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Short communication

Zearalenone metabolism in human placental subcellular organelles, JEG-3 cells, and recombinant CYP19A1

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ABSTRACT

Zearalenone (ZEN) and its derivative, zearalanone (ZAN), are endocrine disruptive mycotoxins produced by *Fusarium* species. We investigated the human placental metabolism of ZEN and ZAN *in vitro* in JEG-3 cells, human term placental subcellular fractions and recombinant enzymes. Human placental enzymes were capable of metabolizing ZEN and ZAN to their primary OH-metabolites which have higher affinity for estrogen receptors than their parent compounds. These metabolites may interfere with physiological placental estrogen signaling and thus disrupt the progress of gestation.

1. Introduction

Zearalenone (ZEN) and its derivatives α- and β-zearalenol (α- & β-ZEL), zearalanone (ZAN) and α- and β-zearalanol (α- & β-ZAL) are mycotoxins produced by *Fusarium* species, such as *Fusarium graminearum* and *Fusarium culmorum* (Figs. 1A and 2A). They are common moulds found in temperate and warm countries, and a frequent contaminant of cereal crops, foods and feeds worldwide posing risks to human and animal health [1,2]. These nonsteroidal estrogenic mycotoxins bind competitively to estrogen receptors [3] and estrogenic effects and infertility caused by ZEN and its metabolites have been demonstrated in pigs, sheep and experimental animals [2,4]. In rats, both ZEN and its metabolites are transferred through the placenta [5]. Although, human exposure to ZEN has been demonstrated via dietary intake, little is known about the health effects except few studies in cancer patients and prepubertal girls [6,7].

In mammals, ZEN is metabolized to two hydroxyl isomers, α-ZEL and β-ZEL by 3α- and 3β-hydroxysteroid dehydrogenase enzymes (HSD) [2,8]. ZEN also undergoes minor metabolism to catechol structures [9]. Zearalenone and its metabolites are conjugated with UDP-glucuronosyltransferases (UGTs) [10–13] and sulfotransferases (SULTs) [14].

During gestation human placenta produces considerable amount of estrogens which are essential for development of the fetus and progress of the pregnancy. Based on mycotoxins estrogenic activity human placental metabolism of ZEN and ZAN was investigated *in vitro* by using chorion carcinoma JEG-3 cells, human term placental subcellular fractions, and recombinant CYP19A1 enzyme.

2. Materials and methods

2.1. Chemicals

Formic acid, ZEN, α-zearalenol (α-ZEL), β-zearalenol (β-ZEL), ZAN, α-zearalanol (α-ZAL), β-zearalanol (β-ZAL), methyl *tert*-buthyl ether (MTBE), (Sigma-Aldrich, USA); D_6-Zearalenone (D_6-ZEN) (TRC, Canada); recombinant human CYP19A1 and reductase enzymes (Gentest/BD Biosciences, USA); acetonitrile (ACN), methanol (J.T. Baker, Holland); NADPH (Roche, Germany).

2.2. Cell culture

Mycoplasma negative tested JEG-3 (ATCC HTB-36) cells were cultured as previously described [15]. JEG-3 cells (2 million cells/dish, three dishes/dose level) were treated with 10, 30 and 100 nM

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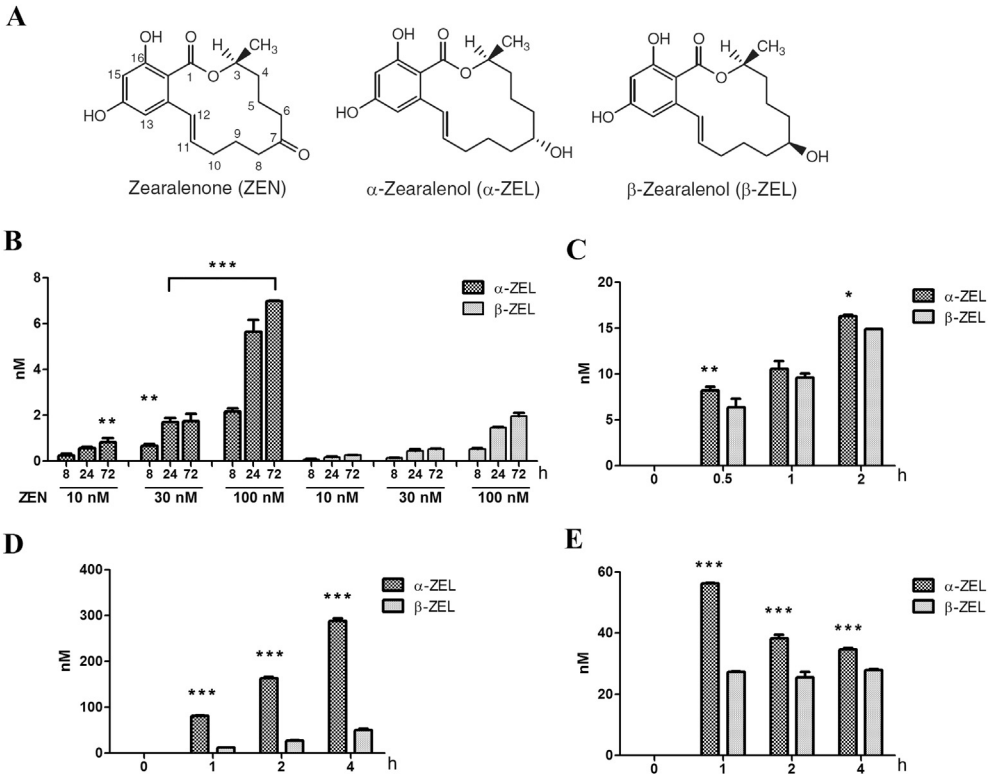

Fig. 1. A) Chemical structures of zearalenone (ZEN), α-zearalenol (α-ZEL) and β-zearalenol (β-ZEL). B) JEG-3 cells: metabolism of 10–100 nM ZEN after 8, 24 and 72 h incubations. C) 2 μM ZEN metabolism by recombinant CYP19A1 enzyme. D) 2 μM ZEN metabolism by placental cytosolic fraction. E) 2 μM ZEN metabolism by placental microsomes. Values shown are mean ± SD. Significant differences $^{*}p < 0.05$ $^{**}p < 0.01$ $^{***}p < 0.001$ (α-ZEL *vs.* β-ZEL, ANOVA with Bonferroni test).

ZEN or ZAN and 0.001% DMSO in controls for 0, 8, 24 and 72 h. The cytotoxicity of ZEN and ZAN treatment on JEG-3 cells was analyzed using the MTT assay [16], with final concentrations in the range 0.01–10 μM.

2.3. Recombinant CYP19A1 and reductase, and placental extract incubations

The incubations were conducted as previously described [16]. Briefly, the incubates contained 100 mM potassium phosphate buffer, pH 7.4, 2.5 mM $MgCl_2$, 2 μM ZEN or ZAN, 10 pmol of recombinant enzyme or 1 mg of placental cytosolic or microsomal protein fractions, 0.5 mM NADPH and H_2O up to 500 μl. The incubation times were 0–4 h in 37 °C.

2.4. Liquid chromatography mass spectrometric (LC-MS) detection of ZEN and metabolites

Cell culture mediums, supernatants from placental incubations and recombinant enzymes (500 μl) were extracted with 2 ml of MTBE and evaporated to dryness in an evaporating centrifuge. Evaporates were reconstituted in 100 μl of 20% methanol containing 50 nM internal standard D_6-ZEN. In LC-MS [16], the injection volume was 10 μl with a flow rate of 0.2 ml/min, column temperature 30 °C, mobile phase (0.1% formic acid and ACN). The gradient elution (ACN), was linearly increased from 10% to 90% in 9 min and then returned to 10%. The run time was 13 min with MS detection in the negative ionization mode analyzing the following parent ions and fragments (retention time in brackets): ZEN *m/z* 317 → 273, 299 (8.0 min); α- and β-ZEL *m/z* 319 → 275, 301 (7.3 min and 6.8 min); ZAN *m/z* 321 → 275, 301 (8.0 min); α- and β-ZAL *m/z* 321 → 277, 303 (7.2 min and 6.7 min) and D_6-ZEN *m/z* 324 → 279, 305 (8.0 min).

3. Results and discussion

ZAN is reduced from ZEN mainly in hepatic and intestinal cells [17]. We found that human placental enzymes cannot reduce ZEN to produce ZAN. Both ZEN and ZAN were metabolized to their α- and β-OH-metabolites by JEG-3 cells, human placental subcellular fractions and recombinant CYP19A1 enzyme. In all experiments, the α-OH-metabolite was the predominant species produced from both ZEN and ZAN (Figs. 1 and 2).

In JEG-3 cells, the formation of α-ZAL was approximately 10 times higher than α-ZEL, while β-ZEL and β-ZAL formation remained at the same level (Figs. 1B and 2B). The metabolite formations from both ZEN and ZAN were dose and time dependent. The metabolism of ZAN, and partly ZEN, became saturated in JEG-3 cells after 24 h exposure (Figs. 1B and 2B). Neither ZEN nor ZAN were cytotoxic to JEG-3 cells exposed at up to 10 μM concentrations (MTT assay, data not shown).

The human placenta produces considerable quantities of estrogens during pregnancy with reduced estrogen levels often associated with miscarriage [18]. Since CYP19A1 is highly expressed in human placenta and possesses reductive properties, we tested its capability to metabolize ZEN and ZAN. The metabolic outcome was similar to that seen in JEG-3 cells. Approximately two times more ZAN was metabolized to α-ZAL than ZEN was to α-ZEL (Figs. 1C and 2C). However, the difference in the amounts of α-OH- and β-OH-metabolites produced by CYP19A1 were not so obvious as in the JEG-3 cells or placental subcellular organelles indicating that ZEN and ZAN were also being metabolized by other reductase enzymes in placenta, such as HSD3B1 [2,8,17]. However, the recombinant reductase enzyme did not metabolize ZEN or ZAN to their hydroxyl

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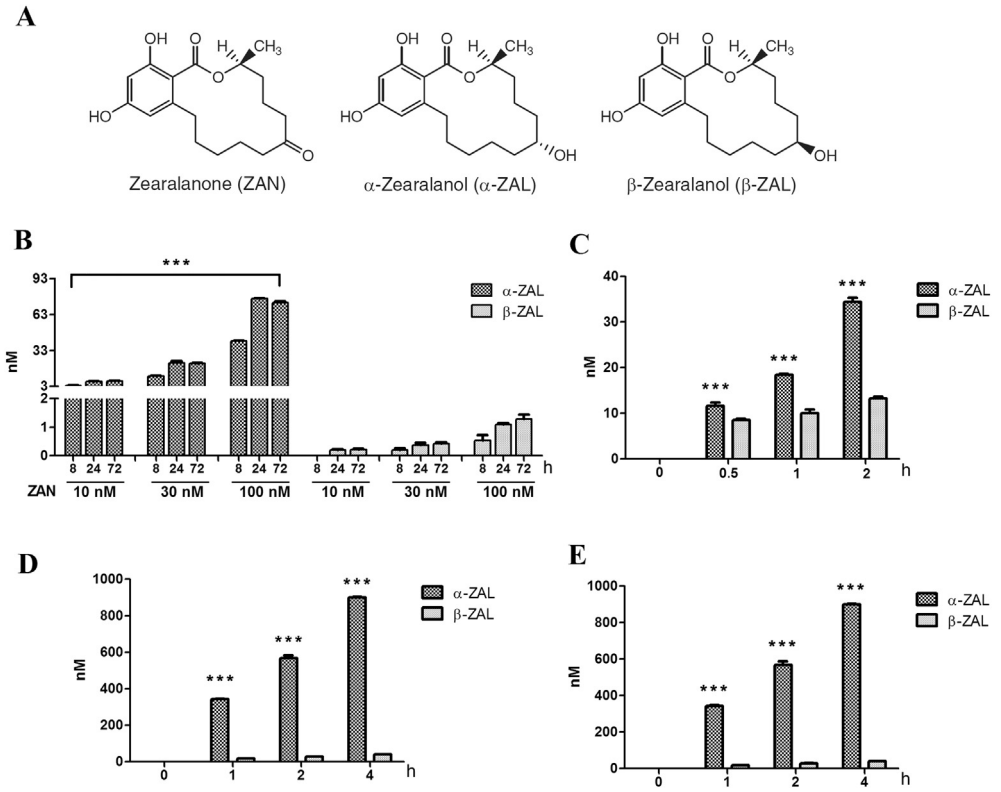

Fig. 2. A) Chemical structures of zearalanone (ZAN), α-zearalanol (α-ZAL) and β-zearalanol (β-ZAL). B) JEG-3 cells: metabolism of 10–100 nM ZAN after 8, 24 and 72 h incubations. C) 2 μM ZAN metabolism by recombinant CYP19A1 enzyme. D) 2 μM ZAN metabolism by placental cytosolic fraction. E) 2 μM ZAN metabolism by placental microsomes. Values shown are mean ± SD. Significant differences $^{***}p < 0.001$ (α-ZAL *vs.* β-ZAL, ANOVA with Bonferroni test).

metabolites (data not shown).

The only difference to the above experiments was observed in the test with ZEN and placental microsomes (Fig. 1E). The formation of α-ZEL was highest after 1 h incubation thereafter declining in the next 4 h. The decrease in the α-metabolite formation is thought to result partly from placental conjugation reactions with microsomal UGTs or SULTs [11–14].

The binding affinity of ZEN and its derivatives for estrogen receptors has been evaluated and ranked in the following order α-ZEL > α-ZAL > β-ZAL > ZEN > β-ZEL [3,17,19,20]. In addition, we noted that ZEN and ZAN metabolism in human placenta showed a preference for the production of the α-OH-metabolite, a compound which has a higher binding affinity for estrogen receptors than the parent compounds. This could be anticipated to interfere with estrogen signaling in the placenta and exert endocrine disruptive effects in the developing fetus.

In conclusion, human placenta is capable of metabolizing ZEN and ZAN to their OH-metabolites, predominantly to the α-OH-metabolite. In addition, CYP19A1 – but not the reductase enzyme alone – can metabolize ZEN and ZAN to their OH-metabolites.

Conflict of interest

The authors report no conflict of interest.

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