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IMPACT OF *FUSARIUM* MYCOTOXINS ON *IN VITRO* ACTIVITY OF MAJOR HEPATIC CYTOCHROME P450 BIOTRANSFORMATION ENZYMES IN PIGS

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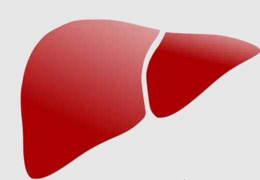
Introduction

Cytochrome P450 enzymes (CYP450) are catalytic oxido-reductases capable of metabolising a wide variety of endogenous and xenobiotic compounds. A principal function of these CYP450 is to improve elimination of such substances by biotransformation to more polar and water soluble metabolites. However, some xenobiotics can inhibit or induce CYP450 activity, and co-ingestion of these compounds with substrate drugs can lead to an altered disposition of these substrate drugs. This has been associated with a number of clinically relevant drug-drug or drug-food interactions. Nonetheless, regarding drug-food contaminant interactions, literature reports are scarce. Mycotoxins are highly prevalent food and feed contaminants produced by several fungal species[1]. Pigs are very sensitive to the toxic effects of mycotoxins, in particular deoxynivalenol (DON) and zearalenone (ZEA). Moreover, the inhibitory impact of T-2 toxin (T-2) on the hepatic CYP3A activity in pigs was previously demonstrated[2]. In addition, the similarities between porcine and human CYP450 enzymes suggest that the pig can serve as a suitable animal model for drug metabolism and safety studies in humans[3,4]. Therefore, the aim of the study was to investigate the impact of DON, ZEA, T-2 and fumonsin B1 (FB1) on six important drug metabolising CYP450 enzymes in a porcine *in vitro* model.

METHODOLOGY

Experimental methods

Mycotoxins ZEA, DON, FB1, T-2 + Substrates midazolam, tolbutamide, coumarin, dextromethorphan, chlorzoxazone, phenacetin

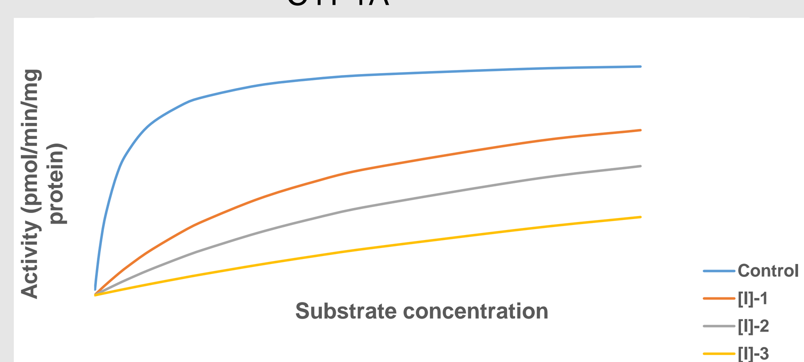


Microsomal CYP3A CYP2C CYP2A CYP2D CYP2E CYP1A

LC-MS analysis of metabolites (Schelstraete et al., submitted)

Selection of mycotoxins-substrate pairs

Mycotoxin + Substrate



Inhibition profile analysis

- **Using nonlinear regression analysis with Sigmaplot® version 13**
- **Eight different inhibition profiles fitted for each selected mycotoxin-substrate pair**
- **Determination of the model parameters: Km (Michaelis-Menten constant), Vmax (maximal biotransformation rate), Ki (inhibitory constant) and modification constant α and β where applicable**
- **Selection of model based on Akaike information criterion (AIC), passing statistical tests for homogenity of residuals and significance of the parameter estimates.**
- **Based on Ki values, *in vivo* direct inhibition potential of the mycotoxins was estimated, taking into account maximal guidance contamination levels of mycotoxins and daily feed intake of pigs.**

RESULTS

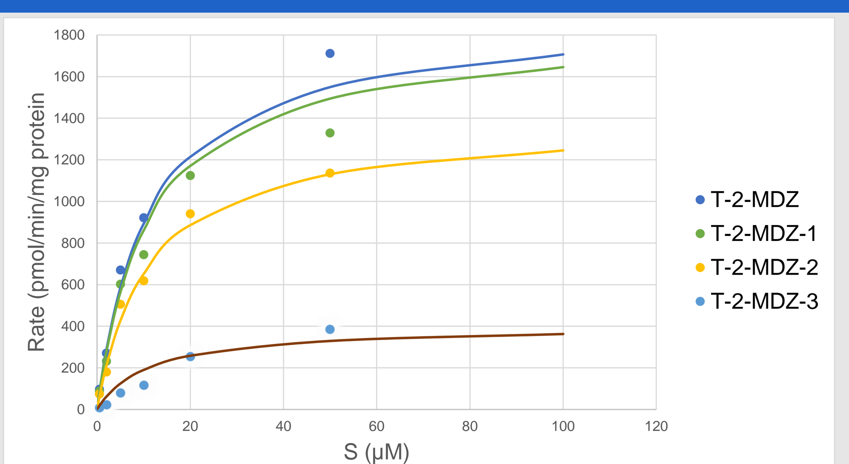


Fig 1. Non-competitive inhibition of T-2 and midazolam after pre-incubation of microsomes with T-2. Concentrations were 0 µM (T-2-MDZ), 1 µM (T-2-MDZ-1), 10 µM (T-2-MDZ-2) and 100 µM (T-2-MDZ-3). Km= 11.3 ± 1.32 µM; Vmax= 1,899 ± 88.0 pmol/min/mg protein; Ki= 27 ± 3.97 µM.

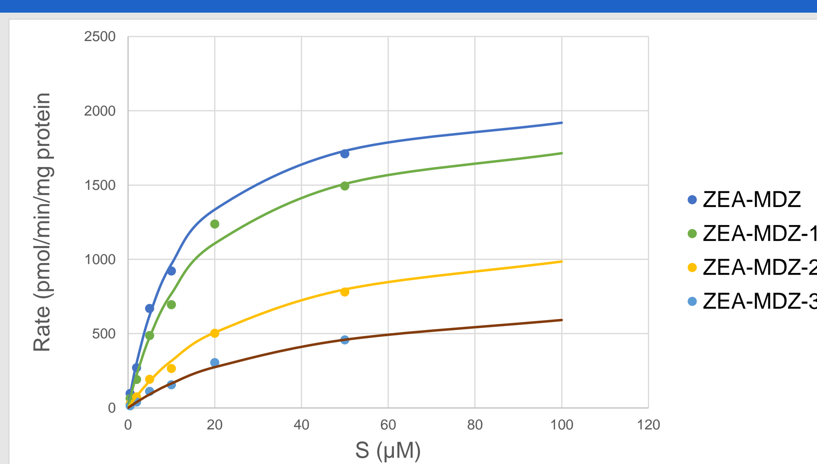


Fig 2. Mixed partial inhibition of ZEA and midazolam, Concentrations were 0 µM (ZEA-MDZ), 0.5 µM (ZEA-MDZ-1), 5 µM (ZEA-MDZ-2) and 20 µM (ZEA-MDZ-3). Km= 12.3 ± 1.1 µM; Vmax= 2,155 ± 73.2 pmol/min/mg protein; Ki= 1.1 ± 0.22 µM; α= 3.8 ± 1.43; β= 0.3 ± 6.37*10^{-2}.

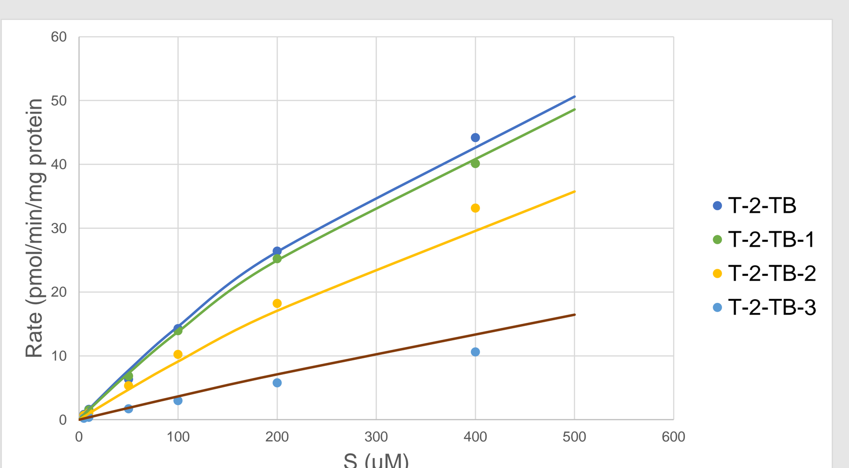


Fig 3. Full competitive inhibition of T-2 and tolbutamide (TB). Concentrations were 0 µM (T-2-TB), 1 µM (T-2-TB-1), 10 µM (T-2-TB-2) and 50 µM (T-2-TB-3). Km= 805 ± 150.0 µM; Vmax= 132 ± 17.9 pmol/min/mg protein; Ki= 14.8 ± 1.45 µM.

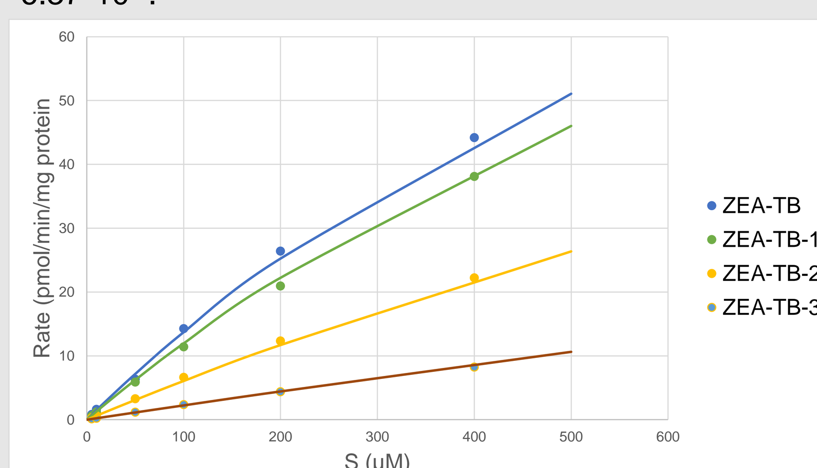


Fig 4. Partial competitive inhibition of ZEA and tolbutamide (TB). Concentrations were 0 µM (ZEA-TB), 0.1 µM (ZEA-TB-1), 1 µM (ZEA-TB-2) and 10 µM (ZEA-TB-3). Km= 1,074 ± 137.9 µM; Vmax= 160.8± 15.98 pmol/min/mg protein; Ki= 0.5 ± 4.27*10^{-2} µM; α= 9.5 ± 1.05.

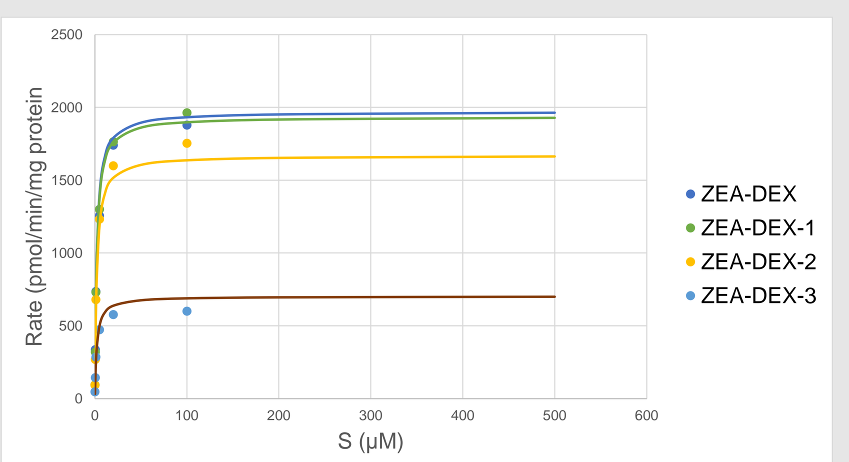


Fig 5. Non-competitive inhibition of ZEA and dextromethorphan (DEX). Concentrations were 0 µM (ZEA-DEX), 1 µM (ZEA-DEX-1), 10 µM (ZEA-DEX-2) and 100 µM (ZEA-DEX-3). Km= 2.0 ± 0.17 µM; Vmax= 1,971± 39.7 pmol/min/mg protein; Ki= 55.4 ± 5.77 µM.

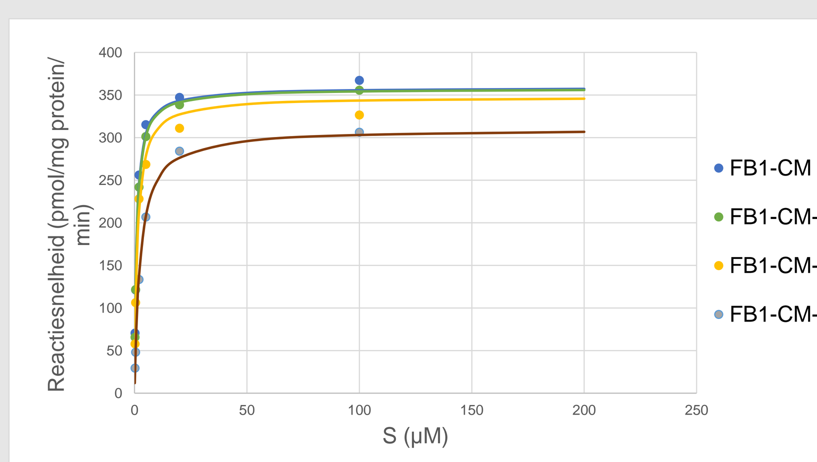


Fig 6. Full mixed inhibition of FB1 and coumarin (CM). Concentrations were 0 µM (FB1-CM), 0.1 µM (FB1-CM-1), 1 µM (FB1-CM-2) and 5 µM (FB1-CM-3). Km= 0.9 ± 5.10*10^{-2} µM; Vmax= 359 ± 4.1 pmol/min/mg protein; Ki= 2.3 ± 0.34 µM; α= 14 ± 4.3.

CONCLUSIONS

- **ZEA can directly inhibit CYP3A, CYP2C and CYP2D enzymes with high potency towards CYP3A and CYP2C as reflected in their Ki values (1.1 and 0.5 µM respectively).**
- **FB1 is a potent inhibitor of coumarin hydroxylase indicating inhibition potential towards CYP2A.**
- **T-2 is a time dependent inhibitor of CYP3A and a competitive inhibitor for CYP2C.**
- **DON could not inhibit any of the reactions significantly.**
- **Based on the volume of distribution, daily feed intake, maximal guidance levels of mycotoxins and assuming complete oral bioavailability, expected *in vivo* mycotoxin plasma concentrations are 20-400 times lower then Ki values. Therefore, direct inhibition *in vivo* seems unlikely. However, hepatic cellular concentrations can be higher then estimated plasma concentrations. In addition, the micro-environment can influence interactions between toxin and enzyme significantly. Moreover, mycotoxins can possibly alter CYP450 *in vivo* by exerting a regulatory effect, depending on the mycotoxin.**
- **Future trials are needed to investigate the *in vivo* impact**

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