Evidence of Microbiome–Drug Interaction between the Antimalarial Lumefantrine and Gut Microbiota in Mice

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Abstract. The antimalarial drug lumefantrine exhibits erratic pharmacokinetics. Intersubject variability might be attributed, in part, to differences in gut microbiome–mediated drug metabolism. We assessed lumefantrine disposition in healthy mice stratified by enterotype to explore associations between the gut microbiota and lumefantrine pharmacokinetics. Gut microbiota enterotypes were classified according to abundance and diversity indices from 16S rRNA sequencing. Pharmacokinetic parameters were computed using noncompartmental analysis. Two distinct enterotypes were identified. Maximal concentration (Cmax) and total drug exposure measured as the area under the drug concentration–time curve (AUC0–24) differed significantly between the groups. The mean and standard deviation of Cmax were 660 ± 220 ng/mL versus 390 ± 59 ng/mL (P = 0.02), and AUC0–24 was 9,600 ± 2,800 versus 5,800 ± 810 ng × h/mL (P = 0.01). In healthy mice intragastrically dosed with the antimalarial drug lumefantrine in combination with artemether, lumefantrine exposure was associated with gut bacterial community structure. Studies of xenobiotic–microbiota interactions can inform drug posology and elucidate mechanisms of drug disposition.

INTRODUCTION

Pharmacokinetics (PK) of some orally administered medications are partially modulated by commensal organisms in the gut via microbial expression of drug-metabolizing enzymes, production of interfering substrates, or effects on drug absorption.1-4 The antimalarial drug lumefantrine (LF), an arylaminolcohol, exhibits highly variable interindividual PK with up to 16-fold differences in exposure.5 Variation is due in part to irregular absorption which is known to be mitigated by coadministration with fatty food. We hypothesized that the intestinal microbiome may play an additional, in part, to differences in gut microbiome–mediated drug metabolism. We assessed lumefantrine disposition in healthy mice stratified by enterotype to explore associations between the gut microbiota and lumefantrine pharmacokinetics. Gut microbiota enterotypes were classified according to abundance and diversity indices from 16S rRNA sequencing. Pharmacokinetic parameters were computed using noncompartmental analysis. Two distinct enterotypes were identified. Maximal concentration (Cmax) and total drug exposure measured as the area under the drug concentration–time curve (AUC0–24) differed significantly between the groups. The mean and standard deviation of Cmax were 660 ± 220 ng/mL versus 390 ± 59 ng/mL (P = 0.02), and AUC0–24 was 9,600 ± 2,800 versus 5,800 ± 810 ng × h/mL (P = 0.01). In healthy mice intragastrically dosed with the antimalarial drug lumefantrine in combination with artemether, lumefantrine exposure was associated with gut bacterial community structure. Studies of xenobiotic–microbiota interactions can inform drug posology and elucidate mechanisms of drug disposition.

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WinNonlin (Certara, Princeton, NJ), and statistical analyses were carried out using Stata 14 (StataCorp, College Station, TX).

Mice from vendors E and C had significantly higher taxonomic abundance than those from J and T (Figure 1A), and weighted UniFrac $\beta$ diversity was lowest (i.e., similarity greatest) for E and C pairs, and J and T pairs (Figure 1B). The dendrogram of the UniFrac distance matrix revealed hierarchical clustering of the same pairs (E and C, J and T), overall conforming to two distinct fecal enterotypes. Mice from cohorts E and C had greater LF exposure than those from cohorts J and T (Figure 2). Means and standard deviations (SDs) of $C_{\text{max}}$ were 660 ± 220 ng/mL in cohorts E and C compared with 390 ± 59 ng/mL in cohorts J and T ($P = 0.02$), and the estimated $\text{AUC}_{0-24}$ was 9,600 ± 2,800 compared with 5,800 ± 810 ng × h/mL, respectively ($P = 0.01$).

We measured the disposition of intragastrically dosed LF in isogenic mice with structurally distinct enterotypes and identified associations between drug PK and the gut microbial community structure. These findings support a possible contribution of the gut microbiota to the high intersubject variability frequently observed in PK studies of LF.

Direct and indirect gut microbiota effects on the absorption, distribution, metabolism, and/or elimination of LF may account for the observed differences in drug exposure. The excretion of LF relies on glucuronidation which is potentially impacted by bacterial commensal secretion of $\beta$-glucuronidases, and the drug’s chemical structure renders it sensitive to

**Figure 1.** Computational analysis of microbiota data reveals partitioning of Envigo (E) with Charles River (C) cohorts and Jackson Laboratory (J) with Taconic (T) cohorts into distinct clusters. (A) Alpha diversity of mice measured by observed operational taxonomic units (OTU). (B) Beta diversity comparisons between mice from different vendors measured by weighted UniFrac, with comparisons between enterotype clusters. Data are means and standard errors cumulative of two experiments for $n = 5-6$ mice per cohort. Data were analyzed by one-way analysis of variance with Tukey’s multiple comparisons test. (C) Dendrogram of the weighted UniFrac distance matrix reveals hierarchical clustering of E/C and J/T cohorts at the second and third bifurcations. Leaves represent individual mice. Clustering was independent of cage assignment. $^*P < 0.05$, $^{**}P < 0.01$, and $^{****}P < 0.0001$. ns = not significant.

**Figure 2.** Lumefantrine pharmacokinetics are associated with gut microbiota composition in healthy mice. Mice were administered lumefantrine (150 µg/g) in combination with artemether (25 µg/g) by gavage at time 0. (A) Concentration–time profiles stratified by enterotype. (B and C) Mean and standard deviation peak concentrations ($C_{\text{max}}$) and areas under the drug concentration–time curve ($\text{AUC}_{0-24}$) were significantly higher in the Envigo (E) and Charles River (C) cohorts than those in the Jackson Laboratory (J) and Taconic (T) cohorts. $\text{AUC}_{0-24}$ was measured using sparse data methods, therefore individual subject data are not computed. $^*P \leq 0.02$. 

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bacterial lyases that cleave carbon–nitrogen bonds.\textsuperscript{5,11,12} The biomass of the intestinal microbiota may make a discernible contribution to drug transit or sequestration, constituting a compartment into which small molecules might distribute. Indirect effects of gut commensals on drug transporter expression or luminal pH have been hypothesized to impede or facilitate absorption.\textsuperscript{4} Limitations to this study include the relative paucity of PK time points which precluded reliable estimations of oral clearance or apparent volume of distribution, or the evaluation of the interaction between enterotype and individual PK parameters.

Acute and chronic malnutrition are common in patients with malaria, and the accompanying alterations to gut microbial communities\textsuperscript{13,14} may affect drug PK. Antimalarial drug PK and pharmacodynamics differ between healthy and infected individuals\textsuperscript{5}; gut microbiome differences are one potential contributor.

The characterization of gut microbial effects on PK of orally administered agents could inform drug posology and improve models of drug disposition by helping to account for interindividual variability. Future experiments that incorporate gnotobiotic controls and fecal transplantation, and studies in human subjects, could go further toward establishing causal and clinically relevant linkages between the gut microbiome and drug PK.

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