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Gene expression kinetics of renal transporters induced by ochratoxin A in male and female F344 rats

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ABSTRACT

Ochratoxin A (OTA) is a mycotoxin that contaminates foodstuffs. The most relevant concern is its high kidney carcinogenicity in male rats and its unclear mechanism of action. It has been hypothesized that variations in transport mechanisms in kidney cells may be the reason of different sex-dependent sensitivities towards OTA. The aim of this study was to analyze, by RT- qPCR, renal transporters expression in 15-week-old male (M) and female (F) F344 rats at basal level and after single oral OTA administration (0.50 mg/kg bw). Temporal profiles (24h, 48h, 72h, 96h, 1 and 2 months) were studied per sex and transporter. The reference gene for all comparisons was Ppia. At basal level, sex differences were confirmed for Oatp1, Bcrp (M>F) and Oat2 (F>M). OTA tended to inhibit the expression of almost all transporters in both sexes, but clearly induced the expression of Oat2 in males. Regarding time profiles, the highest sex differences involved Oat (Slc22) transporters: Oat2, Oat3 and Oat5 expression showed a significant increase in males (24h) while Oat1, Oat2 and Oat5 level decreased in females (48h). Overall, basal sex differences in F344 rats and the specific sex-dependent response to OTA of Oat2 might contribute to high kidney damage in male rats.

1. Introduction

Ochratoxin A (OTA) is a secondary metabolite produced by different fungal species of the genera *Aspergillus* and *Penicillium* (mainly by *A. ochraceus* and *P. verrucosum*). This mycotoxin can contaminate a great variety of vegetal products, especially grains. Due to the fact that OTA is thermostable, it can enter the food chain through raw or processed products as well as through animal-origin products from livestock fed with contaminated feed (EFSA, 2006). Exposure to OTA is a worldwide phenomenon, as evidenced by its detection in human sera in many countries (Duarte et al., 2011; Märtlbauer et al., 2009; Soto et al., 2016).

OTA is a potent nephrotoxic agent (EFSA, 2006; IARC, 1993; Lock and Hard, 2004) with clear carcinogenic effects in rodents. Unfortunately its mechanism of action (MoA) as a carcinogen is still unclear. In 2008, the World Health Organization (WHO, 2008) proposed 5 hypotheses as main contributors (total or partial) to the MoA of OTA: 1) Genotoxicity from direct interaction of OTA or a reactive metabolite with DNA; 2) Generation of tumors secondary

Abbreviations: 18S, 18S ribosomal RNA; Abc, ATP-binding cassette; Actb, actin beta; Bcrp, breast cancer resistance protein; bw, body weight; cDNA, complementary DNA (deoxyribonucleic acid); Cmax, maximum concentration; C_t, threshold cycle; CV, coefficient of variation; EFSA, European Food Safety Authority; F, female; F344, Fisher 344; Gapdh, glyceraldehyde-3-phosphate dehydrogenase; IARC, International Agency for Research on Cancer; M, male; mRNA, messenger RNA (ribonucleic acid); MIQE, Minimum Information for Publication of Quantitative Real-Time PCR Experiments; MoA, mechanism of action; Mrp, multidrug resistance protein; $NaHCO_3$, sodium hydrogen carbonate; NTP, National Toxicology Program; Oat, organic anion transporter; Oatp, organic anion transporter polypeptide; OTA, ochratoxin A; qPCR, quantitative polymerase chain reaction; Pept, oligopeptide transporter; Ppia, peptidylprolyl isomerase A (cyclophilin A); Q, relative quantification level; RT, reverse transcription; RT-qPCR, reverse transcription quantitative real-time polymerase chain reaction; SD, standard deviation; Slc, solute carrier; SV, stability value; *Taq*, *Thermus aquaticus*; Ubc, ubiquitin C; WHO, World Health Organization.

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to chronic renal toxicity and compensatory cell proliferation; 3) Mitochondrial dysfunction leading to oxidative stress and indirect induction of DNA damage; 4) Generation of tumors secondary to inhibition of phenylalanine-tRNA^{Phe}-synthetase and protein synthesis and 5) Disruption of cell–cell signaling pathways and the process of cell division.

Since then several reviews have been devoted to this issue: oxidative stress, genotoxic and non-genotoxic mechanisms are still proposed as the main mechanisms. However, the most plausible situation seems to point towards a complex MoA that combines some of the main effects described to date (Heussner and Bingle, 2015; Kőszegi and Poór, 2016; Limonciel and Jennings, 2014; Malir et al., 2016).

Another important issue is the marked sex specificity of OTA carcinogenicity in rodents. In B6C3F male mice, OTA was considered a renal carcinogen while it was considered hepatocarcinogenic for females of the same strain (Bendele et al., 1985). In the most comprehensive carcinogenicity study carried out with rats of both sexes, male F344 rats presented a much higher incidence of carcinomas at 2 years than F344 females (males: 16/51, 30/50; females: 1/50, 3/50 for the doses of 70 and 210 μg/kg/day, respectively) (NTP, 1989). The sex specific carcinogenicity was again confirmed in a lifespan study carried out with male and female rats of the strains Dark Agouti and Lewis (Castegnaro et al., 1998; Pfohl-Leszkowicz et al., 1998; Son et al., 2003).

OTA kidney tumor formation in male rats has been suggested to be related with the chemically-induced alpha-2u-globulin nephropathy; a mechanism regarded as specific for male rats but not considered a predictor of carcinogenic risk to humans. Whilst this concept has not been completely rejected (Mantle and Nagy, 2008), it has been demonstrated that OTA kidney lesions are different from alpha-2u-globulin nephropathy (Rasonyi et al., 1999).

It has been hypothesized that the first key event in the mechanism of OTA nephrocarcinogenicity is the cellular uptake of OTA in the kidney due to the presence of active transporters, whose sex-hormone-dependent expression may account for site-specific accumulation of OTA, particularly in male rat kidney (Dietrich et al., 2005; EFSA, 2006; Mally, 2012). In several short-term and long-term (7 days - 2 years) toxicogenomic *in vivo* studies performed in male rats of different strains, gene expression analysis showed that genes involved in transport were among the most affected by OTA treatment, and that downregulation was the predominant effect (Arbillaga et al., 2008; Marin-Kuan et al., 2006; Stemmer et al., 2007). In a 10 days study performed in male Wistar rats, changes in the gene expression of some renal transporters of the Oat family have been described as having dual effect depending on the dose given: upregulation at low doses (25, 50 μg/kg bw) and downregulation at high dose (500 μg/kg bw) (Zlender et al., 2009).

Thus, taking into account that marked sex differences in sensitivity towards OTA nephrocarcinogenicity exist and that transport mechanisms in renal cells might explain these differences, the main objective of this study was to evaluate, by reverse transcription quantitative real-time polymerase chain reaction (RT-qPCR), the sex- and time-dependent expression response of renal proteins involved in OTA transport after a single oral administration of 0.50 mg OTA/kg bw. This dose was selected because it is slightly higher than the doses used in carcinogenicity studies (Castegnaro et al., 1998; Mantle et al., 2005; NTP, 1989) and was considered not to cause clinical alterations related to renal dysfunction after single oral dose administration (Vettorazzi et al., 2009).

For that purpose, different transporter families were selected after a bibliographic review as the most interesting proteins in terms of OTA toxicity evaluation and/or previously reported sex differences. According to this, in the present study the following families of transporters were selected: Slc22 (Oat1, Oat2, Oat3, Oat5 and Oat8), ATP binding cassette (Abc) (Mrp2, Mrp4 and Bcrp), Slco1 (Oatp1, Oatp2) and Slc15 (Pept1 and Pept2). Table 1 summarizes the basal sex differences described in different strains of rats and evidences of their capacity to transport OTA. Fig. 1 shows membrane localization, transport direction of the selected transporters and basal gender differences according to literature. It should be noted that several transporters have been more often described to be more expressed in male than in female of different rat stains (Oat1, Oat3, Mrp2, Mrp4, Bcrp and Oatp1) while others have been

Table 1
Main protein families involved in OTA transport in kidney cells of different rat strains. For each transporter basal sex differences in rats have been reviewed. Evidences for OTA-transport have been collected regardless the animal species.

	Name		Basal sex differences	OTA transport evidences
	Official	Others		
Oat1	Slc22a6	Orctl1, Paht, Roat1	M > F or M = F [a, b]	Rat Oat1/*Xenopus oocytes* [c] Rabbit Oat1/Cultured cells [d]
Oat2	Slc22a7		F > M [a, e]	Mouse kidney Oat2/*Xenopus* oocytes [f]
Oat3	Slc22a8	Roct	M > F or M = F [a, b, g]	Rabbit Oat3/Cultured cells [d] Rat Oat3/*Xenopus* oocytes [h] Human Oat3/*Xenopus* oocytes [i]
Oat5	Slc22a9	Slc22a19, Slc22a24	F > M [a, j]	Rat Oat5/*Xenopus* oocytes [k] Mouse Oat5/*Xenopus* oocytes [l] Mouse Oat5/Xenopus oocytes [m]
Oat8	Slc22a25	Ust, Ust1r, Slc22a9	M > F [n]	Rat Oat8/*Xenopus* oocytes [n]
Mrp2	Abcc2	Cmoat	M > F [o]	Human Mrp2/HEK293 transfected cells [p] Transepithelial passage in Caco2 cells [q, r]
Mrp4	Abcc4		M > F [a]	
Bcrp	Abcg2	Bcrp1	M > F [a]	Transepithelial passage in Caco2 cells [r]
Oatp1	Slco1a1	Slc21a1, Slc21a3	M > F [a, s]	Rat liver Oatp1/CHO transfected cells [t] Rat liver Oatp/*Xenopus* oocytes [u]
Oatp2	Slco1a2	Slc21a5, Slco1a4	M = F [g, s]	Mouse brain Oatp1a4/HEK293 transfected cells [v]
Pept1	Slc15a1		M > F or M = F [a, w]	
Pept2	Slc15a2		F > M [a, w]	

Abc: ATP-binding cassette; *Bcrp*: breast cancer resistance protein; *F*: female; *M*: male; *Mrp*: multidrug resistance protein; *Oat*: organic anion transporter; *Oatp*: organic anion transporter polypeptide; *Pept*: oligopeptide transporter; *Slc*: solute carrier.
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