Beta-Glucuronidase activity of gut microbiome and enterohepatic recirculation of mycophenolic acid in kidney transplant recipients

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INTRODUCTION

- The gastrointestinal (GI) system hosts a wide variety of gut microbiota that exhibit enzymatic activities related to the biotransformation of xenobiotic compounds. This includes pharmaceutical products which can affect human health.
- Mycophenolate mofetil (MMF) is an important immunosuppressant commonly prescribed post organ transplantation (tx). Once ingested, MMF is bioactivated by the esterase enzymes in the GI tract and liver into mycophenolic acid (MPA). The therapeutic index of this drug is very narrow, and the concentration of the active component mycophenolic acid (MPA) is highly variable. Glucuronic acid is added to MPA forming mycophenolate glucuronide (MPAG) which has greater solubility than MPA allowing MPAG to be eliminated by biliary excretion.
- This active form undergoes extensive enterohepatic recirculation (EHR) via bacterial β-glucuronidase (BGUS) enzymes which reactivate MPA from MPAG within the GI tract. BGUS enzymes remove the glucuronide from MPAG to create MPA, which in turn is recirculated. MMF causes severe gastrointestinal toxicity in about 45% of recipients, so dosage is carefully controlled and can be affected by this enterohepatic recirculation.
- Previous research shows that there could be an association between microbiome diversity in the stool bacteria, and MPA enterohepatic recycling.
- Previous research has also shown that certain bacteria species commonly found in the gut microbiome express BGUS enzymes and could potentially be linked to the variability of MPA concentration.

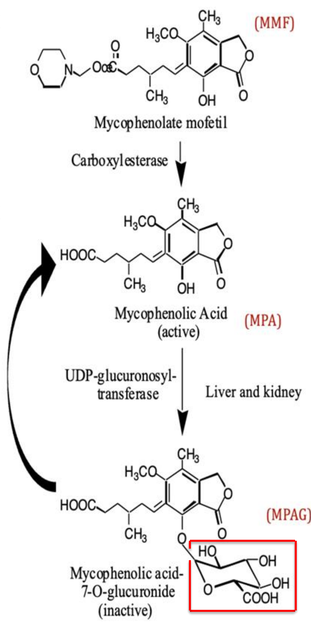

Figure 1: Metabolic pathway of Mycophenolate (MMF). Glucuronide moiety in red box.

METHODS

MISSION Study

- 84 patients who had undergone kidney transplant received MMF via their treatment plan and stool samples were collected at various time points for microbiome research
- 47 patients were in the cross-sectional cohort and 37 were in the prospective cohort
- For the prospective cohort, samples were collected at 1 week post-transplant, at time of MMF PK, 4 and 6 months post-tx. Participants were enrolled at time of transplant between 2020-2023. For the cross-sectional cohort, samples were collected at the time of MMF PK only. Participants were those who received a kidney transplant between January 1, 2012 and 2019
- Stool samples were collected from each patient and sequenced
- A 12-hour pharmacokinetics analysis was performed on the samples from both groups to determine drug concentrations
- Enterohepatic recirculation (EHR) was calculated via the area under the curve (AUC) as the ratio of partial AUC 5 to 12hr to total AUC 0 to 12hr.

Patient Samples

- Stool samples from 3 patients were taken from freezer and split into 2 groups: autoclaved for 1 hour and non-autoclaved (left in the fridge)
- BGUS activity was then assessed using an Abcam kit

Bioinformatics

- Microbiome shotgun data were processed with HUMAnN3 and differentially abundant taxa associated with EHR adjusted for cohort were identified with MaAsLin2. We further adjusted for age, sex, diet and standardized creatinine clearance using zero inflated Poisson regression. In an exploratory analysis stratified by cohort, we compared the relative abundances of 12 known BGUS producers across EHR levels using the Wilcoxon sum rank test.

Results: MISSION STUDY

Mission Study PK Cohorts Descriptive Statistics

Variable	N (CS/PS)	CS	PS	p-value
Demographics				
Male, n(%)	47/37	36 (76.6)	25 (67.57)	0.3569
White, n(%)	47/37	33 (70.21)	26 (70.27)	0.6941
Hispanic or Latino, n(%)	47/37	2 (4.26)	4 (10.81)	0.6139
Age at PK in years, mean(sd)	47/37	56.85 (12.81)	53.49 (14.15)	0.257
Weight at PK in kg, mean(sd)	47/37	87.94 (20.67)	86.36 (16.64)	0.706
Height at PK in cm, mean(sd)	47/37	172.91 (10.79)	171.88 (11.14)	0.6706
Medications				
PPI use, n(%)	47/37	5 (10.67)	11 (29.73)	0.027
Antifungal use, n(%)	47/37	1 (2.13)	3 (8.11)	0.2013
Antibiotic use, n(%)	47/37	26 (55.32)	34 (91.89)	0.0002
Antiviral use, n(%)	47/37	3 (6.38)	36 (97.3)	<0.001
Steroid use, n(%)	47/37	7 (14.89)	22 (59.46)	<0.001
Lab Values				
eGFR (ml/min/1.73m2), mean(sd)	47/37	60.95 (11.66)	54.94 (10.95)	0.0184
eGFR, Epi_RF_Cr2021, mean(sd)	47/37	69.79 (18.18)	59.21 (15.31)	0.0058
cbc_hgb, mean(sd)	47/37	13.48 (1.171)	11.53 (1.73)	<0.001
cbc_hct, mean(sd)	47/37	41.61 (4.74)	36.15 (5.32)	<0.001
Dietary Values				
Energy in kcal, mean(sd)	41/24	1939.99 (747.23)	2077.14 (594.91)	0.4458
Total fat in grams, mean(sd)	41/24	83.51 (46.17)	92.72 (32.44)	0.3936
Total carbohydrates in grams, mean(sd)	41/24	229.18 (121.76)	224.77 (78.22)	0.8596
Total protein in grams, mean(sd)	41/24	72.53 (32.03)	91.41 (29.02)	0.0208
Total dietary fiber in grams, mean(sd)	41/24	17.12 (9.49)	16.09 (6.47)	0.6388
HEI 2015 total score, mean(sd)	41/24	47.95 (10.27)	48.99 (10.99)	0.7041

Table 1. Mission study PK cohorts descriptive statistics. Outlines the demographics, medications, lab values, and dietary values of all 84 participants in both the cross sectional (CS) as well as prospective (PS) cohorts. P-values were calculated based on the difference in values between the cohorts.

Variability in MPA Enterohepatic Recirculation Among KTRs

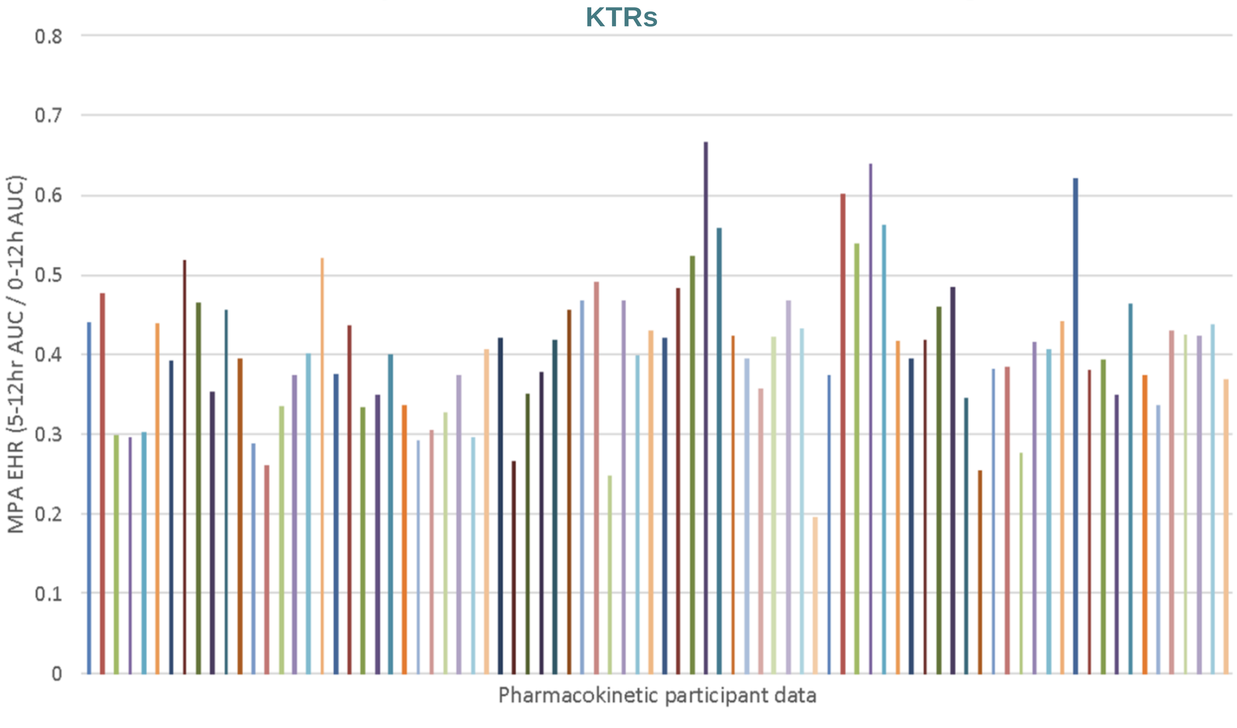

Figure 2. Enterohepatic recirculation variability among KTRs. Enterohepatic recirculation (EHR) calculated by the area under the curve (AUC) of MPA concentration for hours 5-12 and hours 0-12.

Significant Taxa after adjusting for MPA EHR and Cohort

Bacterial taxa (species level)	Prevalence	p-value
Faecalibacillus intestinalis	13.1%	<0.05
Ruminococcus bromii	16.7%	<0.05
Blautia obeum	63.1%	<0.05
Parabacteroides distasonis	36.9%	<0.05
Gordonibacter pamelaeae	23.8%	<0.05
Acidaminococcus intestini	11.9%	<0.05

Table 2. Significant bacterial taxa present in stool microbiome samples from KTRs. Statistically significant associations between species-level taxa and PK metrics were found after adjusting for MPA EHR as well as cohort covariates.

Amount of BGUS Activity Active vs. Inactive Bacteria

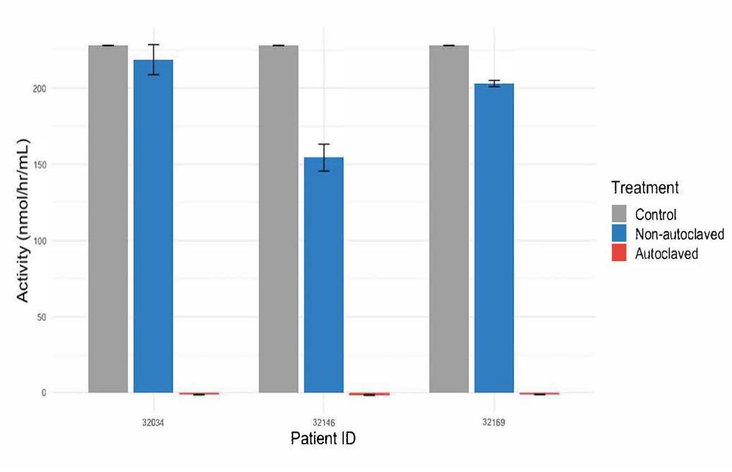

Figure 3. In-vitro BGUS activity for active and inactive gut bacteria. BGUS activity is decreased in bacterially inactive (red) stool samples compared to active (blue) samples. Positive control (gray) was provided in the kit. Participants underwent a pharmacokinetic (PK) study and microbiome stool collection between 30-60 days post-kidney-transplant, N = 3.

Conclusions

Conclusions:

- There is extensive variation in the amount of EHR within kidney transplant patients, potentially due to variability in the gut microbiome
- Based on microbiome data, there exists an association between certain species-level bacterial taxa and PK parameters
- BGUS activity is much lower after bacteria are inactivated

Future Directions:

- Run a panel of 8 selected model organisms to examine BGUS activity
- Analyze stored model organism aliquots with mass spectrometry validated assay to confirm MPAG to MPA metabolism

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