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Ochratoxin A transport by the human breast cancer resistance protein (BCRP), multidrug resistance protein 2 (MRP2), and organic anion-transporting polypeptides 1A2, 1B1 and 2B1

Xiaozhe Qi [a], Els Wagenaar [b], Wentao Xu [a,c], Kunlun Huang [a,c,*], Alfred H. Schinkel [b,**]

[a] Beijing Advanced Innovation Center for Food Nutrition and Human Health, College of Food Science & Nutritional Engineering, China Agricultural University, Beijing 100083, China
[b] Division of Molecular Oncology, The Netherlands Cancer Institute, Plesmanlaan 121, 1066CX Amsterdam, The Netherlands
[c] Beijing Laboratory for Food Quality and Safety, College of Food Science and Nutritional Engineering, China Agricultural University, Beijing, 100083, China

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ABSTRACT

Ochratoxin A (OTA) is a fungal secondary metabolite that can contaminate various foods. OTA has several toxic effects like nephrotoxicity, hepatotoxicity, and neurotoxicity in different animal species, but its mechanisms of toxicity are still unclear. How OTA accumulates in kidney, liver, and brain is as yet unknown, but transmembrane transport proteins are likely involved. We studied transport of OTA *in vitro*, using polarized MDCKII cells transduced with cDNAs of the efflux transporters mouse (m)Bcrp, human (h)BCRP, mMrp2, or hMRP2, and HEK293 cells overexpressing cDNAs of the human uptake transporters OATP1A2, OATP1B1, OATP1B3, or OATP2B1 at pH 7.4 and 6.4. MDCKII-mBcrp cells were more resistant to OTA toxicity than MDCKII parental and hBCRP-transduced cells. Transepithelial transport experiments showed some apically directed transport by MDCKII-mBcrp cells at pH 7.4, whereas both mBcrp and hBCRP clearly transported OTA at pH 6.4. There was modest transport of OTA by mMrp2 and hMRP2 only at pH 6.4. OATP1A2 and OATP2B1 mediated uptake of OTA both at pH 7.4 and 6.4, but OATP1B1 only at pH 7.4. There was no detectable transport of OTA by OATP1B3. Our data indicate that human BCRP and MRP2 can mediate elimination of OTA from cells, thus reducing OTA toxicity. On the other hand, human OATP1A2, OATP1B1, and OATP2B1 can mediate cellular uptake of OTA, which could aggravate OTA toxicity.

1. Introduction

The dietary toxin Ochratoxin A (OTA) is a secondary metabolite of fungi including *Aspergillus ochraceus*, *A. niger* and *A. carbonarius*, *Penicillium verrucosum*, and various other *Penicillium*, *Petromyces*, and *Neopetromyces* species. Food and beverages such as pork, poultry, dairy, chocolate, wine, beer, coffee, grape juice, dried fruit and spices, can be contaminated with OTA (Hope and Hope, 2012). OTA is a pentaketide derived from the dihydrocoumarin family coupled to β-phenylalanine (Fig. 1). OTA has weak acidic properties. The pKa values are in the ranges 4.2–4.4 and 7.0–7.3 for the carboxyl group of the phenylalanine moiety and the phenolic hydroxyl group of the isocoumarin part, respectively (Valenta, 1998). This means that under neutral and slightly acidic conditions most OTA is present as an organic anion (Fig. 1). OTA has several toxic effects, for instance, nephrotoxicity, hepatotoxicity, neurotoxicity, teratogenicity and immunotoxicity in various animal species. The main target organ of OTA in humans is kidney. However, its toxicity mechanisms are still unclear. How OTA accumulates in the kidney, liver, and brain is an unsolved puzzle, which could contribute to the various effects of OTA toxicity.

Given its predominantly charged nature, transmembrane transporters are likely important for OTA uptake, accumulation and efflux. The breast cancer resistance protein BCRP (also known as ABCG2) is an ATP-binding cassette (ABC) transmembrane drug transporter which can actively pump a wide variety of drugs and dietary toxins out of cells. It is located apically in epithelia of the kidney, intestine, and placenta and in the bile canalicular membrane of hepatocytes, as well as in the luminal membrane of endothelial cells at various blood-tissue barriers. BCRP can thus reduce tissue and systemic uptake of xenotoxins, and mediate their extrusion from the body by pumping them into the urine, intestinal lumen and bile (Jonker et al., 2005; Chen et al., 2016). Multidrug resistance-associated protein 2 (MRP2/ABCC2) is another member of the ABC transporter family. MRP2 is apically expressed in the (canalicular) hepatocyte plasma membrane, renal proximal tubules and small intestinal enterocytes, where it is

* Correspondence to: K. Huang, Beijing Advanced Innovation Center for Food Nutrition and Human Health, College of Food Science & Nutritional Engineering, China Agricultural University, Beijing, 100083, China.
** Corresponding author.
E-mail addresses: foodsafety66@cau.edu.cn (K. Huang), a.schinkel@nki.nl (A.H. Schinkel).

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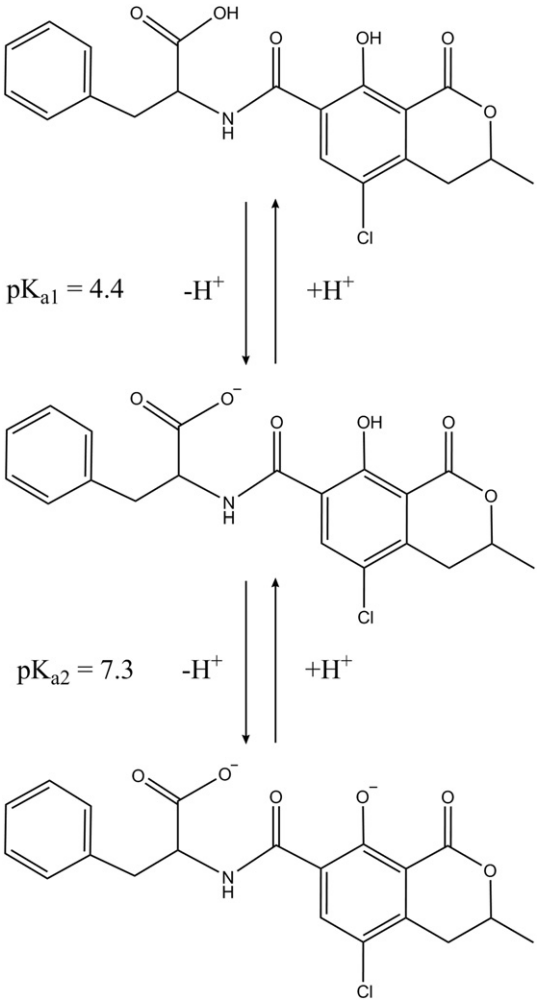

Fig. 1. Chemical structure of ochratoxin A and its monoanionic and dianionic forms. Associated pK_a values are indicated.

particularly well placed to play a role in the (restricted) oral bioavailability and elimination of toxins, drugs, and their phase II metabolites by extruding them into bile, urine and intestinal lumen (Chen et al., 2016).

Organic anion-transporting polypeptides (OATP, SLCO) are transmembrane transporters that primarily mediate the cellular uptake of a broad range of organic endogenous and exogenous compounds (Tang et al., 2013; Stieger and Hagenbuch, 2014). Among OATP transporters, the 1A and 1B subfamilies have broad substrate specificities. They are mainly expressed in the liver, kidney and small intestine. OATP2B1 is mainly expressed in the liver and small intestine. When expressed in the sinusoidal membrane of hepatocytes, these transporters mediate the hepatic uptake of many *endo*- and xenobiotics. OATPs apically expressed in the kidney may mediate (re-)uptake of their substrates from the urine.

Previous studies showed that the organic anion transporters hOAT1 (SLC22A6) and hOAT3 (SLC22A8) mediate high-affinity transmembrane transport of OTA across the basolateral side of the renal proximal tubule *in vitro* (Kusuhara et al., 1999; Jung et al., 2001). *In vivo* studies showed that in rats gavaged with low doses of OTA, the expression of Oat1 and Oat3 in the kidney increased, which may enhance OTA accumulation in this tissue and excretion into urine (Žlender et al., 2006; Zlender et al., 2009). Human BCRP and MRP2 have been implicated in OTA transport (Berger et al., 2003; Schrickx et al., 2006) based on *in vitro* studies. A few studies were also reported on organic anion transporting polypeptides. Kontaxi et al. identified OTA as a substrate for rat Oatp1a1, as analyzed in rat hepatocytes and Oatp1a1-cRNA-injected *Xenopus laevis* oocytes (Kontaxi et al., 1996). Moreover, mouse Oatp1a4, and rat Oatp1a1, Oatp1a3v1 and Oatp1a3v2 mediate transport of OTA *in vitro* (Takeuchi et al., 2001; Iwakiri et al., 2008; Ose et al., 2010). However, to the best of our knowledge no human OATPs have been analyzed for transport of OTA.

In this study we investigated whether human BCRP and MRP2 mediate OTA efflux from cells and whether human OATPs mediate OTA uptake into cells. For this purpose we used cell lines engineered to specifically express these transporters by stable transduction or transformation and various transport assessment techniques. Insight into the transmembrane transport of OTA by OATP, BCRP, and MRP2 could help us to understand how OTA enters into and goes out of cells in different tissues such as kidney and liver, resulting in aggravation or reduction of OTA toxicity in these organs.

2. Materials and methods

2.1. Chemicals

OTA was extracted and purified from corn particle medium and then analyzed by HPLC as described previously (Liang, 2008). [^{3}H]-OTA (26.0 Ci/mmol) was from Campro Scientific (Veenendaal, the Netherlands). Ko143 was from Tocris Bioscience (Bristol, UK). Cyclosporin A was provided by the Pharmacy department of the Antoni van Leeuwenhoek Hospital (Amsterdam, the Netherlands). Sodium butyrate was from Sigma–Aldrich Chemie (Taufkirchen, Germany). Dulbecco's Modified Eagle's medium (DMEM) medium was from Life Technologies (Carlsbad, USA). Fetal bovine serum (FBS) was from Gibco (Waltham, USA).

2.2. Cell culture

Polarized canine kidney MDCKII cells and subclones stably transduced with mBcrp, hBCRP, mMrp2, or hMRP2 cDNA and HEK293 cells and subclones transduced with OATP1A2, OATP1B1, OATP1B3, or OATP2B1 cDNA were grown in DMEM supplemented with 10% FBS, penicillin G (100 U/mL), streptomycin (100 μg/mL), at 37 °C in a humidified atmosphere containing 95% air and 5% CO_2. OATP1A2-, 1B1-, and 1B3-overexpressing HEK293 cells were kind gifts of Prof. Werner Siegmund and Dr. Markus Keiser (University of Greifswald, Greifswald, Germany), and OATP2B1-overexpressing cells were a kind gift of Prof. Per Artursson and Dr. Maria Karlgren (Uppsala University, Uppsala, Sweden). Cells were passaged every 3–4 days. Unless indicated otherwise, all cell growth and experiments were performed at (physiological) pH 7.4.

2.3. Sulforhodamine B (SRB) growth inhibition assay

MDCKII cells and subclones expressing mBcrp or hBCRP cDNA were seeded in 96-well plates (160–300 cells per well, depending on the growth rate) and allowed to attach and grow for 24 h. A series of concentrations of OTA with or without 2 μM Ko143 was then added to the wells and further growth to near-confluency for cells without OTA was allowed for 5 days. On day 6, cells were fixed by adding 50% trichloroacetic acid to the media for 1 h at 4 °C. After fixation, cells were rinsed 3 times with distilled water, and air-dried at room temperature. The fixed cells were stained for 1 h with sulforhodamine B (SRB) dye dissolved in 1% acetic acid (v/v) to a final concentration of 0.4% (w/v). The dye that was bound to the proteins was finally dissolved in 100 μL of 10 mM Tris (without pH adjustment). After incubation for 10 min at room temperature while shaking, the absorbance was measured at 510 nm using a Tecan plate reader (Männedorf, Switzerland).