NOTE

Toxicology

Altered hepatic cytochrome P450 expression in cats after chronic exposure to decabromodiphenyl ether (BDE-209)

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ABSTRACT. The knowledge of cytochrome P450 (CYP) expression involved in chemical exposure are necessary in clinical applications for the medication and prediction of adverse effects. The aim of this study was to evaluate the mRNA expression of CYP1–CYP3 families in cats exposed to BDE-209 for one year. All selected CYP isoforms showed no significant difference in mRNA expressions between control and exposure groups, however, CYP3A12 and CYP3A131 revealed tend to be two times higher in the exposure group compared to control group. The present results indicate that the chronic exposure of BDE209 could not alter CYP expression in the liver of cats. This result considered caused by the deficiency of CYP2B subfamily which is major metabolism enzyme of polybrominated diphenyl ethers (PBDEs) in cat.

KEY WORDS: BDE-209, cat, chronic exposure, cytochrome P450, mRNA expression

Cytochrome P450 (CYP) is a superfamily of heme-containing enzymes that comprise more than 70% of all phase I xenobiotic metabolizing enzymes [27]. They are predominantly expressed in the liver, but they also appear in the small intestine, lungs, placenta, brain and kidney [28]. The CYPs, particularly families 1, 2 and 3, are associated with metabolism of exogenous and endogenous substances involving cellular functions [44, 45]. In human, CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4, and CYP3A5 are most important and can metabolize 90% of clinical used drugs to hydroxylated metabolites [23, 34, 43]. These metabolites are primarily inactive, but some are active metabolites that have greater adverse effects than parent compounds. In addition, the adverse drug interactions related to CYP inhibitors or inducers are caused by foods, drugs and abundant environmental chemicals [23, 45]. Therefore, the information on CYP expression pattern in individual chemical exposure are useful and essential in clinical applications for the drug treatment and prediction of exposure effects.

Polybrominated diphenyl ethers (PBDEs) are a group of flame-retardants used in numerous household and industrial products [17]. They consist of 209 possible congeners that are classified by the number of bromine atoms in the molecule [32, 33]. Due to their structures and chemical properties, PBDEs have persisted in environment, accumulate through food chains, and disturb endocrine systems in animals and humans [13, 38]. These compounds have been considered as one of the most harmful organic substances and the production of some congeners (penta-BDE and octa-BDE) has been phased out [17, 32], while the deca-BDE (BDE-209) has been generally observed in house dust, egg, poultry meat and human serum [3, 24, 37]. However, the toxicity, metabolic process, and CYP expressed in the liver involved in BDE-209 exposure are not clearly understand in several species, even rat and human [20, 35, 42]. Cats are one of most popular companions for human and they commonly share the habitats with

human being. The statistical correlations between indoor dust and cat serum levels of chemicals, such as phthalates, BDE-47, BDE-99, BDE-153 and BDE-209, were reported in many studies [4, 12, 29]. These findings have supported the hypothesis that dust is a significant exposure route for cats. The studies have suggested that pet cats are suitable bio-sentinel for human exposure to household chemicals including PBDEs [7, 8, 19, 26]. Feline hyperthyroidism associated with increased PBDE levels in cat serum have been reported worldwide [26, 30, 40], but the information on CYP expression in cat exposed to PBDE has not been provided. The objective of this study was therefore to investigate the CYP mRNA expression in cats treated BDE-209 for long-term exposure.

BDE-209 exposure test was ethically approved by Hokkaido University (approval number 14-0054 and 14015) and were performed using six male cats (13 months old, 3–4 kg, *Felis catus*). The animals were purchased from Kitayama Labes Co., Ltd. (Nagano, Japan) and fed by 80 g of cat foods contained about 3,917 kcal/kg of energy including; 44.5% protein, 9.7% fat, and 2.3% fiber (Nosan Co., Yokohama, Japan) and unlimited water supply in accordance with the guideline of the Association for Assessment and Accreditation of Laboratory Animal Care International at the Faculty of Veterinary Medicine, Hokkaido University, Japan. The cat foods were analyzed and confirmed that no PBDEs contamination. After three months' acclimatization, they were divided into two groups: control (n=3) and exposure (n=3). The exposure group was orally treated by powder BDE-209 in capsule (7 mg/kg bw/week; purity: ≥99%; Wellington Laboratories Inc., Ontario, Canada), while the control group was given by capsule only. This exposure dose was adjusted that referred to environmental exposure levels in the previous studies [25, 26], and dosed every week. After one-year exposure, the cats were anesthetized with pentobarbital and euthanized by KCl injection. The liver sample used for gene expression studies were preserved in RNAlater (Sigma-Aldrich Co., St. Louis, MO, USA).

Total RNA were extracted from liver tissues of the control and BDE-209 exposed cats using TRI Reagent® (SIGMA Life Science, St. Louis, MO, USA) and NucleoSpin® kit (MACHEREY-NAGEL, Düren, Germany), and synthesized for cDNA with the ReverTra Ace® qPCR RT Master Mix with gDNA Remover (TOYOBO Co., Ltd., Life Science Department, Osaka, Japan). CYP1–CYP3 primer sets designed in our previous study [16] are shown in Table 1. The qRT-PCR (StepOnePlus Real-Time PCR system, Applied biosystems, Foster City, CA, USA) was performed by using 10 μl PCR reaction mixture containing Fast SYBR Green Master Mix (Applied biosystems, Foster City, CA, USA), forward and reverse primers (Thermo Fisher Scientific, Life Technologies Japan Ltd., Tokyo, Japan), and cDNA of liver sample. The qPCR condition was carried out at 95°C for 20 sec, followed by 40 cycles of 95°C for 3 sec, 60°C for 30 sec. The quantification of the transcripts was performed by the ΔΔCT method [22] normalized with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and beta-actin (ACTB). A statistical analysis was performed by using JMP Pro13 (SAS institute, Cary, NC, USA). CYP mRNA expressions of the control and exposure groups were confirmed for normality using the Shapiro-Wilk test and tested for homogeneity of variance using the Levene's test. Wilcoxon test and student's *t*-test were used to determine the significant differences between the control and exposure groups. *P* value <0.05 was considered statistically significant.

We analyzed the mRNA expressions of 17 CYP isoforms, including CYP1A1, CYP1A2, CYP1B1, CYP2A13, CYP2B11, CYP2C41, CYP2D6, CYP2E1, CYP2E2, CYP2F2, CYP2F5, CYP2J2, CYP2S1, CYP2U1, CYP3A12, CYP3A131 and CYP3A132, in the livers of the control and BDE-209 exposure cats, but hepatic CYP2B11 mRNA expression could not detect in this study. This finding similar to the previous studies [16, 31]. A deficiency of CYP2B in the cat liver may relate to the potential

Table 1. Primer sets for quantitative RT-PCR analysis

Gene	Accession number	Forward primer	Reverse primer	Product size (bp)
GAPDH	NM_001009307.1	5′-GGTGATGCTGGTGCTGAGTA-3′	5′-GTCATGAGTCCCTCCACGAT-3′	266
ACTB	XM_023244304.1	5′-ATCAAGGAGAAGCTGTGCTACGT-3′	5′-CGTTGCCGATGGTGATCA-3′	124
CYP1A1	NM_001024859.1	5′-CTCCTGGCCTCCTTTGTCTT-3′	5′-CACCAGATGTGGGTTCTTCC-3′	150
CYP1A2	NM_001048013.1	5′-GCACCTGCCTCTACAGTAGTTGA-3′	5′-GTGGGTTCTTCCCCAAGGTC-3′	200
CYP1B1	XM_023251947.1	5′-TGTTAACCAGTGGTCCGTGA-3′	5′-AGAGCTGCATCTTGGAGAGC-3′	179
CYP2A13	XM_003997781.5	5′-ACCTTCGACTGGCTCTTCAA-3′	5′-GAAGAAGGTGGGATCGATGA-3′	204
CYP2B11	XM_023249708.1	5′-TTCTGCCTCTGTGGGAGAGT-3′	5′-AATTTGAGGTGGAGAGCCAGG-3′	456
CYP2C41	XM_019813568.2	5′-CAGCAGGAAAACGGATTTGT-3′	5′-CCCTTCCTCACAAAGGAACA-3′	195
CYP2D6	NM_001195406.1	5′-AACCTGCTGCAGATGGACTT-3′	5′-GCAGTGTCCTCGCTGTGATA-3′	164
CYP2E1	NM_001048012.1	5′-AGGACCGGTTAGAGATGCCT-3′	5′-CAGGGAACTCCTGGTTGTCA-3′	189
CYP2E2	NM_001048010.1	5′-TCTGCATGGCTATAAGGCGG-3′	5′-CGGAATGCCTCCATCAGGAA-3′	240
CYP2F2	XM_023245944.1	5′-GGTCCACACCACCGAATCTT-3′	5′-ATAAGGAGCCCGTAACGCAG-3′	254
CYP2F5	XM_006941235.4	5′-GCGACTACCCCACCTTTCTC-3′	5′-GGCTTGCCTTCAGTTTTCCG-3′	196
CYP2J2	XM_019839081.2	5′-CCATCTGGACTTTGAGCGGT-3′	5′-GAGTTGTAGGGCGGTTCACA-3′	173
CYP2S1	XM_011289895.3	5′-AACTCCCCTTGATGTGCAAC-3′	5′-GCGAGAGAGAGGGTATGCTG-3′	226
CYP2U1	XM_019829131.2	5′-ACGTGCCCAAGAAGAGAGTG-3′	5′-CACTTTGGTCCCCATTCCGT-3′	198
CYP3A12	XM_019820248.1	5′-TATTCATTCCCCAAGGGACA-3′	5′-TTTGGGTCCAGTTCCAAAAG-3′	164
CYP3A131	NM_001246278	5′-CTTGCCCTTGTCACACTCCT-3′	5′-TAATTGGGACCGGATAGGCTG-3′	392
CYP3A132	NM_001246271	5′-AACTTGCCCTTGTCAGAGTCC-3′	5′-GTCTATGGGAGCCAGATAGGCTG-3′	397

toxicity risk of some drugs and pollutants, because CYP2B play a role in metabolism of 2–10% of drugs (ex. cyclophosphamide, propofol and ketamine) [14] and environmental contaminants including 2,2`,4,4`-tetrabromodiphenyl ether (BDE-47) and 2,2`,4,4`,5-pentabromodiphenyl ether (BDE-99) in humans [10, 11].

The comparison of relative mRNA expressions of detected CYP isoforms in the livers between the control and BDE209 exposure groups are given in the Fig. 1 and Supplementary Table 1. Although the mRNA expressions of all CYP isoforms were not statistically different between control and exposure groups, CYP3A12 (1.8- to 1.9-fold) and CYP3A131 (2.0-fold) tended to be higher in the exposure group compared with those in the control group. We recently reported that CYP3A is dominant subfamily in the liver [16] and it could play a major role in hepatic xenobiotic metabolism for cats [15, 16]. However, the liver microsome in cats revealed lower CYP3A activity than in humans and dogs and the relationship between molecular structure and metabolic activity is still largely unknown in cats [39]. Thus, the studies on feline CYP3A activity and the selectivity of CYP3A for BDE-209 metabolism are also necessary. CYP3A4 was suggested to be the specific enzyme responsible for the biotransformation of OH-PBDEs (3'-OH-BDE-7, 4'-OH-BDE-17 and 3-OH-BDE-47) in pig liver [21]. In rats, CYP3A1 was the most active in BDE-99 metabolism (400 pmol/min/mg protein) compared to BDE-47 (60 pmol/min/mg protein) [9]. Moreover, the statistical correlations among the hepatic expression levels of CYP1B1, CYP3A12 and CYP3A131 were noticed (Spearman's rank coefficients=1, $P=$ <0.0001) that indicated the trends of higher CYP3A12 and CYP3A131 expressions may strongly relate to the lower trend of CYP1B1 expression level in the BDE-209 exposed group. Comparing the control and exposure groups, the relative fold change of most CYP1 and CYP2 families ranged from 0.7 to 1, while the lowest relative fold change of CYP1B1 (0.2-fold) suggested that CYP1B1 mRNA expression was likely to be inhibited by BDE-209 (Fig. 1 and Supplementary Table 2). Previous evidence revealed that CYP1B1 has been extremely active in the bioactivation of polycyclic aromatic hydrocarbons, such as tetrachlorodibenzo-*p*-dioxin, in mouse embryo fibroblast and human mammary epithelial cells [1, 18]. Also, our *in vivo* study presented the up-regulation of CYP1B1 mRNA expression in the liver (37.7-fold) and other tissues of cats exposed to PCBs compared to the control cats [16]. However, no such changes in CYP1B1 mRNA expression were noted in human mammary carcinoma cells exposed to low-dose PBDEs, including BDE-47, BDE-99, BDE-153, BDE-183 or BDE-209 [2]. In addition, the CYP1B1 mRNA down-regulation by BDE-209 has not been reported so far.

The studies on CYP expressions related to BDE-209 exposure are partly summarized and compared to our study in the Table 2. The up-regulations of CYP1A2 and CYP3A4 genes were observed in human hepatocytes exposed to BDE-209 for the periods of 24–72 hr [35]. In Sprague-Dawley rats, oral BDE-209 exposure (100 mg/kg) for three months could up-regulate CYP2B1 mRNA expression in the liver [41] and the protein expressions of CYP1A2, CYP2B1 and CYP3A1 were also induced by oral BDE-209 exposure (100–600 mg/kg) for one month [20], however, the sub-chronic BDE-209 exposure study (90 days) at a lower doses (10–50 mg/kg) was not found any inductions of CYP expression in the liver [42]. The study of Sun *et al.* (2020) suggested that BDE-
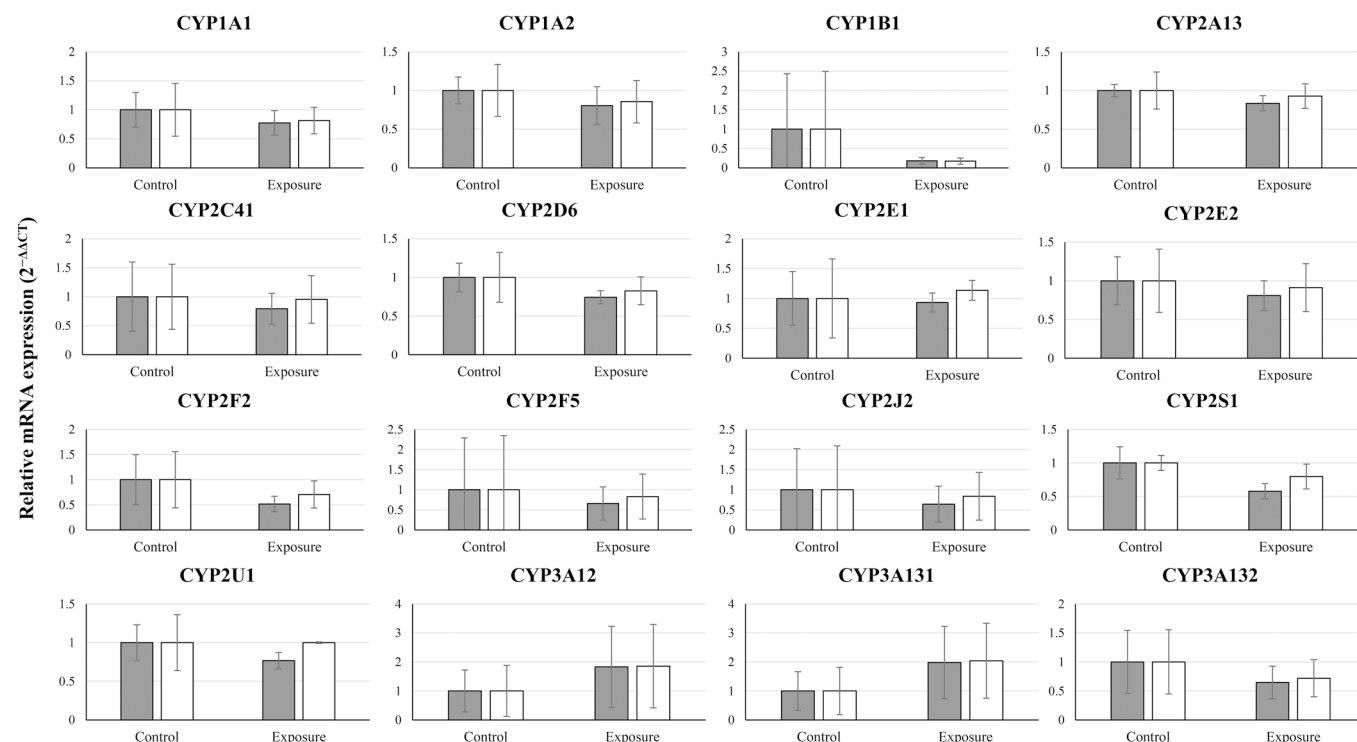
Fig. 1. Comparison of relative cytochrome P450 (CYP) mRNA expression ($2^{-\Delta\Delta CT}$, mean ± SD) in the liver of control and BDE-209 exposed cats (Reference genes: glyceraldehyde-3-phosphate dehydrogenase [GAPDH; grey] and beta-actin [ACTB; white]).

Table 2. Comparison of the studies on cytochrome P450 (CYP) expressions related to BDE-209 exposure compared to this study

Experiment	Stapleton *et al.*, 2009	Lee *et al.*, 2010	Wang *et al.*, 2012	Sun *et al.*, 2020	This study
Animal	Human hepatocyte	Sprague-Dawley rat	Sprague-Dawley rat	Sprague-Dawley rat	Cat
Sex	Male and Female	Male	Male	Male	Male
Age	38–61 years old	10 days old	21 days old	42 days old	13–14 months old
Chemical	BDE-209	BDE-209	BDE-209	BDE-209	BDE-209
Dose	10 μM	100–600 mg/kg	10–50 mg/kg	5–500 mg/kg	7 mg/kg/week
Time of exposure	24–72 hr	32 days	90 days	28 days	1 year
Route of exposure	Direct incubation	Oral	Oral	Oral	Oral
CYP expression	CYP1A2 and CYP3A4 (up-regulation)	CYP1A2, CYP2B1 and CYP3A1 (up-regulation)	No significantly different	CYP3A1 and CYP3A2 (down-regulation)	No significantly different

209 could inhibit the pregnane X receptor (PXR) and constitutive androstane receptor (CAR) expression contributing to CYP3A suppression and impaired hepatic metabolic capacity in rat exposed to BDE-209 at the middle and high doses (50 and 500 mg/kg), but not at the low dose (5 mg/kg) [36]. In addition to the differences in age and species, these studies implied that administered dose and exposure time may be important factors for BDE-209 induced CYP expression in the liver. Because of its large molecule, BDE-209 is poorly absorbed by oral route (less than 1% of the administered dose), slightly accumulated in the liver, and excreted almost completely through the feces [6]. In our study, BDE-209 can be detected in the serum and liver (unpublished data), but there is no report of BDE-209 absorption/excretion rate in cat. Consequently, the administrated dose (7 mg/kg/week) in the present study could not induce CYP1-3 mRNA expressions in cats. The extent of CYP expression is not involved in only toxic effects, but also connected the metabolism capacity for BDE-209 in each species. Nevertheless, the *in vitro* biotransformation of BDE-209 using the liver of ring-billed gulls (*Larus delawarensis*) suggested that CYP-mediated metabolism is a minor metabolic pathway for BDE-209 [5]. Therefore, the information on other phase I and II enzymes-mediated BDE-209 metabolism is also critical to understand the complete risks associated with BDE-209 exposure in cats.

In conclusion, the chronic exposure of BDE-209 could not induce CYP1–CYP3 mRNA expression in the liver of cats. Since the increased prevalence of BDE-209 levels in cats and household environment, the evidence and further study on toxicokinetic process related to the potential health risk of this environmental contaminant are needed.

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