TRANSACTIVATION POTENTIALS OF CONSTITUTIVE ANDROSTANE/ACTIVE RECEPTOR BY PERSISTENT ORGANIC POLLUTANTS AND BROMINATED FLAME RETARDANTS: A COMPARATIVE STUDY OF BAIKAL SEAL (*PUSA SIBIRICA*) AND MOUSE

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Abstract

To characterize ligand dependent transcriptional activation of constitutive androstane/active receptor (CAR) in aquatic mammals, transactivation potentials of Baikal seal (*Pusa sibirica*) CAR by environmental pollutants, including persistent organic pollutants (POPs) and brominated flame retardants (BFRs) were investigated using an *in vitro* reporter gene assay, and compared with those of mouse CAR. Measurement of luciferase reporter gene activities in MCF-7 cells, where the seal CAR expression plasmid was transfected, demonstrated that the seal CAR was activated by POPs, such as a technical mixture of polychlorinated biphenyls (PCBs), and individual PCB congeners, 1,1,1-trichloro-2,2-bis (*p*-chlorophenyl)ethane (*p,p'*-DDT) and its metabolite, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (*p,p'*-DDE) and *trans*-nonachlor. As for BFRs, treatment with some polybrominated diphenyl ether (PBDE) congeners weakly deactivated both Baikal seal and mouse CARs. Moreover, seal CAR was activated by hexabromocyclododecanes (HBCDs), while mouse CAR was not. The interspecies comparison of lowest observable effect levels (LOELs) for CAR transactivation by each compound revealed that the seal CAR responded more sensitively to PCBs than mouse CAR. These results indicate that the ligand profile of CAR for transactivation is species-specific and results derived from rodents and human CAR may not be applicable to the risk assessment in wild species.

Introduction

Aquatic mammals including seals and cetaceans accumulate persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethane and its metabolites (DDTs) through the food chain. Since POPs are widely spread in the environment of Lake Baikal, Russia, Baikal seals (*Pusa sibirica*), a top predator species are highly contaminated by POPs.[1-3] In addition, world wide contamination by brominated flame retardants (BFRs) such as polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecanes (HBCDs) is of growing concern because of the global distribution and potential toxic effects to wildlife. However, little information is available on the potential risk and effects of POPs and BFRs in Baikal seal.

Constitutive androstane/active receptor (CAR) plays an important role in the transcriptional activation of multiple xenochemical metabolizing enzymes such as cytochrome P450 (CYP) 2B, 2C and 3A in response to phenobarbital (PB)-type chemicals including *ortho*-chlorine substituted PCB congeners and DDTs in rodent species.[4,5] The CAR target genes can potentially regulate physiological conditions through the metabolism of endogenous substrates such as steroid and thyroid hormones, bile acids.[6-8]

Therefore, identification of ligands that can potentially modulate the CAR-mediated signaling pathways may provide valuable information on the risk and effects of exposure to PB-type xenochemicals. We have succeeded in isolating CAR cDNA in Baikal seal, suggesting that CAR gene is conserved in aquatic mammals as well as rodents and human. In addition, results of *in vitro* transactivation assay using classical CAR ligands, such as androsta(e)nol, estrogens, TCPOBOP and CITCO, were different between mouse and seal CARs.[9] However, potential of POPs and BFRs for CAR activation was not fully understood. To characterize ligand dependent transcriptional activation of Baikal seal CAR, the present study investigated transactivation potential of the CAR by POPs and BFRs using the *in vitro* reporter gene assay that we have previously constructed, and the ligand profile was compared with that of mouse CAR.

Materials and Methods

The CAR expression plasmids, pcDNA3.2TOPO-bsCAR and pcDNA3.2TOPO-mCAR were constructed using Baikal seal and mouse CAR cDNAs, respectively. To construct pGL3-$(NR1)_3$-Luc luciferase reporter plasmid, the complementary oligonucleotide containing three copies of the PB responsive enhancer module NR1 site (5'-AGAATCTGTACTTTCCTGACCTTGGCAC-3') sequence was synthesized and inserted into *Kpn*I/*Xho*I site of the pGL3-Promoter Vector.

Human breast cancer cell line, MCF-7 cells was seeded into 24-well plates (10^4 cells/well) and cultured overnight in phenol red-free Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% double charcoal/dextran treated fetal bovine serum (CDFBS). Cells were co-transfected with 100ng of pGL3-$(NR1)_3$-Luc, 200ng of CAR expression plasmid and 10ng of phRL-TK control vector as an internal standard using Lipofectamine2000 and incubated for 5 hrs. Cells were then washed by phenol red-free DMEM and further incubated for 24 hrs in 10% CDFBS DMEM containing various concentrations of PCBs, DDTs, *trans*-nonachlor, PBDEs and HBCDs. Luciferase activity was measured using a Dual Luciferase Reporter Assay System. The luciferase activities normalized against *Renilla* luciferase activities of an internal control phRL-TK vector were determined from the measurements in at least three independent transfections.

Results and discussion

A technical PCB mixture, Kanechlor500 induced Baikal seal CAR-mediated transcriptional activity in a dose dependent manner (Fig. 1). Mouse CAR was also activated by the PCB mixture, but the fold induction of transactivation by mouse CAR was lower than that of Baikal seal (Fig. 1). In addition, transactivation by 10 PCB congeners that have been detected in the tissue of Baikal seal was investigated. Baikal seal was activated by these PCB congeners as well as mouse CAR (Fig. 1). Lowest observable effect levels (LOELs) for CAR transactivation by each compound was estimated. The results revealed that the LOEL for Kanechlor500, PCB85, PCB99, PCB101, PCB105, PCB138, PCB153 and PCB180 in seal CAR were lower than those of mouse CAR. This result suggests that Baikal seal is more sensitive to these PCBs than mouse.

Treatment by other POPs, *p,p'*-DDE, *p,p'*-DDT, and *trans*-nonachlor also activated both Baikal seal and mouse CARs at similar concentration levels (Fig. 2). This result indicates that seal and mouse similarly respond to these environmental chemicals.

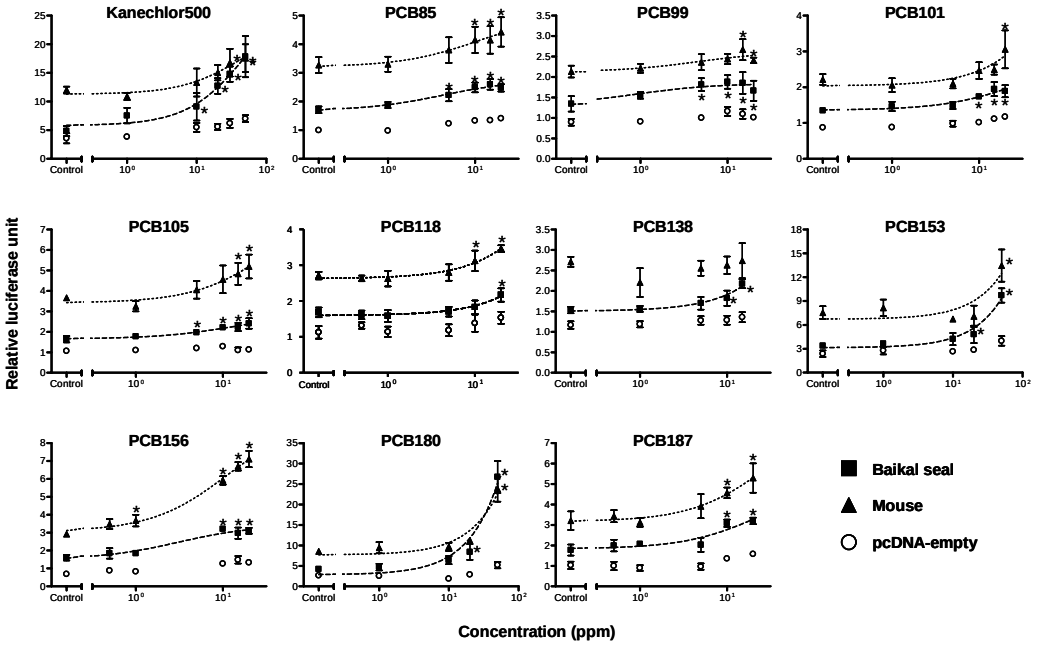

Fig. 1. Transactivation of Baikal seal and mouse CARs by a technical PCB mixture (Kanechlor500) and individual PCB congeners. All data of transactivation are expressed as relative luciferase unit (firefly/*Renilla* luciferase activity) in Baikal seal CAR (square), mouse CAR (triangle) and no CAR (pcDNA-empty; circle) expressed cells. Asterisk indicates a statistical difference from control by one-way ANOVA with Dunnett's post-hoc test ($p < 0.05$).

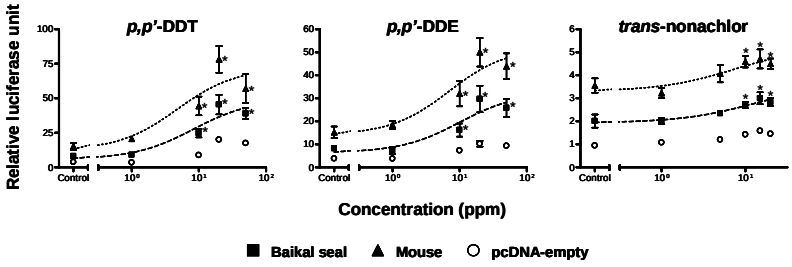

Fig. 2. Transactivation of Baikal seal and mouse CARs by DDT compounds and *trans*-nonachlor.. All data of transactivation are expressed as relative luciferase unit (firefly/*Renilla* luciferase activity) in Baikal seal CAR (square), mouse CAR (triangle) and no CAR (pcDNA-empty; circle) expressed cells. Asterisk indicates a statistical difference from control by one-way ANOVA with Dunnett's post-hoc test ($p < 0.05$).

We investigated CAR transactivation potencies of BFRs including six PBDE congeners and a HBCDs mixture. The result showed that only BDE99 weakly suppressed Baikal seal CAR transactivation at more than 10ppm, but mouse CAR activation was reduced by BDE100, 154 and 183 (Fig. 3). Treatment with other PBDE congeners caused no significant response for both seal and mouse CARs. On the other hand, Baikal seal CAR was activated by a treatment with HBCDs at more than 10ppm, whereas mouse CAR activity was decreased at lower concentration (Fig. 3). Recent studies indicated that CYP2B and 3A are induced by only high dosage of PBDEs and HBCD in rat,[10,11] although no direct evidence of CYP2B and 3A induction through CAR activation by BFRs was provided. Our results imply that these BFRs may potentially alter the transcription of these CYPs through CAR activation in mammalian species. However, considering that the hepatic concentrations of these BFRs in aquatic mammals are generally less than ppm level, the possibility that BFRs are involved in the disruption of CAR-mediated signaling pathways may be low in the liver of Baikal seal.

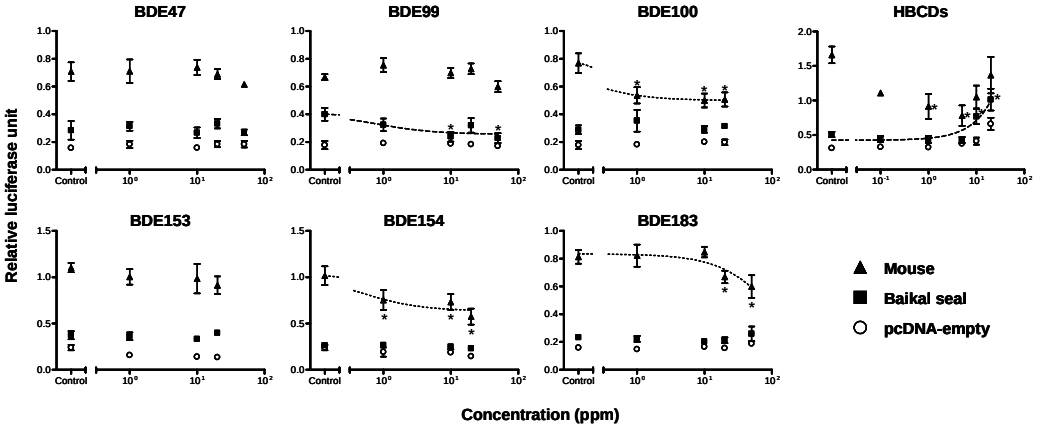

Fig. 3. Transactivation of Baikal seal and mouse CARs by BFRs. All data of transactivation are expressed as relative luciferase unit (firefly/*Renilla* luciferase activity) in Baikal seal CAR (square), mouse CAR (triangle) and no CAR (pcDNA-empty; circle) expressed cells. Asterisk indicates a statistical difference from control by one-way ANOVA with Dunnett's post-hoc test ($p < 0.05$).

In conclusion, the ligand profile of seal CAR and susceptibility to the CAR activation by individual ligands appeared to be different from those of other mammalian CARs. This implies that the results derived from experiments focusing on rodents and human CARs could not simply be extrapolated into CAR-mediated responses in wildlife.

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