I-screen: In vitro platform to study human gut microbiota induced drug metabolism and molecular transformations

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INTRODUCTION

> Our gut microbiome plays a major role in the metabolism of xenobiotics and is a relatively underexplored, but essential, field of study in pharmacology and toxicology.

> Gut microbiota can directly metabolize xenobiotics into active, inactive or toxic metabolites, thereby influencing pharmacokinetics, efficacy and toxicity profiles of prescribed drugs.

AIM

> To study microbiota induced drug metabolic transformation of drugs in vitro, TNO has developed the i-screen platform, a translational intestinal screening multi-well platform simulating the human colonic microbiota conditions.

METHODS

> The metabolic capacity of gut microbiota was investigated by incubating 12 drugs (Table 1) potentially susceptible to microbial metabolism in i-screen using pooled human colonic microbiota for 24 hours under fully anaerobic conditions [1] (Figure 1), followed by LC-HRMS analysis of the samples. This allowed for investigation of different microbial transformations that may occur in the human gut.

***Figure** 1. Schematic Representation of (Personalized) I-screen 96-well Format*

> Furthermore, a large scale incubation (35 mL) with risperidone was performed for 24 hours to obtain larger quantities of the reduced metabolite. Isolation of this metabolite via semi-preparative HPLC was followed by both qualitative and quantitative NMR analysis. NMR spectra were recorded on a Bruker Avance 600 MHz equipped with a 1.7 mm TCI Cryo probe. 2D data were recorded using the standard pulse sequences provided by Bruker. Quantitation of NMR isolates was performed by external calibration against the ^{1}H NMR spectrum of a 5 mM benzoic acid standard.

RESULTS

> Among the 12 drugs examined, five were demonstrated to generate metabolites (risperidone, sulindac, sulfinpyrazone, nizatidine and sulfasalazine). One (metronidazole) was observed to decline in incubations however no metabolites were detectable, and the other 6 were not shown to generate metabolites (omeprazole, simvastatin, levodopa, acetaminophen, zonisamide and dapsone). Notably, the conversion of risperidone and sulindac were complete after 24 hours of incubation.

> These are all metabolic transformations known to occur in human in vivo.

***Table 1.** List of 12 Investigated Drugs Potentially Susceptible to Microbial Metabolism in I-screen*

Compound	Molecular Transformation	Formed Metabolite
Risperidone	N-O reduction	Imine intermediate → Reduced metabolite, complete conversion
Sulindac	S-oxide reduction	Deoxysulindac, complete conversion
Sulfinpyrazone	S-oxide reduction	Deoxysulfinpyrazone
Nizatidine	N-O reduction	Deoxynizatidine
Sulfasalazine	Reduction	Reductively cleaved metabolite of sulfasalazine
Metronidazole	Decline of parent	No metabolites detectable
Omeprazole, Simvastatin, Levodopa, Acetaminophen, Zonisamide, Dapsone	No decline of parent, no metabolites detectable	

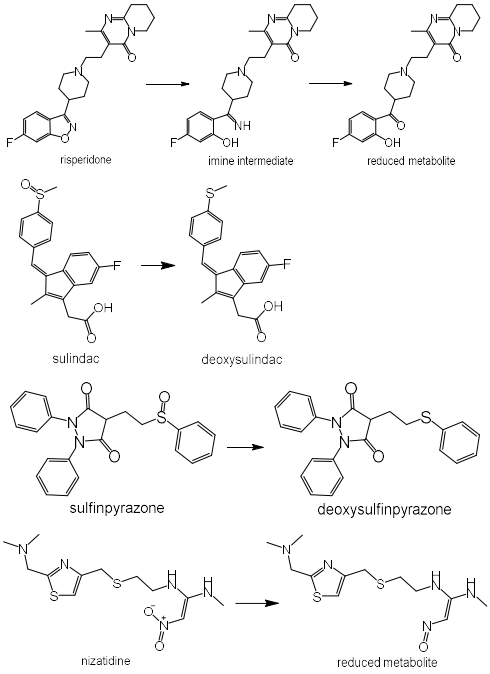

***Figure 2.** Summary of Metabolic Reactions Observed in I-screen*

RESULTS

In the 1H spectrum of the isolated metabolite all of the aliphatic resonances of risperidone are essentially unchanged while the chemical shifts of the aromatic resonances have changed significantly from those observed in the parent. These spectral changes are consistent with the known ring open 2-hydroxybenzoyl metabolite of risperidone but they are not sufficient for a definitive structural assignment. In order to conclusively establish the structure of the metabolite heteronuclear 1H-13C HMBC data was required. 1H chemical shift and coupling patterns established the 8.02 ppm resonance as H28. Within the 1H-13C HMBC data set there is a cross peak from the 1H 8.02 ppm resonance to a 13C resonance at 207.5 ppm which is assigned as the C19 carbon of the ketone. This NMR data, in conjunction with the MS data, establishes the structure of the isolated metabolite as the ring opened hydroxybenzoyl metabolite of risperidone. The concentration of the NMR solution of the metabolite was 8.6 mM as determined by qNMR.

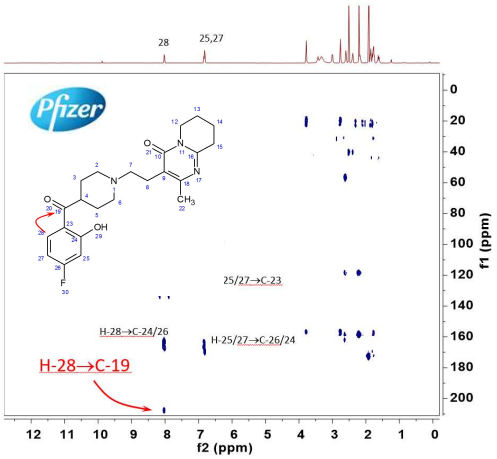

***Figure 3.** NMR of Reduced Risperidone Metabolite ^{1}H and ^{1}H-^{13}C HMBC*

CONCLUSIONS

> We identified different types of drug transformations that may occur in the human gut and are known to occur in vivo.

> We demonstrated that isolates from the i-screen platform are sufficient in both quantity and quality for 1D and 2D NMR analysis.

> We here present an in vitro tool containing human colonic microbiota to study microbiota driven metabolism of drugs.

FUTURE PERSPECTIVES

> We will further explore the potential for quantitively translating the in vitro data to the in vivo situation

> Further effort will be made to investigate inter-individual differences towards personalized i-screen applications

REFERENCES

[1] *Ladirat et al. 2013*