CONVERSION OF DRUG METABOLITES BACK TO PARENT DRUGS BY HUMAN GUT MICROBIOTA IN AN EX VIVO FERMENTATION SCREENING PLATFORM

Visit us at booth #21

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INTRODUCTION

- Human gut microbiota play an important role in drug kinetics and metabolism by activating, inactivating, or even toxifying orally administered drugs.
- Metabolism of selected drugs by pooled microbiota in the ex vivo fermentation platform has been show before (van de Steeg et al., 2018), but conversion of metabolites back to parent (as part of enterohepatic circulation) is currently not highly studied.

AIM

- To study and quantify the fate of drug metabolites (generated in the liver) upon exposure to gut microbiota in the i-screen platform, a translational ex vivo fermentation platform simulating the human colonic microbiota conditions.

***Figure** 1. Graphical abstract of ex vivo fermentation platform to study deconjugation of drug metabolites by the human microbiome.*

METHODS

- A set of 8 drug metabolites (acetaminophen sulphate, mycophenolic acid glucuronide, sertraline-N-CO-glucuronide, benzydamine N-oxide, imipramine N-oxide, SN38 glucuronide (SN38G), raloxifene 4'-glucuronide and raloxifene 6'-glucuronide) was incubated for 6 and 24 hours with human colonic microbiota (pool of 7 healthy adult fecal samples) under strictly anaerobic conditions [1,2].
- In order to determine the kinetics of the metabolic conversion, SN38G was applied as a model drug. Linearity of the metabolic conversion was studied over 6h, and kinetics of the metabolic conversion was studied at multiple concentrations of SN38G (5, 25, 50 and 100 µM). All samples were analyzed using high-performance liquid chromatograph.

REFERENCES

[1] Ladirat SE et al. J. Micr. Methods (2013) 92: 387-397
[2] Van de Steeg E et al. Drug Metab Dispos (2018) 46: 1596-1607

RESULTS

- Previously we have shown that out of 12 parent drugs 5 were metabolized in i-screen experiments and specific reduction metabolites could be shown (microbiota-induced metabolism was demonstrated for risperidone, sulindac, sulfinpyrazone, nizatidine and sulfasalazine).[2]
- Here we have analyzed the capability of adult human colon microbiota to metabolize drug metabolites generated in the liver back to their respective parent drugs, since these drug metabolites may through biliary excretion end up in the GI–tract in vivo.

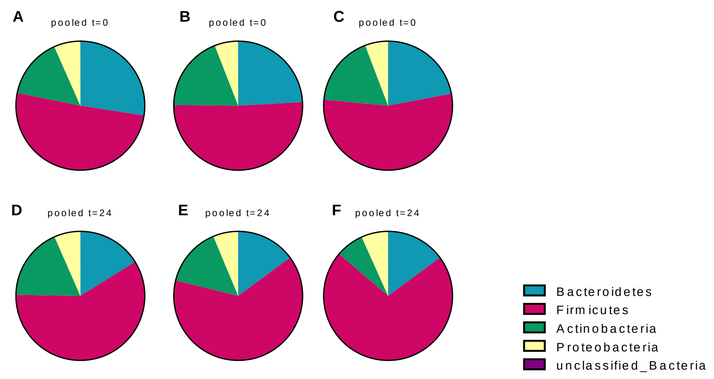

***Figure 2.** Microbial composition of pooled human colon microbiota before (t=0) and after ex vivo fermentation in i-screen for 24 hours (t=24). Intra-experimental variation is shown by presenting A, B and C and D, E and F representing triplicate incubations of one experiment. Data are presented as mean relative abundance of the individual microbial species (n=3).*

***Table 1.** Overview of metabolic faith of 8 Investigated Drug metabolites Susceptible to Microbial Metabolism in I-screen.*

Substrate (metabolite)	Product (parent drug)	Reaction Type	Observation
Acetaminophen sulfate	Acetaminophen	Phenol sulfate hydrolysis	• Reaction was complete at 6h • No reaction in microbiota free incubation
Mycophenolic Acid Glucuronide	Mycophenolic Acid	Acyl glucuronide hydrolysis	• Reaction occurs in 6h and continued over 24h • No reaction in microbiota free incubation
Sertraline-N-Carbamoyl Glucuronide	Sertraline	Carbamoyl glucuronide hydrolysis	• Very fast reaction, within minutes after incubation • Reaction was complete after 6h • Sertraline continued to decline in gut microbiota between 6 and 24h
Benzydamine N-Oxide	Benzydamine	N-Oxide Reduction	• Reaction was complete after 6h • Some conversion to parent in absence of gut microbiota suggesting instability • Benzydamine continued to decline in gut microbiota between 6 and 24h
Imipramine N-Oxide	Imipramine	N-Oxide Reduction	• Reaction was complete after 6h • Some conversion to parent in absence of gut microbiota suggesting instability • Imipramine was further metabolized by gut microbiota between 6 and 24h
SN-38 Glucuronide	SN-38	Phenol Glucuronide Hydrolysis	• Reaction occurs in 6 h and continued over 24h • No reaction in microbiota free incubation
Raloxifene 4'-Glucuronide	Raloxifene	Phenol Glucuronide Hydrolysis	• Reaction occurs in 6 h and continued over 24h • No reaction in microbiota free incubation
Raloxifene 6-Glucuronide	Raloxifene	Phenol Glucuronide Hydrolysis	• Reaction occurs in 6 h and continued over 24h • No reaction in microbiota free incubation

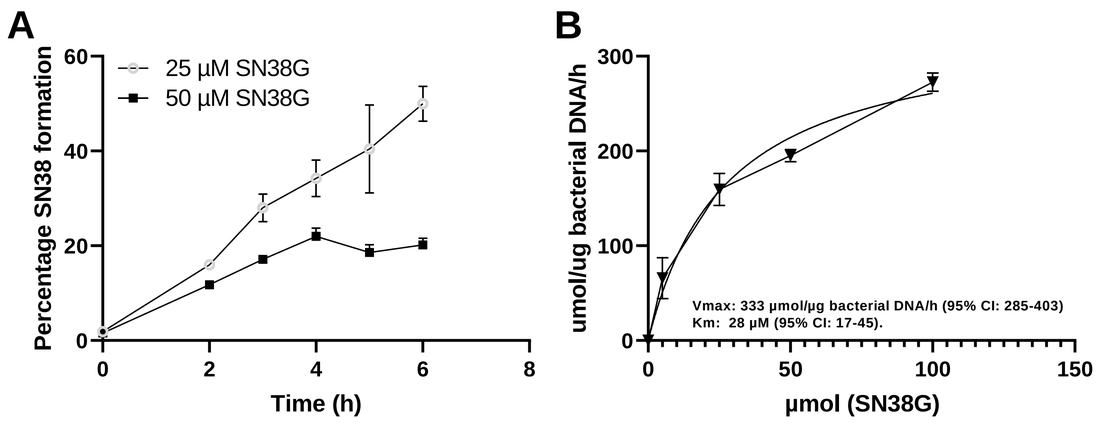

***Figure 3. (A)** Metabolic fate of SN38 glucuronide in ex vivo fermentation platform over time (applying 10-fold diluted microbiome stocks, **(B)** Concentration dependent metabolic conversion of SN38G to SN38 by human microbiota (3h of incubation with 10-fold diluted microbiome stocks).*

CONCLUSIONS

- Enterohepatic circulation (EHC) of drugs involves the following processes: metabolite formation (generally in the liver), secretion into the bile, then back-converted in the GI-tract and (potentially) re-absorbed.
- All tested drug metabolites showed microbiota-based conversion to parent drug within 6h, demonstrating different reaction types including phenol sulfate hydrolysis, phenol glucuronide hydrolysis, acyl glucuronide hydrolysis, carbamoyl glucuronide hydrolysis, and N-oxide reduction.
- Deconjugation of SN38G into SN38 was linear up to 4h of incubation, with an average Vmax of 333 µmol/µg bacterial DNA/h (95% confidence interval (CI): 285-403) and average Km of 28 µM (95% CI: 17-45).
- The i-screen platform can serve as an *in vitro* system to quantify metabolic conversion of drugs by gut microbiota.

FUTURE PERSPECTIVES

- Investigating inter-individual differences in colonic metabolism (personalized medicine).
- Modulation of the microbiome to influence these metabolic processes offers interesting new treatment options.
- Combining data from different in vitro and ex vivo platforms to predict and model EHC by physiologically-based pharmacokinetic modeling.