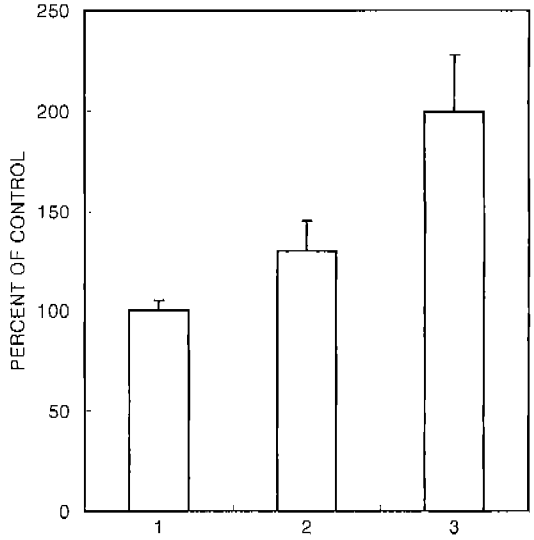

Fig. 1. Quantitation of the CYP1A1 mRNA expression by 3-MC and TCDD stimulation in the perfused male rat liver. CYP1A1 mRNA was measured by RTPCR followed by agarose gel electrophoresis. 341bp PCR product was quantified by image analysis system as described in Methods. Data represent mean±S.E.(n=5).
1: 0.01% DMSO in KHB, 2: 10^{-9} M 3-MC, 3: 10^{-9} M TCDD

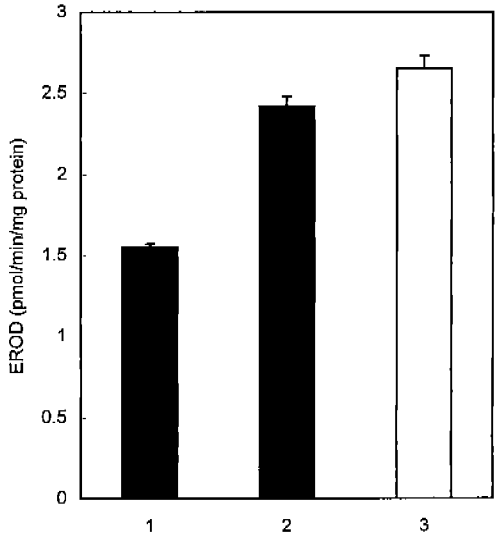

Fig. 2. EROD activity by 3-MC and TCDD stimulation in the perfused male rat liver. Fluorometry was carried out to measure EROD activity in microsome containing 80 μg of total protein that was prepared from female rat liver was perfused with various chemicals as described in Methods. Data represent mean±S.E.(n=6).
1: 0.01% DMSO in KHB, 2: 10^{-9} M 3-MC, 3: 10^{-9} M TCDD

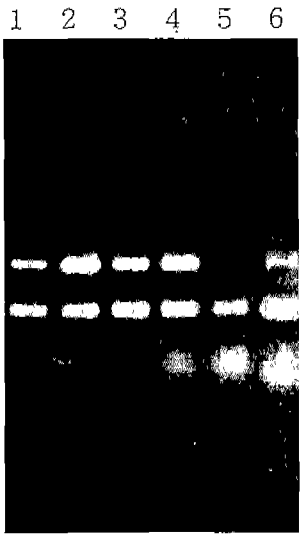

Fig. 3. Effect of morin and estrogen on the CYP1A1 mRNA expression by TCDD stimulation in the perfused male rat liver. Livers of S.D. female rats were perfused with 10^{-6} M morin or 10^{-8} M estradiol for 1.5hrs and then perfused with 10^{-9} M TCDD for 4 hrs and analysed by RTPCR as described in methods. Upper band shows 341bps PCR products of CYP1A 1 and lower band shows 153bps PCR products of β-Actin.
lane 1: 0.01% DMSO in KHB
lane 2: 10-9M TCDD
lane 3: 10^{-6} M Morin
lane 4: 10^{-8} M Estradiol
lane 5: 10^{-6} M Morin → 10^{-9} M TCDD
lane 6: 10^{-8} M Estradiol → 10^{-9} M TCDD

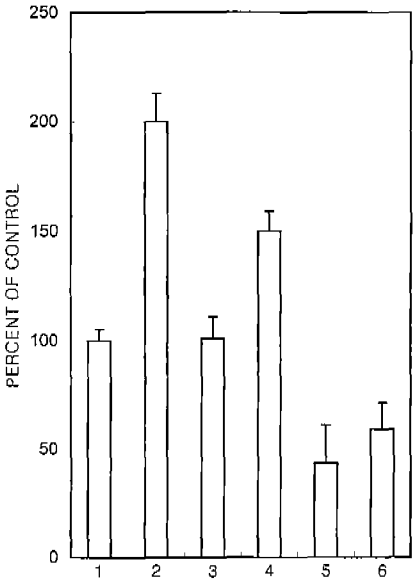

Fig. 4. Quantitation of morin and estrogen effect on the CYP 1A1 mRNA expression by TCDD stimulation in the perfused male rat liver. CYP1A1 mRNA was measured by RTPCR followed by agarose gel electrophoresis. 341bp PCR product was quantified by image analysis system as described in Methods. Data represent mean±S.E.(n=6).
1: 0.01% DMSO in KHB
2: 10^{-9} M TCDD
3: 10^{-6} M Morin
4: 10^{-8} M Estradiol
5: 10^{-6} M Morin → 10^{-9} M TCDD
6: 10^{-8} M Estradiol → 10^{-9} M TCDD

liver. 10nM Estradiol alone perfusion showed slight increases in CYP1A1 mRNA level over that of control male liver, but when 10 nM estradiol was perfused concomitantly with 1nM TCDD, CYP1A1mRNA level was decreased compared to that of 1nM TCDD alone perfused male liver (Fig. 3, 4). This data suggested that female sex hormone, 17β-estradiol antagonized the effect of TCDD on the *CYP1A1* gene expression. At the moment, mechanism of action of estrogen on *CYP1A1* expression is not certain, and it might or might not be mediated through estrogen receptor system.

Effects of morin and estrogen on 3MC stimulated CYP1A1 mRNA

1 nM 3MC perfusion into male rat liver showed slight increase in CYP1A1 mRNA (130% that of control) over control liver. This 3MC stimulated CYP1A1 mRNA level in male rat liver was decreased by either estradiol or morin concomitant treatment with 3MC (Fig. 5, 6). When we compare this result with that has been reported (Ahn and Sheen, 1998), 3MC or TCDD treated female rat liver showed slightly better responsiveness to arylhydrocarbon than male rat liver. However estrogen and flavonoid inhibition on arylhydrocarbon induced CYP1A1 mRNA level in male rat liver was more prominent than that has been shown in female rat liver.

Effects of morin and estrogen on TCDD stimulated EROD activity

Male rat liver seems to show low basal level of EROD activity which was 1.55 pmole/min/mg protein. 1 nM TCDD perfusion into male liver showed increase in EROD activity to be 170% that of control. This is quite different from the female liver based on result reported by Ahn and Sheen (1998). Morin or esradiol perfusion into male liver showed slight increase in EROD activity compared to that of control liver. Especially morin showed stronger effect than 3MC when it was given alone, although the mechanism of action remains to be uncovered. When either estradiol or morin was administered concomitantly with TCDD, EROD activity was decreased but the EROD activity was still higher than that of control liver (Fig. 7). This shows estrogen and morin anto-

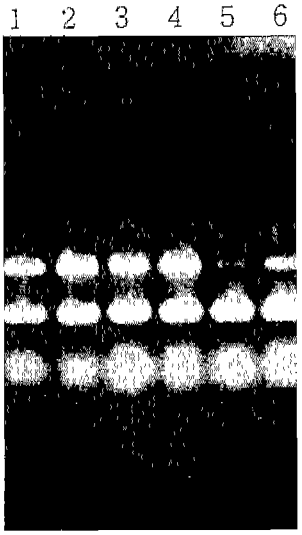

Fig. 5. Effect of morin and estrogen on the CYP1A1 mRNA expression by 3-MC stimulation in the perfused male rat liver. Livers of S.D. female rats were perfused with 10^{-6} M morin or 10^{-8} M estradiol for 1.5 hrs and then perfused with 10^{-9} M 3-MC for 4 hrs and analysed by RTPCR as described in methods. Upper band shows 341bps PCR products of CYP1A1 and lower band shows 153bps PCR products of β-Actin.
lane 1: 0.01% DMSO in KHB
lane 2: 10^{-9} M 3-MC
lane 3: 10^{-6} M Morin
lane 4: 10^{-8} M Estradiol
lane 5: 10^{-6} M Morin → 10^{-9} M 3-MC
lane 6: 10^{-8} M Estradiol → 10^{-9} M 3-MC

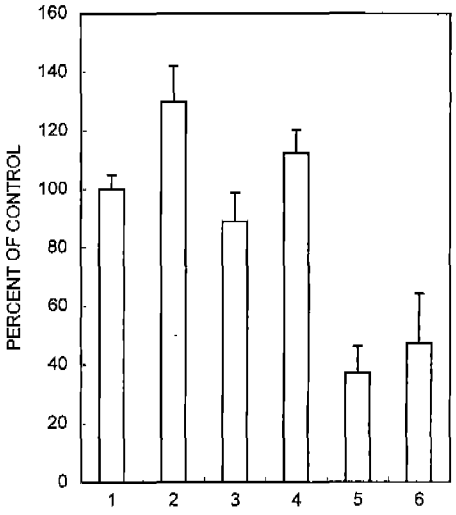

Fig. 6. Quantitation of morin and estrogen effect on the CYP 1A1 mRNA expression by 3-MC stimulation in the perfused male rat liver. CYP1A1 mRNA was measured by RTPCR followed by agarose gel electrophoresis. 341bp PCR product was quantified by image analysis system as described in Methods. Data represent mean ± S.E.(n=4).
1: 0.01% DMSO in KHB, 2: 10^{-9} M 3-MC,
3: 10^{-6} M Morin, 4: 10^{-8} M Estradiol,
5: 10^{-6} M Morin → 10^{-9} M 3-MC,
6: 10^{-8} M Estradiol → 10^{-9} M 3-MC

gonize the effect of TCDD on EROD activity, but their effects was not strong as shown in female liver (Ahn and Sheen, 1998).

Effects of morin and estrogen on 3MC stimulated EROD activity

1 nM 3MC perfusion into male rat liver showed increase in EROD activity by one and half fold of control rat, which shows similar magnitude of stimulation to that of TCDD treatment (Fig. 8). In the case of CYP1A1 mRNA level, TCDD treatment showed stronger effect than 3MC, but EROD activity showed same response to 3MC and TCDD. Estradiol or morin treatment along with 3MC showed inhibitory effect on 3MC stimulated EROD down to control level. Whereas TCDD stimulated EROD activity was not inhibited to control level when estrogen or morin was given concomitantly with TCDD (Fig. 7). This shows estrogen and morin antogonize the effect of 3MC on the stimulation of EROD activity. There is little information about how estrogen affects drug metabolism in terms of CYP1A1 gene expression and EROD activity. Although there are some reports showing that arylhydrocarbon antagonize the estrogen action. In mice and rats, TCDD exposure counteracts the effects of estrogens, such as 17β-estradiol, on uterine hypertrophy, peroxidase activity, ER binding activity, progesterone receptor binding activity, and epidermal growth factor binding activity (Astroff and Safe, 1990). In human mammary cell lines, TCDD exposure results in decreased secretion of tissue plasminogen activator (Gierthy *et al.*, 1987), and decreased secretion of estrogen induced proteins, such as cathepsin D (Krishinan *et al.*, 1994). TCDD exposure also blocks the estradiol dependent cell proliferation response (Gierthy *et al.*, 1993), and occurrence of multicellular foci in postconfluent cultures of human mammary cell line, MCF-7 (Spink *et al.*, 1994).

The flavonoids display a remarkable array of biochemical and pharmacological actions of some of which suggests that certain members of this group of compounds may significantly affect the function of multiple mammalian cellular system. Especially they are known to modulate activities of monooxygenase of drug metabolism (Canivenc-Lavier *et al.*, 1996). Earlier studies demonstrated that synthetic flavonoids inhibit the microsomal enzymatic activity of drug metabolism and later study also showed that hydroxyl group of flavonoids was

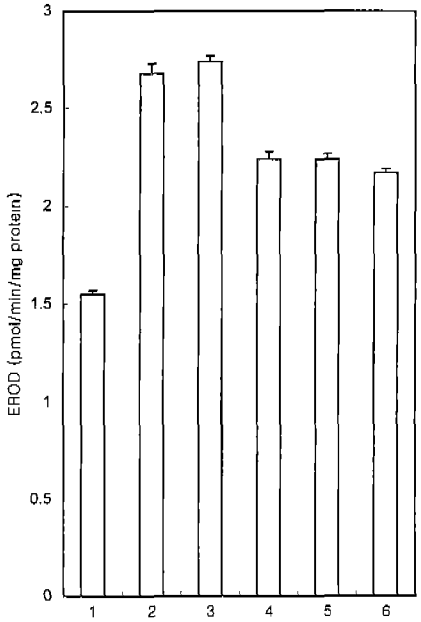

Fig. 7. Effect of morin and estrogen on the EROD activity by TCDD stimulation in the perfused male rat liver. Fluorometry was carried out to measure EROD activity in microsome containing 80 μg of total protein that was prepared from female rat liver was perfused with various chemicals as described in Methods.
1: 0.01% DMSO in KHB. Data represent mean± S.E.(n=5)
2: 10^{-9} M TCDD
3: 10^{-6} M Morin
4: 10^{-8} M Estradiol
5: 10^{-6} M Morin → 10^{-9} M TCDD
6: 10^{-8} M Estradiol → 10^{-9} M TCDD

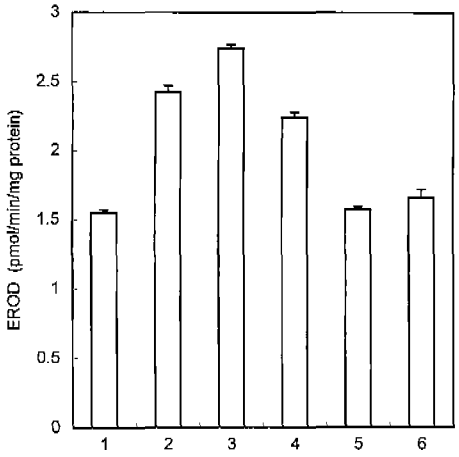

Fig. 8. Effect of morin and estrogen on the EROD activity by 3-MC stimulation in the perfused male rat liver. Fluorometry was carried out to measure EROD activity in microsome containing 80 μg of total protein that was prepared from female rat liver was perfused with various chemicals as described in Methods. Data represent mean± S.E.(n=4).
1: 0.01% DMSO in KHB
2: 10^{-9} M 3-MC
3: 10^{-6} M Morin
4: 10^{-8} M Estradiol
5: 10^{-6} M Morin → 10^{-9} M 3-MC
6: 10^{-8} M Estradiol → 10^{-9} M 3-MC

important for the inhibition of hydroxylation of benzo (a)pyrene whereas flavonoid without hydroxyl group increase in the hydroxylation of benzo(a)pyrene (Buening *et al.*, 1981). It is intriguing to know the mechanism of action of flavonoids in drug metabolizing enzymes. Previous studies demonstrated that 7,8-benzoflavone seemed to increase the interaction of cytochrome P450 dependent NADPH reductase and cytochrome P450 (Sousa and Marletta, 1985). This study showed hydroxylated flavonoids such as morin inhibited the 3MC or TCDD stimulated ethoxyresorufin O-deethylase and CYP1A1 mRNA level. However, further studies of regulation of gene expression of cytochrome P4501A would be necessary to know the mechanism of action of hydroxylated flavonoids on drug metabolizing enzymes.

ACKNOWLEDGEMENTS

The present study was supported by 1998 Research Grant from the Research Institute of Pharmaceutical Science, College of Pharmacy, Ewha Womans University.

REFERENCE

Ahn, M. and Sheen, Y. Y. (1998). Comparison between TCDD and 3MC action on *CYP1A1* expression and EROD activity in the isolated perfused male rat liver. *J. Tox. Pub. Health* **14**, in press.

Astroff, B. and Safe, S. (1990). 2,3,7,8-tetrachlorodibenzo-p-dioxin as an antiestrogen; effect on rat uterine peroxidase activity. *Biochem Pharmacol* **39**, 485-488.

Bucning, M. K., Chang, R. L., Huang, M. T., Forther, J. G., Wood, A. W. and Conney, A. H. (1981). Activation and inhibition of benzo(a)pyrene and aflatoxin B_1 metabolism in human liver microsomes by naturally occuring flavonoids, *Cancer Research,* **41**, 67-72.

Canivenc-Lavier, M. C., Bentejac, M., Miller, M. L., Leclerc, J., Siess, M. H., Latruffe, N. and Suschetet, M. (1996). Differential effects of nonhydroxylated flavonoids as inducers of cytochrome P450 IA and IIB isozymes in rat liver, *Toxicol. Appl. Pharmacol.,* **136**, 348-53.

Carrier, F., Chang, C. Y., Duh, J. L., Nebert, D. W. and Puga, A. (1994). Interaction of the regulatory domains of the murine CYP1A1 gene with two DNA-binding proteins in addition to the Ah receptor and the Ah receptor nuclear translocator (ARNT), *Biochem. Pharmacol.,* **48**, 1767-1778.

Chomczynski, P. and Sacchi, N. (1987). Single-step method

of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction, *Anal. Biochem.*, **162**, 156-159.

Chou, M. W., Heflich, R. H. and Fu, P. P. (1986). Metabolism of 1-nitrobenzo(a)pyrene by rat liver microsomes to potent mutagenic metabolites, *Carcinogenesis*, **7**, 1837-1844.

Denison, M. S., Fisher, J. M. and Whitlock, J. P. Jr. (1988). The DNA recognition site for the dioxin-Ah receptor complex. Nucleotide sequence and functional analysis, *J. Biol. Chem.*, **263**, 17221-17224.

Dolwick, K. M., Swanson, H. I. and Brandfield, C. A. (1993). In vitro analysis of Ah receptor domains involved in ligand-sactivated DNA recognition, *Pro. Nath. Acad. Sci. USA.*, **90**, 8566-9570.

Gierthy, J. F., Lincoln, D. W., Gillespie, M. B., Seeger, J. I., Martinez, H. L. and Kurmar, S. A. (1987). Suppression of estrogenregulated extracellular plasminogen activator activity of MCF-7 cells by 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Cancer Res.* **47**, 6198-6203.

Gierthy, J. F., Bennett, J. A., Bradly, L. M. and Cutler, D. S. (1993). Correlation of in vitro and in vivo growth suppression of MCF-7 human breast cancer by 2.3.7.8-tetrachlorodibenzo-p-dioxin. *Cancer Res.* **53**, 3149-3153.

Gonzalez, F. J. (1988). The molecular biology of cytochrome P450s. *Pharmacol. Rev.*, **40**, 243-288.

Ko, H. P., Okino, S. T., Ma, Q. and Whitlock, J. P. Jr. (1996). Dioxin-induced CYP1A1 transcription in vivo; the aromatic hydrocarbon receptor mediates transactivation, enhancer-promoter communication, and changes in chromatin structure, *Mol. Cell. Biol.*, **16**, 430-436.

Krishnan, V, Wang, X. and Safe, S. (1994). ERISPI complexes mediate estrogeninduced cathepsin D gene expression in MCF-7 human breast cancer cells. *J Biol Chem* **269**, 15912-15917.

Mason, G. G., Witte, A. M., Whitelaw, M. L., Antonsson, C., McGire, J., Wilhelmsson, A., Poellinger, L. and Gustafsson, J. A. (1994). Purification of the DNA binding form of dioxin receptor, *J. Biol. Chem.*, **269**, 4438-4449.

Miksicek, R. J. (1995). Estrogenic flavonoids; Structural requirments for biological activity, *Proc. Soc. Exp. Biol. Med.*, **208**, 44-50.

Pendurthi, U. R., Okino, S. T. and Tukey, R. H. (1993). Accumulation of the nuclear dioxin (Ah) receptor and transcriptional activation of the mouse Cyp1a-1 and Cyp1a-2 genes, *Arch. Biochem. Biophys.*, **306**, 65-69.

Poland, A. and Glover, E. (1974). Comparison of 2,3,7,8-tetrachlorodibenzo-p-dioxin, a potent inducer of arylhydrocarbon hydroxylase, with 3-methylcholanthrene, *Mol. Pharmacol.*, **10**, 349-359.

Riddick, D. S., Huang, Y., Harper, P. A. and Okey, A. B. (1994). 2,3,7,8-tetrachlorodibenzo-p-dioxin versus 3-methylcholanthrene; Comparative studies of Ah receptor binding, transformation and indudtion of CYP1A1, *J. Biol. Chem.*, **269**, 12118-12128.

Rowlands, J. C., McEwan, I. J. and Gustafsson, J. A. (1996). Transactivation by the human aryl hydrocarbon receptor and arylhydrocarbon receptor nuclear translocator proteins: Direct interaction with basal transcription factors, *Mol. Pharmacol.*, **50**, 528-548.

Sousa, R. L. and Marletta, M. A. (1985). Inhibition of cytochrome P450 activity in rat liver microsomes by the naturally occuring flavonoid, quercetin, *Arch. Biochem. Biophys.*, **240**, 345-357.

Sousa, R. L. and Marletta, M. A. (1985). Inhibition of cytochrome P-450 activity in rat liver microsomes by the naturally occurring flavonoid, quercetin, *Arch. Biochem. Biophys.*, **240**, 345-357.

Spink, D. C., Johnson, J. A., Connor, S. P., Aldous, K. M. and Gierthy, J. F. (1994). Stimulation of 17-estradiol metabolism in MCF-7 cells by bromochloro and chloromethyl substituted dibenzo p-dioxins and dibenzofurans: correlation with antiestrogenic activity. *J. Toxicol. Environ. Health.* **41**, 451-466.

Yang, C. S., Strickhart, F. S. and Kicha, L. P. (1978). Interaction between NADPH-cytochrome P450 reductase and hepatic microsomes, *Biochim. Biophys. Acta.*, **509**, 326-337.