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A different kinetic profile of ochratoxin A in mature male rats

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ABSTRACT

Ochratoxin A (OTA) is a mycotoxin that causes renal tumors in rodents, particularly in male rats. The present work explored the impact of gender and age on OTA toxicokinetics in F344 rats after a single oral dose (0.5 mg/kg b.w.). OTA plasma concentrations were analysed with a validated HPLC-FLD method and a population approach (NONMEM VI) was used to perform the kinetic analysis and the one year exposure simulation (0.21 mg/kg daily). Maximum observed OTA concentration ($CMAX_{obs}$) was at 2 h in all groups except in mature females (6 h). Mature females reached higher $CMAX_{obs}$ than males of the same age. Apparent volume of distribution, but not apparent total plasma clearance, increased significantly with body weight ($P < 0.01$) resulting in the following values for the terminal plasma half life (h) in males: 219 (young), 264 (matures) and females: 191 (young), 205 (matures). In addition mature males showed a significant lower relative bioavailability. The simulation showed similar plasma concentrations in males and females after two-months. Thus, toxicokinetic does not seem to explain sex-differences in toxicity in long-term studies. However, the age and weight should be taken into account in short-term toxicological studies if sex-differences are studied.

1. Introduction

Ochratoxin A (OTA) is a fungal metabolite produced by several species of *Aspergillus* and *Penicillium* which can contaminate a great variety of foods. Contamination of cereals, pulses, dried fruit, coffee, beer, grape juice, dry vine fruits, wine, cocoa, nuts and spices has been reported from all over the world (EFSA, 2006). Although at very low concentrations, human exposure is continuous and it occurs through consumption of the aforementioned food commodities or products from animals that retain ochratoxin A in their tissues after being fed with contaminated feed (NTP, 1989). Some studies suggested that OTA may be involved in the Balkan Endemic Nephropathy (BEN) and it has been associated with an increased incidence of urinary tract tumors in humans (Pfohl-Leszkowicz et al., 2002) but there is already a lack of epidemiological evidence.

OTA has been found to be a potent renal toxin in all of the animal species tested with considerable differences between species, the pig and chicken being the most sensitive. It has also been described as being immunotoxic, teratogenic, hepatotoxic (Kuiper-Goodman and Scott, 1989) and neurotoxic (Belmadani et al., 1998; Sava et al., 2006). There is a great deal of evidence of renal carcinogenicity in rats and mice with marked differences in sex specificity. The NTP (1989), in a two year gavage study, concluded that there was a *clear evidence of carcinogenic activity* for male and female F344 rats. However, there was a much higher incidence of renal tubule adenomas or carcinomas in male rats than in females. Consistent with these results, Castegnaro et al. (1998) also observed a higher incidence of renal adenocarcinomas, levels of DNA adduct and multifocal tubular epithelial karyomegalies in male kidneys. However they found some differences between Dark Agouti and Lewis rats. The carcinogenicity sex-specificity of OTA has also been described in $B6C3F_1$ mice (Bendele et al., 1985). The results of this study indicate that OTA is a renal carcinogen specifically in male mice whereas it is a hepatic carcinogen in

Abbreviations: −2LL, −2 × log (likelihood); BEN, Balkan Endemic Nephropathy; CMAX, maximum concentration; $CMAX_{obs}$, maximum observed concentration; CV(%), coefficient of variation; CL, total plasma clearance; *F*, relative bioavailability; F344, Fisher 344 rats; HPLC-FLD, high pressure liquid chromatography equipped with a fluorescence detector; IAV, inter-animal variability; i.v, intravenous; K_A, first order rate constant of absorption; LOD, limit of detection; LOQ, limit of quantification; OTA, ochratoxin A; *Q*, distribution clearance; TWI, tolerable weekly intake; *V*, apparent volume of distribution of the central compartment; V_T, apparent volume of distribution of the peripheral compartment; RSD, relative standard deviation; WGT, body weight.

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females. Taking into account these studies, the EFSA (2006) stated in its report that male animals are more sensitive than females and rats are considerably more sensitive than mice. Due to the aforementioned reasons, this mycotoxin has been classified as a possible human carcinogen by the International Agency for Research on Cancer (IARC, 1993) and a tolerable weekly intake (TWI) of 120 ng/kg b.w. has been established by EFSA (2006).

As commented above, there are considerable sex-differences in OTA acute and chronic nephrocarcinogenicity. The IPCS (1990) recommended the elucidation of the mechanism of sex-differences in renal, neoplasic and non-neoplasic disease, caused by ochratoxin A in experimental animals. Further understanding this problem will aid the extrapolation to human situation and risk assessment. Both toxicokinetic (the changes of concentrations of a compound in the organism over time) and toxicodynamic (the dynamic interactions of a compound with biological targets and their downstream biological effects) factors determine the toxicity of OTA (for a review see Ringot et al., 2006), and may account for these differences. With respect to toxicokinetics, numerous studies have been performed with different species and the kinetic parameters have been determined after oral and i.v. administration of the mycotoxin (for a review see Galtier, 1991 and Dietrich et al., 2005). Nevertheless, the discrepancies and inaccuracies found in the reference literature make comparisons between studies and the extrapolation to the human situation impossible (Dietrich et al., 2005). According to these authors, a principal problem in the determination of the kinetic parameters in most studies to date is that the number of timepoints in the very early phase of the kinetic experiments is generally too limited to allow an adequate description of the absorption, distribution and elimination of the ochratoxin A. Moreover, these authors also highlighted that the lack of published raw data prevents analyzing them with different mathematical methods; therefore, direct comparison among the different kinetic published data is not possible.

The kinetic profile of ochratoxin A in rat after oral administration was first studied by Suzuki et al. (1977) and Galtier et al. (1979) but the experiments were only performed in male Wistar and Sprague-Dawley rats respectively (Table 1). The first kinetic study performed using oral administration in animals of both genders was published by Hagelberg et al. (1989). They completed the kinetic study with both sexes in rhesus monkey and quail, not finding any differences between males and females. Unfortunately, their experiment performed in Wistar rats was only carried out in males. Dietrich et al. (2005) pointed some possible differences when comparing two different studies performed in male (Galtier et al., 1979) and female (Li et al., 1997) Sprague-Dawley rats after i.v. injection. More recently Zepnik et al. (2003) found higher OTA concentrations in kidneys of male F344 rats compared to their females. They concluded that their results, may explain, at least in part, organ and gender-specific toxicity of OTA. However, this study was mainly focused in OTA and its metabolites elimination and excretion, so early timepoints were not obtained.

In none of the studies performed to date was the age of the rats taken into account or analyzed, and this should be an important factor if sex-differences are studied. Indeed, no kinetic studies have been specifically performed to investigate sex- and age-related toxicity of OTA, and several signs seem to indicate that both sex and age may play an important role when analyzing OTA toxicological data. Male rats are clearly more sensitive than females (NTP, 1989). In a repeated oral dose study, old female SPF Wag/Rij rats were more sensitive to OTA toxicity than the young adult females (Dortant et al., 2001). In addition to this, a recently published study (Mantle and Nagy, 2008) relates gender differences in rat nephrocarcinogenicity to OTA binding to α2u-globulin, a protein specific to adult male rats.

For all these reasons, the first objective of the present work was to develop a pharmacokinetic study capable of describing adequately the time course of OTA in plasma after its oral administration, at the dose of 0.5 mg/kg b.w dissolved in $NaHCO_3$ (0.1 M pH 7.4), to young and mature F344 rats from both sexes. The second objective was to explore the impact of gender and age on the absorption and disposition characteristics of OTA. For this purpose, some interesting aspects, not taken into account in previous OTA kinetic studies, have been considered. First, rats were not fasted before the administration, as is usual in single oral dose kinetic studies. This decision was taken partly to mimic what occurs during long-term carcinogenicity studies in which rats are maintained with diet *ad libitum*. Secondly, a high number of early timepoints have been used in the in vivo experimental design to allow a better description of the kinetic curve. Finally, the concentrations in plasma of OTA in each animal have been analyzed. This last aspect was attained by using a validated HPLC-FLD method that needs only a small amount of sample to quantify OTA in plasma, and by performing a kinetic analysis using a population modelling approach that preserves individuality of each animal.

Finally, a simulation, assuming a continuous once daily administration of 0.21 mg/kg during one year period, has been performed in order to better understand what might happen with the plasmatic OTA levels during carcinogenic studies.

2. Materials and methods

2.1. Reagents

OTA was purchased from Sigma (Steinheim, Germany). For the animal oral administration, OTA was dissolved in 0.1 M $NaHCO_3$ (Sodium Hydrogen Carbonate powder. Riedel-deHaën, Seelze, Germany) and adjusted to pH 7.4 with HCl. OTA solutions were prepared in chemical safety cabinet and were always manipulated with double gloves and filtering masks FFP3. For the retro-orbital blood collection, animals were anesthetized with isoflurane (IsoFlo®, Veterinaria Esteve, Barcelona, Spain). All reagents used for the HPLC analysis were of pro-analysis grade. Sodium acetate and phosphoric acid were purchased from Panreac (Barcelona, Spain). Acetonitrile and methanol HPLC grade were obtained from Sigma Aldrich (Steinheim, Germany). The reagents used for OTA extraction and calibration standards were: absolute ethanol UV–IR-HPLC PAI and trichloroacetic acid 20% w/v, both from Panreac (Barcelona, Spain) and normal saline solution 0.9% from Braun (Barcelona, Spain). HPLC water from Millipore's Milli-Q System was used throughout the analysis.

Table 1
Principal kinetic studies performed to date in rats.

References	Strain	Sex	Age (weeks)	Weight (g)	Fasted before administration	Dose (mg/kg b.w.)	Administration	Vehicle
Suzuki et al. 1977	Wistar	m	Adults*	200–250	n.r.	15	p.o.	$NaHCO_3$
Galtier et al. 1979	Sprague-Dawley	m	n.r.	250	Yes	2.5	p.o. i.v.	$NaHCO_3$
Hagelberg et al. 1989	Wistar	m	n.r.	250–300	Yes	0.05	p.o. i.v.	$NaHCO_3$
Li et al. 1997	Sprague-Dawley	f	Adults*	270–350	n.r.	~0.33	i.v.	Ethanol/saline
Zepnik et al. 2003	F344	m	8	236–306	n.r.	0.5	p.o.	Corn oil
		f	8	152–188	n.r.	0.5	p.o.	Corn oil

f: female; m: male; n.r.: not reported; p.o.: oral; i.v.:intravenous.
* Exact weeks not reported.