Toxicology in Vitro 28 (2014) 432–441

Contents lists available at ScienceDirect

Toxicology in Vitro

journal homepage: www.elsevier.com/locate/toxinvit

A novel MDCKII in vitro model for assessing ABCG2-drug interactions and regulation of ABCG2 transport activity in the caprine mammary gland by environmental pollutants and pesticides

Sandra Halwachs *, Louise Wassermann, Walther Honscha

Institute of Pharmacology, Pharmacy and Toxicology, Faculty of Veterinary Medicine, Universität Leipzig, An den Tierkliniken 15, 04103 Leipzig, Germany

ARTICLE INFO

Article history:
Received 17 June 2013
Accepted 23 December 2013
Available online 3 January 2014

Keywords:
Caprine ABCG2
Mammary gland
Aryl hydrocarbon receptor (AhR)
Dioxin
Pesticides
MDCKII cells

ABSTRACT

The ABC efflux transporter ABCG2 represents the main route for active secretion of xenobiotics into milk. Thus, ABCG2 regulation by aryl hydrocarbon receptor (AhR) ligands including ubiquitously environmental pollutants is of great toxicological relevance. However, no adequate in vitro model is as yet available to study AhR-dependent ABCG2 regulation in dairy animals. In this study, we therefore systematically investigated the effect of various environmental contaminants and pesticides on ABCG2 efflux activity in MDCKII cells stably expressing mammary ABCG2 from dairy goats. The AhR-agonists TCDD, Aroclor 1254, prochloraz, and iprodione caused a dose- and time-dependent increase in EROD activity. Moreover, TCDD and prochloraz significantly stimulated ABCG2 transport activity through a dose- and time-dependent induction of transporter gene expression. AhR inhibitors like CH 223191 significantly reversed TCDD- and prochloraz-induced stimulation of ABCG2 efflux activity. In contrast, non-AhR activators such as PCB 101 had no significant effect on EROD activity, ABCG2 gene expression or transporter activity. As we identified various anthelmintics including monepantel as potential ABCG2 substrates this regulatory mechanism may result in increased milk residues of potentially harmful xenobiotics. Thus, MDCKII-cABCG2 cells may represent a suitable in vitro model to study mammary ABCG2 secretory activity and its potential regulation by AhR-activating contaminants.

1. Introduction

Exposure of the general population to environmental pollutants like dioxins and pesticides is of central public concern due to risk of serious adverse health effects. Emission of polychlorinated dibenzodioxins (PCDDs) including the most toxic congener 2,3,7,8-tetrachlordibenzo-*p*-dioxin (TCDD) and polychlorinated biphenyls (PCBs) mainly occurs by industrial processes like waste incineration (Scrogi, 2008). Due to their high lipid solubility and low biodegradability, PCDDs and PCBs accumulate in organisms throughout the food chain. Human exposure to these pollutants thereby occurs mainly through food from animal sources particularly dairy products (Baars et al., 2004). Dairy animals are exposed to PCDDs and PCBs through feed or soil contamination resulting in relevant PCDD levels in edible ruminant tissues and milk (Malisch, 2000; Schulz et al., 2005). Various PCBs exert biological effects similar to TCDD including reproductive or dermal toxicity and are therefore designated as "dioxin-like" PCBs (Larsen, 2006). Toxicity of dioxins is predominantly mediated by the aryl hydrocarbon receptor (AhR or dioxin receptor), a ligand-activated transcription factor of the basic helix-loop-helix/PER-ARNT-SIM (bHLH/PAS) family of transcriptional regulators (Puga et al., 2009). Upon ligand binding the AhR is translocated from the cytosol into the nucleus followed by heterodimerisation with another PAS protein designated AhR nuclear translocator (ARNT). The AhR/ARNT complex binds to consensus sequences termed dioxin response elements (DRE) in the 5′-untranslated region (5′-UTR) of target genes. This mechanism subsequently induces regulation of target gene expression like cytochrome P450 1A1 (CYP1A1) (Puga et al., 2009). Interestingly, some widely used pesticides including the imidazole fungicide prochloraz or the dicarboximide fungicide iprodione have been recently identified as AhR inducers in different cell lines (Long et al., 2003; Halwachs et al., 2013). Similar to dioxin-like contaminants,

Abbreviations: AhR, aryl hydrocarbon receptor; ABZSO, albendazole sulfoxide; ARNT, aryl hydrocarbon receptor nuclear translocator; CALUX assay, chemical activated luciferase gene expression assay; CYP1A1, cytochrome P450 1A1; DRE, dioxin response element; MDCKII cells, Madin-Darby canine kidney II cells; MDR1, multi-drug resistance 1 carrier; MNP, monepantel; monepantel sulfon, $MNPSO_2$; PBMECs, primary bovine mammary epithelial cells; PCBs, polychlorinated biphenyls; PCDD, polychlorinated dibenzodioxins; TCDD, 2,3,7,8-tetrachlordibenzo-*p*-dioxin; TBZSO, triclabendazole sulfoxide.

* Corresponding author. Tel.: +49 (0) 341 9738142; fax: +49 (0) 341 9738149.
E-mail addresses: halwachs@vetmed.uni-leipzig.de (S. Halwachs), wassermann@vetmed.uni-leipzig.de (L. Wassermann), honscha@vetmed.uni-leipzig.de (W. Honscha).

0887-2333/$ - see front matter © 2014 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.tiv.2013.12.015

the extensive use of pesticides in agriculture causes exposure of the general population to these AhR-inducing compounds. Human exposure to both compounds occurs mainly through consumption of fruit and vegetables (Claeys et al., 2011). Additionally, metabolism studies indicated potential prochloraz residue levels of $\geqslant$0.01 mg/kg (EFSA, 2011) and iprodione residue levels of 0.05 mg/kg edible tissues (FAO, 1995) in dairy animals. Overall, it is apparent that regulation of xenobiotic metabolising enzymes like CYP1A as an adaptive response to chemical exposure has so far been extensively investigated (Schrenk, 1998; Long et al., 2003). However, the complex mechanisms by which AhR-activating contaminants display their toxic effects are still poorly understood.

One of the possible effects of chronic exposure to AhR-inducing contaminants may be alteration of ABCG2 secretory activity in the mammary gland. The 72 kDa ABCG2 protein also referred to as breast cancer resistance protein (BCRP) belongs to the ATP-binding cassette (ABC) family of efflux transporters (Doyle et al., 1998). In the mammary gland, ABCG2 is expressed at the apical membrane of alveolar epithelial cells and is induced during lactation in humans (Jonker et al., 2005) and in dairy animals (Lindner et al., 2013). However, the biological meaning of ABCG2 secretory activity remains unclear (Jonker et al., 2005). The mammary ABCG2 transporter is rather of high toxicological relevance as it mediates excretion of various drugs including the fluoroquinolone antibiotic enrofloxacin and toxins like aflatoxin B_1 or the dietary carcinogen 2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhiP) (van Herwaarden et al., 2006; Pulido et al., 2006; Wassermann et al., 2013b). Interestingly, the 5′-untranslated region (5′-UTR) of bovine and caprine (Wassermann et al., 2013a) as well as human (Bailey-Dell et al., 2001) mammary ABCG2 exhibits DRE motifs. Moreover, induction of ABCG2 gene expression and functional transporter activity by TCDD was shown in human breast cancer MCF-7 (Tan et al., 2010) and primary bovine mammary epithelial cells (PBMECs) (Halwachs et al., 2013). Thus, chronic exposure to AhR-activating contaminants may enhance ABCG2-mediated xenobiotic secretion in the mammary gland. This may result in a potential health risk for consumers of dairy products through formation of relevant residues of likely harmful drugs and toxins. However, no adequate in vitro model is so far available to efficiently investigate the effect of AhR-inducing xenobiotics on ABCG2 secretory activity in the mammary gland of dairy animals. In pharmacokinetic studies, dairy goats often provide a model for milk-producing ruminants to detect potential milk residues of drugs or pesticides. In our laboratory, we recently generated MDCKII cells stably expressing full-length caprine mammary ABCG2 that exhibits a 5′-UTR DRE motif (Wassermann et al., 2013a). Thus, we aimed to validate this novel cell culture model as a suitable in vitro screening tool to simultaneously identify potential ABCG2 substrates and dioxin-like modulators of mammary ABCG2 activity. In this study, we therefore systematically examined ABCG2-drug interactions and regulation of functional ABCG2 transport activity by AhR-activating contaminants in MDCKII-cABCG2 cells.

2. Materials and methods

2.1. Chemicals

All chemicals including the anthelmintic drugs triclabendazole sulfoxide (TBZSO), levamisole hydrochloride and albendazole sulfoxide (ABZSO) as well as Aroclor 1254, PCB 101, prochloraz, iprodione, tolclofos-methyl, media and supplements were obtained from Sigma–Aldrich (Deisenhofen, Germany) unless stated otherwise. TCDD was purchased from AccuStandard (New Haven, CT, USA). Purity of TCDD, PCB 101, prochloraz, and iprodione was greater than 99% as indicated by HPLC analysis. Monepantel (MNP) and monepantel sulfon ($MNPSO_2$) were kindly provided by Novartis Animal Health Inc. (Basel, Switzerland) with purity of 98.4 ± 0.4% and 99.0 ± 0.2%, respectively. All other substances were obtained at analytical grade.

2.2. Cell culture

Madin-Darby canine kidney (MDCKII) cells stably expressing full-length bovine (bABCG2), ovine (oABCG2) or caprine (cABCG2) ABCG2 as well as Mock-transfected control cells (MDCKII-Mock) lacking ruminant ABCG2 expression were generated recently in our laboratory (Wassermann et al., 2013a). The full-length mammary bABCG2, oABCG2 or cABCG2 cDNA clones consist of the open reading frame (ORF) sequence as well as an 5′-untranslated region (5′-UTR) where regulatory elements are located. Thus, the core 5′-CGTG-3′-motif for the AhR/ARNT complex binding site (DRE) was identified in the 5′-UTR of the bovine and caprine mammary ABCG2 (Wassermann et al., 2013a). Cells were maintained in MEM with Earle's Salts (PAA, Coelbe, Germany) supplemented with 10% (v/v) fetal calf serum (Gibco Invitrogen, Karlsruhe, Germany), 1% (v/v) non-essential amino acids (PAA), 100 U/ml penicillin and 100 µg/ml streptomycin (PAA) in a humidified incubator with 5% CO_2 at 37 °C.

2.3. Quantitative RT-PCR of caprine ABCG2 gene expression

Total RNA was prepared from 4×10^6 MDCKII-cABCG2 cells using the RNeasy Mini system including DNase digestion with RNase-free DNase (Quiagen, Hilden, Germany) according to the manufacturer's instructions. Total RNA (1 µg) was used to synthesize cDNA under standard conditions employing the First Strand cDNA Synthesis Kit for RT-PCR (Fermentas, St. Leon-Rot, Germany) with 20 U of reverse transcriptase (M-MuLV RT) and 25 µg oligo $(dT)_{18}$ primer. Quantitative PCR of 1 µl cDNA was carried out using DreamTaq PCR Master Mix (Fermentas) including 0.2 mM mixed dNTPs as well as 1.5 U DreamTaq DNA polymerase. Caprine ABCG2 was detected with 0.4 pmol of ruminant specific sense (5′-TGATGAAGCTAACGAGACCGAAGAG-3′) and antisense (5′-GGAAGCTTCTTCCTTCT CTGATCCCC-3′) primers and the respective TaqMan probe (5′-FAM-AGTTTTATGTCAA CTCCTCCTTCTTCAAGG-BHQ-1–3′). For ruminant ß-actin 0.4 pmol of specific sense (5′-CGACATCCGCAAGGACCTCT-3′) and antisense (5′-TACT-TGCGCTCAGGGGG CGC-3′) primers and the respective TaqMan probe (5′-ROX-CGGCATCGCGGACAGGATGCAGA AAGAGAT-BHQ-2–3′) were used. All specific primers and probes were designed using gene bank entries and Primer Select (DNASTAR Inc.). PCR was performed for 40 cycles with an initial denaturation step of 30 s at 94 °C followed by annealing and extension at 61 °C for 45 s in a real-time DNA thermal cycler (Agilent technologies, Waldbronn, Germany). The amplified cDNA was quantified using the Mx3000P QPCR system (Agilent technologies). Caprine ABCG2 mRNA expression was normalized to β-actin levels as internal control and relative carrier gene expression was calculated by the $2^{-\Delta\Delta CT}$ method, taking the cABCG2 PCR efficacy into account.

2.4. Pre-treatment of MDCKII-bABCG2, MDCKII-oABCG2, MDCKII-cABCG2 or MDCKII-Mock cells

The test chemicals MNP, $MNPSO_2$, TBZSO and prochloraz were prepared in ethanol. Stock solutions of iprodione and tolclofos-methyl were made in methanol. ABZSO, Aroclor 1254 or PCB 101 were prepared in DMSO. TCDD was prepared in toluene and levamisole hydrochloride in Milli Q. For measurements of ABCG2-drug interactions, MDCKII-ABCG2 clones were incubated for 4 h with MNP (32; 320; 1600 nM), $MNPSO_2$ (0.12; 1.22; 6.10 µM), TBZSO