Lack of synergistic nephrotoxicity between vancomycin and piperacillin/tazobactam in a rat model and a confirmatory cellular model

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Received 8 October 2019; returned 3 November 2019; revised 18 December 2019; accepted 19 December 2019

Background: Vancomycin and piperacillin/tazobactam are reported in clinical studies to increase acute kidney injury (AKI). However, no clinical study has demonstrated synergistic toxicity, only that serum creatinine increases.

Objectives: To clarify the potential for synergistic toxicity between vancomycin, piperacillin/tazobactam and vancomycin + piperacillin/tazobactam treatments by quantifying kidney injury in a translational rat model of AKI and using cell studies.

Methods: (i) Male Sprague–Dawley rats ($n=32$) received saline, vancomycin 150 mg/kg/day intravenously, piperacillin/tazobactam 1400 mg/kg/day intraperitoneally or vancomycin + piperacillin/tazobactam for 3 days. Urinary biomarkers and histopathology were analysed. (ii) Cellular injury was assessed in NRK-52E cells using alamarBlue®.

Results: Urinary output increased from Day –1 to Day 1 with vancomycin but only after Day 2 for vancomycin + piperacillin/tazobactam-treated rats. Plasma creatinine was elevated from baseline with vancomycin by Day 2 and only by Day 4 for vancomycin + piperacillin/tazobactam. Urinary KIM-1 and clusterin were increased with vancomycin from Day 1 versus controls ($P<0.001$) and only on Day 3 with vancomycin + piperacillin/tazobactam ($P<0.001$, KIM-1; $P<0.05$, clusterin). The histopathology injury score was elevated only in the vancomycin group when compared with piperacillin/tazobactam as a control ($P=0.04$) and generally not so with vancomycin + piperacillin/tazobactam. In NRK-52E cells, vancomycin induced cell death with high doses (IC_{50} 48.76 mg/mL) but piperacillin/tazobactam did not, and vancomycin + piperacillin/tazobactam was similar to vancomycin.

Conclusions: All groups treated with vancomycin demonstrated AKI; however, vancomycin + piperacillin/tazobactam was not worse than vancomycin. Histopathology suggested that piperacillin/tazobactam did not worsen vancomycin-induced AKI and may even be protective.

Introduction

Vancomycin and piperacillin/tazobactam are two of the most commonly utilized antibiotics in hospitalized patients.¹ Meta-analyses² compiling over 25 000 patients suggest that vancomycin + piperacillin/tazobactam increases acute kidney injury (AKI) by an absolute of 9% against vancomycin + comparators with mean ORs ranging from 1.6- to 3.6-fold higher risk. This suggested that synergistic toxicity is concerning, and several experts recommend avoiding vancomycin + piperacillin/tazobactam^{3,4} even though vancomycin + piperacillin/tazobactam is a drug of choice in numerous national guidelines. Also concerning, other alternatives for piperacillin/tazobactam have equal limitations: cefepime is associated with dose-dependent neurotoxicity,⁵ fluoroquinolones have numerous black-box warnings,⁶ carbapenems promote broad antibiotic resistance,⁷ and aminoglycosides cause worse AKI.⁸ Thus, defining the AKI profile for vancomycin + piperacillin/tazobactam is imperative; even moderate AKI increases mortality and prolongs hospitalization.^{9,10}

Despite recommendations to avoid vancomycin + piperacillin/tazobactam, the biological plausibility of the increased renal damage has not been established¹¹. Previous studies have relied on serum creatinine (SCr) as an AKI surrogate. While SCr is easily measured, it is neither highly sensitive nor specific for AKI. False positives for AKI exist because SCr transit is defined by secretion and re-absorption (in addition to free filtration)¹² and SCr can be elevated for reasons unrelated to filtration even when the kidney is not injured because of drug competition for renal tubular secretion.¹³ Unlike vancomycin, which causes acute tubular necrosis via oxidative stress at the renal proximal tubule or uromodulin interaction/cast formation,^{14,15} piperacillin/tazobactam rarely causes AKI.^{14,16} To date, only acute interstitial nephritis (AIN) has been cited with piperacillin/tazobactam. Given that the absolute increase in the incidence of AKI in patients with vancomycin + piperacillin/tazobactam is very high (9%),² it is extremely unlikely that the rare AIN drives toxicity. Furthermore, no clinical study has demonstrated that synergistic toxicity occurs because of these mechanisms, only that SCr increases.

While SCr increases are not pathognomonic for AKI, novel urinary biomarkers may be more sensitive and specific for AKI. Additionally, the rat is an excellent model because kidney biomarkers are conserved between rats and humans.^{17,18} Further validating the rat-human translational link, urinary biomarkers are qualified for rat¹⁹ and human drug trials²⁰ by the FDA for drug-induced AKI. We have previously demonstrated that urinary kidney injury molecule-1 (KIM-1) and osteopontin (OPN) predict histopathological damage in a rat model of vancomycin-induced kidney injury (VIKI).^{21,22} This study sought to clarify the potential for synergistic toxicity between vancomycin, piperacillin/tazobactam and vancomycin + piperacillin/tazobactam treatments by quantifying kidney injury in a translational rat model of AKI and using cell studies.

Materials and methods

Chemicals and reagents

Treatments were clinical grade vancomycin (Hospira, Lake Forrest, IL, USA), piperacillin/tazobactam and cefepime (Apotex Corporation, Weston, FL, USA), gentamicin sulphate USP (Medisca, Plattsburgh, NY, USA) and normal

saline (Veterinary 0.9% Sodium Chloride Injection USP, Abbott Laboratories, North Chicago, IL, USA). For LC-MS/MS, creatinine pharmaceutical secondary standard certified reference material (Sigma-Aldrich), creatinine-d3 (Cayman Chemicals, Ann Arbor, MI, USA), LC-MS/MS grade acetonitrile and methanol (VWR International, Radnor, PA, USA), formic acid (Fisher Scientific, Waltham, MA, USA) and frozen, non-medicated, non-immunized, pooled Sprague-Dawley rat EDTA plasma (BioreclamationIVT, Westbury, NY, USA) were used. Other materials were similar to our previous reports.^{21,22}

Experimental design and animals

The animal toxicology study was conducted at Midwestern University (IACUC #2295) in compliance with the National Research Council's publication, the Guide.²³

Male Sprague-Dawley rats (290–320 g, Envigo, Indianapolis, IN, USA) were randomized to receive vancomycin 150 mg/kg/day ($n=8$), piperacillin/tazobactam 1400 mg/kg/day ($n=8$) and vancomycin + piperacillin/tazobactam at the same doses ($n=10$) or normal saline ($n=6$, Figure 1a). Surgeries were as previously described.²² The vancomycin dose was chosen based on previous studies^{21,24,25} and to approximate the human dose (30 mg/kg/day) allometrically scaled for the rat [i.e. 30 mg/kg $\times$ 6.2 (rat factor) = 186 mg/kg].²⁶ The piperacillin/tazobactam dose was chosen to approximate the human dose (225 mg/kg/day) allometrically scaled for the rat (i.e. 225 mg/kg $\times$ 6.2 = 1395 mg/kg). Vancomycin was given IV via the left jugular catheter as this has been shown to result in VIKI in our model, and piperacillin/tazobactam was given IP to extend residence time. Animals were placed in metabolic cages on Day -1 and from Day 1 to Day 3 (Figure 1b). Rats received the first dose on Day 1. In addition to saline as a control, animals served as their own controls, and each study day was compared with Day -1. Rats were housed in a light- and temperature-controlled room for the duration of the study and allowed free access to water and food, including the time in which they resided in metabolic cages (Lab Products Inc., Seaford, DE, USA).

Blood and urine collection

Blood samples (0.125 mL, maximum 15 samples/animal) were drawn from a right-sided jugular vein catheter in a sedation-free manner when possible,^{21,22} and plasma was stored at -80°C (Figure 1b). Urine was collected continuously, aliquoted every 24 h and centrifuged (400 g, 5 min), and the supernatant was stored at -80°C .

Figure 1. Experimental design. (a) Flow chart of animal dosing. (b) Timeline of the experiments. VAN, vancomycin; TZP, piperacillin/tazobactam.

Plasma creatinine

Plasma concentrations of creatinine were assayed by LC–MS/MS similarly to a method previously described.²⁷ The internal standard was isotope-labelled creatinine-d3 (0.1 mg/mL Cre-d3, Cayman Chemical, Ann Arbor, MI, USA). Creatinine was detected using Q1/Q3 ion transitions at mass-to-charge (m/z) ratios 114.1/44.3 amu as quantifier and 114.1/43.4 amu as qualifier. Cr-d3 internal standard was detected at m/z 117.09/89.2 amu. The assay was linear between concentrations of 0.31 and 40 mg/dL ($R^2 = 0.999$). We calculated the endogenous creatinine concentration in the blank plasma from the linear equation of the standard curve and adjusted the standard curve to reflect the true creatinine concentration in the sample. The intra- and inter-assay precisions were <2%. Greater than 92% accuracy was observed in all standards tested, with an overall mean assay accuracy of 99.8%. For plasma creatinine, baseline was Day 1 (post-surgery).

Urinary biomarkers

Urine samples were analysed using MILLIPLEX® MAP Rat Kidney Toxicity Magnetic Bead Panel 1 (EMD Millipore Corporation, Charles, MO, USA), according to the manufacturer's protocol as previously described.^{28,29} MILLIPLEX® Analyst v5.1 Flex software was used to calculate analyte concentrations. For urinary biomarkers, baseline was Day –1 (pre-surgery).

Histopathology

Animals were euthanized via exsanguination while under anaesthesia. Kidneys were harvested and washed in cold isotonic saline. The left kidney was preserved in 10% formalin solution; the right kidney was flash frozen in liquid nitrogen. Histopathological scoring was performed by IDEXX BioAnalytics (West Sacramento, CA, USA) on paraffin-embedded haematoxylin and eosin-stained kidney sections as previously described.²² In brief the PSTC Standardized Kidney Histopathology Lexicon was utilized with categorical scoring according to grades from 0 to 5 (0, no observable; 1, minimal; 2, mild; 3, moderate; 4, marked; and 5, severe pathology).³⁰ Composite scores for each animal were the highest ordinal score from any kidney site.³⁰

Cell viability

Normal rat kidney epithelial cells (NRK-52E, ATCC® CRL1571™)³¹ were cultured in DMEM (GenClone, Genesee Scientific) with 5% bovine calf serum in 5% CO₂ at 37°C. Penicillin and streptomycin were not included in the medium. Cells were either sub-cultured or received fresh growth medium two or three times per week. Cells were used between passages 14 and 30. NRK-52E cells were plated in 96-well half-volume black plates in DMEM. The next day the medium was changed to 10 mM HEPES-buffered Hanks' balanced salt solution (HHBS) in the absence of serum. Drugs were dissolved in normal saline and added to the cells. Cefepime was a negative control and gentamicin, a known nephrotoxin,³² a positive control. After 24 h of incubation with antibiotics at 37°C, 5% CO₂, cell viability was assessed using alamarBlue® (5 µL/well, Invitrogen). Cells remained in the same drug–HHBS solution until the end of the experiment. Plates were read at 48 h (24 h after the addition of alamarBlue®) using an Enspire Multimode Plate reader (Perkin Elmer) with Ex530/Em590 filters. Results were analysed by normalizing the cell metabolism of antibiotic-treated cells to saline controls and expressed as relative fluorescence units. Drug concentrations were transformed to log base 10. Sigmoidal three-parameter dose–response curves were generated after 48 h of incubation with antibiotics alone or combined with vancomycin at a concentration below its IC₅₀ (vancomycin, 1 mg/mL). The relative toxicity was compared using IC₅₀ values (the drug concentration resulting in 50% of maximal reduction in cell viability) obtained using GraphPad Prism version 7.02 (La Jolla, CA, USA).

Statistical analysis

For the rat studies, most statistical analyses were performed using Stata IC 15.1 (except where specifically noted). Decisions to perform analyses with repeated-measures ANOVA or mixed models were based on the Breusch–Pagan/Cook–Weisberg test for heteroscedasticity on the ANOVA model. Departure from constant variance at $P < 0.05$ served as a trigger to use a mixed model. Plasma creatinine, urinary output and urinary biomarker elevations were compared across groups using a mixed-effects, restricted maximum likelihood estimation regression, with repeated measures occurring over days; measures were repeated at the level of the individual rat. Additionally, LOESS models with 95% CI were created using R version 3.5.1³³ and the package ggplot2³⁴ to circumvent fit assumptions. From the mixed model, contrasts of the marginal linear predictions³⁵ facilitated comparisons of treatment groups versus controls of saline and pre-treatment values (i.e. Day –1). Ordinal logistic regression was used to classify the ordered log-odds of being in a higher histopathological scoring group according to treatment group. Logistic regression was utilized to determine the odds of having a histopathological score ≥ 2 when treatment groups were compared with saline or piperacillin/tazobactam. All tests were two-tailed, with an *a priori* level of statistical significance set at an α of 0.05.

For the *in vitro* studies, graphics were generated and inferential statistics were performed in GraphPad Prism version 7.02 (La Jolla, CA, USA) and R 3.5.1. Mean and SD were calculated for triplicate wells. Comparison of IC₅₀ values across treatment groups was facilitated by constraining a shared bottom value between 0 and 0.3 and a top of 1 for all groups. The extra sum of squares *F*-test was utilized to compare independent fits with a global fit, whereby a conservative α level > 0.2 defined IC₅₀ values that did not differ.

Results

Rat model

In all, 32 rats completed the protocol (Figure 1). Mean plasma creatinine at baseline was 0.51 ± 0.03 mg/dL and did not differ between the groups (Figure 2a). Plasma creatinine rose for vancomycin on Days 2, 3 and 4 from baseline and for vancomycin + piperacillin/tazobactam on Day 4 ($P < 0.02$ for all), but no treatment group was different from saline. The overall mean total urine output on Day –1 was 6.4 ± 1.9 mL (Figure 2b). Mean total urine output significantly increased on Day 1 following the first dose in the vancomycin-treated animals ($P < 0.02$) compared with baseline and saline-treated animals and remained elevated over 3 days. On the final day urine output began to fall for vancomycin-treated animals. There was a gradual increase in the mean total urine output in the vancomycin + piperacillin/tazobactam-treated animals compared with baseline and saline-treated animals that was significant on Days 2 and 3 ($P = 0.007$).

The constant variance assumption was not upheld in repeated-measures ANOVA for KIM-1, clusterin or OPN ($P < 0.001$ for all). Therefore, the mixed model was utilized. Urinary KIM-1 and clusterin differed between the treatment groups as a function of time, whereas OPN did not (Tables S1 and S2, available as Supplementary data at JAC Online). Changes in these biomarkers over time for each treatment group are graphically displayed in Figure 2(c–f). In the vancomycin group, urinary KIM-1 and clusterin were increased on Days 1, 2 and 3 when compared with saline ($P < 0.001$ for all; Table S1). For vancomycin + piperacillin/tazobactam versus saline, KIM-1 and clusterin elevations were seen only after 3 days of treatment ($P < 0.001$ and $P = 0.04$, respectively); however, both were elevated at Day 3 compared with baseline

Figure 2. Plasma and urinary biomarker levels in the rat after treatment with vancomycin (VAN) or piperacillin/tazobactam (TZP) alone or in combination with vancomycin (VAN+TZP). (a) Plasma creatinine change from baseline, (b) urinary output, (c) urinary KIM-1, (d) urinary clusterin and (e) urinary osteopontin levels in animals treated with saline ($n=6$), TZP ($n=8$), VAN ($n=8$) and VAN+TZP ($n=10$). Values are expressed as mean $\pm$ SD; * $P<0.01$ versus saline; # $P<0.05$ versus saline; ^ $P<0.02$ versus baseline. (f) Predictive margins with 95% CI of KIM-1 over 3 experimental days according to treatment group. Mild horizontal perturbation/jitter was applied to the points to enhance visualization. The top line (blue, triangles) is VAN, the intermediate line (red, diamonds) is VAN+TZP and the bottom lines are TZP (green, circles) and saline (black, squares). KIM-1, kidney injury molecule-1; OPN, osteopontin. Plasma creatinine is presented as change from baseline for ease of interpretation, but statistics were performed on the raw data. This figure appears in colour in the online version of JAC and in black and white in the printed version of JAC.

values ($P<0.001$). Similar findings were obtained with the LOESS model and non-overlapping CIs (Figure 2f). OPN did not increase between treatment groups or treatment days (Figure 2e, $P>0.05$ for all).

Ordinal histopathological scores were increased after treatment with vancomycin, piperacillin/tazobactam and vancomycin + piperacillin/tazobactam compared with the controls (Figure 3a). Control animals that received normal saline displayed normal kidney tissue histology (Figure 3b). When piperacillin/tazobactam was used as the referent group, histopathological scores were

significantly elevated in the vancomycin-treated group (score 2.16, $P=0.044$), significantly lower in the saline group (score -2.8 , $P=0.02$), and not different in the vancomycin + piperacillin/tazobactam group (score 0.29, $P=0.76$). In the model to predict histopathological score ≥ 2 , the saline group accurately classified the absence of injury (Table 1). Treatment with vancomycin was associated with a borderline increased risk of injury (OR 11.67, $P=0.058$, 95% CI 0.922–147.56). On the other hand, vancomycin + piperacillin/tazobactam treatment was not associated with injury (OR 1.11, $P=0.91$, 95% CI 0.16–7.5). The presence of casts was

Figure 3. Histopathological assessment of rat kidney tissue after treatment with vancomycin (VAN) alone, piperacillin/tazobactam (TZP) alone or TZP in combination with VAN (VAN+TZP). (a) Quantitative analysis of histopathology score in kidney tissues of rats. Values are expressed as mean ± SD; * $P < 0.044$ versus saline. Representative photomicrographs of haematoxylin and eosin-stained rat kidney sections. (b) Normal histology of kidney tissue in control rats administered normal saline, (c) tubular casts (arrow, $\times 100$) in cortex of rats treated with TZP 1400 mg/kg IP once daily for 3 days, (d) tubular dilatation, tubular degeneration (arrows) and tubular basophilia (asterisk, $\times 100$) in cortex of rats treated with VAN 150 mg/kg IV once daily for 3 days, and (e) tubular dilatation, tubular degeneration (arrows, $\times 100$) in cortex of rats treated with VAN+TZP once daily for 3 days. This figure appears in colour in the online version of JAC and in black and white in the printed version of JAC.

observed in all, but vancomycin, either alone or in combination with piperacillin/tazobactam had a higher incidence of casts. A representative cast is visualized in a stained kidney section from a piperacillin/tazobactam-treated rat (Figure 3c). More casts were observed among vancomycin (eight casts) and vancomycin + piperacillin/tazobactam (nine casts) groups compared with either saline (two casts) or piperacillin/tazobactam groups (four casts). Tubular dilatation and tubular basophilia were observed in all treatment groups but absent in controls. Notably, tubular basophilia and tubular dilatation were evident in every single kidney section in the vancomycin group ($n = 8/8$, Figure 3d). However, these findings were less common among the piperacillin/tazobactam and vancomycin + piperacillin/tazobactam groups ($n = 2$ and $4/8$ and $n = 2$ and $4/10$, respectively), suggesting possible nephron

Table 1. Ordered logistic regression on Day 4 histopathology score when comparing with either saline or piperacillin/tazobactam

Comparison	Coefficient	P value	Lower 95% CI	Upper 95% CI
Groups compared with saline				
TZP	2.834	0.020	0.438	5.230
VAN	4.993	≤ 0.001	2.287	7.699
VAN+TZP	3.123	0.010	0.753	5.494
Groups compared with TZP				
saline	-2.834	0.020	-5.230	-0.438
VAN	2.159	0.044	0.061	4.257
VAN+TZP	0.290	0.755	-1.526	2.105

VAN, vancomycin; TZP, piperacillin/tazobactam.

Figure 4. Serum-deprived normal rat kidney NRK-52E epithelial cell viability after treatment with vancomycin (VAN), piperacillin/tazobactam (TZP), cefepime (FEP) and gentamicin (GEN) alone (a) or in combination with 1 mg/mL vancomycin (b) for 48 h using the alamarBlue[®] assay. The results presented were obtained from a single experiment in triplicate but are representative of experiments conducted under different conditions. Values are expressed as mean $\pm$ SD. RFUs, relative fluorescence units. This figure appears in colour in the online version of *JAC* and in black and white in the printed version of *JAC*.

protection. Tubular degeneration was observed only among vancomycin and vancomycin + piperacillin/tazobactam groups but not in control and piperacillin/tazobactam groups. Tubular degeneration was more pronounced in rats treated with vancomycin (Figure 3d) compared with the vancomycin + piperacillin/tazobactam group (Figure 3e).

NRK-52E cell experiments

In serum-deprived NRK-52E cells, treatment with vancomycin alone produced cell death (IC_{50} 48.76 mg/mL, Figure 4a and b). Treatment with piperacillin/tazobactam and cefepime in the absence of vancomycin produced no cellular death (Figure 4a). When vancomycin was combined with fixed concentrations of piperacillin/tazobactam or cefepime, the degree of cellular death was not different compared with vancomycin alone (Figure 4b, $P > 0.2$). Gentamicin produced significant cellular death (IC_{50} 11.29 mg/mL). However, when gentamicin was combined with vancomycin, the IC_{50} of 6.98 mg/mL did not differ compared with gentamicin alone ($P > 0.2$).

Discussion

While a growing number of clinical studies have reported that vancomycin + piperacillin/tazobactam is associated with increased rates of AKI as measured by SCr, we demonstrated that VIKI from vancomycin + piperacillin/tazobactam is not worse than vancomycin alone. In fact, our rat data suggest that piperacillin/tazobactam may partially protect against VIKI. These data question the biological plausibility of vancomycin + piperacillin/tazobactam resulting in increased kidney toxicity. To the best of our knowledge, this is the first study investigating the effect of vancomycin + piperacillin/tazobactam on kidney injury using translational models focused on histopathology and biomarkers capable of discerning direct toxicity (as opposed to SCr as a surrogate).

Our experimental animal data are somewhat consistent with a previous animal study.¹⁵ In that study, vancomycin was administered at 25 mg/day IP for 2 days (which equates to ~ 1000 mg/kg/

day or an allometrically scaled dose of ~ 80 mg/kg/day)²⁶ and acute tubular necrosis was observed with granular material in the tubular lumen and cast formation. Human vancomycin doses are ~ 60 mg/kg/day even at the upper end of the dosing range.³⁶ This investigation also employed a very low dose of piperacillin/tazobactam at 100 mg/kg/day IP for 2 days (which is equivalent to 8.2 mg/kg/day human dose).²⁶ A standard human dose for a 70 kg patient is ~ 190 mg/kg/day.³⁷ Thus, it was possible that high-dose vancomycin and a low dose of piperacillin/tazobactam was the reason for seeing identical toxicity between vancomycin and vancomycin + piperacillin/tazobactam in their study. In the present study, we allometrically scaled vancomycin and piperacillin/tazobactam to the human dose and did not observe an increase in nephrotoxicity with vancomycin + piperacillin/tazobactam compared with vancomycin alone, demonstrating that adding piperacillin/tazobactam did not worsen VIKI and may even be protective. This protective effect of piperacillin/tazobactam against vancomycin nephrotoxicity could be due to the sodium load associated with it, as observed with ticarcillin sodium.^{38,39}

We demonstrated that vancomycin resulted in cell death in NRK-52E cells. The addition of piperacillin/tazobactam to vancomycin did not affect cell death. Similar results were obtained using other cell lines (HEK-293 and MDCK.2) under different culture conditions (data not shown). Notably, the IC_{50} of vancomycin from our study is higher than that reported by other groups in kidney cell lines,^{40–42} but we used clinical grade vancomycin in our cell studies. The pH of the final solution from clinical grade vancomycin should be less toxic to cells.⁴³ This removes a variable that could cause cellular death by means other than drug toxicity. Regardless, we did obtain a dose-response toxicity curve with vancomycin alone.

Mechanistically, it is well established that VIKI is caused by oxidative stress¹⁴ and uromodulin interaction/cast formation.¹⁵ Piperacillin can rarely cause AIN.¹⁶ However, no clinical study has demonstrated that synergistic toxicity occurs because of these mechanisms. It is notable that piperacillin/tazobactam on its own is not generally a nephrotoxin. Prospective randomized controlled trial data support the idea that piperacillin/tazobactam alone is

not overtly damaging to the kidney. For instance, when piperacillin/tazobactam was compared with meropenem/vaborbactam in 545 patients with urinary tract infections, piperacillin/tazobactam was reported to increase SCr at a rate of 0.4% versus 0% for meropenem/vaborbactam.⁴⁴ In a separate trial comparing piperacillin/tazobactam versus meropenem, piperacillin/tazobactam only resulted in $n=1$ non-fatal serious adverse event versus $n=0$ for meropenem.⁴⁵ At least some large retrospective studies have also failed to find differences in AKI rates (with SCr as a surrogate).^{46,47} Given our findings and these others, it remains possible that the reported increase in AKI with vancomycin + piperacillin/tazobactam from the clinical setting is a false positive from an imperfect surrogate of kidney injury (i.e. creatinine).

Clinical studies generally utilize the RIFLE or the AKIN definitions to classify AKI according to SCr.⁴⁸ SCr is non-specific for kidney injury.¹² However, in retrospective studies, SCr is often the only reliable variable to assess a patient's kidney function. It is possible that competition for secretion or re-absorption can affect SCr with vancomycin + piperacillin/tazobactam treatment, while kidney function (as measured by glomerular filtration rate) remains unaffected.^{49,50} Yet in our rat study plasma creatinine followed similar trends but lagged 2 days behind urinary output changes and 1 day behind urinary biomarker changes. Because of the study design, we did not obtain blood at Day -1, and thus it is difficult to understand the full relationship between urinary biomarkers and plasma creatinine in our study. An interaction between vancomycin and piperacillin/tazobactam at the level of tubular secretion may not explain the late elevation of SCr for vancomycin + piperacillin/tazobactam. It may be that short-term piperacillin/tazobactam use is protective, but prolonged use triggers other mechanisms. Notably, we have only studied a total of 4 days of combined vancomycin + piperacillin/tazobactam, and longer study durations are necessary.

Novel biomarkers are qualified for use in preclinical animal¹⁹ and clinical human²⁰ studies by the FDA to assess for drug-induced AKI, and by the EMA and the Japanese Pharmaceuticals and Medical Devices Agency for pre-clinical rodent studies.¹⁹ Urinary biomarker data from this study are interesting because: (i) KIM-1 and clusterin were recently demonstrated to be the most sensitive biomarkers for predicting VIKI defined by histopathology;⁵¹ and (ii) multiple samples over time facilitate temporal investigation of toxicity after renal insult. KIM-1 is highly sensitive and specific for proximal tubule damage, the locale of VIKI.²¹ KIM-1 is highly conserved between rats and humans, and thus the predictive capacity of rat KIM-1 for VIKI is compelling. A human homologue, KIM-1b, is structurally similar except for the cytoplasmic domain.⁵² Clusterin is present in kidney tubules, is anti-apoptotic and confers cell protection.⁵³ As it is not filtered through the glomeruli, elevation should indicate tubular damage.⁵³

Several limitations bear mention. These data are from a healthy rat model; however, the rat model is a well-accepted surrogate for human pathobiology.²⁰ We have previously demonstrated that the pharmacokinetic/toxicodynamic index between this predictive rat model (i.e. AUC of 482 mg·h/L over 24 h)⁵⁴ matches that seen in prospective human studies.⁵⁵ Histopathology was elevated for all three groups compared with saline; however, only vancomycin was elevated compared with piperacillin/tazobactam. This may reflect the inherent subjectivity of histopathology as the control

group was identified to the pathologist (per standard practice⁵⁶); however, the pathologist was blinded to treatments received. Urinary biomarkers may be less biased. We have not yet conducted drug assays, though these experiments are planned. Finally, while we saw signal for KIM-1 and clusterin in our study, OPN did not differ, but it is also not the best biomarker for VIKI⁵¹ or proximal tubule necrosis.⁵⁷

In conclusion, vancomycin + piperacillin/tazobactam did not cause more kidney injury than vancomycin alone as evidenced by a translational rat model measuring plasma creatinine, urinary output, urinary biomarkers and histopathology. Cellular studies supported these conclusions. Novel urinary biomarkers may aid in determining whether higher rates of kidney injury with vancomycin + piperacillin/tazobactam are realized in clinical studies.

Funding

This work was supported by internal funding from Midwestern University to the Pharmacometrics Center of Excellence and via equipment usage at the Midwestern University CORE center.

Transparency declarations

K.J.D. has received research support from Merck & Co., Inc. and Pfizer, Inc. M.H.S. reports having received a research grant from Nevakar, Inc. unrelated to the current work. All other authors: none to declare.

Supplementary data

Tables S1 and S2 are available as [Supplementary data](#) at JAC Online.

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