Effects of Obesity and Sex on Antimicrobial Pharmacokinetics and Acute Kidney Injury: Validation of a Preclinical Model

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Obese patients may be at a greater risk for acute kidney injury (AKI) with the use of certain antimicrobial agents that are dose by weight. Current preclinical models of AKI utilize the male rat within a narrow weight range that limits extrapolation of the generated results. We evaluated the pharmacokinetics and AKI potential of gentamicin in 14-week-old diet-induced obesity-prone (n = 40) and obesity-resistant (n = 40) rats of both sexes. Single daily doses of gentamicin (12.5, 18.75, or 25 mg/kg of body weight) or saline (control) were administered intraperitoneally for 14 doses. Blood samples were collected after doses 1, 7, and 14, assayed by liquid chromatography-tandem mass spectrometry (LC-MS/MS), and analyzed using a nonparametric population pharmacokinetic approach for gentamicin. Urine was collected after doses 1, 3, and 5 and assayed for kidney injury molecule 1 (KIM-1) and neutrophil gelatinase-associated lipocalin (NGAL) and normalized to creatinine (Cr) values. Histology was performed on all animals, and the degree of proximal tubular injury was graded. The mean (minimum, maximum) weight of the rats was 330 (136, 580) g. NGAL/Cr predicted AKI better than did KIM-1/Cr and was detectable in male rats after dose 1 and in obesity-prone female rats after dose 5. Proximal tubular injury by histology was significantly higher in male than in female rats. A significant relationship between the gentamicin area under the curve from zero to 24 hours (AUC₀–₂₄) estimates and the maximum NGAL/Cr ratio was observed. This preclinical model has the potential to aid with dose extrapolation for body size and improve assessment of the toxicology potential of antimicrobials in development.

The prevalence of obesity in the U.S. adult population is estimated to be approximately 36% (1, 2). This high prevalence of obesity should raise concerns about our current drug-dosing approach, which broadly includes use of flat-fixed doses (regardless of weight) and weight-based pharmacokinetics and pharmacodynamics due to nonproportional increases in drug clearance with body weight in obese adults (3). Specifically, obesity is known to be an excellent surrogate of kidney function, and so obesity, they have not been used sufficiently for toxicology testing. The diet-induced obese rat model has been shown to mimic changes in kidney function seen in human obesity more closely than do the genetically modified rat models (12). In addition, changes in kidney function are most often evaluated using the endogenous biomarker creatinine, which may not be sensitive for early detection of AKI (13). In the past decade, kidney injury molecule 1 (KIM-1) and neutrophil gelatinase-associated lipocalin (NGAL) have been identified as sensitive endogenous biomarkers of AKI (13). Commerically available assays are also now available for both KIM-1 and NGAL for clinical use.

Taken together, a clear opportunity exists to improve the clinical translational potential of toxicology data generated from the rat by (i) using animals of both sexes, (ii) evaluating a wider weight distribution, and (iii) detecting AKI with sensitive biomarkers. Hence, the current study was performed to validate a rat model of obesity as a system to compare the effects of obesity and sex on drug-induced AKI. Gentamicin was selected as the test drug since it is dosed clinically based on weight and is associated with AKI.

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the International Genetic Standard (IGS) breeding program (14, 18–23). The present study compares the pharmacokinetics, AKI potential, and histopathological differences of gentamicin in IGS male and female rats (Charles River, Wilmington, MA) that were obesity prone (OP) and obesity resistant (OR).

MATERIALS AND METHODS

Animals. All animal preparation, monitoring, and recovery procedures were approved by the institutional animal care and use committee prior to study initiation. OP and OR rats of both sexes were obtained from Charles River Laboratories (Troy, NY) and maintained individually in a climate-controlled vivarium at 21°C on a 12-h light/12-h dark cycle with food and water ad libitum. Details regarding the development of this strain have been reviewed (24). Briefly, the OP and OR outbred line have a fully functioning leptin receptor and were developed from a Crl:CD(SD) line. Obesity is induced in this model through maintenance of animals on a high-fat diet. White and Lee have previously detailed the system for development and maintenance of the Crl:CD(SD) IGS breeding system (Charles River, Wilmington, MA) (24). The rats were 10 weeks of age at acquisition, and both OP and OR rats were maintained on a 60% fat diet (RD12492; Research Diets, New Brunswick, NJ) for 6 weeks. Animals were weighed each week during acclimation (initial 4 weeks) and daily during the experimental dosing phase (final 2 weeks).

Drug acquisition and dosing. A single batch (lot 90-433-DK) of gentamicin sulfate (injectation, USP; Hospira, Lake Forest, IL) was used for this study. An initial group of male rats (5 obese and 5 lean) given a daily dose of 50 mg/kg of body weight were studied, but this experiment was terminated prematurely due to morbidity (see Results). Consequently, the highest tested dose was reduced by 50%. Animals were weighd daily prior to the single daily intraperitoneal (i.p.) injection of three weight-based dosing groups given gentamicin (12.5, 18.75, or 25 mg/kg) and one group given normal saline (control, dose volume matched) for 14 days. Eighty rats were studied, which permitted allocation of 5 rats to each of 16 possible groups, composed of 2 sexes (male and female), 2 weight groups (OP and OR), and 4 dosing groups (control included).

Blood and urine sampling. Blood samples were collected from the lateral tail vein at baseline and 0.25, 0.5, 0.75, 1.0, 3.0, 6.0, and 24 h after dose 1, dose 7, and dose 14. Blood was collected from 2 animals (from the same group) in a time-staggered manner, for example, rat 1 at the 0.25, 0.75, and 3.0 h time points, from rat 2 at the 0.5, 1.0, and 6.0 h time points, and from both rats at baseline and the 24-hour time point. Serum was separated from whole blood and stored frozen at −70°C until analysis. Urine was collected by housing 2 animals per treatment group individually in metabolic cages for 24 h after dose 1, dose 3, and dose 5. The urine volume was measured, and aliquots were stored frozen at −70°C until analysis was performed.

AKI biomarker assays. Enzyme-linked immunosorbert assay (ELISA) 96-well kits for rat KIM-1 (Argutus Medical, Dublin, Ireland) and rat NGAL (ALPCO, Salem, NH) were used to measure biomarker concentrations in urine. A commercially available quantitative colorimetric assay was used to measure creatinine concentrations in urine and serum (Cayman Chemicals, Ann Arbor, MI). All assays of biomarkers were performed per the manufacturer’s instructions. All measurements were read using the Tecan Infinite 200 PRO plate reader system (Männedorf, Switzerland).

Gentamicin assay. Serum samples were assayed for gentamicin by a liquid chromatography-tandem mass spectrometry (LC-MS/MS) approach using an API 4000 triple-quadrupole mass spectrometer (Applied Biosystem/MDS Sciex, Carlsbad, CA). For preparation of the stock solution, gentamicin (Sigma, St. Louis, MO) was weighed and dissolved with 50% methanol to get 5 mg/ml of gentamicin. The working solution of gentamicin was prepared freshly with serum by dilution of the stock solution to obtain two sets of calibration curve samples ranging from 7.8 to 500 μg/ml and 0.039 to 5 μg/ml, respectively, for quantification of high and low levels of gentamicin in sera of experimental animals. Serum (10 μl) from experimental animals was deproteinated using a 40% methanol and 4% trifluoroacetic acid solution. Samples were separated using an Oasis HLB online column, 4.6 mm by 20 mm by 25 μm (Waters, Milford, MA) with a two-flow channel selection valve system for both extraction and chromatographic analysis. The mobile phase (MP) was switched between two solutions of either 0.2% formic acid (MP-A) or methanol containing 0.2% formic acid (MP-B) at specified time points to create a gradient elution. Specifically, the gradient elution was achieved by initiating with 60/40% MP-A/MP-B, 15/85% MP-A/MP-B from 0.1 to 1.50 min, and 4/96% MP-A/MP-B from 1.51 to 4.50 min and returning to 40% MP-B at 4.51 min. The column effluent was switched into waste for the first 40 s and then into ion source for 1 min, with gentamicin elution at 1.06 min. The tandem mass spectrometer was operated in positive electrospray mode with the ion spray voltage set at 2.5 kV and temperature at 600°C. The collision gas (CAD), curtain gas, and ion source gas 1 and gas 2 were set to 3, 30, 35, and 40 liters/min, respectively. Gentamicin was detected using multiple-reaction monitoring and quantitation achieved through monitoring of mass transition 464.4/332.2 (m/z). All data were acquired and processed with Analyst 1.4.2 software (Applied Biosystem/MDS Sciex, Carlsbad, CA). The limit of detection was 20 pg, and limit of quantitation was 2.5 ng/ml.

Pharmacokinetic analysis. Nonparametric population pharmacokinetic (POP-PK) system analysis was performed using PMetrics version 0.25, developed by Michael Neely and colleagues at the Laboratory of Applied Pharmacokinetics (LPK), University of Southern California (25). POP-PK modeling was achieved with PMetrics using the nonparametric adaptive grid (NPAG) algorithm with adaptive λ. The natural log concentration-time profile of gentamicin was suggestive of a two-compartment model. Therefore, the initial exploratory approach included modeling the concentration-time profile of gentamicin using a two-compartment model. The model included bolus input, $K_{e}$ as the rate constant of systemic absorption (peritoneum to central), $V_{c}$ as the volume of the central compartment, CL as the total clearance from the central compartment, $K_{tp}$ as the transfer rate constant from the central to peripheral compartment, and $K_{cp}$ as the transfer rate constant between the peripheral and central compartment. The model was executed as an algebraic solution. Discrimination between the initial and alternate models (higher and lower complexity) was accomplished by the rule of parsimony based on Akaake’s information criterion (AIC) (26). Adaptive-λ, comparable to an additive and proportional error variance model, was used to define residual variability. The additive component (standard deviation [SD] intercept) and the proportional component (SD slope) was fit for the population. The initial estimate for the SD intercept was 0.00025 (lower limit of quantification [LLOQ]), and the SD slope was 0.10.

Stepwise covariate-Bayesian posterior parameter linear regressions (forward and backward) were performed using the PMstep subroutine within PMetrics. Significant covariates were introduced into the model in a stepwise fashion, and final model selection was based on goodness of fit and AIC. Evaluation of the final POP-PK model was performed using diagnostic plots. The diagnostic plots included population and individual predicted versus observed plots, residuals versus time, and residuals versus individual predicted concentration. Visual predictive check of the final POP-PK model was performed by first simulating (SIMrun) of 1,000 subjects for each rat using a semiparametric approach followed by overlaying (SIMparse and PMdiag) the 5th, 25th, 50th, 75th, and 95th confidence intervals (CI) over time on the observed data. Finally, an integrated pharmacokinetic-pharmacodynamic model comparing the area under the concentration-time curve integrated to 24 h (AUC0–24) to biomarkers of AKI was tested. Additional details regarding the procedures outlined above can be retrieved through the PMetrics manual, tutorials, and forum available through the LPK website (27).

Histopathology. Animals were sacrificed by a regulated carbon dioxide (7.5%) euthanasia procedure, and necropsies were performed on all rats. Harvested kidneys were weighed, washed twice with cold phosphate-buffered saline, and stored in 10% buffered formalin. Tissue samples were...
submitted for slide preparation and microscopic evaluation by a treatment dose-blinded veterinary pathologist (Taconic, Rockville, MD). Kidney injury was evaluated using a modified grading scale based on the percentage of total proximal tubules demonstrating desquamation and necrosis, where 0 corresponds to proximal tubules within normal limits, 1 corresponds to >0% to <10%, 2 corresponds to 10% to 30%, 3 corresponds to >30% to 50%, 4 corresponds to >50 to 70%, 5 corresponds to >70 to 90%, and 6 corresponds to >90% (22).

Data analysis. Parametric and nonparametric statistical analyses were performed using StataSE Version 11 (StataCorp, College Station, TX). As stated earlier, the POP-PK analysis was performed as an integrated model (all time points included). Given that the study included repeated measurements over time, multivariate and multilevel mixed-effects linear regression models were also used to assess the sources of variation for animal weight and biomarker concentrations over time. The mixed-effects models were fitted by restricted maximum likelihood using the mixed-effects model function (xtmixed) in StataSE. Separate fixed-effect models were fitted by adding covariates to assess individual significance before inclusion in the multivariable model. Discrimination between models was based on AIC using postestimation statistics. The final model incorporated the significant covariates and the random effects. Histopathology grading scores were compared between sex and weight groups by gentamicin dose using the Kruskal-Wallis rank test. A P value of <0.05 was considered statistically significant.

RESULTS

Weight distribution and changes over time. Male OP-rats in the 50-mg/kg dosing group demonstrated >15% loss of weight, and 2 rats demonstrated signs of neuromuscular blockade within 20 min of gentamicin injection that lasted for 15 to 20 min. As a result, the study protocol was modified to study lower doses, and the following data reflect those generated with the exclusion of the initial 50-mg/kg group. The mean weight (coefficient of variation [CV]) for the overall population was 330 g (32.7%) with a minimum and maximum weight of 136 g and 580 g, respectively. The mean weight (CV) by group just prior to receipt of dose 1 of gentamicin or saline control was 454 g (15.5%) for obese males, 342 g (17.2%) for lean males, 268 g (14.8%) for obese females, and 206 g (10.0%) for lean females. Male and female rats gained approximately 40% and 20%, respectively, in weight from acquisition through the acclimation period. However, male rats lost weight while female rats maintained weight during the 14-day treatment period with gentamicin (Fig. 1). Mixed-effects modeling demonstrated a mean (95% CI) loss of 1.25 (0.33, 2.17) g per mg/kg dose of gentamicin and loss of 4.77 (4.42, 5.12) g per day of gentamicin treatment among male rats. This independent relationship of dose and treatment duration on weight loss was not observed with female rats.

Gentamicin concentration-time profile. The maximum gentamicin serum concentration was observed 0.5 h after intraperitoneal injection. The mean (SD) concentrations after dose 1 at 0.5 h (C_{0.5}) by dosage were 81.9 (30.3) mg/liter, 86.3 (38.6) mg/liter, and 121 (85.1) mg/liter for the 12.5-, 18.75-, and 25-mg/kg groups, respectively. The observed concentrations with each dosing regimen for the entire population over time are illustrated in Fig. 2A. The natural logarithm concentration-time profile declined in a biphasic manner with a rapid decline over the first 3 h followed by a slower decline between 3 and 24 h as illustrated in Fig. 2B. The mean (SD) concentrations at 3 h (C_{3}) by dose were 3.12 (4.40) mg/liter, 4.33 (5.46) mg/liter, and 10.5 (13.2) mg/liter for the 12.5-, 18.75-, and 25-mg/kg groups, respectively. The mean concentrations 24 h (C_{24}) after dose 1 ranged between 0.290 mg/liter and 1.62 mg/liter.

Gentamicin pharmacokinetic parameters. The initial structural model provided a good fit to the data, and exploration of lower and more complex models did not improve the fit or reduce the AIC (Fig. 3). A summary of the pharmacokinetic parameters, covariance matrix, and covariate assessment is provided in Table 1. The population K_{p}, V_{c}, CL, K_{cp}, and K_{pc} estimates were a median of 2.194 h^{-1}, 26 ml, 28 ml/h, 1.342 h^{-1}, and 0.068 h^{-1} respectively. Weight was identified as a significant (P = 0.030) covariate of V_{c}, while no significant covariate of CL was observed. Obese group classification, sex, and time were all observed to be significant (P < 0.013) independent covariates of K_{pc}. However, a stepwise inclusion of these covariates into the structural model mar-

FIG 1 Change in weight of male and female rats over the 14-day experimental period based on the reference weight at day zero (gentamicin dose initiation).
ginally improved the individual model predicted versus observed concentration fit but worsened the population model predicted versus the observed concentration fit. The AIC was also not reduced with the inclusion of these covariates. As a consequence, no covariates were included in the final model.

**Comparison of AKI biomarkers.** A good linear relationship ($R^2 = 0.70$) between creatinine (Cr) excretion in urine and weight was observed, with a slope of 0.035 mg of Cr/g of rat weight with a nonsignificant constant. No significant change ($P = 0.53$) in the KIM-1/Cr ratio was observed in the female rat groups. In contrast, a median 2- to 3-fold change in KIM-1/Cr was observed among the male obese and lean rat groups after dose 3 of gentamicin. However, no significant difference in the KIM-1/Cr ratio was noted between the dose levels of gentamicin. Compared to KIM-1/Cr, NGAL/Cr ratios were a median 1.6-fold (OR) and 4.4-fold (OP) higher in urine after dose 1 (18.75 mg/kg) of gentamicin in male rats. In contrast, only the obese female rats had a >2-fold increase in NGAL/Cr from baseline after dose 5 of the 25-mg/kg gentamicin regimen. No quantitative relationships between changes in KIM-1/Cr and NGAL/Cr over drug exposure and treatment duration were observed. Serum Cr concentrations were a median 2-fold higher after dose 7 and 4.8-fold higher after dose 14 in the male rats compared to baseline. In contrast, serum creatinine concentrations were a median 1.8-fold higher in female rats after dose 14. An integrated population pharmacokinetic-pharmacodynamic model could not be fit to each biomarker, but a relationship of the maximum NGAL/Cr ratio to model-predicted $\text{AUC}_{0-24}$ values was observed. As shown in Fig. 4, the maximum NGAL/Cr ratio fit well to a sigmoidal function to the model-estimated $\text{AUC}_{0-24}$ values, with a 50% effective concentration (EC$_{50}$) of 138 mg · h/liter.

**Histopathology.** Differences in the gross pathology of gentamicin-treated rats by sex were evident during the necropsies, with...
TABLE 1 Population pharmacokinetic parameter estimates, covariance, and significant covariates identified for each parameter for the final two-compartment model describing gentamicin concentration-time data

<table>
<thead>
<tr>
<th>Statistic</th>
<th>K_e (h⁻¹)</th>
<th>V_e (liter)</th>
<th>CL (liter/h)</th>
<th>K_pe (h⁻¹)</th>
<th>K_pe (h⁻¹)</th>
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<tr>
<td>Mean</td>
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<td>0.032</td>
<td>0.032</td>
<td>1.089</td>
<td>0.234</td>
</tr>
<tr>
<td>SD</td>
<td>3.210</td>
<td>0.024</td>
<td>0.045</td>
<td>0.775</td>
<td>0.532</td>
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<tr>
<td>Median</td>
<td>2.194</td>
<td>0.026</td>
<td>0.028</td>
<td>1.343</td>
<td>0.068</td>
</tr>
</tbody>
</table>

Covariance matrix

<table>
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<th>Covariates</th>
<th>K_e (h⁻¹)</th>
<th>V_e (liter)</th>
<th>CL (liter/h)</th>
<th>K_pe (h⁻¹)</th>
<th>K_pe (h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K_e (h⁻¹)</td>
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<td>0.012388</td>
<td>−0.00374</td>
<td>0.346865</td>
<td>0.95299</td>
</tr>
<tr>
<td>V_e (liter)</td>
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<td>0.000575</td>
<td>−0.00513</td>
<td>−0.00602</td>
<td>0.001841</td>
</tr>
<tr>
<td>CL (liter/h)</td>
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<td>0.000513</td>
<td>0.002007</td>
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<td>0.002794</td>
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<td>K_pe (h⁻¹)</td>
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<td>−0.00602</td>
<td>−0.00835</td>
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<td>0.1169</td>
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<tr>
<td>K_pe (h⁻¹)</td>
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<td>0.001841</td>
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Covariates

<table>
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<tr>
<th>Covariates</th>
<th>(P value)</th>
<th>Wt (g)</th>
<th>OP (yes, no)</th>
<th>Sex (male, female)</th>
<th>Time (day)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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Note: NS, not significant.

kidneys appearing markedly blanched in males. The fractional weight of kidneys to body weight was 13 to 46% higher in OR than in OP control rats. The mean (95% CI) slope of the natural logarithm plot of kidney weight to rat weight for control rats was 0.76 (0.43 to 1.1) (P < 0.001 and R² = 0.72). The fractional weight of the kidneys was 30 to 80% higher (P < 0.0001) in gentamicin-treated male rats than in control rats but not (P = 0.23) for female gentamicin-treated versus control rats. Female rats had lower (grades 1 or 2) histology scores (P < 0.0001) than male rats (grades 3 to 5) that were treated with gentamicin (Fig. 5). However, there was no clear influence of the gentamicin dose on the histology score. Most of the gentamicin-treated kidney sections displayed a minimal (female) to mild to moderate (male), multifocal, interstitial inflammatory cell infiltration composed of primarily lymphocytes. These interstitial infiltrations were primarily confined to the renal cortex and were often visualized surrounding occasional small vascular channels or forming nodules adjacent to glomeruli. There was no indication of any glomerular changes, including glomerular sclerosis or periglomerular fibrosis, occurring within any of the kidney sections evaluated.

DISCUSSION

The current investigation evaluated the pharmacokinetics and toxicology of 12.5- to 25-mg/kg once-daily doses of gentamicin in a well-defined rat model (Charles River, Wilmington, MA) with a wider weight distribution than typically utilized in toxicology models (9). Our experiments yielded pharmacokinetic data that mimicked the concentration-time profile of gentamicin observed in humans across a wide body size (2). In humans, use of 7-mg/kg doses is associated with concentrations in serum at the end of 1-hour intravenous infusion of approximately 40 mg/liter, which decline biphasically to 5 to 10 mg/liter over 3 h, with an approximate mean AUC₀⁻二十四 of 120 mg · h/liter in a typical 80-kg individual (28). Our observed higher C₀⁻二十四 concentrations in rats are a reflection of bolus injection with a biphasic decline to comparable concentrations postdistribution. The observed and population AUC₀⁻二十四 estimated values in the current study are also in line with values that would be expected in patients treated with 7 to 10 mg/kg of gentamicin (28). The observed difference in the degree of AKI between the groups was also consistent with historic data (14, 19). However, the effects of sex outweighed the influence of obesity on the development of gentamicin-induced AKI. These sex-based differences have historically been attributed to differences in body size and to composition-, pharmacokinetics-, and strain-based differences (10, 14, 21, 23). However, the current data suggest that these observed sex-based differences may be independent of body size and pharmacokinetics. Herein we explain the basis of these conclusions and the potential role of this model for future studies.

Our weight-based dose fractionation design permitted comparison of groups that received variable absolute gentamicin doses but achieved highly comparable exposures (Fig. 6). For example, the OP female group achieved absolute gentamicin exposures similar to those of OR males, but the degree of AKI by histology was clearly greater in the male group (Fig. 5). This difference between the sexes was also evident by the urine biomarkers of AKI and serum creatinine. Although NGAL/Cr was more sensitive than KIM-1/Cr, higher concentrations of these biomarkers were observed in males than in females. In addition, NGAL/Cr was detectable after the first gentamicin dose in male rats but was detectable with the highest-dose level in obese female rats only after the fifth dose. Finally, histology demonstrated definitively that on average, gentamicin-treated female rats had 10 to 30% proximal tubular injury, in contrast to male rats, who had 50 to 70% proximal tubular injury despite comparable exposure.

Our findings in the male rats are concordant with those of Corcoran and Salazar, who pioneered the evaluation of drug-induced organ injury in the obese overfed rat (18, 19). They previously demonstrated AKI in male rats within 5 days of treatment with gentamicin at 30 mg/kg administered i.p. every 12 h (19). The model was established by assigning weanling male rats to either an energy-dense diet (obese group) or a standard diet (lean group) over 52 weeks (19). This diet led to an average 1.8-fold difference in the fractional weight of kidneys to body weight.

![FIG 4 Scatter and sigmoidal-fit plot of the relationship between the maximum observed ratio of urine neutrophil gelatinase-associated lipocalin to creatinine (NGAL:Cr) over the gentamicin area under the concentration-time curve integrated from time zero to 24 h (AUC₀⁻二十四).](http://aac.asm.org/Downloaded-from)
in weight between the obese and the lean male rats. In contrast, our model utilized a standardized energy-dense diet in both groups, tested both sexes, and established a 2.2-fold mean difference in weight between groups. This difference in weight was established over a much shorter time (14 weeks versus 52 weeks) and eliminated the influence of diet as an extrinsic confounder when comparing the treatment groups. We also used younger rats, which is relevant given that age can also affect the toxicology potential of xenobiotics (29). Hence, this model has the potential to define the interaction of age, sex, and obesity on toxicology in the future. Finally, this model used IGS rats, which permits the replication and confirmation of our results by other research groups.

As with any study, our work has important limitations that should be considered. First, we did not evaluate the impact of dosing frequency on the occurrence of gentamicin-induced AKI. That is, we did not complete a dose fractionation (by frequency) study to better define the potential relationship between alternate gentamicin pharmacokinetic parameters such as the maximum drug concentration in serum ($C_{\text{max}}$) and $C_{\text{min}}$ on the occurrence of AKI. Second, we evaluated only one time point for histology (after dose 14) and 3 early time points (after doses 1, 3, and 5) for AKI biomarkers. Our failure to tease out a well-defined exposure-toxicity relationship with histology and biomarker data was likely a consequence of this limited evaluation period and range of exposures. We demonstrated a relationship between the maximum NGAL:Cr ratio and AUC$_{0-24}$ but the limited number of measurements in the upper exposure range warrants caution when interpreting this finding. Finally and most importantly, we did not evaluate the mechanistic pathway for this sex-based difference in gentamicin-induced AKI. Previous investigations have also shown this sexual dimorphism in drug-induced AKI but have also not been able to identify a clear mechanistic explanation for this finding (10).

Conflicting data thus far have suggested that this sexual dimorphism in aminoglycoside-induced AKI is rat strain specific. Bennett et al. first observed a higher probability of AKI in male than in female Fischer 344 rats treated with gentamicin (14). Subsequently, Hottendorf and Williams suggested that the inbred Fischer 344 rats were more prone to this sex-based difference than the outbred Sprague-Dawley rat (21). Reinhard et al. compared the toxicity of tobramycin at 30, 60, or 120 mg/kg twice daily administered to 8 week-old male Fischer 344 (203 ± 2 g) and Sprague-Dawley (346 ± 6 g) rats (23). This study did not compare the two sexes but observed a significant difference between the strains with the 60- and 120-mg/kg twice-daily administration but not with the 30-mg/kg twice-daily dose (23). The degree of AKI was greater in Fischer 344 than in Sprague-Dawley rats despite receipt of lower absolute doses (23). Finally, Goodrich and Hottendorf compared both male and female rats (10 to 11 weeks of age) of both Fischer 344 and Sprague-Dawley strains to evaluate tobramycin-induced AKI (20). In this previous study, the Sprague-Dawley rats received a dose of 90 mg/kg twice daily, while the Fischer 344 rats received a dose of 30 mg/kg twice daily (20). A sex-based difference was observed in the Fischer 344 group but not in the Sprague-Dawley group (20). These previous experiments did not quantitatively characterize the pharmacokinetics of aminoglycosides or use a dose fractionation design to yield comparable exposures between these groups (14, 20, 21, 23). So, it is not clear if accurate comparisons of toxicity differences by sex were made from this historical data set, and this suggests that the question of potential strain-based differences in aminoglycoside-induced AKI is far from resolved. Furthermore, we evaluated gentamicin, which is considered to have a higher AKI potential than tobramycin (21). Our observed sex-based difference in this out-
bred rat model derived from the Sprague-Dawley strain occurred despite use of substantially lower doses (12.5 to 25 mg/kg/day) than those studied historically (60 to 240 mg/kg/day) (18–21, 23). Although our findings are not entirely congruent with these historic animal data, they are consistent with the observations from a large sample of clinical patients (15). Whether or not this sex-based finding extends to other xenobiotics, it is reasonable to at least evaluate the potential for differences in toxicity between the sexes across weight with preclinical experiments (10). This is especially true of new molecular entities such as the neoglycosides that are in development in response to rising Gram-negative organism resistance (30). Evaluation of this potential and of sex-based differences for AKI should also be performed for other common antimicrobials such as vancomycin, telavancin, colistin, and polymyxin B. Our development of a novel toxicology model creates an opportunity to reappraise the toxicology potential of drugs that are dosed on weight and eliminated by the kidneys. Although our evaluation was limited to the kidneys, the influence of obesity and sex on drug dosing and toxicology is likely to extend to other target organs. Exploration of this model to evaluate the multiorgan toxicology potential of xenobiotics across body size is warranted. The commercial availability of this obese, overfed rat model provides a rapid, simple, and potentially cost-effective platform to ascertain the influence of body size on antimicrobial pharmacokinetics and toxicology.

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