Association between MPA Pharmacokinetics and in-vitro Mycophenolic Glucuronide turnover by gut microbiota of Kidney transplant recipients

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INTRODUCTION

- Mycophenolate mofetil (MMF) is used in >90% of kidney transplant recipients (KTRs).
- Its inactive metabolite, MPAG, is de-glucuronidated by bacterial beta-glucuronidase (BGUS) in the gut and the active metabolite MPA is reabsorbed back into the blood in a process know as enterohepatic recirculation (EHR).
- EHR leads to a secondary MPA peak, increasing MPA blood concentrations, enhancing immunosuppression and possibly toxicity in KTRs.

HYPOTHESIS

- We hypothesized that KTRs with extensive EHR in-vivo would have a gut microbiome with higher MPAG to MPA conversion and performed an anaerobic, in-vitro assay of MPAG conversion to MPA by the stool microbiome.

METHODS

- Participants underwent a pharmacokinetic (PK) study and microbiome stool collection post-kidney-transplant in the Microbiome and Immunosuppression in Kidney Transplantation (MISSION) study.
- Stool samples were exposed to 100ug/mL MPAG diluted in 7mL of Yeast extracts-Casein hydrolysate-fatty acids (YCFA) medium broth under anaerobic conditions in triplicate. The resulting mixture was incubated under anaerobic conditions with aliquots collected at 0, 0.5, 1,1.5 and 2 hours (Fig. 1).
- Aliquots were stored at -80ºC prior to assessing MPA concentrations in triplicate using a mass spectrometry validated assay.
- The V4 region of the 16S rRNA gene was amplified and sequenced on the Illumina MiSeq platform, then analyzed using the QIIME2 pipeline.

RESULTS

Table 1. Demographic characteristics of study participants

Demographics and Pharmacokinetic data (N=9)	
Age at transplant, mean (sd)	55.9 (16.0)
Male , n (%)	6 (67%)
Black or African American, n(%)	3 (33%)
BMI, mean (sd)	27.9 (4.2)
Living Donor, n(%)	5 (55%)
MPA AUC, mean (sd)	0.07 (0.02)
MPA % EHR, mean (sd)	38.0 (0.07)

*The MPA % EHR was defined as MPA AUC5-12 hour / AUC0-12 hour x 100.

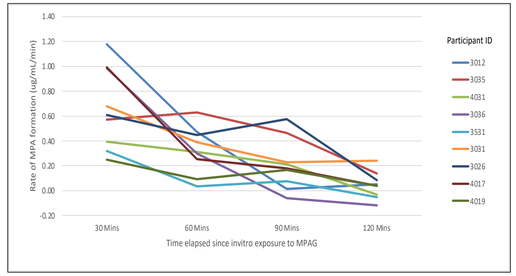

Figure 1: Rate of MPA formation after exposure to MPAG in-vitro. The rate of MPA formation was calculated as (C*i*-C*o*)/(T*i*-T*o*), where *i* represent the timepoint of interest and *o* represents baseline.

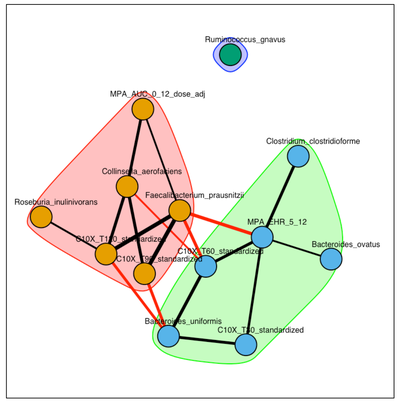

Figure 2: Correlation network map between in-vivo MPA pharmacokinetics (MPA EHR and AUC), in-vitro MPA turnover rates and the relative abundance of pre-specified beta-glucuronidase (BGUS) producing bacteria. The color nodes (orange vs blue vs green) indicate group membership to a specific cluster based on the correlation network. A black line indicates a correlation with another node within the cluster, whereas a red line indicates a correlation with a node outside of the cluster.

Table 2. Correlation analysis between in-vivo PK data and in-vitro MPA assay

	MPA dose adjusted AUC (0-12hours)	MPA %EHR (5-12hours)
Stool MPA concentration at T0	*0.85	0.40
In vitro MPA formation rate at 30 mins	-0.05	-0.36
In vitro MPA formation rate at 60 mins	-0.20	-0.43
In vitro MPA formation rate at 90 mins	-0.20	-0.21
In vitro MPA formation rate at 120 mins	-0.21	-0.19

*The p-value for the Pearson correlation coefficient was < 0.05

- The patient demographics and PK data used in this experiment are shown in Table 1.
- The rate of MPA formation was calculated as (C*i*-C*o*)/(T*i*-T*o*), where *i* represent the timepoint of interest and *o* represents baseline. The rate of MPA formation was highest in the first 30 minutes of the assay and steadily decreased until the 120 mins timepoint (Fig 1).
- We implemented the Louvain Modularity Maximization (LMM) algorithm for community detection, focusing on the correlation between in-vitro MPA levels, MPA AUC, MPA EHR (defined as MPA AUC5-12 hour / AUC0-12 hour x 100) and the relative abundance of BGUS producing bacteria present in more than 10% of the samples (Threshold R=0.3).
- A Pearson correlation analysis between in-vitro MPA levels and PK data showed a positive correlation between MPA AUC and stool MPA concentrations at T (R = 0.85, p-value < 0.05, Table 2).
- Further correlation network analyses suggested that the relative abundance of *B. uniformis*, *B. ovatus* and and *C. clostridioforme* was correlated with invitro MPA formation rate over 30 and 60 minutes, as well as MPA EHR (FIG 2).

CONCLUSIONS

- Our preliminary findings suggest that the relative abundance of specific BGUS producing bacteria are correlated with the rate of MPA formation in an in-vitro setting.
- These data may have implications for the impact of the gut microbiome composition on MPA EHR for KTRs and should be replicated in larger experiments.

Acknowledgements: This study was supported in part by NIH/NIAID grant **5R01AI140303,** the Hennepin Healthcare Research Institute grant, and the Center for Chronic Disease Reduction and Equity Promotion Across Minnesota under Award Number **P50MD017342**