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Evaluation of Vancomycin Exposures Associated with Elevations in Novel Urinary Biomarkers of Acute Kidney Injury in Vancomycin-Treated Rats

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Vancomycin has been associated with acute kidney injury (AKI). However, the pharmacokinetic/toxicodynamic relationship for AKI is not well defined. Allometrically scaled vancomycin exposures were used to assess the relationship between vancomycin exposure and AKI. Male Sprague-Dawley rats received clinical-grade vancomycin in normal saline (NS) as intraperitoneal (i.p.) injections for 24- to 72-h durations with doses ranging 0 to 200 mg/kg of body weight divided once or twice daily. Urine was collected over the protocol's final 24 h. Renal histopathology was qualitatively scored. Urinary biomarkers (e.g., cystatin C, clusterin, kidney injury molecule 1 [KIM-1], osteopontin, lipocalin 2/neutrophil gelatinase-associated lipocalin 2) were assayed using a Luminex xMAP system. Plasma vancomycin concentrations were assayed by high-performance liquid chromatography with UV detection. A three-compartment vancomycin pharmacokinetic model was fit to the data with the Pmetrics package for R. The exposure-response in the first 24 h was evaluated using Spearman's nonparametric correlation coefficient (r_s) values for the area under the concentration-time curve during the first 24 h (AUC_{0-24}), the maximum concentration in plasma during the first 24 h ($C_{max_{0-24}}$), and the lowest (minimum) concentration in plasma after the dose closest to 24 h ($C_{min_{0-24}}$). A total of 52 rats received vancomycin ($n = 42$) or NS ($n = 10$). The strongest exposure-response correlations were observed between AUC_{0-24} and $C_{max_{0-24}}$ and urinary AKI biomarkers. Exposure-response correlations (r_s values) for AUC_{0-24}, $C_{max_{0-24}}$, and $C_{min_{0-24}}$ were 0.37, 0.39, and 0.22, respectively, for clusterin; 0.42, 0.45, and 0.26, respectively, for KIM-1; and 0.52, 0.55, and 0.42, respectively, for osteopontin. However, no differences in histopathological scores were observed. Optimal sampling times after administration of the i.p. dose were 0.25, 0.75, 2.75, and 8 h for the once-daily dosing schemes and 0.25, 1.25, 14.5, and 17.25 h for the twice-daily dosing schemes. Our observations suggest that AUC_{0-24} or $C_{max_{0-24}}$ correlates with increases in urinary AKI biomarkers.

Acute kidney injury (AKI) is a major contributor to patient morbidity and mortality in the hospital setting (1, 2). While the etiology of AKI is multifactorial, many cases among hospital patients are related to medication exposure (2). Not surprisingly, the risk of drug-induced AKI is highest among critically ill, hospitalized patients (3), who carry multiple risk factors for the development and progression of AKI. Vancomycin, the antibiotic most frequently administered in the hospital setting (4), has been implicated as a cause of AKI in a number of clinical (5–7) and animal (8–10) studies. Among the clinical studies, the incidence of vancomycin-induced AKI has been associated with higher doses of vancomycin (11, 12), increasing numbers of vancomycin doses (12), and elevated trough concentrations (7, 13).

While a number of clinical studies have shown that more intensive vancomycin dosing regimens are associated with an increased risk of AKI, these studies could only suggest an association with kidney injury. As these studies were largely observational in nature, it is difficult to discern if the association was reflective of a true effect or was biased due to confounders. For example, patient confounder factors, such as severity of illness, residence in an intensive care unit, and concurrent receipt of nephrotoxins, may influence the vancomycin exposure-response profile that best predicts clinical AKI.

Animal systems are ideally suited to define exposure-response relationships, as they provide flexibility to titrate dosing groups and minimize the influence of external covariates on the observed results. To date, animal models of AKI have confirmed that vancomycin is a nephrotoxin. Dose-ranging studies have revealed that an increase in the vancomycin dose and an increase in the duration of treatment in rats are associated with increases in histopathological damage and elevations in novel urinary biomarkers of AKI (8–10). However, a prospectively derived ex-

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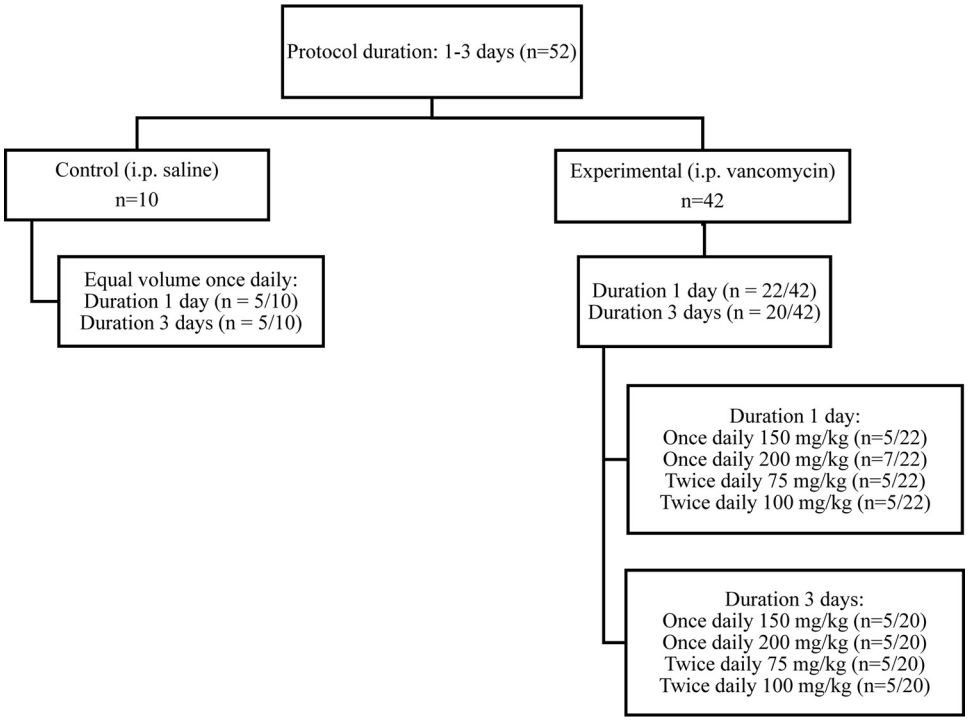

FIG 1 Allocation of experimental animals across experimental protocols. The experimental flow diagram of animal allocation is according to the duration of exposure and the dose administered.

posure-AKI (i.e., a pharmacokinetic [PK]/toxicodynamic [TD]) threshold for vancomycin has not been defined. Being cognizant of this knowledge gap, we sought to evaluate vancomycin exposure-response relationships for elevations in several sensitive urinary AKI biomarkers in a paired PK/TD animal study of vancomycin-induced AKI. Secondarily, we sought to define the optimal sampling times for determination of the PKs of vancomycin. These data are important for future animal work, as they will reduce the number of blood draws needed to estimate the vancomycin exposure profile with low bias and high precision.

MATERIALS AND METHODS

This PK/TD study was conducted at Midwestern University, in Downers Grove, IL. The study methods were reviewed and approved by the Midwestern University Institutional Animal Care and Use Committee (IACUC; protocol number 2295).

Experimental design and animals. The PK/TD experimental design is summarized in Fig. 1. Animals were divided between experimental (i.e., vancomycin-treated) and control protocol arms. Experimental animals received intraperitoneal (i.p.) injections of clinical-grade vancomycin (Hospira, Lake Forest, IL) in normal saline (NS), and control animals received equivalent volumes of NS as i.p. injections. Experimental and control animals were further enrolled into protocols with 1- and 3-day durations. Finally, experimental animals were divided into allometrically scaled daily doses (i.e., 150 and 200 mg/kg of body weight) and dose fractionation (i.e., once or twice daily) groups. All together there were 2 control groups and 8 treatment groups. The rationale for the choice of these doses was partially based on previous studies that found that these doses produce some renal dysfunction while avoiding overt kidney failure or death in animals (8, 9, 14–16). Likewise, when the principle of allometry is applied, the human equivalent daily dose of vancomycin for rat doses of 150 and 200 mg/kg would approximate to 24.2 and 32.3 mg/kg, respectively, which are doses that are routinely administered according to current clinical practice guidelines (17, 18).

Male Sprague-Dawley rats (weight, 250 to 350 g; Harlan, Indianapolis, IN, USA) were maintained in plastic cages on a 12-h light/12-h dark cycle. For all experiments, vancomycin-treated animals ($n = 5$ to 7 per protocol) received i.p. injections of clinical-grade vancomycin at doses of either 150 or 200 mg/kg/day as a 100-mg/ml solution in NS. On the final day of each experimental or control protocol condition, urine samples were collected over 24 h. All animals were allowed free access to water at all times. Food was also available *ad libitum*, except during the period in which the urine samples were being collected. Data were analyzed for all animals enrolled in each treatment protocol. When animals contributed some predictor data (e.g., vancomycin concentrations) but died prior to urine collection, urinary biomarker outcomes were treated as missing data.

Blood and urine sampling. Blood samples were withdrawn from internal jugular vein (i.j.) catheters, which were locked with heparin saline solution (100 IU/ml) when not in use. Catheters were surgically implanted while the animals were under ketamine (100 mg/kg) and xylazine (10 mg/kg) anesthesia. Animals were allowed to recover for 24 h prior to withdrawal of blood samples. Blood samples (0.25-ml aliquots) were collected after an experimental dose 24 h before the protocol end (i.e., on day 1 or day 3, depending on the protocol). A maximum of 8 samples were taken within a 24-h period from any animal. Blood samples were taken 0, 5, 15, and 30 min and 1, 2, 4, and 24 h postdose. Time zero corresponded to the time immediately prior to administration of the given dose. For animals who received twice-daily dosing, to maintain total blood sampling limits, the sample collected 5 min after the first dose on the sampling day was omitted to obtain a sample 30 min after the second dose (12.5 h after collection of the sample at time zero). Equivalent volumes of NS were administered via the i.j. catheter after withdrawal of each blood sample. Urine was collected during the 24-h period prior to terminal blood sampling and euthanasia. Animals were placed in metabolic cages for fasting urine collection and quantitation, as previously described (19–21). Urine

was collected via metabolic cage (catalogue number 650-0350; Nalgene, Rochester, NY). Animals were placed in the metabolic cage after collection of the sample at 120 min and momentarily removed for collection of other blood samples up until collection of the terminal sample at 24 h. Urine was collected under ambient conditions, and the urine volume was measured at the end of the 22-h residence. Notably, kidney injury molecule 1 (KIM-1), cystatin C, osteopontin (OPN), and neutrophil gelatinase-associated lipocalin 2 (NGAL) levels are stable throughout this time period under ambient conditions. Urine was collected from each animal and centrifuged at 400 × *g* for 5 min. After the centrifugation step, the entire amount of urine (i.e., the urine pooled over 22 h) from each animal was individually stored at −80°C for batch analysis. Animal urine was not pooled by dose group: each animal's urine was matched with their own PK exposure data.

Chemicals and reagents. Vancomycin hydrochloride for injection (lot number 343748E03) was obtained commercially (Hospira, Lake Forest, IL). As previously described (22), vancomycin hydrochloride USP with a purity of 99.3% was obtained commercially (Enzo Life Science, CA) for the generation of standard curves using high-performance liquid chromatography (HPLC) with UV detection. Caffeine (purity, 99.7%; Alfa Aesar, Ward Hill, MA, USA), acetonitrile, and methanol were from purchased from VWR International (Radnor, PA). Formic acid was obtained from Fisher Scientific (Waltham, MA). All solvents used were of HPLC or liquid chromatography-tandem mass spectrometry grade. Frozen, nonmedicated, nonimmunized, pooled plasma (anticoagulated with disodium EDTA) from Sprague-Dawley rats was procured (BioreclamationIVT, Westbury, NY) for calibration of standard curves.

Determination of vancomycin concentrations in plasma. Blood from vancomycin-treated animals was immediately transferred to disodium EDTA-treated Eppendorf microcentrifuge tubes and centrifuged (Eppendorf, Hauppauge, NY) at 3,000 rpm for 10 min. Plasma was separated as a supernatant and was then stored at −80°C for further analysis. Plasma samples underwent phospholipid removal and filtration using Phree columns (Phenomenex, Torrance, CA, USA). Vancomycin was eluted using methanol. The eluted samples were analyzed using a previously reported HPLC method with UV detection (22). Samples were analyzed using a gradient HPLC technique using a Kinetex biphenyl column (particle size, 2.6 μm; 50 by 3 mm; Phenomenex, Torrance, CA, USA). The mobile phase consisted of acetonitrile and water containing 0.1% formic acid, while the UV detector was set at 198 nm. Caffeine was used as the internal standard. The linear range of the assay was 3.0 to 74.9 μg/ml. The coefficient of variation (CV) for intra-assay precision ranged from 2.45 to 15.16%, and the CV for interday assay precision ranged from 2.17 to 15.82%; all of these values met the assay standards (23). The interday accuracy and precision ranges of the assay were 97.4 to 118.5% and 2.18 to 15.81%, respectively.

Determination of urinary biomarkers of AKI. Urine samples were analyzed for biomarker and creatinine content. Sample aliquots were processed and stored as described above. Urine aliquots were analyzed in batches to determine the concentrations of clusterin, cystatin C, KIM-1, NGAL, and OPN. Biomarkers were assayed using the microsphere-based Luminex xMAP technology (Austin, TX) as described previously (19–21). Urine was aliquoted into 96-well plates supplied with Milliplex MAP rat kidney toxicity magnetic bead panels 1 and 2 (EMD Millipore Corporation, Charles, MO). Samples were prepared and analyzed according to the manufacturer's recommended protocol. The urine creatinine concentration was determined by a colorimetric assay (Cayman Chemical, Ann Arbor, MI). Urine was diluted 1:50; aliquoted into 96-well plates supplied with the kit, which were analyzed according to the manufacturer's instructions; and read at 485 nm on a Beckman Coulter DTX 880 multimode detector (Beckman Coulter, Inc., CA).

Evaluation of histopathological evidence of renal cell damage. After terminal urine and blood sampling, rats were humanely euthanized by exsanguination from the right atrium while they were under anesthesia (induced by the intraperitoneal injection of 100 mg/kg ketamine and 10 mg/kg xylazine). Necropsies were performed on all rats, and kidneys were harvested for postmortem examination. Each animal's kidneys were removed, briefly washed in cold isotonic saline, and preserved in 10% formalin solution for histologic examination. Histopathological analysis was conducted by Charles River Pathology Associates (Wilmington, MA), using light microscopy on hematoxylin and eosin-stained, paraffin-embedded renal specimens. Pathologists did not have access to any of the TD data concerning surrogate measures of kidney function (i.e., urinary biomarkers) or vancomycin PK exposure data (i.e., the area under the concentration-time curve during the first 24 h [AUC_{0-24}], the maximum concentration in plasma during the first 24 h [$C_{max_{0-24}}$], or the the lowest [minimum] concentration in plasma after the dose closest to 24 h [$C_{min_{0-24}}$]); pathologists were informed only of nominal treatment group status (i.e., the dose [in milligrams per kilogram]). Categorical scoring was according to the histopathological lexicon from the Critical Path Institute's Predictive Safety Testing Consortium Nephrotoxicity Working Group (8, 24–27). Evidence of histopathological damage was rated on an ordinal scale ranging from 0 to 5, where the grades for pathological lesions were 0 for no observable pathology, 1 for minimal pathology, 2 for mild pathology, 3 for moderate pathology, 4 for marked pathology, and 5 for severe pathology; this scale has been validated elsewhere (8, 28). The composite score for an individual animal was calculated as the highest ordinal score for any histopathological process at any kidney site (28).

Vancomycin pharmacokinetic model and exposure determination. A three-compartment structural model of vancomycin disposition accounting for the rate of absorption from the peritoneal space to the central compartment (K_a) was fit to the PK data. The PK model was parameterized with K_a, the volume of distribution (V), the intercompartmental transfer rates between the central and peripheral compartments (K_{12} and K_{21}), and the total elimination rate (k_{el}), which were estimated as apparent parameters (i.e., V/F and k_{el}/F, where F is bioavailability) and solved algebraically.

The nonparametric adaptive grid (NPAG) algorithm (29–31) with adaptive gamma implemented within the Pmetrics package (version 1.4.0; Laboratory of Applied Pharmacokinetics and Bioinformatics, Los Angeles, CA) for R (32) was used for all PK model-fitting procedures, as previously described (33–36). There were 80,021 initial grid points. The inverse of the estimated assay variance was used as the first estimate for parameter weighting. Final parameter weighting was accomplished using gamma, a multiplicative observation error model to capture process noise (i.e., error = standard deviation [SD] · gamma), with the initial gamma value being 3. Assay error (SD) was accounted for using an error polynomial as a function of the measured concentration, Y (i.e., $SD = C_0 + C_1 \cdot Y$), with inputs of 1.5 and 0.08, respectively. Comparative performance evaluation was completed using Akaike's information criterion, a regression of observed versus predicted concentrations, visual plots of PK parameter-covariate regressions, and the rule of parsimony. The best-fit model from the iterative model-fitting process was utilized to obtain median MAP Bayesian vancomycin exposure estimates. Population predictions were utilized only in sampling time simulations performed by use of the MMopt command within Pmetrics, as described below (see "Calculation of optimal sampling time points for analysis of vancomycin PKs in the rat" below). All exposure-response indices were calculated from individual Bayesian posterior predicted concentrations, as described below (see "Estimation of PK exposure profiles and statistical analysis" below).

Estimation of PK exposure profiles and statistical analysis. Individual vancomycin exposures for the first 24 h (i.e., AUC_{0-24}, $C_{max_{0-24}}$, and $C_{min_{0-24}}$) were determined for each rat using individual median Bayesian posterior parameter values to calculate plasma vancomycin concentrations every 5 min. Bayesian posterior parameter value distributions were calculated in Pmetrics from the final population model (see Table 2) and each animal's measured vancomycin concentrations, dosing history, and weight. The highest concentration values within the first 24 h were used to determine $C_{max_{0-24}}$, and the lowest concentration observed between 11 and 24 h postdose was used to determine $C_{min_{0-24}}$ across all animals in

TABLE 1 Summary of animal weight loss, urine output, histopathology scores, and vancomycin exposure estimates[a]

Characteristic	One-day protocol			Three-day protocol		
	Control	Vancomycin	*P* value	Control	Vancomycin	*P* value
No. of animals in group/total no. of animals	5/52	22/52		5/52	20/52	
Mean ± SD wt loss (g)	25.6 ± 4.0	21.5 ± 9.4	0.35	26.6 ± 3.6	25.3 ± 12.7	0.82
Mean ± SD urine output (ml)	NR	10.6 ± 5.3[b]	—[c]	13.6 ± 2.3	12.8 ± 6.7[d]	0.79
Median (IQR) worst composite score	2 (2–2)	2 (1–2)	0.68	1 (1–2)	2 (1–3)	0.41
Median (IQR) AUC_{0-24} (μg · h/ml)	0	112 (62.2–292)	—	0	202 (83.1–248)	—
Median (IQR) $C_{max_{0-24}}$ (μg/ml)	0	17.5 (8.7–57.6)	—	0	26.4 (13.9–51.8)	—
Median (IQR) $C_{min_{0-24}}$ (μg/ml)	0	0.04 (0.0–2.5)	—	0	1.8 (0.0–3.2)	—

[a] Urinary, histopathological, and PK exposure endpoints were stratified by experimental condition (i.e., vancomycin or saline control) and protocol duration (1 or 3 days). Abbreviations: AUC_{0-24}, area under the concentration time curve during the first 24 h; $C_{max_{0-24}}$, maximal concentration during the first 24 h; $C_{min_{0-24}}$, lowest (minimum) concentration after the dose closest to 24 h; IQR, interquartile range. NR, data were not recorded.
[b] Data are for 21 rats.
[c] —, statistical testing against a zero value was not performed.
[d] Data are for 17 rats.

order to capture the lowest concentration within the first 24 h after i.p. administration. The trapezoidal rule was used to determine AUC_{0-24}. The variability of each PK exposure measure was calculated as the CV by dividing the standard deviation by the mean of the given exposure measure.

Association of PK measures with urinary AKI biomarkers and renal histopathology. All statistical analyses, except where indicated, were completed using Intercooled Stata (version 14.0; StataCorp, College Station, TX). PK/TD exposure-response relationships were assessed for vancomycin exposure profiles (i.e., PKs) and (i) urinary biomarkers of AKI and (ii) composite histopathological damage (i.e., TD). Relationships between exposure and renal endpoints were assessed using Spearman's rank coefficient.

Statistical analyses for between-treatment-group comparisons. Renal histopathological score, urine output, pharmacokinetic exposure measures (e.g., AUC_{0-24}, $C_{max_{0-24}}$, and $C_{min_{0-24}}$), and body weight loss were analyzed according to vancomycin dose, frequency, and duration. Differences were evaluated using either the Student *t* test or the Wilcoxon rank sum test, as appropriate. All statistical tests were two-tailed, with an *a priori* level of alpha set at 0.05 for statistical significance.

Calculation of optimal sampling time points for analysis of vancomycin PKs in the rat. Once a finalized population PK model was fitted using the NPAG algorithm, the population model consisting of discrete support points (up to one point per animal was included in the model) was leveraged to identify optimal sampling times using the MMopt command within Pmetrics (37). A support point is a collection of values for each parameter in the model and an associated probability of those values, based on how well they predict the observed vancomycin concentrations in the study population. Each support point in the population model can generate a time-concentration profile for vancomycin for a given dosage regimen or regimens for which optimal sampling times are desired. The MMopt algorithm finds the desired number of optimal sampling times (typically, 1 to 4) where the profiles from each support point are maximally separated. In this way, the Bayesian risk of misclassifying a new animal as the wrong support point or combination of support points is minimized. We evaluated 1 to 4 samples for each dose fractionation scheme.

RESULTS

Demographics of animal cohort. Figure 1 displays the disposition of the 52 rats according to treatment group, fractionation scheme, and duration of exposure. In total, four vancomycin-treated animals ($n = 1$ from the 150-mg/kg group and $n = 3$ from the 200-mg/kg group) were euthanized early according to IACUC protocols, and thus, they contributed only partially complete PK and TD data. Of the 52 animals evaluated, 38 vancomycin-treated animals and 10 control animals contributed complete PK and TD data. Differences in animal weight loss postcatheterization according to vancomycin or control status and protocol duration are shown in Table 1. Mean weight loss was not significantly different between vancomycin-treated and control animals overall (23.3 g versus 26.1 g; $P = 0.44$). Likewise, animal weight loss did not differ between vancomycin-treated and control animals according to protocol duration ($P = 0.35$ and $P = 0.82$ for the 1- and 3-day-protocol groups, respectively).

Urine output, concentrations of urinary AKI biomarkers, and renal histopathology. Urinary output measurements are summarized in Table 1 and were stratified according to vancomycin treatment or control status and protocol duration. On the final protocol day, the overall mean ± SD urine output was 11.8 ± 5.7 ml ($n = 43$). Mean total urine output did not differ between vancomycin-treated and control animals overall (11.6 ml versus 13.6 ml; $P = 0.46$). Likewise, mean urine output was not different between vancomycin-treated and control animals in the 3-day-protocol group (12.8 ml versus 13.6 ml; $P = 0.79$). Urine creatinine concentrations did not differ significantly between vancomycin-treated and control animals ($P = 0.28$). The range of concentrations of nearly all sensitive urinary AKI biomarkers evaluated (with the exception of the osteopontin concentration) were higher among vancomycin-treated animals in the 3-day-protocol group than control animals and vancomycin-treated animals in the 1-day-protocol group (see Table S1 in the supplemental material). The worst ordinal histopathology score data are also summarized in Table 1 and were stratified according to vancomycin treatment or control status and protocol duration. The highest observed composite histopathology scores did not differ according to treatment group ($P = 0.69$), nor did they differ according to protocol duration (Table 1).

Vancomycin PK model and exposure measure determination. The final population PK model had 34 support points defining a 3-compartment model fitted to 276 vancomycin concentrations in 42 animals. Bias, imprecision, and the coefficient of determination for observations versus Bayesian posterior predictions were −0.12 μg/ml, 1.01 $μg^2/ml^2$, and 98.5%, respectively (Fig. 2). For the purposes of determining optimal sampling times, the population mean, median, and dispersion metrics are shown in Table 2, while the interparameter covariances (i.e., the covariance matrix in lower triangular form) for each of the PK model

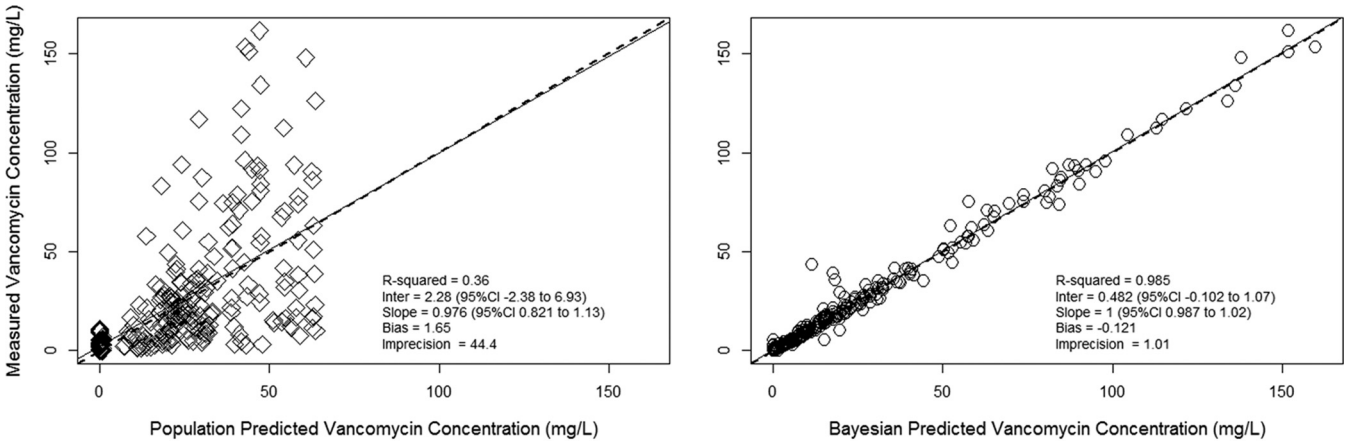

FIG 2 Population and individual goodness-of-fit plots for the three-compartment PK model. The final three-compartment population model parameters are provided in Table 2. Vancomycin observations ($n = 276$) were obtained from 42 animals (male Sprague-Dawley rats). (Left) Population model goodness-of-fit plot with diamonds; (right) individual Bayesian posterior model goodness-of-fit plot with circles.

parameters are shown in Table 3. Figure 2 displays the population and individual posterior Bayesian observed versus predicted plots.

The calculated Bayesian posterior vancomycin exposure estimates are summarized in Table 1 according to treatment status and protocol duration. Among the vancomycin-treated animals, the mean AUC_{0-24}, $C_{max_{0-24}}$, and $C_{min_{0-24}}$ were 202 μg · h/ml, 38.7 μg/ml, and 1.64 μg/ml, respectively. Overall, significant inter-animal exposure variability was observed. The CVs among vancomycin-treated animals for AUC_{0-24}, $C_{max_{0-24}}$, and $C_{min_{0-24}}$ were 114%, 118%, and 131%, respectively. The estimated population mean absorption rate constant (K_a) was prolonged at 1.5 h^{-1} (Table 2). The Bayesian posterior predicted concentrations captured the majority of the observed concentrations with high accuracy and low bias (Fig. 2 and 3).

PK/TD correlations. Since vancomycin exposure metrics were not uniformly distributed, nonparametric exposure-response correlations were generated. Urine creatinine concentrations were low for all study animals and did not significantly correlate with vancomycin exposure metrics ($P = 0.17$, 0.27, and 0.10 for the AUC_{0-24}, $C_{max_{0-24}}$, and $C_{min_{0-24}}$ correlations, respectively; see Fig. S1 in the supplemental material). Sensitive urinary biomarkers of AKI correlated with vancomycin exposure metrics (i.e., AUC_{0-24}, $C_{max_{0-24}}$, and $C_{min_{0-24}}$), as shown in Table 4. Of the urinary AKI biomarkers tested, osteopontin, KIM-1, and clusterin exhibited the highest correlations with vancomycin AUC_{0-24} and $C_{max_{0-24}}$ ($P \leq 0.05$ for all comparisons). Vancomycin exposure-response plots are shown in Fig. 4 for clusterin, KIM-1, and osteopontin. The highest correlations were observed between AUC_{0-24} and $C_{min_{0-24}}$ and each of these respective biomarkers. None of the vancomycin exposure measures (AUC_{0-24}, $C_{max_{0-24}}$, or $C_{min_{0-24}}$) were significantly correlated with the worst ordinal renal histopathology scores measured (Spearman's nonparametric correlation coefficient [r_s] = 0.18, 0.11, and 0.09, respectively). Of note, vancomycin exposure measures were highly intercorrelated across all doses and dosing frequencies, with AUC_{0-24} and $C_{max_{0-24}}$ being the most highly intercorrelated ($r_s = 0.898$; $P < 0.0001$), followed by AUC_{0-24} and $C_{min_{0-24}}$ ($r_s = 0.783$; $P < 0.0001$) and $C_{max_{0-24}}$ and $C_{min_{0-24}}$ ($r_s = 0.579$; $P < 0.0001$).

Optimal sampling times for PK analysis in the rat. For animals receiving vancomycin once every 24 h, the four most informative time points to obtain samples for PK analysis were 0.25, 0.75, 2.75, and 8 h postdose. The Bayes risk of misclassification increased marginally (11.2%) when the number of samples was reduced to 3 at 0.25, 2.75, and 8 h postdose. Alternatively, for animals receiving vancomycin every 12 h, the four most informative time points to obtain samples for PK analysis were 0.25 and

TABLE 2 Final three-compartment population PK model estimates[a]

Parameter	Mean	Median	SD	CV (%)
K_a (h^{-1})	1.5	1.0	1.3	84.8
V_0/F (liter/0.3 kg)	0.3	0.3	0.2	54.0
k_{el}/F (h^{-1})	1.3	0.9	0.9	73.8
K_{12} (h^{-1})	4.8	1.9	5.3	110.3
K_{21} (h^{-1})	4.2	1.6	5.3	124.8

[a] Mean and median population pharmacokinetic parameters along with their attendant measures of dispersion are presented. The PK model was derived from 276 observations in 42 animals. Abbreviations: CV, coefficient of variation; K_a, intercompartmental absorption rate from the peritoneal space to the central compartment; k_{el}, central compartment elimination rate; K_{12} and K_{21}, intercompartmental mass transfer rates between the central and peripheral compartments; V_0, volume of distribution standardized to a 0.3-kg body weight; F, apparent bioavailability of clearance and volume, which was not estimated [i.e., $CL/F = (V_0 \cdot k_{el})/F$].

TABLE 3 Covariance matrix in lower triangular form of the final pharmacokinetic model[a]

Parameter	Covariance				
	k_{el} (h^{-1})	V_0/F (liter/0.3 kg)	K_a (h^{-1})	K_{12} (h^{-1})	K_{21} (h^{-1})
k_{el} (h^{-1})	0.866				
V_0/F (liter/0.3 kg)	−0.052	0.0228			
K_a (h^{-1})	−0.344	0.006	1.566		
K_{12} (h^{-1})	0.971	0.043	−1.473	28.248	
K_{21} (h^{-1})	1.418	0.207	1.102	−4.086	27.62759

[a] Abbreviations: K_a, intercompartmental absorption rate from the peritoneal space to the central compartment; k_{el}, central compartment elimination rate; K_{12} and K_{21}, intercompartmental mass transfer rates between the central and peripheral compartments; V_0, volume of distribution standardized to s 0.3-kg body weight; F, apparent bioavailability of clearance and volume, which was not estimated [i.e., $CL/F = (V_0 \cdot k_{el})/F$].

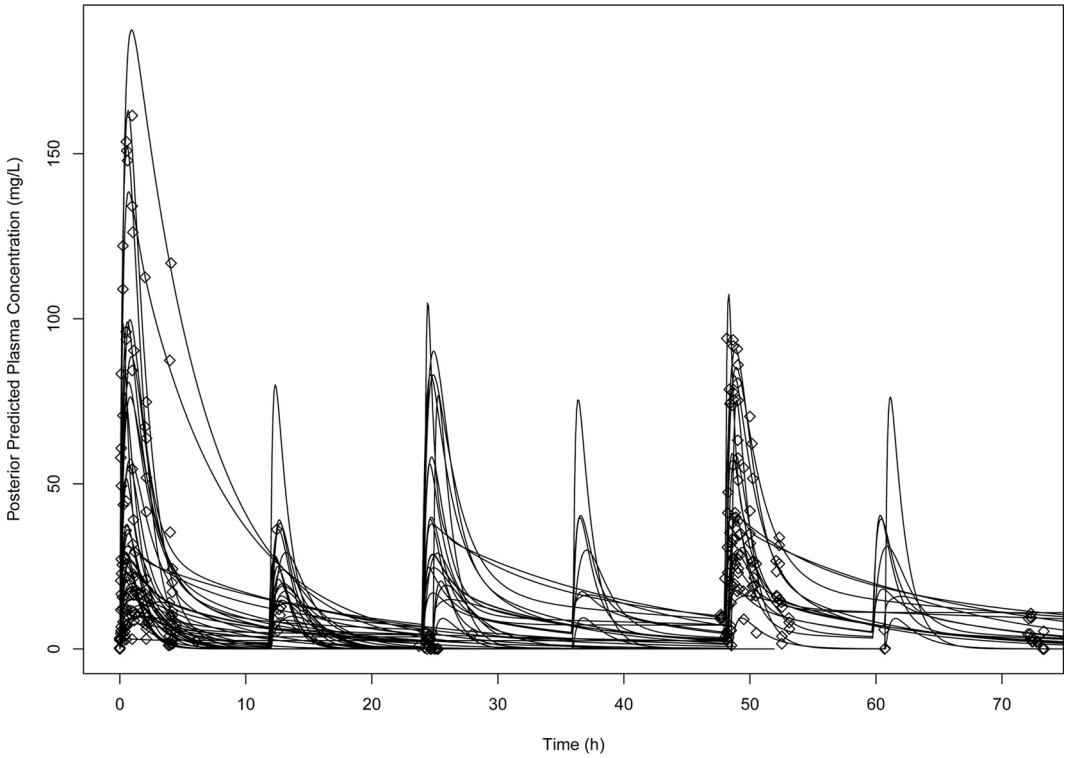

FIG 3 Concentration-time profile of vancomycin in rats according to individual Bayesian posterior predictions. Maximum *a posteriori* Bayesian estimated concentration-time profiles (lines) were fitted to the observed data (diamonds).

1.25 after the first dose and 2.5 and 5.25 h after the second dose. The Bayes risk of misclassification increased very slightly (9.8%) when the number of samples was reduced to 3 at 0.25, 13, and 16.25 h postdose. Reducing the number of sampling times to 1 or 2 time points increased the Bayes risk of misclassification by >25% compared to that for the four-sample design, irrespective of the dosing schedule. Thus, in the future, sampling times for PK analysis in the rat after i.p. dosing may reasonably be reduced to 3 optimal time points according to the dose fractionation scheme employed.

TABLE 4 Correlations between urinary biomarkers of renal injury and vancomycin PK exposure[a]

Urinary biomarker	Association measure	AUC_{0-24}	$C_{max_{0-24}}$	$C_{min_{0-24}}$
Clusterin (ng/ml)	r_s	0.372	0.393	0.225
	P value for r_s	**0.009**	**0.006**	0.125
Cystatin C (ng/ml)	r_s	0.033	0.076	0.126
	P value for r_s	0.822	0.610	0.393
KIM-1 (ng/ml)	r_s	0.422	0.453	0.259
	P value for r_s	**0.003**	**0.001**	0.076
Osteopontin (ng/ml)	r_s	0.517	0.546	0.419
	P value for r_s	**0.0002**	**0.0001**	**0.003**
Lipocalin-2/NGAL (ng/ml)	r_s	0.263	0.271	0.306
	P value for r_s	0.071	0.062	**0.035**

[a] Measures of association between log exposure (i.e., AUC_{0-24}, $C_{max_{0-24}}$, $C_{min_{0-24}}$) and the level of expression of urinary biomarkers. Bold, correlations indicate significance at the level with a *P* value of <0.05. Abbreviations: AUC_{0-24}, area under the concentration-time curve during the first 24 h; $C_{max_{0-24}}$, maximal concentration during the first 24 h; $C_{min_{0-24}}$, lowest concentration after the dose closest to 24 h; KIM-1, kidney injury molecule 1; r_s, Spearman's nonparametric correlation coefficient.

DISCUSSION

Our study attempted to link PK exposures to vancomycin with elevations in sensitive renal biomarkers in a rat model of nephrotoxicity. While no notable relationships between vancomycin exposure over 24 or 72 h and renal histopathology were observed, our study demonstrated that urinary biomarkers increased as a function of vancomycin duration and intensity. Increasing vancomycin exposures in the form of AUC_{0-24}, $C_{max_{0-24}}$, and $C_{min_{0-24}}$ were all positively correlated with increased concentrations of several urinary biomarkers of AKI. While there was a positive correlation between all PK parameters and urinary biomarkers, the visual exposure-urinary biomarker relationships (Fig. 4) clearly demonstrated that the relationship between increasing urinary biomarkers and exposure was most pronounced for AUC_{0-24} and $C_{max_{0-24}}$. Our observations support an exposure-response relationship for vancomycin and AKI, a relationship that is critical to elucidate, as vancomycin is the single most common antibiotic administered to hospitalized patients in the United States (4). These data lay the foundation for future experiments that will further clarify the exact PK/TD exposure profile within a larger population treated for longer durations with higher doses. To facilitate the execution of these future studies, this study also provided critical information on the optimal times of sample collection for PK analysis to minimize bias in exposure estimation with the fewest rat blood draws when combined with a population modeling approach.

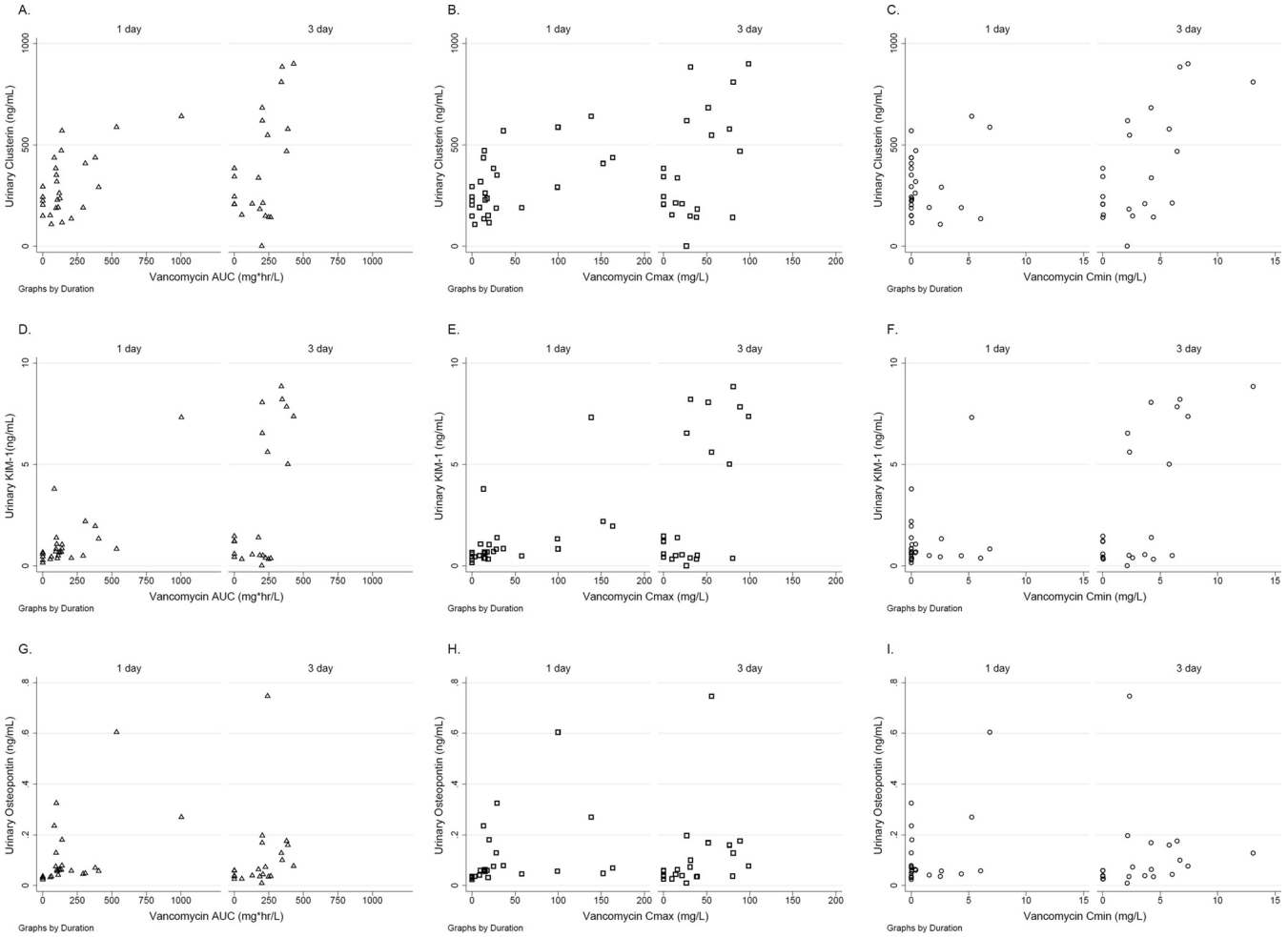

FIG 4 Exposure-response for vancomycin and early urinary biomarkers of acute kidney injury. Sensitive urinary biomarkers of AKI compared to vancomycin exposure measures were stratified by protocol duration (in days). (A to C) Clusterin expression according to vancomycin exposure measures; (D to F) KIM-1 expression according to vancomycin exposure measures; (G to I) osteopontin expression according to vancomycin exposure measures.

Our results are generally consistent with those of other preclinical studies that have assessed the relationship between vancomycin and urinary AKI biomarkers. Similar to our findings, Fuchs et al. noted increased concentrations of KIM-1, clusterin, and osteopontin among Wistar rats treated with increasing doses of vancomycin, especially when the daily dose was in excess of 200 mg/kg/day (9). Vaidya et al. also observed that vancomycin produced dose-dependent increases in KIM-1 concentrations over time (8). The authors observed male Han Wistar rats receiving 3, 7, and 14 days of treatment and noted increased expression of KIM-1 with total doses of 140 mg/kg as early as 3 days (8). We observed a similar increase in KIM-1 concentrations among male Sprague-Dawley rats even after only 1 day of treatment with doses of up to 200 mg/kg/day. In light of these findings, we believe that our results reflect the low end of the exposure-response curve. Although we administered allometrically scaled doses (i.e., equivalent doses in humans of approximately 25 to 30 mg/kg/day), the actual amount of vancomycin reaching the rat plasma was likely limited by less than 100% bioavailability after intraperitoneal injection. Furthermore, the durations were relatively short compared to those of typical regimens prescribed to human patients.

Our findings and the findings of others are biologically plausible. In our study, we observed a relationship between the intensity and duration of vancomycin exposure and the elevation in the concentrations of urinary AKI biomarkers, such as KIM-1, which are localized to the proximal tubule. Increases in KIM-1 urinary expression greater than 1.87-fold compared with that for the controls has over 95% specificity for AKI (8). Ample preclinical (8, 9, 38) and clinical (39, 40) data demonstrate that vancomycin can induce proximal tubular damage. Although the exact mechanism by which vancomycin causes proximal tubule damage remains unclear, data suggest that this damage may be due to oxidative stress and mitochondrial dysfunction. In their study of the genomic and transcriptomic response within renal cells among vancomycin-treated BALB/c mice, Dieterich et al. observed a significant downregulation of antioxidant gene expression and an upregulation in oxidative stress and organelle dysfunction markers (41). Similarly, King and Smith observed increased ATP and oxygen consumption concurrently with the proliferation of proximal tubule cells in their study of vancomycin-treated porcine ($LLC\text{-}PK_1$) cells (42). Further, multiple groups have demonstrated that antioxidants mitigate the damage done by vancomy-

cin (43–47). As vancomycin is thought to be an oxidative stressor within the proximal tubule, increases in local vancomycin exposures within proximal tubule cells should precede elevations in sensitive biomarkers of AKI. Our observation that increasing exposures are correlated with the rise of novel AKI biomarkers is thus highly biologically plausible.

While we noted elevations in several novel urinary AKI biomarkers, we did not observe a relationship between exposure and histopathological changes. Of note, we did not observe frank histopathological damage among the experimental animals, and the urinary biomarker elevations that we observed did not reach the levels seen in other investigations (8, 9, 15, 16). However, it is important to note that the study protocols used in studies producing frank histopathological damage were longer and the doses were higher than those in the present study. Thus, the doses used in our study likely led only to the low end of the toxic range (i.e., the first bend in a sigmoidal curve). As such, we did not fit an exposure-response model to the data, as more robust sampling of the upper bend in the curve is needed for proper fitting of sigmoidal exposure-response relationships.

Limitations to our study should be considered. First, we observed high degrees of variability in vancomycin exposures among vancomycin-treated animals. The variability seen in our study, as with the variabilities seen in other studies (41), was thought to be inherent because of the i.p. administration route. We utilized vancomycin concentrations of 100 mg/ml, but we did not evaluate any pH-dependent solubility issues that may have occurred with i.p. dosing. Certainly, precipitation of the drug in the peritoneal cavity is possible (48, 49), which may have contributed to the intra-animal variability in absorption. However, the increased variability in vancomycin exposures seen within our study allowed a wide range of drug exposures. Even with high correlations between the pharmacokinetic parameters, some separation was attained, which suggests that the vancomycin AUC_{0-24} or $C_{max_{0-24}}$ is likely the important target for avoiding kidney injury. Additionally, i.p. dosing provides a depot effect and allows vancomycin concentration-time profiles to more closely mimic those in humans in comparison to the profiles obtained if the doses were simply administered as an intravenous bolus. Previous investigations of the influence of the vancomycin dose on the concentrations of urinary AKI biomarkers in animal models did not evaluate the variability in exposure, as vancomycin PKs either were not evaluated (9) or were not evaluated within the same animals in which TD was measured (41). The variability in absorption and bioavailability due to i.p. administration of vancomycin (an increase in the 50% lethal dose of up to 7-fold has been observed when vancomycin was given i.p. [14, 48, 49]) presents a significant challenge when attempting to make comparisons on a by-dose basis. Thus, our analysis is unique in that paired PKs and TD were measured in the same animals. Accordingly, our analysis was based on exposure as opposed to a strict evaluation by dose category.

We have demonstrated that higher vancomycin exposures are correlated with increasing concentrations of urinary AKI biomarkers. Our preliminary PK/TD correlations support the suggestion that AUC_{0-24} and $C_{max_{0-24}}$ are most closely associated with the rise of urinary markers of renal insult. Further work is required to further delineate the exact PK/TD profile that produces AKI in a rat model. Clarification of the most predictive profile will have implications for the design of future experiments designed to minimize AKI after administration of humanized exposures.

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