RESEARCH ARTICLE

Gastrointestinal transit mathematical model in mice treated with antibiotics

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ABSTRACT

Fecal pharmacokinetics is crucial in developing treatment design and evaluating gastrointestinal motility; however, it has not been yet elucidated. This study aimed to elucidate the fecal pharmacokinetics in mice orally administered vancomycin and establish a pharmacokinetic model with interpretable system parameters. In this study, we quantified the antibiotic concentrations in fecal samples collected at high frequency from C57BL/6J mice treated with single oral doses of low and high (1 and 20 mg/mL) concentrations of vancomycin. Samples were taken at approximately 4-hour intervals after administration of antibiotics, making it possible to track the dynamics of vancomycin in the feces with high resolution. Mice structurally pool contents in the stomach and cecum, so we constructed an intestinal transit model that compartmentalizes these organs. Two models were built based on the functional form of gastric content elimination, and physiological parameters such as gastric emptying and intestinal transit time were estimated using high-resolution actual data from each mouse. Fortunately, both models were suitable for evaluating the antibiotic concentrations in feces. By simulation, we confirmed that our estimates of model parameters, which are quite difficult to measure experimentally, are satisfactory. Importantly, this study is applicable to fundamental research relating to pharmacokinetics in the gastrointestinal tract.

NEW & NOTEWORTHY

This study tracked the pharmacokinetics of orally administered vancomycin by measuring its concentration in feces and described it using a mathematical model based on the physiological

characteristics of mice to replicate these dynamics. As a predictive model, it allows for estimation of drug dynamics outside of the sampling time and extrapolation to individuals with different physiological characteristics.

Keywords: Fecal pharmacokinetics; Antibiotics; Compartment model; Solid phase extraction

INTRODUCTION

The process by which orally administered substances pass through the gastrointestinal (GI) tract towards their defecation as excrements has been comprehensively analyzed using various measurement techniques. Particularly, GI tract pharmacokinetics are considered to be critical in the oral administration of solid drugs (1) and have provided optimal data towards therapeutic interventions. While utilizing antibiotics to target gut bacteria, treatment interventions can be established by measuring the drug in feces as an estimate for the gut concentrations (2). Glycopeptide antibiotic vancomycin is widely utilized for mitigating infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) (3), and its concentration in the feces has implications in optimal therapeutic design as it is fairly absorbed by the human GI tract and mainly excreted via defecation (2, 4). Fecal vancomycin concentrations and frequency of defecation have been measured in patients with *Clostridium difficile* infection, and the relationship between dose and actual drug concentration in the body has been discussed (2, 5). Clarification of pharmacokinetics in the GI tract, including the time the gut is exposed to the antibiotic and concentration transitions in the GI tract, is important in determining the optimal dose and duration of administration in therapy that recovers and maintains the gut homeostasis.

Pharmacokinetics in plasma has been described by using physiologically based pharmacokinetic (PBPK) models, which have provided useful information for the design of preclinical or clinical trials for decision making in drug discovery (6). PBPK models describe pharmacokinetics in the body as organ-specific distribution and loss, and therefore provide useful parameters for understanding the kinetic mechanisms underlying observed drug concentrations (7). In addition, PBPK modeling can be extrapolated across dose levels, formulations, and species (8, 9).

Nevertheless, no study has provided mathematical models describing fecal concentrations of drugs, even those with simple kinetics, which are not absorbed in the GI tract. Previously, pharmacokinetic parameters have been evaluated from longitudinal data on fecal drug concentrations using nonparametric methods (10, 11). While these analyses are suitable for describing individual data, they cannot be extrapolated to individuals with different physiological conditions (12) and lack general insight into digestive tract motility.

In this study, excrement samples of mice orally administered with vancomycin were collected severally at hour intervals to analyze the antibiotic concentration in the excrements. The purpose of this study is to model temporal variations in antibiotic concentrations in feces with physiologically interpretable parameters.

MATERIALS AND METHODS

Reagents

Vancomycin hydrochloride (VMC) was purchased from FUJIFILM Wako Pure Chemical Corporation (Biochemistry grade, Osaka, Japan). Oasis HLB 96-well plate (60mg) used as solid phase extraction (SPE) was purchased from Waters (Milford, MA, USA). Distilled water was obtained from Millipore Milli-Q water-purification system (MSD K.K., Tokyo, Japan). Formic acid (>98%, LC/MS grade) was purchased from MSD K.K. (Tokyo, Japan). Acetonitrile (LC/MS grade) was purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). An EDTA-Na2 solution (0.5 M) was purchased from Merck (Darmstadt, Germany). An EDTA extraction solution was prepared by mixing pH 4.0 citrate buffer and acetonitrile at 55:45 [v/v] and adding 0.5 M of the EDTA-Na2 solution to a final concentration of 0.2% [v/v].

Mice and fecal sample collection

All experiments were performed with 8-weeks old C57BL/6J male specific pathogen free mice, purchased from CLEA Japan. All mice were kept in isolated cages and darkened with plastic sheets at night in a Laboratory Animal Center, Keio University School of Medicine. Mice were provided with food (CL-2, CLEA Japan, Inc.) and water ad libitum. The mouse experiment consisted of three treatment groups of three mice each, depending on the concentration of VMC administered at 12:00 on day 3 (the start of the experiment is set as day 0). The first was the control experimental group (C1, C2, C3), which received only water orally on day 3. The other two groups were the low (L1, L2 and L3) and high (H1, H2 and H3) concentration groups, and these mice received 0.5 mL of VMC dissolved in water at 1 and 20 mg/mL orally on day 3, respectively. Fecal samples were collected every 4h at 12:00, 16:00, 20:00 in day 0, at 0:00, 4:00, 8:00, 12:00, 16:00, 20:00 in day 1 to day 6, at 0:00, 4:00, 8:00, 12:00, 16:00 in day 7, and thereafter at 12:00 every 1 to 7 days until day 39. The samples were snap-frozen in liquid nitrogen prior to storage at -80°C.

Quantification of antibiotics

Preprocessing

Experimental conditions for pretreatment and solid-phase extraction (SPE) followed the study of Opris et al.(13). A piece of weighed fecal sample was dipped in 0.5 mL of the EDTA extraction solution(14), and the feces was ground with pellet pestle homogenizers in microtubes. Then, ultrasonic extraction was performed at 45°C, 40kHz for 10 min, followed by centrifugation at 15,000 rpm for 3 min. The supernatant was collected and diluted to 4 mL with pH 4.0 formic acid buffer.

Solid phase extraction

The HLB cartridge was preconditioned by passing 2 mL of methanol, followed by 2 mL of distilled water. Thereafter, 4 mL of each sample was loaded 1 mL into the cartridge and rinsed with 2 mL of pH 4.0 formic acid buffer. The cartridge was then vacuum dried for 10 minutes, and eluted with 1 mL of 60% [v/v] methanol/water solution(15). Finally, the elution was filtered through a 0.22 μm syringe filter (PVDF membrane, Merck).

For the determination of VMC recovery using the SPE method, the 10, 20, 30, 40, and 50 mg of feces samples of control experimental group were spiked with 2, 4, 6, 8, and 10 μg of VMC prior to extraction, respectively. The VMC recovery was 91.5–138%.

Mass spectrometry

Each solution was analyzed via Liquid chromatography/tandem mass spectrometry (LC/MS/MS) using a Xevo TQD MS (Waters Corporation) coupled with an ACQUITY UPLC H-class (Waters Corporation). The chromatography analysis was performed using an ACQUITY UPLC HSS T3 Column (100 mm length × 3.0 mm i.d., 1.8 μm particle size, Waters Corporation.). The column was maintained at 40°C, and the flow rate and injection volume were 0.3 mL/min and 5 μL, respectively. Acetonitrile (mobile phase A), distilled water (mobile phase B), and distilled water containing 1% [v/v] formic acid (mobile phase C) was used as the mobile phase solutions. The initial compositions of mobile phases A, B, and C were 10, 89, and 1% [v/v/v], respectively, which were maintained for 2 min after the injection. Subsequently, the composition of mobile phase A was increased to 90% at 3 min and maintained for 5 min. Thereafter, it was decreased to 1% and maintained for 5 min to enable for equilibration. Mobile phase C was maintained at 10% for the gradient cycle. The analysis was performed in the positive-electrospray ionization mode. The MS ion-source parameters were as follows: source temperature, 120°C; desolvation temperature, 500°C; capillary voltage, 2.0 kV; desolvation gas flow, 1,000 L/h; cone gas flow, 50 L/h. The data acquisition was performed in the selected-reaction monitoring (SRM) mode. A mass transition ion pair, cone voltage (CV), and collision energy (CE) for the analyte were m/z 725.00 > 144.10, 30 V, and 30 eV, respectively. VMC quantification was performed by using the external calibration method. The calibration standards were prepared immediately before the analysis. The calibration curve exhibited good linearity in the standard concentration range of 0.01–1.0 μg/mL, and the r^2 value was over 0.995. The lowest concentration of the calibration curve was defined to be a quantification limit.

Calculation of concentration

The concentration of antibiotics in fecal samples was calculated by dividing the measurements obtained by using mass spectrometry with LC/MSMS by the wet weight of each fecal sample. The values are means of three replications ± standard deviations of each extraction.

Mathematical model

The intestinal transit model was designed to explain the antibiotic concentration in the feces. In the digestive activity of mice, contents are pooled in the stomach and the cecum. The rate of inflow from the stomach into the cecum and the rate of discharge from the cecum were described by a function using the antibiotic mass in each organ, and the antibiotic concentration in the feces was described as a function of time by solving differential equations given initial conditions.

The antibiotic masses in the stomach and cecum are $M_a(t)$ and $M_b(t)$, respectively. The model was constructed with the following two patterns, depending on how the antibiotic is eliminated in the stomach.

Model1

Model1 assumes that the antibiotic is eliminated from the stomach at a constant rate α [mg/hr] regardless of its concentration in the stomach. Then, the dynamics of the antibiotic mass in the stomach $M_a(t)$ is modeled as follows:

$$\frac{dM_a(t)}{dt} = -\alpha \#(1)$$

Assuming that all of the orally administered antibiotic q [mg] reaches the stomach at time $t = 0$. The initial condition is $M_a(0) = q$, and the differential equation is solved as follows:

$$M_a(t) = q - \alpha t \#(2)$$

Let Δt_1 be the time from when the contents leave the stomach to when they reach the cecum through the small intestine, then $t = \Delta t_1$ is the time for the antibiotics to appear in the cecum. Also, since t_p, the time it takes for all the antibiotic in the stomach to be eliminated, is $t_p = {}^{q}/_{\alpha}$ [hr], when $t \le \Delta t_1 + t_p$, the antibiotic is injected and eliminated simultaneously in the cecum, and when $t > \Delta t_1 + t_p$, only elimination occurs. Therefore, the antibiotic mass in the cecum $M_b(t)$ is as follows:

$$\frac{dM_b(t)}{dt} = \begin{cases} \alpha - k_b M_b(t), & 0 < t - \Delta t_1 \le t_p \\ -k_b M_b(t), & t - \Delta t_1 > t_p \end{cases} \#(3)$$

where k_b is the elimination rate constant. since $M_b(\Delta t_1) = 0$, the differential equation is solved as follows:

$$M_b(t) = \begin{cases} 0, & t - \Delta t_1 \le 0 \\ \frac{\alpha}{k_b}\{1 - \exp(-k_b(t - t_s))\}, & 0 < t - \Delta t_1 \le t_p \\ \frac{\alpha}{k_b}\{\exp(-k_b(t - t_s - t_p)) - \exp(-k_b(t - t_s))\}, & t - \Delta t_1 > t_p \end{cases} \#(4)$$

The value obtained by the experiment in this case is the concentration of antibiotic to feces. Therefore, in order to consider the antibiotic concentration in the digestive tract contents, we introduce a constant A and consider the following antibiotic concentration $C_b(t)$ in the cecum.

$$C_b(t) = \frac{1}{A} M_b(t) \#(5)$$

This parameter A can be interpreted as the mass of the contents of the cecum and can be biologically validated through parameter estimation. Let Δt_2 be the time between leaving the cecum and being eliminated as feces. If the antibiotic concentration in feces, $C(t)$, can be expressed in terms of the antibiotic concentration in the cecum before time Δt_2, then the equation can be expressed as follows:

$$C(t) = C_b(t - \Delta t_2) \#(6)$$

The time t_s for the oral dose to be eliminated as feces can be expressed as $t_s = \Delta t_1 + \Delta t_2$. Finally, the antibiotic concentration in feces can be calculated using the parameters α, k_b, t_s, and A as follows:

$$C(t) = \begin{cases} 0, & t - t_s \le 0 \\ \dfrac{\alpha}{Ak_b}\{1 - \exp(-k_b(t - t_s))\}, & 0 < t - t_s \le t_p \\ \dfrac{\alpha}{Ak_b}\left\{\exp\left(-k_b(t - t_s - t_p)\right) - \exp(-k_b(t - t_s))\right\}, & t - t_s > t_p \end{cases} \#(7)$$

Model2

In Model 2, the contents of the stomach are assumed to be continuously diluted with gastric juice and the antibiotic mass eliminated from the stomach is assumed to be exponentially decaying. If the rate constant for elimination is k_a, the dynamics of the antibiotic mass $M_a(t)$ in the stomach is modeled as follows:

$$\frac{dM_a(t)}{dt} = -k_a M_a(t) \#(8)$$

Assuming that all of the orally administered antibiotic q [mg] reaches the stomach at time $t = 0$. The initial condition is $M_a(0) = q$, and the differential equation is solved as follows:

$$M_a(t) = q\exp(-k_a t) \#(9)$$

The definition of the time related parameters Δt_1, Δt_2, t_p, and t_s is the same as in Model 1. After $t = \Delta t_1$, when the antibiotic reaches the cecum, injection and elimination always occur simultaneously in the cecum. Therefore, the antibiotic mass $M_b(t)$ in the cecum satisfies the following differential equation:

$$\frac{dM_b(t)}{dt} = k_a M_a(t) - k_b M_b(t) \#(10)$$

where k_b is the elimination rate constant from cecum. Since $M_b(\Delta t_1) = 0$, the differential equation is solved as follows:

$$M_b(t) = \begin{cases} 0, & t \le \Delta t_1 \\ \dfrac{k_a q}{k_b - k_a}\{\exp(-k_a(t - \Delta t_1)) - \exp(-k_b(t - \Delta t_1))\}, & t > \Delta t_1 \end{cases} \#(11)$$

where $T' \le t - \Delta t_1$. In the following, $C_b(t)$ and $C(t)$ are defined as in Model 1.

$$C_b(t) = \frac{1}{A} M_b(t) \#(12)$$

$$C(t) = C_b(t - \Delta t_2) \#(13)$$

As in Model 1, denoting $t_s = \Delta t_1 + \Delta t_2$, the final description of the antibiotic concentration in feces in Model 2 is as follows:

$$C(t) = \begin{cases} 0, & t \le t_s \\ \dfrac{k_a q}{A(k_b - k_a)}\{\exp(-k_a(t - \Delta t_1)) - \exp(-k_b(t - \Delta t_1))\}, & t > t_s \end{cases} \#(14)$$

Parameter estimation

Parameters were estimated for each time series data for each mouse. There are four estimated parameters for each of the two models, three of which are common except for the stomach rate constant. For estimation of best fit parameters, we minimized the following loss function, $L(\theta)$, which is given by the squared error of the data and a penalty term of errors in derivatives to control overfitting.

$$L(\theta) = \sum_{i=1}^{M} \left(y(t_i) - \hat{y}^{\theta}(t_i)\right)^2 + \lambda \sum_{i}^{M-1} \left(\frac{y(t_{i+1}) - y(t_i)}{t_{i+1} - t_i} - \left. \frac{d\hat{y}^{\theta}(t)}{dt} \right|_{t=t_i} \right)^2 \#(15)$$

where $y(t_i)$ is the actual measured antibiotic concentration at sampling time t_i and $\hat{y}^{\theta}(t_i)$ is the estimated antibiotic concentration at time t_i when estimated with parameter set θ, and λ is a penalty coefficient to be determined.

Since Model 1 and Model 2 differ only in the way they are eliminated from the stomach, we considered that other common parameters such as k_a, k_b, A and calculated pharmacokinetic parameter C_{max} should be estimated so that the variation in estimation between the models is small. Therefore, the penalty coefficient λ was selected from [0,0.0001,0.0005,0.001,0.005,0.01,0.05,0.1,0.5,1] to minimize the difference between the estimated values of the common parameters (k_a, k_b, A) and C_{max} calculated from them across two models.

Parameter fitting by the least squares method was performed using the curve_fit function defined in the Python scipy library. The fitting range was up to 7 days after antibiotic administration. The two models were evaluated by RMSE as defined below.

$$RMSE = \sqrt{\frac{1}{n}\sum_{i=1}^{n}\left(y(t_i) - \hat{y}(t_i)\right)^2} \#(16)$$

Evaluation of estimated parameters

We analyzed the extent of variation in the estimation results with respect to fluctuations in the observed data. The final evaluation of the estimated parameters for each mouse was performed by analyzing the distribution of the estimated parameters for several synthetically generated time series. The data sets were established for sampling and measurement errors of the actual experiment. The sampling times were extracted from a uniform distribution, considering an initial error of 30 min from the sampling time based on the experimental protocol. Measurements were extracted from a normal distribution with the mean and variance of the three actual calculations. A 100-time series were generated for each mouse, and parameter estimation was performed as well.

RESULTS

Pharmacokinetics of vancomycin in feces

Antibiotic concentrations were quantified in fecal samples obtained from mice treated with the antibiotic vancomycin. The experiment consists of three groups of three treatment group of mice each, depending on the concentration of vancomycin administered on day 3. The first was a control experimental group, which received solely water orally on day 3. While the others were the low (L1, L2 and L3) and high (H1, H2 and H3) concentration groups, which received 0.5mL of 1 mg/mL and 20 mg/mL vancomycin orally, respectively. Feces were sampled at 4h intervals until day 7, and thereafter every 1 to 7 days until day 40. Antibiotic concentrations in the feces were measured using fecal samples after administration of the antibiotic in the low and high concentration groups of mice (see Materials and Methods). The mean and standard deviation of the three measurements using LC-MS/MS per sample are shown in Figure 1. The highest concentration of antibiotics in feces sample of each mouse and the time index for concentration changes are shown in Table 1. The highest concentrations were recorded 8 to 30h after administration, followed by 4 to 12h in the low concentration group and 24 to 48h in the high concentration group prior the decrease below the quantification limit. The time T_{max} to reach the highest concentration and T_{elim} to fall below the antibiotic threshold tended to be slower in the high concentration group. The maximum concentration C_{max} in the high concentration group was 5 to 30 times higher than that in the low concentration group. Consequently, it was confirmed that the fecal concentrations were dose dependent.

Figure 1

Fecal concentration of vancomycin following oral administration to each mouse (L1-L3: mice of low concentration group, H1-H3: mice of high concentration group). Bars represent each mean and standard deviation of three measurements by LC-MS/MS.

Mathematical model

The concentration of antibiotics in the fecal sample is described by using a mathematical model. Oral absorption of drugs is usually composed of three factors as follows: dissolution of the drug in the GI tract, passage of the drug and dosage form, and penetration of the drug through the intestinal epithelium (16). The antibiotic vancomycin used in this study is abnormally absorbed in the GI tract, thus, vancomycin kinetics can be characterized solely by drug dissolution and passage through the GI tract of these factors. Figure 2A illustrates an intestinal transit model that describes the GI transit for vancomycin and its pharmacokinetics in feces. The stomach and cecum are the compartments of the GI tract as the mouse establishes a pouch-like structure with these two organs, and GI transit products are believed to be retained at these two points. The process of flow through each compartment is modeled by the velocity equation.

Two models were designed depending on the pattern of gastric content discharge. In model 1, we proposed that the antibiotic was transferred from the stomach to the cecum at a constant concentration, and the antibiotic concentration in the cecum was approximated using a first order rate equation. The concentration changes in the feces $C(t)$ are finally expressed by the following equation:

$$C_{model1}(t) = \begin{cases} 0, & t - t_s \le 0 \\ \frac{\alpha}{Ak_b}\{1 - \exp(-k_b(t - t_s))\}, & 0 < t - t_s \le t_p \\ \frac{\alpha}{Ak_b}\{\exp(-k_b(t - t_s - t_p)) - \exp(-k_b(t - t_s))\}, & t - t_s > t_p \end{cases} \#(17)$$

where the parameter α is the rate of elimination from the stomach, k_b is the elimination rate constant from the cecum, A represents the mass of the contents of the cecum, t_s represents the intestinal transit time, and t_p represents the time it takes for all the antibiotic in the stomach to be eliminated. The model parameters to be estimated are α, k_b, t_s, and A. The t_p [hr] is calculated from the antibiotic dose q [mg] and parameter α [mg/hr] with $t_p = q/\alpha$.

Model 2 demonstrates the flow of antibiotics as they gradually moving into the cecum relatively to their concentration in the stomach. The concentration variations in the feces $C(t)$ are also expressed by the following equation:

$$C_{model2}(t) = \begin{cases} 0, & t \le t_s \\ \frac{k_a q}{A(k_b - k_a)}\{\exp(-k_a(t - \Delta t_1)) - \exp(-k_b(t - \Delta t_1))\}, & t > t_s \end{cases} \#(18)$$

where the parameters k_b, t_s, A, q are similar as that of the model 1, and k_a is the elimination rate constant of the stomach. The model parameters to be estimated are k_a, k_b, t_s, and A. Figure 2 shows the outline of each model and the fitting results.

For model 1, the fitting error RMSE was smaller for L2, L3, H1 and H3, and for model 2, it was lesser for L1 and H2, and the fitted models differed among individuals.

Figure 2

Intestinal transit model. (A) Outline of 2 types of intestinal transit model. Black line represents function of antibiotic mass, and red line represents function of antibiotic concentration in each compartment. Error bars in the fecal compartment represent actual antibiotic concentrations in the feces. (B,C) Fitting result of model 1(B) and model 2(C). Error bars represent measured antibiotic concentrations in feces, red lines represent fitted results.

Estimated parameters

Estimation was performed on 100 time series data for each mouse, generated for sampling and measurement errors, and evaluated by analyzing the distribution data. The distribution of estimated parameters for each model and mouse is shown in Figure 3. The parameter of model 1, the gastric emptying rate α, showed less variation in the low concentration group mice than in the high concentration group; 0.09±0.02 [mg/hr] in the low concentration group and 0.85±0.39 [mg/hr] in the high concentration group. The respective discharge rate constants k_a and k_b for the stomach and cecum in Model 2 were similar, with values ranging from 0.1 to 0.5 [1/hr]. The elimination rate constant k_b, the intestinal transit time t_s, and cecum capacity A in both models 1 and 2 were estimated to be similar in

model 1 and model 2, with t_s ranging from 3 to 13 [hr] and cecum capacity, A, ranging from 0.2 to 1 [g] in all mice.

Figure 3

Estimated parameters of each model. This shows estimation results from parameter fitting on a 100 time series data generated from actual measurements. The box whiskers indicate the quartiles with the median and the minimum and maximum parameter values. The parameters k_b, t_s and A, which are common to each model, have similar values for each mouse in model 1 and model 2.

DISCUSSION

This is the first study to present the temporal variation of antibiotic concentrations in feces as a mathematical model. Following a detailed time series of fecal concentrations of vancomycin, the dynamics of the antibiotic in the GI tract was generally elucidated.

First, we measured the fecal concentration of antibiotic and found that the highest concentration (C_{max}) was obtained a few hours after administration in all antibiotic-treated mice, followed by an exponential decrease (Figure 1). The maximum and minimum concentration time of the antibiotic tended to be slower in the high concentration group (Table 1). In addition, the concentration of vancomycin in the feces was dose-dependent, which is consistent with the clinical and experimental reports in human (2) and mouse (17), respectively.

Applying a compartmental model design based on the structure of a mouse GI tract, the present study provides an insight into the temporal variation of antibiotic concentrations and GI motility in the GI tract, which is difficult to measure directly. Gastric content expulsion has been reported to depend on the physical properties of food (18) and food intake patterns (1). Two intestinal transit models were designed, depending on whether the rate at which antibiotics are excreted from the stomach is constant or proportional to concentration. In humans, it has been shown that there is an exponential decrease in gastric contents when water or other liquids are ingested, versus a linear phase of decrease when solids are ingested (18). Furthermore, it has been shown that continuous ingestion of food tends to result in an exponential and linear gastric content discharge when there is an interval between intakes (1), and the differences in the fit of each mouse to each model may reflect such individual differences in food intake.

The validity of the results obtained from the models was tested by the distribution of parameters estimated from an artificially generated data set; which indicate that the model parameters common to the two models were constant excretion rate from the cecum k_b [1/hr], GI transit time t_s [hr], cecum capacity A [g] and the only parameter that differs is the rate of expulsion from the stomach. Model 1 entails a constant gastric elimination rate α [mg/hr] and Model 2 demonstrates a constant gastric elimination rate k_a. The validity of this model can be verified by comparing the parameters estimated in this study with previous literature data measured experimentally. First, literature data on GI transit time t_s report a total GI transit time of 6-7h in mice (19), and some studies have shown that vancomycin administration increases this time (20). Estimation of the parameter t_s of the intestinal transit model in

this study also shows consistent results, with the higher concentration groups having larger values on the average. The mass of cecum contents has been shown to vary with diet any content in the same C57BL/6 mice, with an overall range of approximately 100 to 450 [mg] (21). The estimated results of the parameter A in the intestinal transit model ranged from 150 to 470 [mg], except for mouse H3, confirming that the interpretation of mass as cecum contents is valid. Mouse H3 had a smaller maximum concentration than the other two mice in the high-concentration group, and the duration for minimal antibiotic concentration was shorter, suggesting that the cecal mass was estimated to be larger than in the other mice.

The mathematical model introduced in this study is built by compartments based on the internal structure of the mouse. While PBPK models generally allow extrapolation to individuals with different physiological conditions, the compartments should be reconstructed in order to clinically apply this owing to the variation in GI structures between mice and humans. Particularly, the number and function of required compartments is expected to vary as the cecum is a compartment specific to mice, which have a large cecum, whereas in humans, the colon is divided into segments (22, 23).

Importantly, our study has several limitations. First, the sample size is minute to detect statistically significant differences between experimental groups. Second, we did not consider the ingestion and excretion in the current experiment, thus, the estimated parameters could not be tied to them. Furthermore, at this stage, the model can only be applied to substances that are not absorbed through the GI tract, and extensions of the model should be considered for common substances that are metabolized and absorbed in the GI tract. Several PBPK models have been used to demonstrate the absorption of oral drugs in the GI tract and drug concentrations in plasma (16, 24), and it is indispensable to confirm that changes in fecal concentrations are consistent with variations in the fecal compartment in these models in order to validate of the models.

In summary, we consider fluctuations in the concentration of antibiotics in feces to be a matter of substances moving through the intestinal tract, and we have developed a model that describes the rate of movement and concentration in the tract. This model, which provides estimates of the intricate changes in antibiotic concentrations, is anticipated to yield valuable insights into the fluctuation of the gut microbiome. In future studies, we intend to merge this model with compositional data on the gut microbiome to quantitatively examine the correlation between variations in antibiotic concentrations and changes in the microbiome.

GLOSSARY

Abbr. definition

M_a Antibiotic mass in the stomach

M_b Antibiotic mass in the cecum

C_b Antibiotic concentration in the cecum.

C Antibiotic concentration in feces

q Orally administered antibiotic mass

α Rate of elimination from the stomach

k_a Elimination rate constant from the stomach

k_b Elimination rate constant from the cecum

A Mass of the contents of the cecum

t_p Time it takes for all the antibiotic in the stomach to be eliminated

Δt_1 Time for the antibiotics to appear in the cecum the time for the antibiotics to appear in the cecum

Δt_2 Time between leaving the cecum and being eliminated as feces

t_s Intestinal transit time (=The time for the oral dose to be eliminated as feces)

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

DISCLAIMERS

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AUTHOR CONTRIBUTIONS

R.M., L.T., W.S. and M.T. conceived and designed research; R.M., L.T., K.W., S.T., T.K., K.T., S.N., and W.S. performed experiments; R.M. and L.T. analyzed data; R.M., L.T., H.T., W.S. and M.T. interpreted results of experiments; R.M. prepared figures; R.M. and S.T. drafted manuscript; R.M., L.T., H.T., K.W., S.T., T.K., K.T., S.N., W.S. and M.T. edited and revised manuscript; R.M., L.T., H.T., K.W., S.T., T.K., K.T., S.N., W.S. and M.T. approved final version of manuscript.

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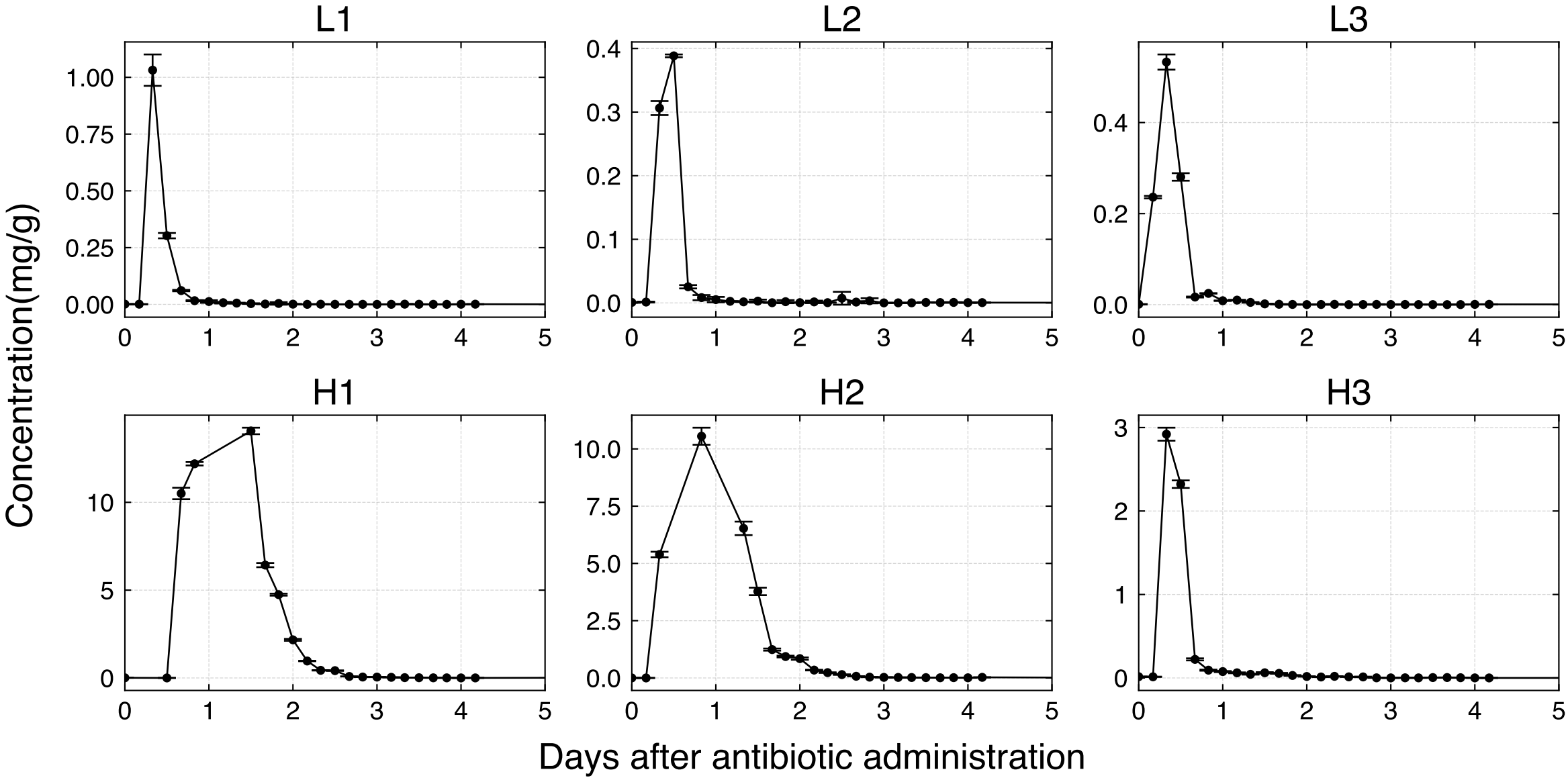
TABLES

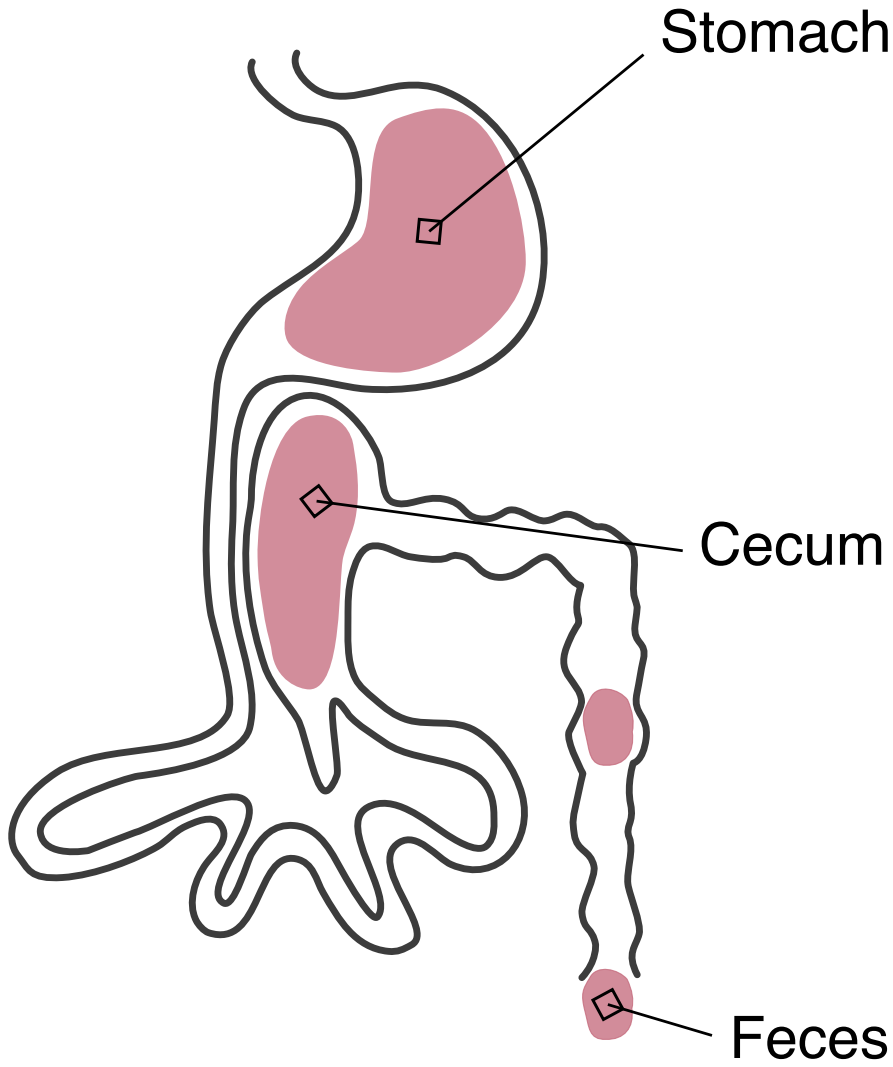
Table 1. Results of antibiotic concentrations in feces

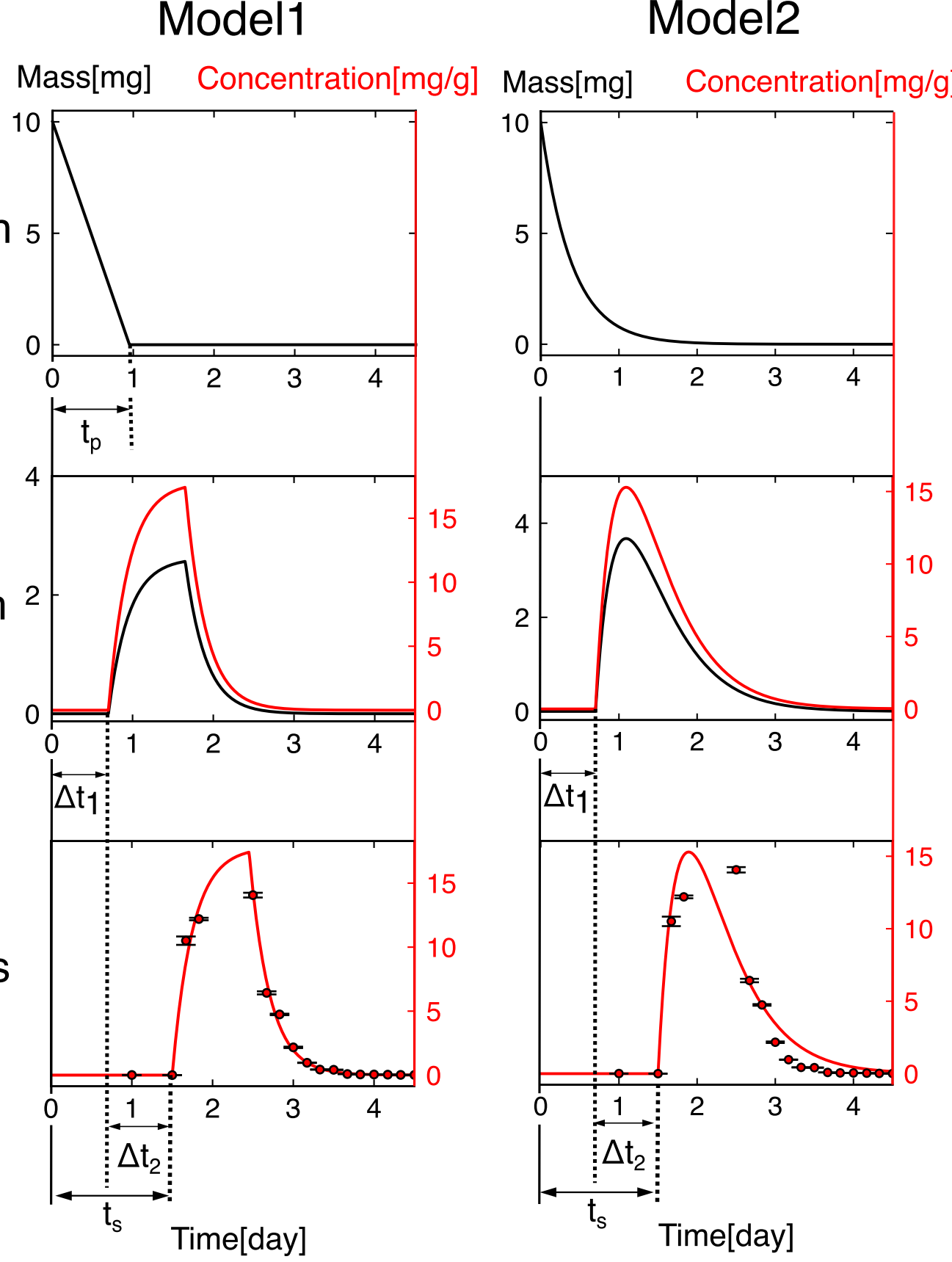
Mouse	C_{max}[mg/g]	T_{app}[hr]	T_{max}[hr]	T_{elim}[hr]
L1	1.03±0.07	8	8	20
L2	0.39±0.00	8	12	16
L3	0.53 ±0.02	4	8	16
H1	14.06±0.19	16	36	72
H2	10.55±0.37	8	20	68
H3	2.92±0.08	8	8	32

C_{max} : The highest concentration (mean ± SD, n=3), T_{app} : Time from antibiotic administration to detection in feces, T_{max} : Time from antibiotic administration to C_{max}, T_{elim} : Time from administration of the antibiotic until the antibiotic is not detected in the feces.

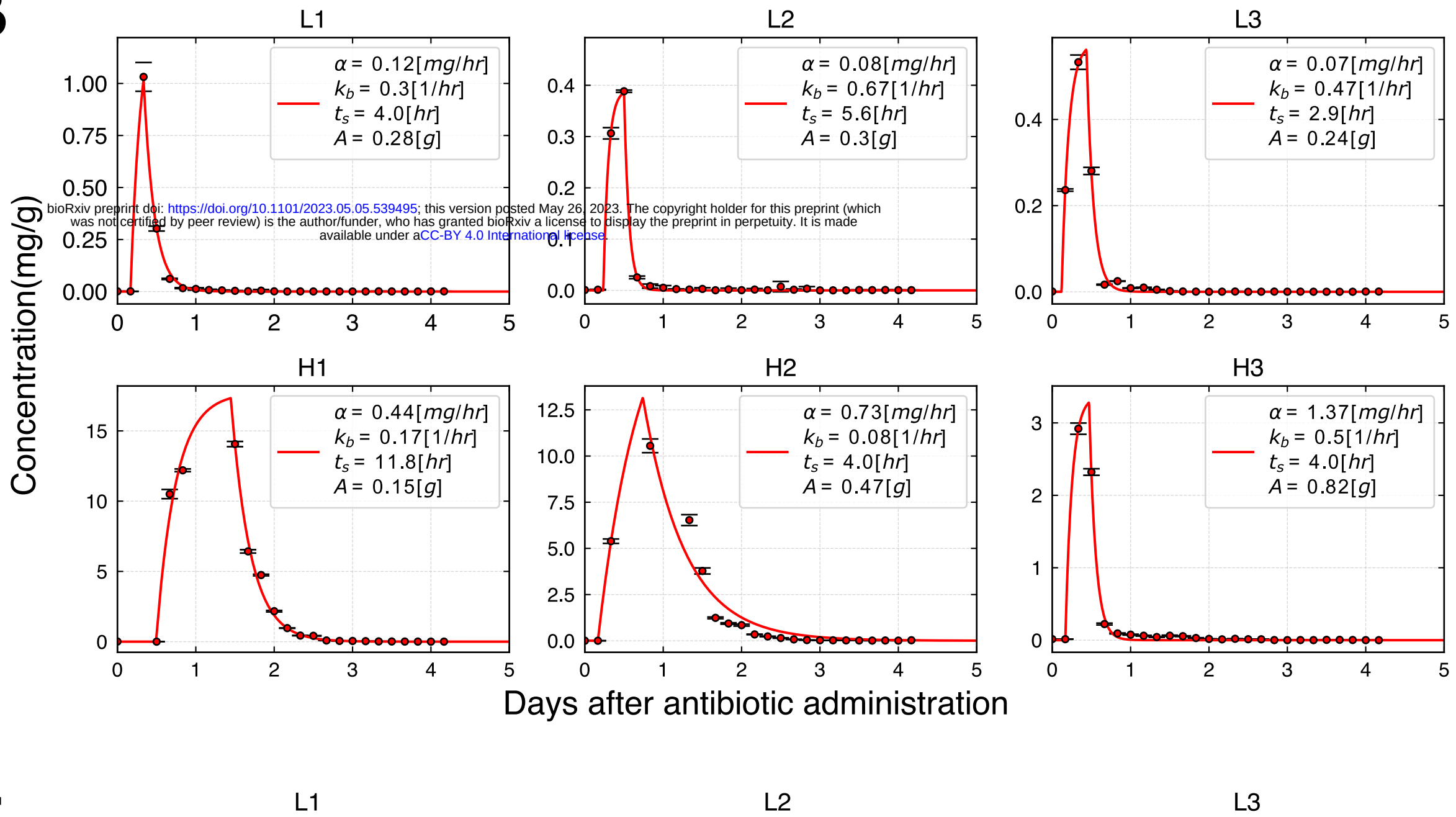

L1
L2
L3
H1
H2
H3
Concentration(mg/g)
Days after antibiotic administration

A

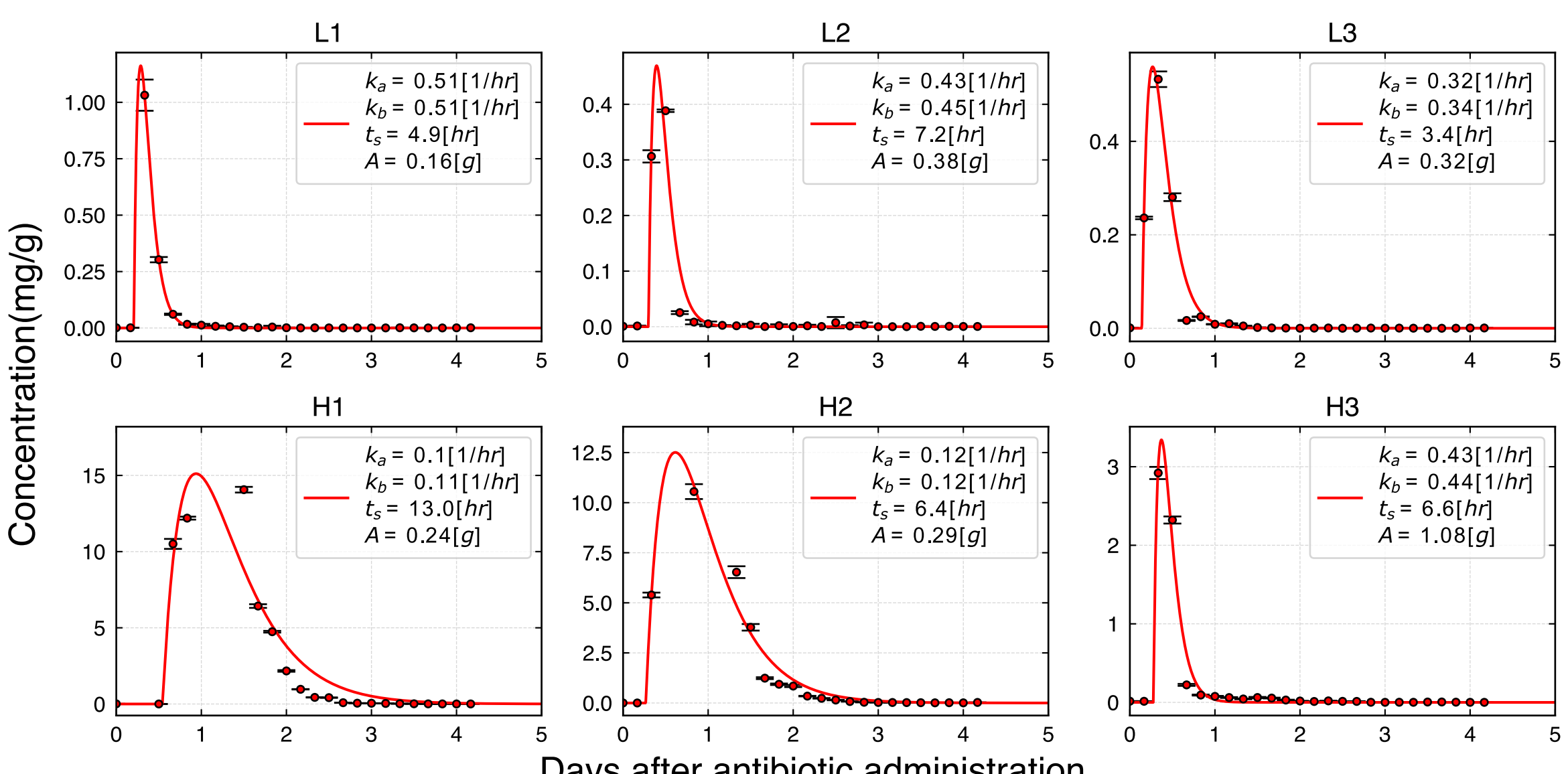


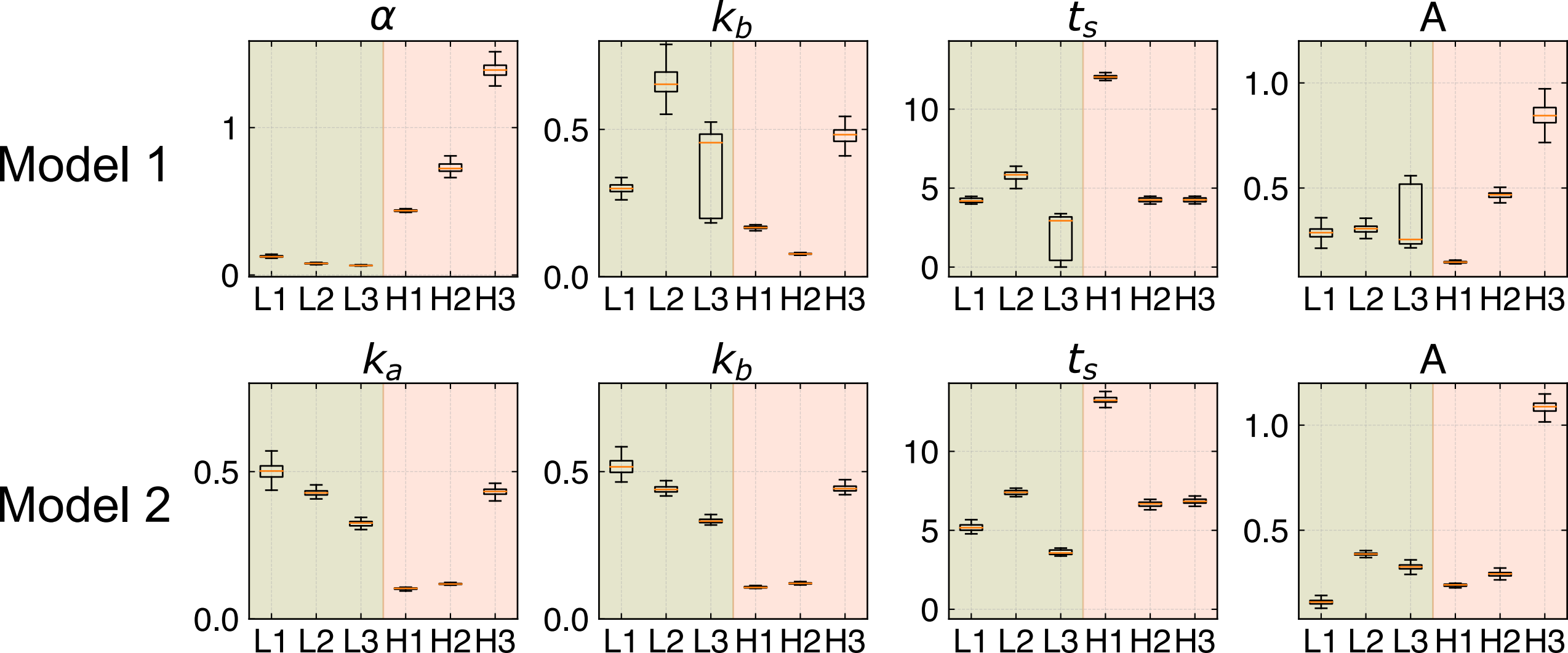


B



C




Model 1
Model 2
α
k_b
t_s
A
k_a
k_b
t_s
A
L1 L2 L3 H1 H2 H3