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INTERACTION BETWEEN *FUSARIUM* MYCOTOXINS AND CYTOCHROME P450 DRUG METABOLIZING ENZYMES/ABC DRUG TRANSPORTERS IN A PORCINE ANIMAL MODEL

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INTRODUCTION AND OBJECTIVES

The cytochrome P450 (CYP450) enzymes and ABC drug transporters in the intestine and liver play a major role in the pharmacokinetics of drugs and other xenobiotics. If feed and food components also affect these enzymes or transporters, then the disposition of drugs can be altered leading to clinically relevant interactions. However, little is known about pharmacokinetic interactions between drugs and food or feed contaminants. Mycotoxins are such contaminants produced by fungi and are the number one threat regarding chronic toxicity. Moreover, our research group has shown a reduction in hepatic CYP3A activity in pigs (Goossens et al., 2013), and a down-regulation of hepatic *CYP3A37*, *CYP1A4*, *CYP1A5* and *MRP2* in broiler chickens after exposure to T-2 toxin (Osselaere et al., 2013). Therefore it is the aim of this project to investigate the influence of oral exposure to *Fusarium* mycotoxins on the pharmacokinetic behavior of drugs which are substrates for these enzymes and transporters.

EXPERIMENTAL SET-UP

In vitro* experiments*:
Different experiments will be set-up (fig 1), The first step is to characterize key hepatic/intestinal metabolizing enzymes and transporters and incubate them with different *Fusarium* mycotoxins. For transporters, fresh tissue as wel as cell cultures will be used to asses their activity and the effect of mycotoxins on the activity. Also the gene expression will be assesed using qPCR.

Feed trial:
Next, a trial with contaminated feed will be carried out and tissue collected to perform the same *in vitro* experiments as before

***In vivo* experiments:**
Mycotoxins with significant effect on CYP450 enzymes/drug transporters will be used to conduct an *in vivo* trial with relevant substrate drugs to determine changes in pharmacokinetic behavior

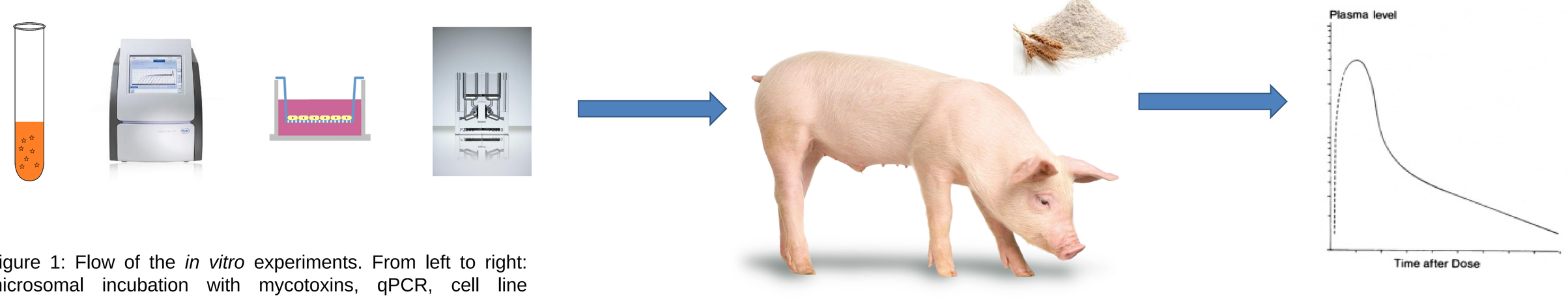

Figure 1: Flow of the *in vitro* experiments. From left to right: microsomal incubation with mycotoxins, qPCR, cell line transport experiments and transporter activity determination with Ussing chambers.

RESULTS

Development and validation of a multiprobe HPLC-MS/MS method to assess CYP450 activity. Validation results are displayed in table 1.

Sample preparation:
•50 µL porcine hepatic microsomes (1.25mg protein/ml); 50 µL probe solution (probe 1-5); 50µL buffer (pH 7.4); 50µL NADPH (5mM); 50µL KCl (1.15%) and 25 µL stop reagent containing the internal standards.
•Samples with different probes are pooled
•Extraction with 5mL ethyl acetate, evaporation to dryness (N_2, 40°C)
•Reconstitution in water/methanol 50/50 (v/v)
Chromatography:
•HPLC instrument: Alliance type 2695 HPLC (Waters)
•HPLC-column: Zorbax Eclipse Plus column (C18, 3.0 x 100 mm, d.p.: 3.5 µm) in combination with a guard column (2.1 x 12.5 mm, d.p.: 5 µm), both from Agilent Technologies (Sint-Katelijne-Waver, Belgium)
•Mobile Phase: (A) 0.1% formic acid in water; (B) 0.1% formic acid in acetonitrile, gradient elution
MS/MS detection:
•MS instrument: Quattro Ultima® triple quadripole MS (Micromass)
•MS conditions: ESI+ (probe 1-3); ESI- (probe 4, 5)
•MRM transitions are depicted in figure 2

Table 1: Validation results for the LOQ, limit of quantification; LOD, limit of detection; Gof, goodness of fit; accuracy and precision; QC, quality control sample

		Probe 1	Probe 2	Probe 3	Probe 4	Probe 5
range (ng/ml)		2-1000	10-1000	2-1000	1-1000	2-1000
Acquisition mode		ESI^+	ESI^+	ESI^+	ESI^-	ESI^-
Accuracy (%)	QC-low	-5.0	7.8	6.3	-5.0	2.5
	QC-mid	-0.2	-1.4	3.4	-0.9	-6.9
	QC-high	3.0	6.5	2.7	4.1	-1.1
Precision within day (%)	QC-low	6.7	0.7	25.0	5.8	14.1
	QC-mid	3.2	5.2	6.5	2.3	4.9
	QC-high	5.1	5.4	4.5	4.3	4.2
Precision between day (%)	QC-low	10.4	7.2	21.9	12.0	9.4
	QC-mid	3.1	7.2	6.5	5.0	7.8
	QC-high	4.9	4.4	14.8	5.8	8.5
LOQ (ppb)		2.00	10.0	2.00	1.00	2.00
LOD (ppb)		0.22	0.59	0.03	0.01	0.12
Gof (%)		2.37	4.63	10.76	7.45	4.22

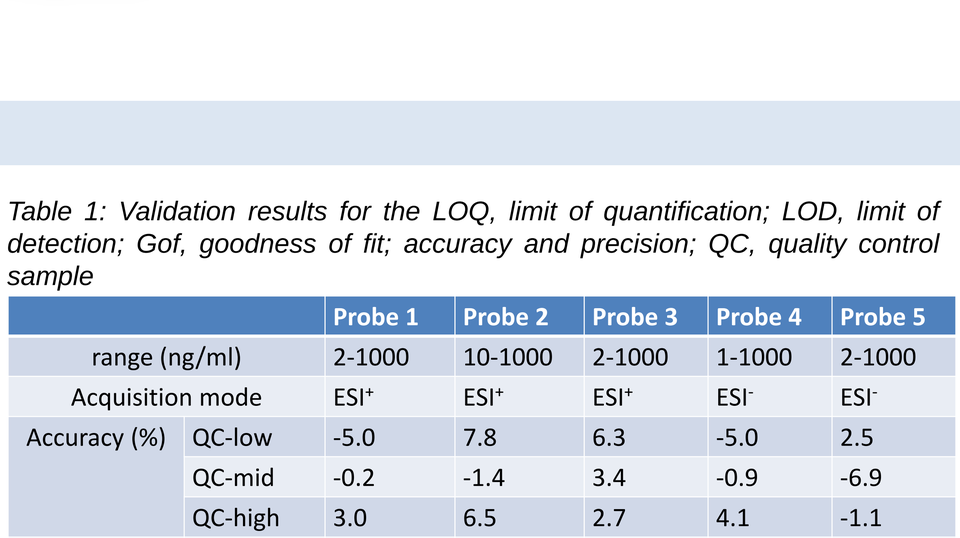

Figure 2: MRM transitions for the different probes. First two rows ESI-, last three rows ESI+. * only one of two transitions depicted for each probe.*

CONCLUSION AND PERSPECTIVES

The validated method is suited for analysis of the microsomal incubation mixtures. Hence, the inhibitory potential of several mycotoxins on CYP450 enzymes can be assessed. Together with the results from the qPCR method (under development), this will give reveal the effect of mycotoxins on drug metabolizing enzymes.

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