PARABENS AND PHTHALATES: THEIR METABOLISM, AHR LIGAND ACTIVITY, ESTROGENIC ACTIVITY, AND P450-INHIBITORY ACTIVITY

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【Abstract】

p-Hydroxyalkylbenzoates (parabens) have antimicrobial activity, and have been widely used as preservatives in food, cosmetic and pharmaceutical products. Phthalate diesters are commonly used as plasticizers for polyvinyl chloride-based products. In this study, we examined the arylhydrocarbon receptor (AhR) ligand activity, estrogen receptor (ER) ligand activity, and chytochrome P450-inhibitory (P450) activity of parabens and phthalates. We also studied the metabolism of parabens and phthalates in vitro in rat. Parabens with a medium-length side chain (C4~C6) showed AhR ligand activity and ER ligand activity, but the phthalates examined here lacked these activities. Parabens and phthalates with a side chain of medium length (C6~C9) showed higher inhibitory effects on P450 activity than did those with a short (C1~C4) side chain. Benzyl-*n*-butylphthalate showed the gratest inhibitory effect on P450 activities among the phthalates tested in this study. It was found that carboxylesterase (CES) involved in the hydrolysis of parabens in liver, intestine and lung. The hydrolytic activity of liver microsomes toward parabens decreased with decreasing side chain length of the parabens. CES in the solubilized fraction of liver microsomes is involved in the hydrolysis of phthalates with the short side chain (C2~C4) , while an enzyme in the insoluble fraction is involved in the hydrolysis of phthalates with a long side chain (C6).

【Introduction】

Parabens have high antimicrobial activity, and have been widely used as preservatives in food, cosmetic and pharmaceutical products. Methyl, ethyl, propyl, butyl and benzyl paraben are commonly used[1]. Parabens are rapidly absorbed, metabolized, and excreted, but it was known that methyl, ethyl, propyl and butyl paraben have weak estrogenic activity[2]. Longer-alkyl-chain parabens exhibit cytotoxity, and their skin penetration efficiency is higher[3,4]. Phthalates have been widely used as plasticizers in blood bags, medical tubing, and so on. Dibutyl phthalate (DBP), di-2-ethylhexyl phthalate (DEHP), monobutyl phthalate (MBP) (a main metabolite of DBP), and mono-2-ehtylhexyl phthalate (MEHP) (a main metabolite of DEHP) disrupt endocrine function[5]. It has been reported that hydrolysis of parabens is catalyzed by CES[3], but the isozyme specificity is unknown. Here we discribe AhR ligand activity, estrogenic activity, chytochrome P450-inhibitory activity and metabolism of various parabens and phthalates.

【Materials and Method】

AhR yeast reporter assay - We used the yeast assay system with integrated human AhR, ARNT gene and plasmid containing XRE and *Lac Z* gene, developed by Miller[6] AhR ligand activity is estimated as β-galactosidase activity.

ER yeast reporter assay - The yeast assay system with integrated human ERα gene and plasmid containing ERE and Lac Z gene, developed by Sumpter 7, was employed. ER ligand activity is estimated as β-galactosidase activity.

Inhibition of P450 activity - Each male Slc:SD rats, 6weeks old, treated with methylcholanthrene (25 mg/kg/day), phenobarbital (80 mg/kg/day), dexamethasone (100 mg/kg/day), or acetone (4.8 mL/kg/day) were used. The inhibitory effects of parabens and phthalates on the activities of ethoxyresorufin-*O*-dealkylase (EROD), methoxyresorufin-*O*-dealkylase(MROD),pentoxyresorufin-*O*-dealkylase(PROD), 7-benzyloxy-4-(trifluoromethyl)coumarin dealkylase (BFCD) and 7-methoxy-4-(trifluoromethyl) coumarin dealkylase (MFCD), 7-ethoxy-4-(trifluoromethyl) coumarin dealkylase (EFCD) were examined.

Metabolism of parabens and phthalates - Male Slc:SD rats, 6 weeks old, were used for the preparation of the microsomes of various organs. Hydrolytic activity towards parabens and phthalates was estimated by measuring the amount of the hydrolysis product by high-performance liquid chromatography. CES in microsomes was solubilized with Triton-X 100.

【Results and Discussion】

AhR yeast reporter assay - AhR ligand activity increased with increasing length of the alkyl chain of parabens. Hexyl paraben showed the highest AhR ligand activity. Parabens with a branched alkyl chain, isobutyl paraben and isoamyl paraben, and benzyl paraben showed relatively high AhR ligand activity.However, *p*-hydroxybenzoic acid, which is the main metabolite of parabens, had no AhR ligand activity. All phthalates examined here lacked AhR ligand activity. These results suggest that the longer-side-chain of parabens show higher AhR ligand activity.

ER yeast reporter assay - ER ligand activity increased with increasing alkyl chain length of parabens. Among parabens with a straight alkyl chain, hexyl paraben showed the gratest ER ligand activity. Isobutyl paraben, isoamyl paraben and benzyl paraben showed relatively high ER ligand activity. *p*-Hydroxybenzoic acid lacked ER ligand activity. In MCF-7 luciferase reporter assay, hexyl paraben, isobutyl paraben and isoamyl paraben also showed high ERα ligand activity. Thus, the longer-alkyl-chain paraben showed the higher ER ligand activity. Phthalates showed no estrogenic activity.

Inhibition of P450 activity - The inhibitory effect of parabens and phthalates on CYP 1A2（MROD）activity increasd with increasing side chain length. Parabens with C6~C9 side chains showed relatively potent on MROD activity. Phthalates with C3~C4 side chains also showed strong inhibitory effects. However, parabens and phthalates with a longer side chain showed weaker inhibitory effects. *p*-Hydroxybenzoic acid did not inhibit

MROD activity, but some monoester phthalates showed a weak inhibitory effect. Parabens also inhibited all the P450 isozyme activities (1A1, 2B, 2C, 2E1, 3A) examined here. These results suggest that parabens with a short side chain (low hydrophobicity) show weak inhibitory effects on P450 activity. Benzyl-*n*-butylphthalate showed the greatest inhibitory effect on P450 activities among the phthalates tested in this study. Phthalates with a side chain of medium length (C3~C4) showed higher inhibitory effects on P450 activities than phthalates with a short (C1, C2) or the long (C6) side chain. All monoesters of these phthalates showed much weaker inhibitory effects than the mother chemicals (Fig.1).

Metabolism of parabens and phthalates - Metabolism of parabens by liver microsomes treated with phenobarbital (PB) and dexamethasone (DEX) was examined. The hydrolytic activity towards 4-nitrophenyl acetate was significantly decreased in DEX-treated microsomes compared with control microsomes, while the hydrolytic activity of 4-methylumberferyl acetate was significantly increased in PB- and DEX-treated microsomes compared with control microsomes. The metabolism of parabens in DEX-treated microsomes was significantly decreased compared with the control. Metabolism of parabens by rat liver microsomes, lung microsomes, intestinal homogenate, pancreas homogenate, plasma and kidney microsomes was examined, and parabens with a short side chain (C2~C4) were well hydrolyzed in liver and lung, with propyl paraben being the best substrate. In intestine, pancreas, plasma and kidney, parabens with a long side chain (C7, C12) were well hydrolyzed. In the case of parabens with shorter alkyl side chains, hydrolytic activity was lower in the intestine, pancreas, plasma and kidney. Hydrolytic activity towards isopropyl paraben, which has a branched side chain, was the lowest. Thus, the hydrolytic activity towards parabens increased with increasing side chain length of parabens in the intestine, which contains only CES2 isozyme, and increased with decreasing side chain length in lung, in which only CES1 isozyme exists. So, CES1 appears to be involved in the hydrolysis of parabens with a short side chain (C2~C4) and, CES2 in the hydrolysis of parabens with a long side chain (longer than C7) In the metabolism of the phthalates by rat liver microsomes, CES in the solubilized fraction of liver microsomes is involved in the hydrolysis of phthalates with a short side chain (C2~C4), and an enzyme in the residual fraction is involved in the hydrolysis of phthalate with a long side chain (C7). In this study, we found that parabens with a medium-length side chain (C4~C6) showed high AhR and ER ligand activities, and parabens with a C6~C9 side chain showed high cytochrome P450- inhibitory activity. The phthalates examined here all lacked AhR and ER ligand activities, but phthalates with a C3~C4 side chain showed high P450-inhibitory effect activity. Parabens are metabolised excluding by CES, which is present at high levels in liver, intestine and lung, while enzymes involved in phthalate hydrolysis exist in both the CES-solubilized fraction and the residual fraction (lipase phase) of liver microsomes (Fig.2).

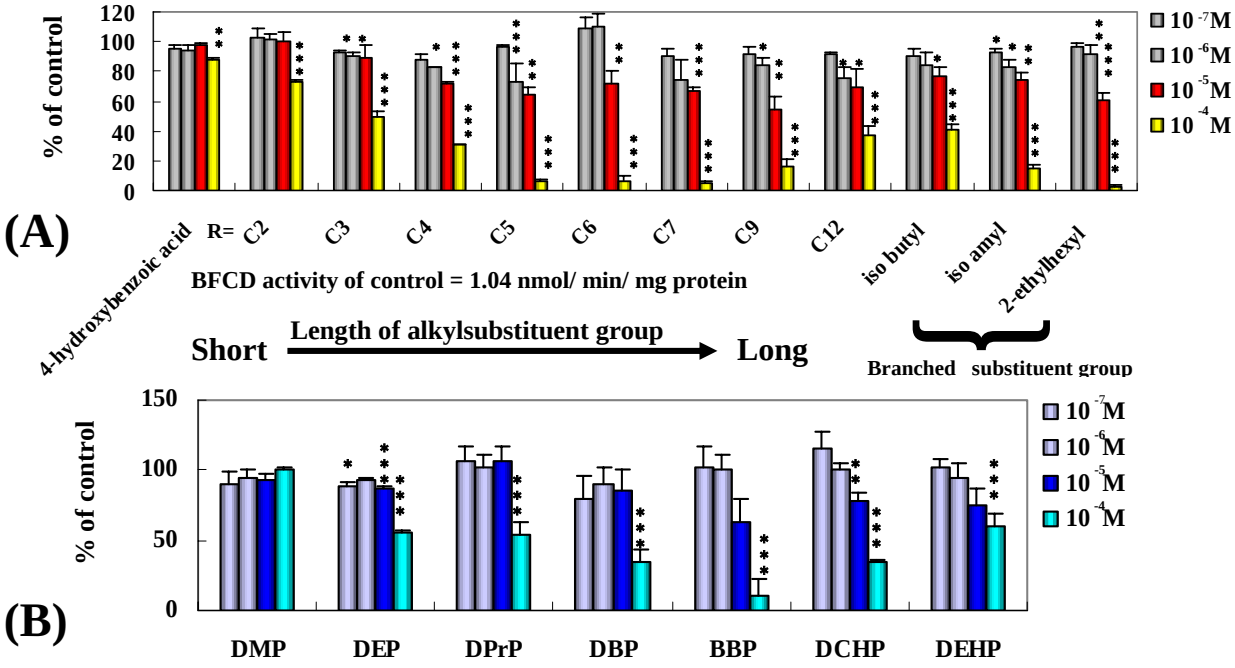

Figure.1 Inhibitory effect of parabens (A) and phthalates (B) on CYP 3A activity in rat liver microsomes.BFCD activity (control activity is adjusted to 100%) in liver microsomes were determined in the presence of various concentration (10^{-7} ~10^{-4} M) of parabens or phthalates. Control activity is determined in the absence of parabens or phthalates in mixtures. (Means±S. D. ＊ P < 0.05, ＊＊ P < 0.01, ＊＊＊ P < 0.001 VS control., n=3)

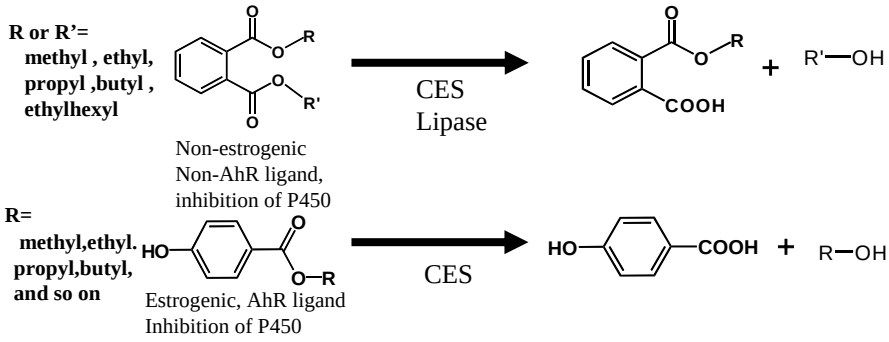

Figure.2 Metabolism pathway of parabens and phthalates

【Acknowledgement】

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【References】

1. Gilliland D, Li Wan Po A, Scott E. J *Appl Bacteriol*.1992; 72: 258-261
2. Routledge EJ, Parker J, Odum J, Ashby J, Sumpter JP.*Toxicol. Appl. Pharmacol.* 1998; 153,12-19
3. Nakagawa Y, Moldeus P. *Biochem. Pharmacol.* 1998; 55: 1907-1914
4. Komatsu H. Suzuki, M *J. Pharmaceutic. Sci.* 1979; 68: 596-598
5. Lau TK, Chu W, Graham N. *Chemosphere* 2005; 60: 1045~1053
6. Miller CA. *J. Biol. Chem.* 1997, 1998; 272: 32824-32829
7. Routledge EJ, and Sumpter JP. *Environ. Toxicol. Chem.* 1996; 15: 241-248