DETOXIFICATION OF BDE99 IS CHANGED BY A COMMON VIRAL INFECTION IN THE MOUSE

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Introduction

Polybrominated diphenyl ethers (PBDEs) are used worldwide in fire protection. Due to their abundant use and their chemical properties PBDEs have been identified as persistent organic pollutants (POPs) and have been found in wildlife and human tissues, not seldom in high levels[1]. Commercially used mixtures are denoted penta-, octa- and deca-BDEs[2]. Deca-BDE is the most commonly used PBDE preparation. However, the most frequently found congeners in human tissues are 2,2',4,4'-tetra-BDE (BDE47), 2,2',4,4',5-penta-BDE (BDE99) and 2,2',4,4'5,5'-hexa-BDE (BDE153). The major human exposure route is supposedly through food intake[1-3] but also indoor environment, i.e. ingestion of house dust, could be an important source[4-6]. PBDEs have low acute toxicity (0.5-5g/kg body wt)[3, 7] but e.g. BDE99 has been reported to cause toxic effects at comparably low doses[1, 2]. The central nervous system, liver, thyroid gland and reproductive organs seem to be primary targets of toxicity and effects on micosomal enzyme activities have been reported[8-10]. Exposure to PBDE is known to stimulate gene expression of the phase I microsomal enzyme CYP2B and related protein synthesis and it has been speculated if also CYP1A1expression is induced by PBDE[11]. Tissue distribution of BDE99 is affected by coxsackievirus B3 (CVB3) infection, possibly due to infection-induced effects on detoxifying enzymes[12]. Coxsackieviruses are non-enveloped viruses with a single-stranded RNA genome that are associated with several human diseases, such as heart diseases, idiopathic chronic pancreatitis and insulin-dependent type I diabetes mellitus (IDDM)[13-16]. A well-characterised experimental mouse model for the study of CVB3 infection shows that the disease development is very similar to that in humans[17, 18]. In this paper, by using this mouse model, we report on the effects of CVB3 infection and PBDE exposure on CYP gene expression and microsomal enzyme activity.

Materials and Methods

Female Balb C mice were purchased from Scanbur B&K and kept under a 12 hour light/dark cycle at a room temperature of 22-23˚C. The mice had free access to food and tap water *ad libitum*. After a two-week acclimatisation period the mice were randomly divided into four groups, (n=5). Both infected and non-infected mice were treated with BDE99. In addition non-infected and infected mice were administrated corn oil to serve as controls. Each mouse was inoculated intraperitoneally (i.p.) with 0.2 ml of a solution containing a myocardidic strain of coxsackievirus B3 (2*103 pfu/0.2 ml) at day 0, orally treated with pure (99%) 2,2',4,4',5-penta-BDE (BDE99) dissolved in corn oil (20 mg/kg bw) at day 1. Purity of BDE99 was analysed with HRGC/HRMS by Oekometric, Germany[19]. The mice were sacrificed at day 3 of the infection.

Table 1. *Primer and probe sequences used for CYP 1A1 and CYP2B.*

CYP1A1	
Reverse primer	5'- GGTGGCTGTTCCTGTGATTCA -3'
Forward primer	5'- AAAGTAGGAGGCAGGCACAATG -3'
Probe	5'- TAGCCAGAAACACAGATC -3'
CYP2B	
Reverse primer	5'- GCCCTTCTCAACAGGACAAATTT -3'
Forward primer	5'- GCCAATGCTTTCACCAAGACA -3'
Probe	5'- TGATCAAAAGTCTGTGGGAAAGCGCAT -3'

Gene expressions of CYP1A1 and CYP2B as well as the corresponding activities of ethoxyresorufin-O-deethylase (EROD) and pentoxyresorufin-O-depentylase (PROD) were measured in the liver. The gene

sequences of CYP2B and CYP1A1 were obtained using the nucleotide search at the National Centre for Biotechnology Information (NCBI) homepage (www.ncbi.nih.gov). Primer and probe sequences were obtained using the Primer Express 2.x program. Primers were purchased from Thermo Electron. Probes were purchased from Tib Molbiol. Isolation of total RNA was made using the Qiagen RNA/DNA Mini kit. For synthesis of cDNA the Omniskript Reverse Transcription kit was used. Primer optimisations were performed using heat-label Uracil-DNA-Glycosylase, RNas inhibitor 2500 U, LightCykler Capillaries, Real-Time PCR. Optimisation was preformed using a non-specific fluorescent probe, LightCycler FastStart DNA Master SYBR Green 1 kit. Primer optimisation was controlled by electrophoreses using agarose gel (3%). Glyceraldehydes-3-phosohate dehydrogenase (GAPDH) was used as the housekeeping gene. The protein concentrations were measured[20] and the PROD and EROD activities measured using a modified method[21] originally described by Pohl and Fouts[22]. The non-parametric Mann-Whitney test was used to test statistical differences between treatment groups. Significance was accepted at $p<0.05$. In order to compare gene expression levels a model based on the cycle threshold-values (CT-values) was used.

Results and Discussion

There were no significant differences in CYP1A1or CYP2B gene expression between infected and non-infected mice, *(figure 1 and 2),* but a tendency of decreased gene expression after infection was more emphasised in CYP2B. Exposure to BDE99 did not induce the CYP1A1 expression but rather seemed to slightly reduce this expression, both infected and non-infected animals. However, BDE99 exposure induces CYP2B expression in infected mice ($p=0.02$) and the same tendency was observed in non-infected mice.

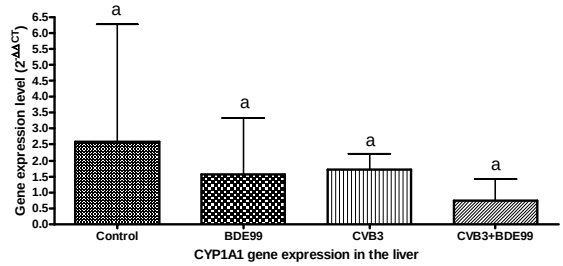

Fig. 1 *Gene expression of CYP1A1 in the liver of untreated mice and mice treated with BDE99 with or without CVB3 infection. The bar charts show the mean value + SD of 2-ΔΔCT in each group. Bars not sharing a letter are significantly different (p<0.05).*

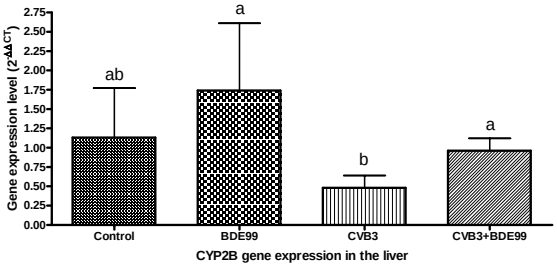

Fig. 2 *Gene expression of CYP2B in the liver of untreated mice and mice treated with BDE99 with or without CVB3 infection. The bar charts show the mean value + SD of 2-ΔΔCT in each group. Bars not sharing a letter are significantly different (p<0.05).*

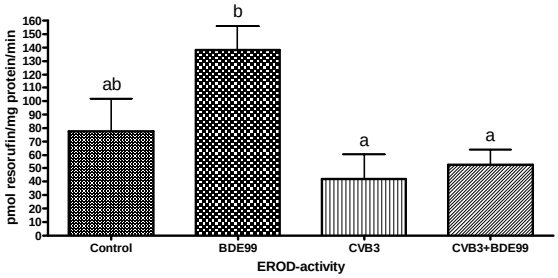

Fig. 3 *EROD activity in the liver of untreated mice and mice treated with BDE99 with or without CVB3 infection. The bar charts show the mean value + SD of PROD activity in each group. Bars not sharing a letter are significantly different (p<0.05).*

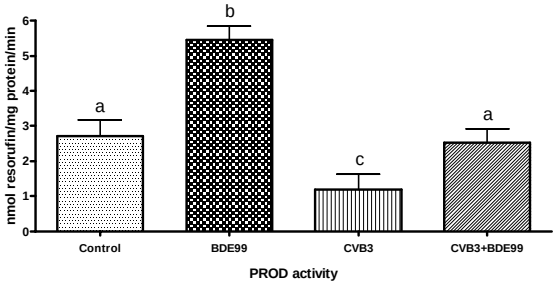

Fig. 4 *PROD activity in the liver of untreated mice and mice treated with BDE99 with or without CVB3 infection. The bar charts show the mean value + SD of EROD activity in each group. Bars not sharing a letter are significantly different (p<0.05).*

Correspondingly to a tendency of a decreased CYP1A1 gene expression the infection decreased the EROD activity. Thus, infected mice exposed to BDE99 showed a lower EROD activity (p=0.01) than non-infected, exposed mice. The same tendency of a decreased activity could be observed in non-exposed mice *(figure 3).* Exposure to BDE99 did not cause any induction of the EROD activity. On the other hand infection caused a decrease in PROD activity; Infected groups, both untreated (p=0.02) and BDE99-treated (p=0.01), showed lower PROD activity than non-infected mice *(figure 4).* Exposure to BDE99 increased PROD activity in both infected mice (p=0.02) and non-infected mice (p=0.01). These results are in line with CYP2B gene expression data.

Infection reduced CYP2B gene expression, although not significantly. Down-regulation of protein synthesis and gene expression as an effect of infection has previously been shown and the mechanism behind this has been discussed[12, 17]. However, infection does not seem to influence the CYP1A1 gene expression, although the gene expression values seemed to be lower in infected mice. Infected mice exposed to BDE99 showed a induction of CYP2B expression and a similar tendency was observed in non-infected mice. The present results were in line with other studies showing CYP2B to be an inducible enzyme that might be involved in the metabolism of PBDEs[11, 23, 24]. Gene expression of CYP1A1 in the various groups showed a somewhat different pattern than CYP2B. BDE99 exposure did not induce CYP1A1 expression. In fact, in both infected and non-infected mice the gene expression of CYP1A1 seemed to be lower after BDE99 exposure. Other studies have questioned whether PBDEs really induce CYP1A1 gene expression[25-28]. Studies showing an increased CYP1A1 gene expression state that BDE99 is one of the least dioxin-like PBDE congeners which implicates that if there is a BDE99 induced CYP1A1gene expression it should be less pronounced than for other PBDEs and observed only at high doses[11, 27].

The regulation of the induction of gene expression has a sensitive on- and off-switch mechanism. After genes have been induced, production of proteins starts within hours. When sufficient amount of proteins have been synthesised, or when the inducer is no longer present, the gene expression is shut down. Thus, gene expression is under sensitive regulation that probably varies during the course of the disease depending on individual differences in host responses, for example cytokine release. Thus, it is unlikely that all individuals within the different test groups show the same gene expression at the same time point. This might explain the large difference in standard deviation in both CYP2B and CYP1A1 data. It is also interesting to note that the difference in deviation seems to be smaller in the EROD and PROD enzyme activity data. This may be explained by a slower infection-induced regulation of synthesis and degradation of the proteins resulting from gene induction[17].

The PROD activity corresponded well to the gene expression of CYP2B. The general effect of infection seems to be a decrease in PROD activity. This general decrease in PROD activity during infection and concomitant exposure to BDE99 has previously been shown[12]. Exposure to BDE99 in non-infected, as well as in infected mice, increased the PROD activity. This corresponded to the gene expression data where BDE99 increased the CYP2B expression in the infected group, the same tendency was also observed in the non-infected group. The observation that PBDEs induce PROD activity in rodents have previously been shown and this increase in PROD activity was dose-dependent[9]. The EROD activity showed no significant induction after BDE99 exposure. This corresponds with the gene expression of CYP1A1. However, infected mice exposed to BDE99 had a reduced EROD activity. It is known that infection down-regulates the synthesis of many proteins because amino acids are used for vital host defence reactions including the immune system[12, 17]. CYP1A1 gene expression and EROD activity are used as biomarkers for ligand/AhR/ARNT mediated toxicity[25, 29]. A lack of EROD induction after PBDE exposure has been shown by *in vitro/ex vivo* studies, as well as an antagonistic behaviour at co-exposure with TCDD[25-28]. It has been suggested that some PBDEs including BDE99 binds to the Ah receptor but are unable to activate the AhR-ARNT-XRE complex with the result that CYP1A1 gene expression is not induced and EROD enzymes not synthesised[25-28]. It is noteworthy that these studies have been performed *in vitro/ex vivo* i.e. in test systems without the regulatory influence of cytokines. Some studies have shown higher EROD activity after oral exposure of PBDEs in rodents[30, 31]. However, these studies have used commercial mixtures instead of pure congeners with a higher risk of contaminants that might have affected the results[1, 2, 11]. In conclusion, infection per se reduced the PROD activity and this was also reflected by a tendency of reduced CYP2B gene expression. This contrasts to CYP1A1 and EROD levels that were hardly affected by the infection.

The gene expression of CYP2B was significantly induced after BDE99 exposure both in infected and non-infected mice and this were also reflected by measured PROD activity. However, BDE99 exposure did not induce CYP1A1 gene expression or EROD activity, which is in accordance with results from other studies[25-28]. Consequently, viral infections may modify the metabolism of PBDEs and probably of other POPs as well, a fact with consequences in the risk assessment of these compounds.

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