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Glucuronidation of Hydroxylated Polychlorinated Biphenyl by *Channel Catfish* Liver

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챠넬메기 간에서 Hydroxylated Polychlorinated Biphenyl의 Glucuronidation

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요 약

Hydroxylated polychlorinated biphenyl (OH-PCBs)는 PCB의 CYP-dependent oxidation의 대사물로서 잠재적 독성이 강하고 지질친화성을 가지며 생물군에 지속적인 축적성을 나타낸다. OH-PCBs의 해독화 효능을 조사하기 위하여 *channel catfish* 간에서 glucuronidation을 통한 해독작용 가능성을 평가하고 biphenyl 구조에 다양한 염소치환의 구조적 차이점에 따른 영향을 비교 분석하여 보았다. Kinetic parameters에서 K_m과 V_{max}는 192~871 μM, 869~1774 pmol/min/mg으로써 4′-OH-PCB35와 4′-OH-PCB69이 가장 높은 속도의 glucuronidation을 나타냈으며, 구조적 차이점에서 phenolic group에 한 개의 염소치환이 존재할 경우보다 두 개의 염소치환이 존재할 경우에 OH-PCBs ($p<0.001$)의 glucuronidation에 대한 V_{max}를 현저하게 낮추는 결과를 보였다.

Key words : glucuronidation, hydroxylated polychlorinated biphenyl

INTRODUCTION

Glucuronidation is catalyzed by a group of endoplasmic reticular membrane-bound enzymes, the UDP-glucuronosyltransferases (UGTs), which transfer a D-glucuronic acid moiety from the co-substrate UDP-glucuronic acid (UDPGA) to a xenobiotic containing a suitable nucleophilic atom such as oxygen, nitrogen and sulfur. The resulting polar glucuronide is readily excreted in bile or urine (Bergman *et al.*, 1994).

Hydroxylated polychlorinated biphenyls (OH-PCBs) are the metabolic products of the biotransformation of polychlorinated biphenyls (PCBs). PCBs are still considered as important environmental contaminants, due to their persistence and ubiquity in the environment (Barrie *et al.*, 1992). Iwata and co-workers (1993) have presented evidence for a net transfer of PCBs from air to ocean surface water, with a strong tendency to increase with increasing latitude as the air and water become colder. These toxic lipophilic metabolites persist in biota and accumulate at higher trophic levels. One route of detoxification is glucuronidation, whose efficiency is influenced by the various

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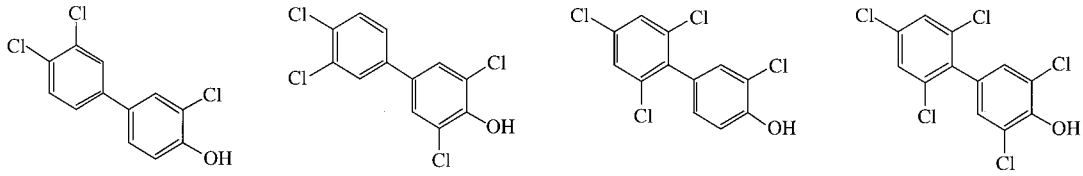

4′-OH-PCB35 4′-OH-PCB79 4′-OH-PCB69 4′-OH-PCB121

Fig. 1. Structures of OH-PCBs used glucuronidation study by catfish liver.

chlorine and hydroxyl substitution patterns possible on the biphenyl structure.

Metabolism of the numerous PCB congeners is mainly via cytochrome P450, mainly through the isozymes CYP1A and CYP 2B, leading to the formation of a OH-PCB or an epoxide. The epoxide may either rearrange to give a OH-PCB or form the glutathione or methyl sulfone conjugate (Norstrom and Muir, 1994; James *et al.*, 2001). The OH-PCBs may be due to the high binding affinity for transthyretin, the thyroxine transport protein, consequently disrupting thyroxine and vitamin A transport (Brouwer and Van den Berg, 1986; Brouwer *et al.*, 1998). This competitive inhibition may be due to the *para*-hydroxy group with adjacent halogen groups on the phenyl ring in both thyroxine and OH-PCBs.

The objective of this study was to investigate the efficacy of detoxification of OH-PCBs by the channel catfish liver and to determine structure-activity relationships with chlorine substitution on phenolic or non-phenolic ring via glucuronidation.

MATERIALS AND METHEDS

1. Chemicals

Uridine 5′-diphosphoglucuronic acid (UDP-GA) was obtained from Sigma (St. Louis, MO). Homogenizing buffer consisted of 1.15% KCl, 0.05M K_3PO_4 pH 7.4, and 0.2 mM phenylmethylsulfonyl fluoride (PMSF), added from concentrated ethanol solution just before use. Resuspension buffer consisted of 0.25 M sucrose, 0.01 M Hepes pH 7.4, 5% glycerol, 0.1 mM dithiothreitol (DTT), 0.1 mM ethylendiamine tetracetic acid (EDTA) and 0.1 mM PMSF. The 4′-OH -PCB35 (4′-hydroxy-3,3′,4-trichlorobiphenyl), 4′-OH -PCB69 (4′-hydroxy-2,3′,4,6-tetrachlorobiphenyl), 4′-OH-PCB79 (4′-hydroxy-3,3′,4,5′-tetrachlorobiphenyl), and 4′-OH-PCB121 (4′-hydroxy-2,3′,4,5′,6-pentachlorobiphenyl) were purchased from AccuStandard (New Haven, CT) or were synthesized by Suzuki coupling (Lehmler and Robertson, 2001). The OH-PCBs are classified in pairs, two OH-PCBs that differ only in the number of chlorine atoms flanking the phenolic group (Fig. 1). Other reagents were the highest grade available from Sigma and Fisher Scientific (Atlanta, GA).

2. Animals

Four *channel catfish* (*Ictalurus punctatus*) were used for this study (two males, H-S41, H-S46 and two females, H-S47 H-S53) with body weight of 2.1 ~ 3.1 kg. All fish were kept in flowing well water and fed regular fish chow diet. Care and treatment of the animals was conducted as per the guidelines of the institutional animal care and use committee. Liver samples were removed within 10 ~ 15 minutes after death, cut into small pieces and frozen in liquid N_2. The samples were subsequently stored at −80°C.

3. Microsome preparation

Hepatic cytosol and microsomes of catfish were prepared by the following method. The catfish was sacrificed after briefly anesthetizing in ice-cold water. The liver was removed and placed in ice-cold ho-

mogenizing buffer. The liver was then minced with scissors and transferred to a Potter-Elvejhem glass homogenizing vessel (James and Little, 1983). The tissue was homogenized with a motor-driven serrated Teflon pestle, using 4 complete up-and-down strokes. The homogenate was then poured into a suitably sized Sorvall polycarbonate centrifuge tube. The homogenates were centrifuged at 10,500 rpm for 20 min in a Sorvall Superspeed RC2-B (Newtown, CT) refrigerated centrifuge to sediment nuclei, cell debris and mitochondria. The supernatant containing the microsomes and cytosol was pipetted into a polycarbonate ultracentrifuge tube, and the tube filled to the shoulder. The supernatant was then centrifuged at 47,500 rpm for 45 min using a Beckman L8-M Ultracentrifuge (Palo Alto, CA). The pellet was resuspended in homogenizing buffer, centrifuged at 47,500 rpm for 30 min, and resuspended again in sucrose storage buffer. Microsomal and cytosol protein were determined according to the method developed by Lowry and co-workers (1951). After division into small aliquots, the samples were purged with N_2 and then stored at −80°C.

4. Glucuronidation assay

The incubation mixture consisted of 0.1 M Tris-Cl buffer (pH 7.6), 5 mM $MgCl_2$, 0.5% Brij-58, 1.5 mM UDPGA, 100 μg catfish microsomal protein, and substrate in a total reaction volume of 0.1 mL. The OH-PCBs were added from methanol solutions and evaporated under nitrogen. Subsequently, the buffer, $MgCl_2$, and water were added in that order and vortex-mixed. After a pre-incubation of 2 minutes, UDPGA was added to initiate the reaction, which was terminated after 30 minutes incubation at 35°C. The reaction was terminated with the addition of a 1 : 1 mixture of 2.5% acetic acid and water, such that the final volume was 0.5 mL. The glucuronide product was extracted with 3.0 mL ethyl acetate. The phases were separated by centrifugation, and duplicate portions of the ethyl acetate phase were counted for quantitation of glucuronide conjugate. Duplicate tubes were used for each measurement. Duplicate values were employed for the rate of conjugate formation at each substrate concentration to calculate kinetic parameters using Prism v4.0 (GraphPad Software, Inc., San Diego, CA). Equations used to fit the data were the Michaelis-Menten model for one-site binding.

RESULTS AND DISCUSSION

The biotransformation of OH-PCBs is very important since not only have these compounds been shown to exert toxic effects, they also tend to persist and accumulate in tissues, especially adipose (Guvenius *et al.*, 2003). While the addition of a phenol group to the parent PCB might be expected to improve aqueous solubility. Van den Hurk and co-workers (2002) have recently demonstrated that several OH-PCBs are more potent inhibitors of UGT than Sulfotransferases (SULT), when 3-OH-B[a]P is the substrate. These studies have been the first to record UGT inhibition by OH-PCBs, with IC_{50}s ranging from 1.17 to 56.4 μM. The strongest inhibition was exhibited by 5-OH-PCB-77 (5-hydroxy-3,3′,4,4′-TCB), a metabolite of a coplanar PCB. These compounds are formed as a result of the oxidation of polychlorinated biphenyls′ (PCBs) by CYP1A and CYP2 or 3′ (James *et al.*, 2001). Organisms may also be exposed to OH-PCBs in the diet, due to the environmental persistence of these compounds.

The Michaelis-Menten kinetic parameters obtained for 4′-OH-PCB35 (K_m, 192.2 μM; V_{max} 988.7 pmol/min/mg), 4′-OH-PCB69 (K_m, 871.1 μM; V_{max} 1773.5 pmol/min/mg), 4′-OH-PCB79 (K_m, 476.4 μM; V_{max} 868.5 pmol/min/mg), and 4′-OH-PCB121 (K_m, 207.3 μM; V_{max} 1046.1 pmol/min/mg) for which glucuronidation has been studied. In the case of 4′-OH-PCB35, the data corresponding to the specific activity at higher substrate concentrations was excluded due to biphasic phenomenon (Fig. 2). The kinetic studies for 4′-OH-PCB69 are incomplete, the biphasic relationship made it difficult to fit the data to the Michaelis-Men-

ten model for H-S41. Thus, at the moment the standard deviation of the estimated parameters for four fishes are particularly high whether this is due to individual variation (Fig. 3).

Interestingly, the biphasic relationship has only been observed with the OH-PCBs with one chlorine substituent flanking the phenolic group (Fig. 2). Thus, the high K_m seen for 4′-OH-PCB69 may be anomalous and a lower estimate may be obtained by conducting the assay at lower substrate concentrations, as was the case for 4′-OH-PCB35 (Fig. 3). The results of this study indicate that microsome and cytosol fractions taken from catfish liver have contaminants which act as UGT inhibitors. It can be postulated that OH-PCBs, possibly derived from coplanar parent congeners, are the contaminants responsible for the marked UGT inhibition observed with 3-OH-B[a]P as substrate (Singh and Wiebel, 1979).

The OH-PCBs may be acting as inhibitors, similar to their potent inhibitory effect on estrogen sulfotransferase (Kester *et al.*, 2000). However, evidence for PCB-O-glucuronide excretion in bile or urine is meager (Connor *et al.*, 1997). The environmental health implication is that organisms exposed to PCBs and which consequently have a circulating pool of protein-bound OH-PCBs, may have a reduced capacity to detoxify OH-PAHs due to UGT inhibition, with a consequent increased risk of carcinogenesis in addition to the endocrine-disrupting effects of PCBs.

In view of the persistence of particular OH-PCBs in biota and the potential for toxicity, it is surprising that few studies have investigated the Phase II conjugation of OH-PCBs. Tampal and co-workers (2002) showed that substitution of chlorine atoms on the *para-* and *meta-* positions on the non-hydroxylated ring greatly lowered the V_{max} of the UGTs present in rat liver. The efficiency of glucuronidation was also lower for OH-PCBs with the *para*-phenol to the dihedral bond. This chlorine substitution pattern and the *para*-phenol are consistent with the persistent OH-PCB types in biota, thus implying that inefficient glucuronidation may be one reason why such xenobiotics accumulate. The lack of large differences in K_m for the various OH-PCBs tested prompted the same

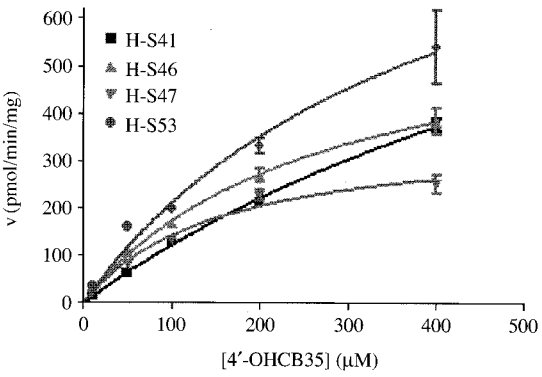

Fig. 2. The Michaelis-Menten plot for the glucuronidation of 4′-OHCB35 by catfish liver.

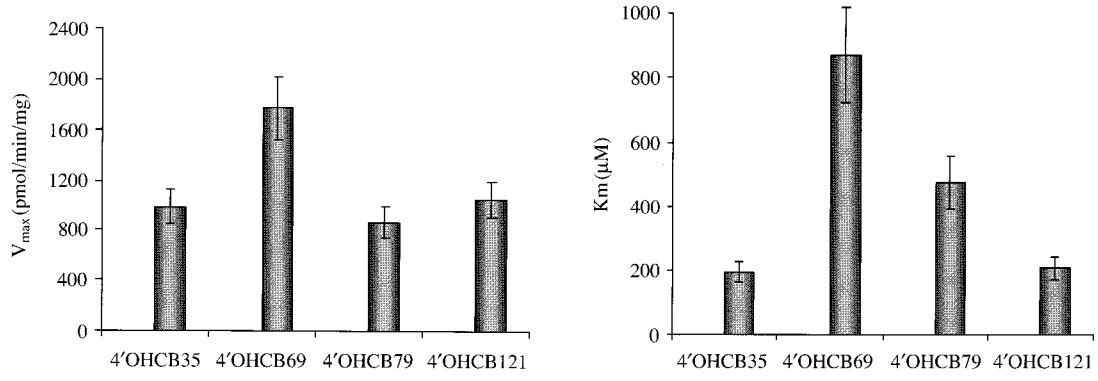

Fig. 3. The estimated kinetic parameters (Mean ± SD) for the glucuronidation of 4 different OH-PCBs by catfish hepatic microsomes (n=4), at [UDPGA]=1.5 mM.

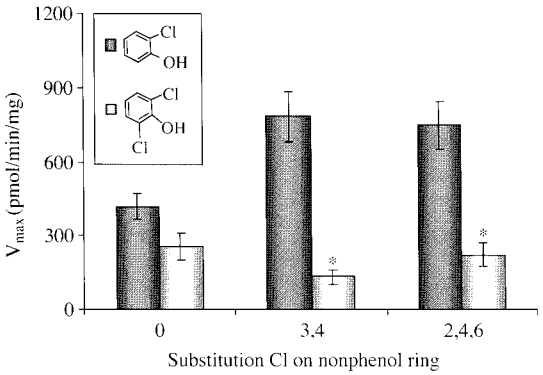

Fig. 4. Differences in V_{max} values for the glucuronidation of OH-PCBs with chlorine substitution on phenolic or non-phenolic ring ($p<0.001$).

researchers to postulate that the *meta*- and *para*-chlorines interfere with product formation or release rather than enzyme affinity. While the extent of ionization and lipophilicity of the compounds could influence their glucuronidation, no relationship could be established between these parameters. The most persistent OH-PCBs in biota have been shown to possess six chlorine substituent or more in humans (Hovander *et al.*, 2002; Sandau *et al.*, 2002). Most of these persistent compounds have a heavily chlorinated phenolic ring.

Daidoji and co-workers (2005) have described the different glucuronidation activities of various rat liver UGT isoforms towards OH-PCBs. The 4-OH-PCBs with no or a single chlorine substituent flanking the phenol appear to be metabolized by UGT2B1, while those with two chlorine substituents on either side of the phenol are preferentially metabolized by UGT1A1. UGT1A6 had higher glucuronidation activity with OH-PCBs whose phenolic group is in the *ortho*- and *meta*-position.

However, steric hindrance around the phenol group was not implicated in low rates of enzyme activity in rat liver (Tampal *et al.*, 2002). The presence of two chlorine substituent flanking the phenol group leads to a decrease V_{max} of hepatic glucuronidation of OH-PCBs. A statistically significant difference (p=0.0008) was found between the $V_{max\ (app)}$ of OH-PCBs grouped according to the number of chlorine substituents flanking the phenyl moiety (Fig. 4.). The two flanking chlorine atoms may make the phenolic group more amenable to glucuronidation, possibly by facilitating the deprotonation of the phenolic oxygen, which is the first step in the conjugation. The conjugation efficiency of 4-hydroxybiphenyl (4-OHBP) was at least an order of magnitude less than all the other OH-PCBs (Yoshimura *et al.*, 1992). It was also much less than glucuronidation efficiencies reported for rat, guinea pig, beagle dog and rhesus monkey liver microsomes.

In summary, the glucuronidation of a series of OH-PCBs was investigated for channel catfish liver. The apparent K_m and V_{max} values ranged from 192~871 μM and 869~1,774 pmol/min/mg protein in liver. The presence of two chlorine substituents flanking the phenolic group seems to lead to a significant decrease in the rate of turnover of the glucuronide product, which is an arrangement typical of OH-PCB congeners detected in plasma and adipose. Thus, one possible reason for the prevalence of certain bioaccumulating and biomagnifying OH-PCBs is poor glucuronidation.

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