Poster Number

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Development of drug metabolism method by human microbiome

Kosuke Nagasaka, Koichi Shiga and Kazunobu Aoyama

DMPK Business Unit, Axcelead Drug Discovery Partners, Inc.

Purpose

Recently, the microbiome has been reported to contribute to drug metabolism and may be involved in inter-individual differences in pharmacological responses. Although the microbiome-mediated deconjugation of glucuronides and sulfates has been revealed, the microbiome-mediated metabolism of parent compounds, such as hydrolysis and reduction, remains to be clarified. Additionally, there is no conventional culture method for anaerobic microbiome evaluation in drug metabolism, though several previous reports have evaluated microbiome-mediated drug metabolism under extremely anaerobic conditions, focusing on specific microbiome strains. Therefore, we propose a standard method for drug metabolism mediated by microbiome.

Materials and Methods

Microbiome Source: Microbiome samples were collected from the feces of five human donors. Each sample was suspended in PBS containing 20% glycerol at a ratio of 1:2.5 and cryopreserved. In preliminary experiments, fresh fecal samples were also collected from C57BL/6 mice.

Assay Conditions: To evaluate the effects of freezing conditions and inter-donor variability, assays were conducted using mGAM medium, which has been reported to support microbiome-mediated drug metabolism. Anaerobic conditions were maintained using AnaeroPack, which generates a low-oxygen environment with approximately 15% CO_2 at 37°C. Drug metabolism was compared across different media, including mGAM, PBS, BHI, and thioglycolate medium.

Compounds and Analytical Methods:Test compounds were prepared at a final concentration of 10 μmol/L in 1% DMSO. Samples were incubated for up to 24 hours. After incubation, the remaining parent compounds and metabolites were quantitated using LC-MS/MS to calculate residual ratios and identify metabolic products.

Question about metabolism evaluation by microbiome…

- How to obtain fecal sample?
- Anaerobic chamber is necessary? How strict?
- How to address donor differences?
- How to select the best medium?
- Impact on human PK?

Results

Fig. 1 Atmospheric effect on drug metabolism

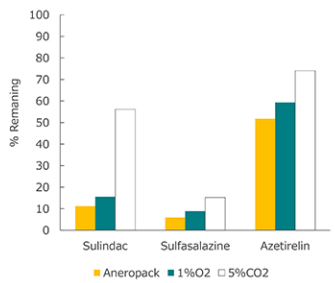


Fig. 2 Inter-donor differences in drug metabolism

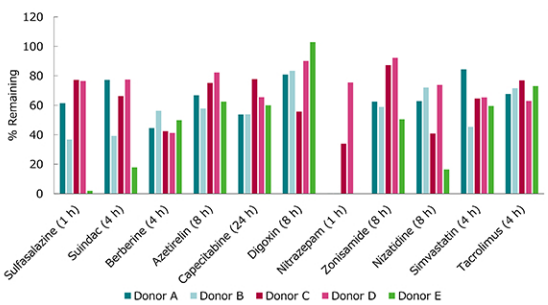
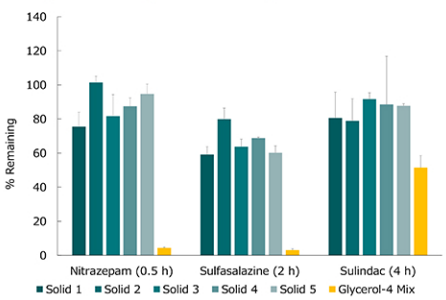


Fig.1) Three atmospheric conditions were compared using mouse feces suspended in PBS, with incubation for 4 hours (Sulindac and Azetirelin) or 0.5 hour (Sulfasalazine), n=2.
Fig.2) Inter-donor differences among five human donors were evaluated under anaerobic conditions established using AnaeroPack.(9.1% feces, n=2).

Fig. 3 Freezing effect on drug metabolism



Solid: frozen in solid state (Medix Biochemica)
Glycerol-4Mix: frozen in suspension with 2.5-fold 20%Glycerol-PBS, 4 donors (CTI Biotech)

Fig. 4 Assay medium effect on drug metabolism

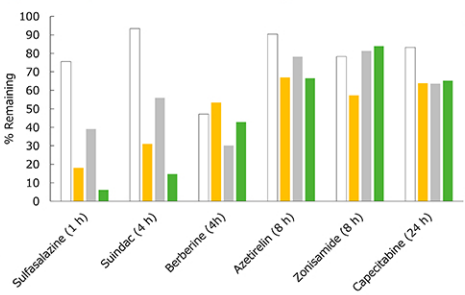


Fig.3) Freezing conditions were compared using human fecal suspensions in mGAM containing 10 μM of test compounds. (9.1% feces, Mean+SD, n=3).
Fig.4) The effect of assay media was tested at 10 μM compounds using PBS and three different media under anaerobic conditions.(9.1% feces, n=2)
Table.1) Metabolite detection using mGAM sample (n=1) at 10 or 100 μM of test compounds.

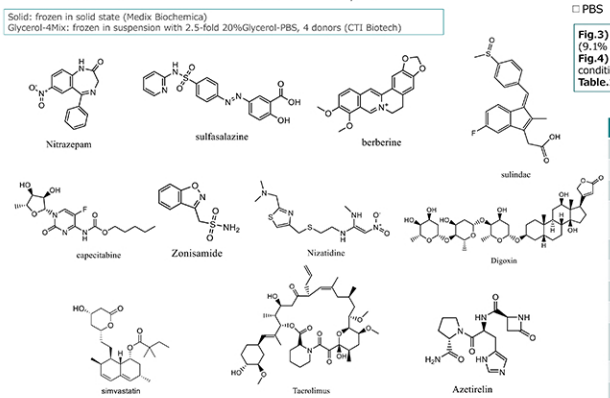


Table 1 Metabolites formed by microbiome

	Estimated reaction Site	Detected metabolites
Nitrazepam	Nitro	Nitro reduction ($-NO_2 \Rightarrow -NH_2$) (+30)
Sulfasalazine	Azo	Azo reduction
Berberine	Tertiary ammonium	+58 (unreported metabolite)
Sulindac	Sulfoxide	Sulfoxide reduction (-16) Oxidation (+16)
Capecitabine	Glycoside	Deglycosylation (-116)
Zonisamide	Isoxazole	Isoxazole cleavage (+3)
Nizatidine	Nitro	Nitro reduction ($-NO_2 \Rightarrow$=NOH)(-16)
Digoxin	Cyclic Alkene	Not detected
Simvastatin	Lactone	Hydroxylation or lactone cleavage (+18) Dehydroxylation(-18)
Tacrolimus	Ketone	Reduction (+2)
Azetirelin	Amide	Not detected (unreported)

Discussion

Fig.1: Oxygen concentration control was important for drug metabolism by microbiome.

Fig.2: Substantial inter-donor variability highlights the importance of donor selection when evaluating microbiome-mediated drug metabolism.
The donor sample with the least metabolizing activity (Donor D) was excluded from the 5-donor samples. The 4-donor pooled sample was used in the following assays.

Fig.3: Glycerol-PBS stock maintains drug metabolism activity.

Fig.4: Both mGAM and thioglycolate media have been shown to be useful for evaluating drug metabolism under anaerobic conditions. The presence of reducing agents in mGAM and thioglycolate, unlike in BHI, may account for the observed differences among the media, possibly due to decreased levels of dissolved oxygen.

Conclusion

We have developed a simple method to evaluate drug metabolism mediated by human microbiome.
We believe that this method can serve as the standard for evaluating microbiome-mediated drug metabolism.

COI disclosure information

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