THE EFFECT OF ENVIRONMENTAL CHEMICALS ON LIVER DRUG METABLIZING ENZYMES OF FETAL AND NEONATAL RATS

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Introduction

Recently, the effects of environmental chemicals on fetuses and neonates have been concerned. It was reported that the fetuses and neonates were more sensitive to the chemicals than mature adults. Because of their developmental plasticity, the environmental chemicals would disrupt epigenetic programming, differentiation control, development of organs and others[1]. Xenobiotic-metabolizing enzyme activity is extremely low in fetal and early neonatal liver, so exposure to foreign chemicals might result in toxicity. Various adverse effects owing to deficiency of drug-metabolizing enzymes have also been reported[2]. Developmental changes of several drug-metabolizing enzymes (cytochrome P450, flavin-containing monooxygenase, glucuronyltransferase and aldehyde oxidase) have been reported[3,4,5]. Lupp *et al.* reported changes in the expression of cytochrome P450 isoforms in neonatal rats[6]. In this study, we examined the developmental changes and induction of drug metabolizing enzymes, cytochome P450 (CYP), in liver by xenobiotics in fetuses or neonates of rats. CYP activities were assayed in terms of alkylresorufin-O-dealkylase activities, and the protein level was determined by Western blotting analysis.

Materials and methods

Animal treatments

Wistar rats (Sic:Wistar/ST), pregnant and neonates were used. The pregnant mothers rats were daily treated with sodium phenobarbital (PB, 80mg/kg *i.p.*), 3-methylcholanthrene (MC, 50mg/kg *p.o.*), dexamethasone (DEX, 100mg/kg *p.o.*), bisphenol A (BPA, 200mg/kg *p.o.*), 1,1,1-trichloro-2,2-bis (*p*-chlorophenyl) ethane (DDT, 100mg/kg *i.p.*), di-*n*-butylphthalate (DBP,100mg/kg *i.p.*) or panacete (vehicle) from PND15 to PND17 of gestation, and sacrificed on PND18 of gestation. While, the neonates were daily treated with PB (80 mg/kg s.c.), MC (50 mg/kg s.c.), DEX (100 mg/kg s.c.), BPA (200mg/kg s.c.), DDT (100mg/kg *s.c.*), DBP (100 mg/kg *s.c.*) or panacete (vehicle) from PND1 to PND3 after birth and sacrificed on PND4.

Measurement of specific activities of CYP isozymes

Specific activities of CYP isozymes were measured using the specific substrates of CYP isozymes, e.g. resorufin ethyl ether for CYP1A1, resorufin methyl ether for CYP1A2, resorufin pentoxy ether for CYP2B, 7-methoxy-4-trifluoromethylcoumarin for CYP2C9 and 7-benzoxy-4-trifluoromethylcoumarin for CYP3A. (EROD, MROD, PROD, BFCD, MFCD) [7].

Measurement of CYP protein expressions

The expression of CYP proteins were evaluated by Western blotting analysis using anti-rat CYP antibodies (CYP1A1, CYP2B1, CYP2C1, and CYP3A2), and the amount of reactive CYP proteins was densitometrically measured by using densitometer MacBAS (Fuji Photo Film Co., Ltd)

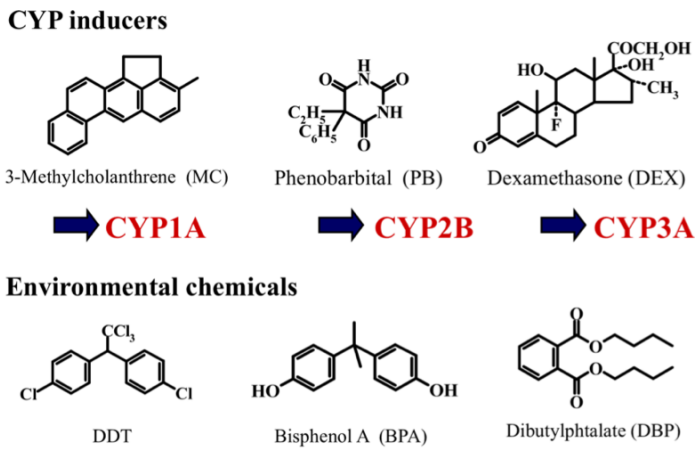

Fig.1 Structure of CYP inducers and the environmental chemicals using this study.

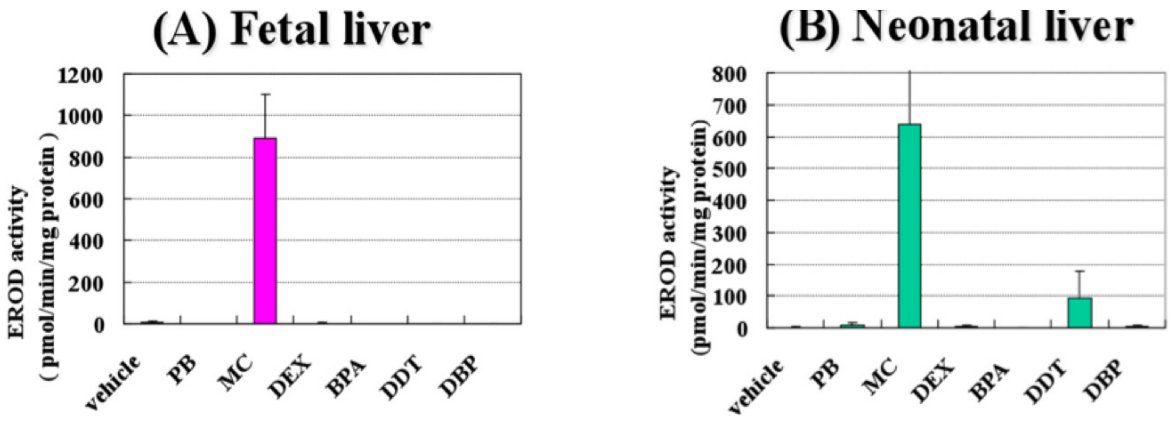

Fig.2 Changes of EROD activity in fetal and neonatal rat liver by xenobiotics treatment.

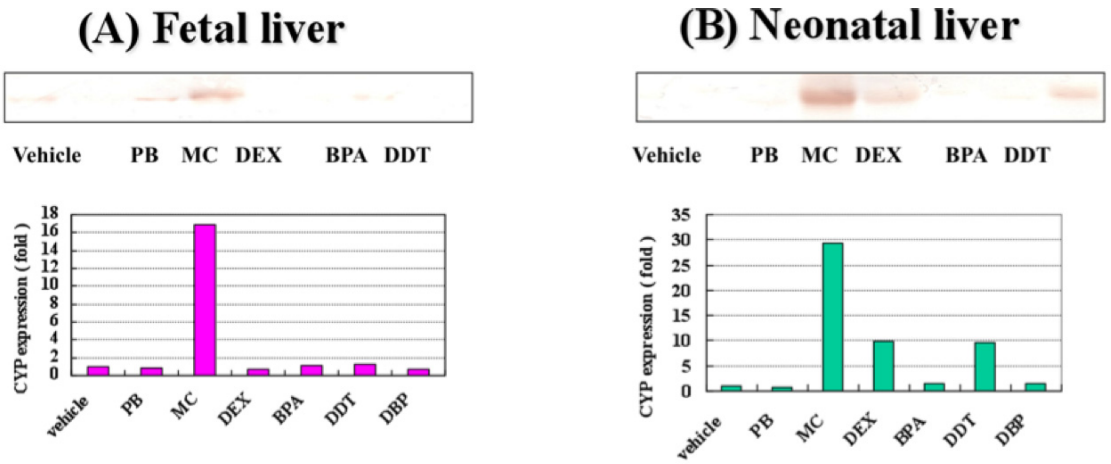

Fig.3 Changes of CYP1A expression on Western blotting in fetal and neonatal rat liver by xenobiotics treatment.

Results and discussion:

The effect of environmental chemicals on the activities of the drug metabolizing enzymes, cytochrome P450 (CYP) and others, in rat fetal and neonatal liver were examined. Little activity of CYPs was found in the liver of rat fetuses and neonates (GD18, PND 1, 3, and 5).

Effect of treatment of CYP inducers or environmental chemicals on the expressions and activities of CYP isozymes in fetal rat liver

The pregnant mothers rats were daily treated with PB, MC, DEX, BPA, DDT and DBP. The typical CYP inducer, PB (CYP2B), MC (CYP1A) and DEX (CYP3A), and the environmental chemicals, BPA, DDT and DBP were showed in Fig.1.

When MC was administrated to pregnant rats (GD 15) and the activity of CYPs in fetal liver were assayed, significant induction of CYP1A1/2 and 3A were observed (Fig. 2A). The induction of their protein expression was confirmed by Western blot analysis (Fig. 3A).

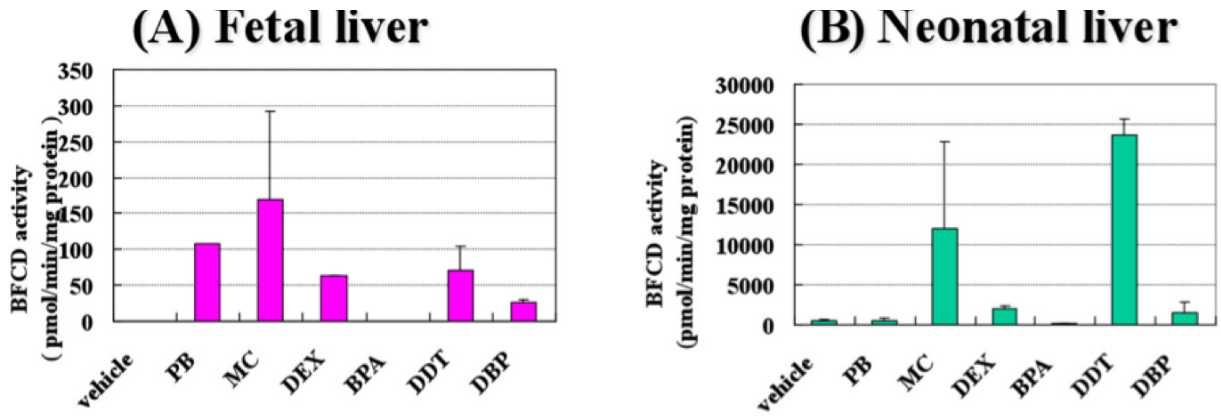

Fig.4 Changes of BFCD activity in fetal and neonatal rat liver by xenobiotics treatment.

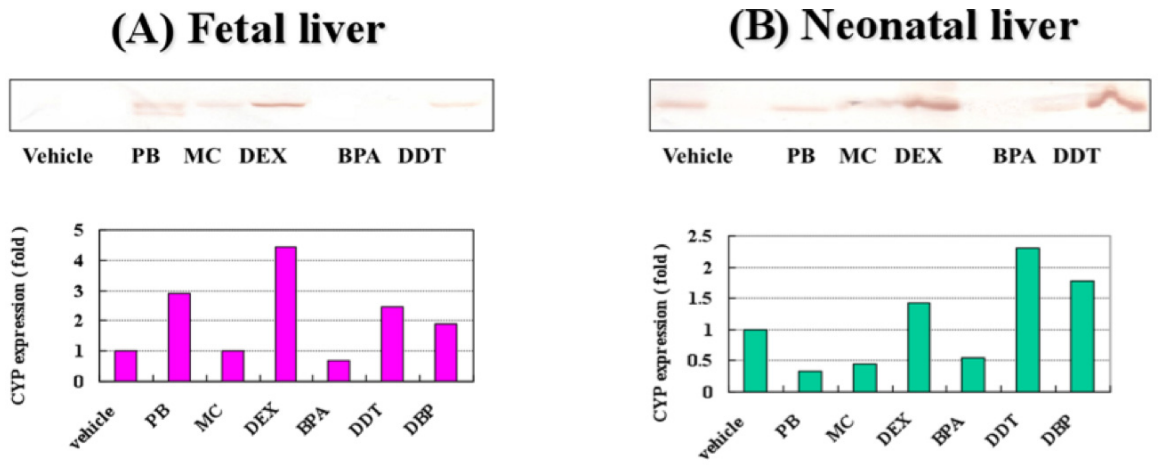

Fig.5 Changes of CYP3A expression on Western blotting in fetal and neonatal rat liver by xenobiotics treatment.

CYP3A proteins were induced by PB, DEX, DDT and DBP (Fig. 4,5). CYP2B and CYP2C were induced by PB and DDT treatment (data not shown).

Effect of treatment of CYP inducers or environmental chemicals on the expressions and activities of CYP isozymes in neonatal rat liver

The administration of PB induced CYP2B in fetal liver. Environmental chemicals, DDT and DBP, induced CYP3A. In neonates, the CYP inducers (MC, PB and DEX) and the environmental chemicals (DDT and DBP) also induced the corresponding CYPs significantly (Fig. 2-5). CYP1A proteins were induced by MC, DEX and DDT treatment (Fig. 2, 4). CYP3A proteins were induced by treatment with typical CYP inducer : DEX and environmental chemicals : DDT and DBP. CYP2B and CYP2C proteins were induced by DDT treatment (data not shown). These level of induction of CYP isozymes by xenobiotics treatments in neonatal liver were higher

than those in fetal liver. So it is important to consider the risk of exposure to xenobiotics on fetal and neonatal stage.

In conclusion, the activities of CYPs in fetuses and neonates of rats were very low, but they were induced by CYP inducers and the environmental chemicals. The results indicated that the fetuses and neonates of rats had the ability of inducing CYPs by xenobiotics and a part of the developmental changes of drug-metabolizing enzymes would be induced by the xenobiotic chemicals.

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References:
1. Gluckman PD, and Hanson MA, (2009); *Early life origins of Human Health and Disease.* (Newnham JP and Ross MG. eds.), pp 1-10, Karger
2. Johnson, T. N.. *Toxicology* 192, 37-48 (2003)
3. Leeder JS, Gaeddigk R, Marcucci KA, Gaedigk A, Vyhlidai CA, Schindel BP, Pearce RE*, J. Pharmacol. Exp. Ther.,* 314, 626-635 (2005)
4. Tayama Y, Miyake K, Sugihara K, Kitamura S, Kobayashi M, Morita S, Ohta S, Kihira K., *Clin Pharmacol Ther.* 81, 567-72 (2007).
5. Tayama Y, Moriyasu A, Sugihara K, Ohta S, Kitamura S., *Drug Metab Pharmacokinet.* 22, 119-24 (2007).
6. Lupp A, Danz M, and Muller, D., *Toxicology*, 206, 427-438 (2005).
7. Burke RE, Fahn S, *Biochem Pharmacol*, 48, 923-936 (1994).