Oxygenator Impact on Ceftolozane and Tazobactam in Extracorporeal Membrane Oxygenation Circuits

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Objectives: To determine the oxygenator impact on alterations of ceftolozane/tazobactam in a contemporary neonatal/pediatric (1/4-inch) and adolescent/adult (3/8-inch) extracorporeal membrane oxygenation circuit including the Quadrox-i oxygenator (Maquet, Wayne, NJ).

Design: A 1/4-inch and 3/8-inch, simulated closed-loop extracorporeal membrane oxygenation circuits were prepared with a Quadrox-i pediatric and Quadrox-i adult oxygenator and blood primed. Additionally, 1/4-inch and 3/8-inch circuits were also prepared without an oxygenator in series. A one-time dose of ceftolozane/tazobactam was administered into the circuits and serial preoxygenator and postoxygenator concentrations were obtained at 5 minutes, 1, 2, 3, 4, 5, 6, and 24-hour time points. Ceftolozane/tazobactam was also maintained in a glass vial and samples were taken from the vial at the same time periods for control purposes to assess for spontaneous drug degradation

Setting: A free-standing extracorporeal membrane oxygenation circuit.

Patients: None.

Interventions: Single-dose administration of ceftolozane/tazobactam into closed-loop extracorporeal membrane oxygenation circuits prepared with and without an oxygenator in series with serial preoxygenator, postoxygenator, and reference samples obtained for concentration determination over a 24-hour study period.

Measurements and Main Results: For the 1/4-inch circuit, there was approximately 92% ceftolozane and 22–25% tazobactam loss with the oxygenator in series and 19–30% ceftolozane and 31–34% tazobactam loss without an oxygenator in series at 24 hours. For the 3/8-inch circuit, there was approximately 85% ceftolozane and 29% tazobactam loss with the oxygenator in series and 25–27% ceftolozane and 23–26% tazobactam loss without an oxygenator in series at 24 hours. The reference ceftolozane and tazobactam concentrations remained relatively constant during the entire study period demonstrating the drug loss in each size of the extracorporeal membrane oxygenation circuit with or without an oxygenator was not a result of spontaneous drug degradation.

Conclusions: This ex vivo investigation demonstrated substantial ceftolozane loss within an extracorporeal membrane oxygenation circuit with an oxygenator in series with both sizes of the Quadrox-i oxygenator at 24 hours and significant ceftolozane loss in the absence of an oxygenator. Tazobactam loss was similar regardless of the presence of an oxygenator. Further evaluations with multiple dose in vitro and in vivo investigations are needed before specific drug dosing recommendations can be made for clinical application with extracorporeal membrane oxygenation. (*Pediatr Crit Care Med* 2019; XX:00–00)

Key Words: ceftolozane; drug sequestration; extracorporeal membrane oxygenation; oxygenator; quadrox; tazobactam

Ceftolozane/tazobactam (CT) is a novel antipseudomonal β-lactam/β-lactamase inhibitor combination with U.S. Food and Drug Administration approval for the treatment of complicated intra-abdominal infections

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and complicated urinary tract infections (1, 2). CT displays bactericidal activity via inhibition of bacterial cell wall synthesis mediated through penicillin-binding proteins (PBPs). Ceftolozane is a potent inhibitor of PBP3 in addition to demonstrating a higher affinity for PBP1b compared with other β-lactam antimicrobials which enhances CTs activity against *Pseudomonas aeruginosa* and some AmpC β-lactamases (1, 2). The β-lactamase inhibitor tazobactam increases the spectrum of ceftolozane to include extended-spectrum β-lactamase-producing Enterobacteriaceae and certain anaerobic organisms (1, 2). As with other β-lactams, the efficacy of CT is pharmacodynamically linked to the time the concentration remains above the minimum inhibitory concentration (MIC), referred to as time above MIC (T > MIC). The minimum target T > MIC for Enterobacteriaceae and *P. aeruginosa* to generate bactericidal activity was determined to be approximately 30% for ceftolozane (1). CT is a promising newer agent against multidrug-resistant gram-negative organisms with the potential for use in a variety of clinical settings. Considering this, insight into the potential changes (i.e., sequestration, binding, and loss) of CT with extracorporeal membrane oxygenation (ECMO) circuitry is crucial to help inform clinicians regarding the potential need for dosing adjustments and additional research opportunities in the realm of extracorporeal therapies.

Most published literature regarding drug changes in ECMO circuits is potentially outdated considering these studies were conducted with legacy equipment and oxygenators (3–6). Until these studies are conducted with contemporary equipment, including vital circuitry components such as the oxygenator, the previous literature may not be relied upon to inform clinicians caring for patients on ECMO. However, a resurgence of research in this area has begun as evidence-based dosing regimens in the setting of ECMO are virtually nonexistent (7–9). Historically, factors such as lipophilicity have been linked to the extent of drug adsorption to the polymers and silicone rubber in the ECMO circuitry components (10–14). Currently, minimal to no data regarding drug changes in contemporary ECMO circuits with either size of the Quadrox-i oxygenator exists (15). Therefore, the purpose of this study was to determine the alterations of CT in a contemporary neonatal/pediatric and adolescent/adult ECMO circuit with and without the Quadrox-i oxygenators in series.

MATERIALS AND METHODS

Our methodology has been previously published (7, 8), but in brief, a 1/4-inch ($n = 1$) and 3/8-inch ($n = 1$), simulated closed-loop ECMO circuits were prepared using custom tubing with 1/4-inch diameter and 3/8-inch thickness, made of polyvinylchloride and superTygon (Medtronic, Minneapolis, MN) and a Quadrox-i Peds and a Quadrox-i Adult membrane oxygenator (Maquet, Wayne, NJ), respectively. Each coated circuit was carbon dioxide primed, evacuated, and then crystalloid primed. After debubbling the circuit, 50 mL of 5% albumin was added. The initial crystalloid/albumin prime was then displaced with the priming solution (human whole blood), sodium bicarbonate, heparin, and calcium gluconate. The circuit pH was adjusted as needed to a range of 7.35–7.45. The closed-loop design was established by connecting the ends of the arterial and venous cannulae to a reservoir bag, allowing continuous flow of the priming fluid around the circuit. Utilizing the simulated closed-loop ECMO circuits, levels of CT were obtained preoxygenator and postoxygenator at the following time intervals; 0–5 minutes (right after drug administration), 1 hour (hr), 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, and 24 hours. Additionally, a 1/4-inch ($n = 1$) and 3/8-inch ($n = 1$), simulated closed-loop ECMO circuits were also prepared without an oxygenator in series and levels of CT were obtained from the same two access ports as stated above to obtain two concentrations at the same time intervals in an effort to determine the impact of the oxygenator on CT alterations within the ECMO circuit. The purpose of obtaining two samples at each time point was done to assess for re-circulation or re-distribution phenomena over the course of the experiment at a given time point that may occur pre and/or post oxygenator and to attempt to determine the degree of oxygenator binding and/or saturation during the experiment. Additionally, a second sample would also allow for a higher degree of confidence in the concentration results and determine if a particular value was a spurious finding. CT was also maintained in a glass vial and samples were taken from the vial at the same time periods (eight total samples) for control purposes to assess for spontaneous drug degradation. A 1.5-gram vial of CT (1 g of ceftolozane and 0.5 g tazobactam) was reconstituted with 10 mL of normal saline to a concentration of 100 mg/mL ceftolozane and 50 mg/mL tazobactam according to labeling instructions. Using the estimated circuit volume of 750 mL for the 1/4-inch circuit and 1,500 mL for the 3/8-inch circuit, 60 mg ceftolozane and 30 mg tazobactam was added to the 1/4-inch circuit and 120 mg ceftolozane and 60 mg tazobactam was added to the 3/8-inch circuit, respectively, for an estimated initial target concentration of 80 mg/L ceftolozane and 40 mg/L tazobactam. Additionally, a 1.5-gram vial of CT was reconstituted with 10 mL of normal saline to a concentration of 100 mg/mL ceftolozane and 50 mg/mL tazobactam and used for control purposes.

Samples for CT determination were collected in regular red top tubes and subsequently taken to the laboratory for immediate processing. Upon receipt in the laboratory, samples were centrifuged within 30 minutes of collection at 3,000 rpm for at least 15 minutes to separate the plasma. Separated plasma was then transferred to a cryovial and stored at –80°C until concentration determination. CT samples were analyzed by validated liquid chromatography tandem mass spectrometry (LC-MS/MS) (U.S. Food and Drug Administration guidelines: www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM070107.pdf) at Atlantic Diagnostic Laboratories (Bensalem, PA). The LC-MS/MS method was accurate and precise at a linearity range of 0.5–100 mg/L with a correlation coefficient (r) of greater than or equal to 0.99 and an inter-day assay variability that was less than 4% across all control samples and an intra-day assay variability that was less than 10% across all control samples.

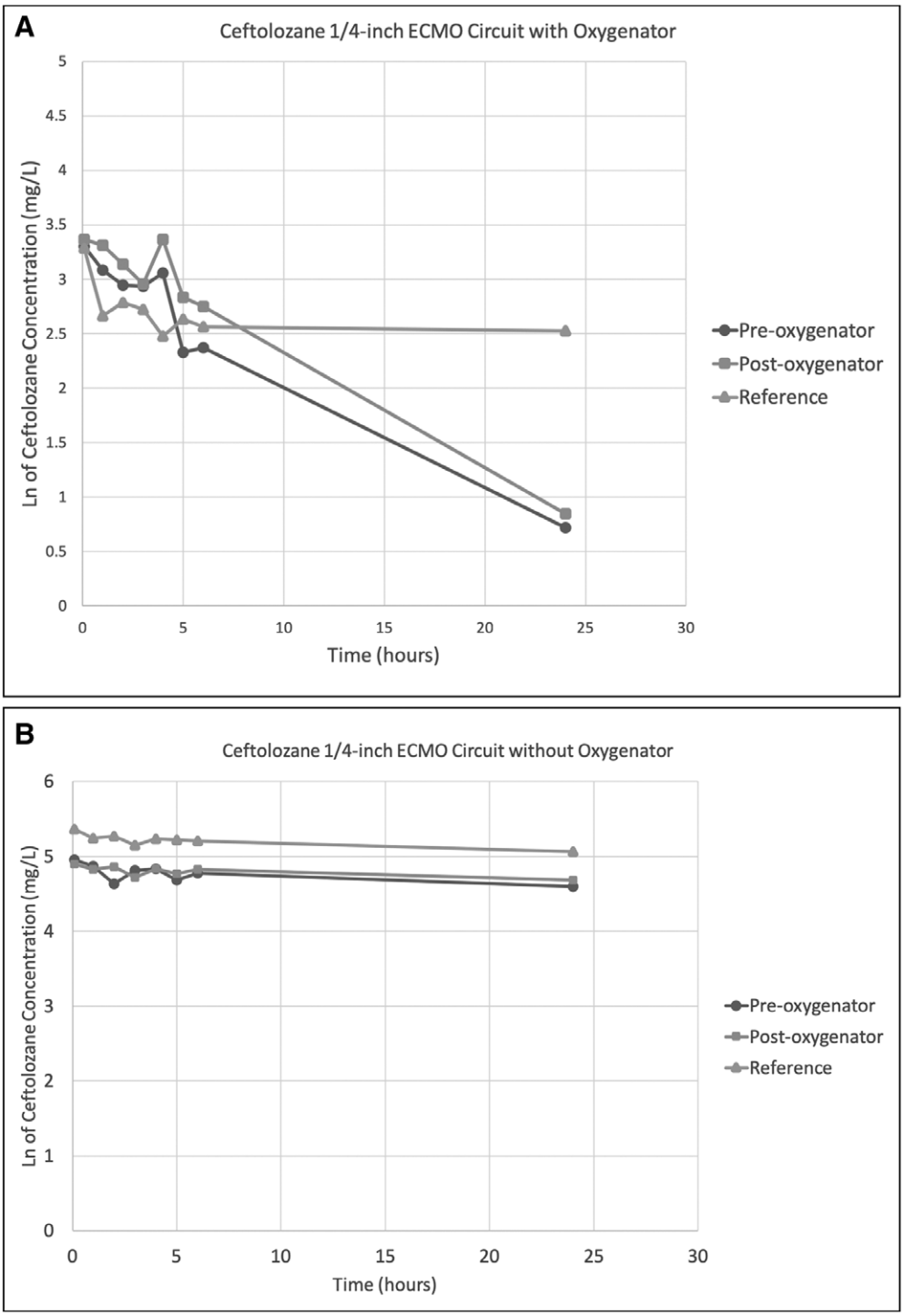

Figure 1. Ceftolozane and tazobactam concentration versus time graphs. **A**, Graph of the natural logarithm of preoxygenator, postoxygenator, and reference ceftolozane concentrations versus time in a 1/4-inch extracorporeal membrane oxygenation (ECMO) circuit with a Quadrox-i pediatric oxygenator. Actual reference sample values are 10^3. **B**, Graph of the natural logarithm of preoxygenator, postoxygenator, and reference ceftolozane concentrations versus time in a 1/4-inch ECMO circuit without a Quadrox-i pediatric oxygenator. Actual reference sample values are 10^3.

CT data were plotted (concentration vs time) and analyzed. To calculate the percentage of drug recovered from the circuit, the drug concentration remaining at each time point and at 24 hours was divided by the total CT concentration in the circuit, as calculated from the administered amount of drug divided by the estimated circuit volume and confirmed with the concentration taken immediately after addition of the CT to the circuit. For purposes of graphing, the reference concentrations were divided by 1,000 to allow for graphing of the reference concentrations on the graph with the preoxygenator and postoxygenator concentrations and the concentrations taken from the ECMO circuit without an oxygenator in series on a similar scale. Therefore, the actual reference concentrations are to the power of 10^3. A paired *t* test and/or analysis of variance testing with a post hoc Tukey test were performed to assess differences in drug concentrations and recovery over the study period in addition to differences between the 1/4-inch and 3/8-inch circuits with and without an oxygenator in series. Statistical significance was defined as a *p* value of less than 0.05. All analyses were performed using IBM SPSS Version 24 (IBM SPSS, Chicago, IL).

RESULTS

The concentration versus time profile for ceftolozane in the 1/4-inch ECMO circuit with the Quadrox-i Peds oxygenator is presented in **Figure 1*A*** and without an oxygenator is presented in **Figure 1*B***. The concentration versus time profile for ceftolozane in the 3/8-inch ECMO circuit with the Quadrox-i Adult oxygenator is presented in **Figure 2*A*** and without an oxygenator is presented in **Figure 2*B***. The concentration versus time profile for tazobactam in the 1/4-inch ECMO circuit with the Quadrox-i Peds oxygenator is presented in **Figure 3*A*** and without an oxygenator is presented in **Figure 3*B***. The concentration versus time profile for tazobactam in the 3/8-inch ECMO circuit with the Quadrox-i Adult oxygenator is presented in **Figure 4*A*** and without an oxygenator is presented in **Figure 4*B***. **Table 1** displays the amount

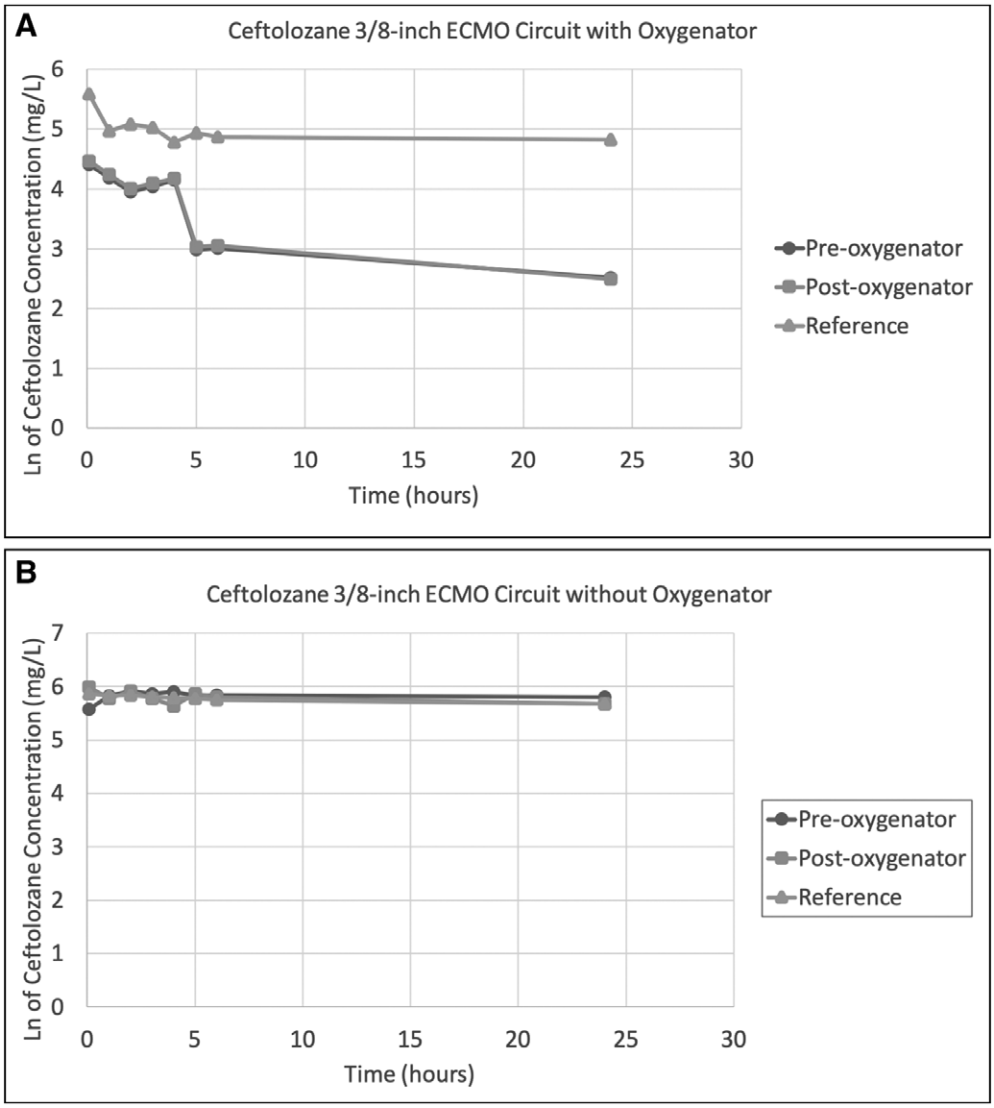

Figure 2. Ceftolozane and tazobactam concentration versus time graphs. **A**, Graph of the natural logarithm of preoxygenator, postoxygenator, and reference ceftolozane concentrations versus time in a 3/8-inch extracorporeal membrane oxygenation (ECMO) circuit with an oxygenator in series. Actual reference sample values are 10^3. **B**, Graph of the natural logarithm of preoxygenator, postoxygenator, and reference ceftolozane concentrations versus time in a 3/8-inch ECMO circuit without an oxygenator in series. Actual reference sample values are 10^3.

of drug loss at 24 hours based on circuit size and the absence or presence of an oxygenator. The reference ceftolozane and tazobactam concentrations remained relatively constant during the entire study period demonstrating the ceftolozane and tazobactam loss in each size of the ECMO circuits with an oxygenator was not a result of spontaneous drug degradation.

DISCUSSION

This investigation demonstrated significant ceftolozane loss over the 24-hour study period between 85% and 92% in a closed-loop 1/4-inch and 3/8-inch ECMO circuits with both sizes of the Quadrox-i oxygenators and approximately 20–30% ceftolozane loss when the oxygenator was taken out of series. Regarding tazobactam, there was approximately a 20–30% loss over the 24-hour study period that was consistent across the four experiments and was not impacted by the presence of an oxygenator. Several factors are implicated regarding drug concentration changes during ECMO including circuit-related factors, drug-related factors, and patient characteristics (16).

The Quadrox-i pediatric and adult oxygenators are composed of a polymethylpentene microporous fiber material and a polyurethane heat exchanger fiber material and have not been widely investigated to understand the potential alterations to drugs within an ECMO circuit (15). The relatively large surface area to size ratio of the oxygenator is one major concern for drug sequestration with ECMO circuitry. Earlier work data found the amount of drug loss via the oxygenator to be minimal in comparison to the conduit tubing (4, 5, 17). Fentanyl loss with (80%) and without (83–86%) an oxygenator (Quadrox D; Maquet) were comparable as was morphine (~40%) with and without and oxygenator in series (3). A more recent investigation evaluated seven different beta-lactam antimicrobials with an oxygenator in series. This work suggested cefotaxime was the only beta-lactam with any significant loss (9). One recognizable concern was addition of seven "similar" antimicrobials into the same circuit. This methodology could have significantly impacted the results due to competitive protein binding and possible saturation with lipophilic circuitry components. Additionally, the lack of albumin in the circuit prime could have also impacted the amount of drug loss which is in conflict with the amount of ceftolozane loss in this investigation, our previous work with ceftaroline, and previous investigations by Shekar et al (7, 18). These are all important consideration and when conducting drug alteration studies, it may be prudent to conduct each study initially as a single drug experiment to exclude any potential competitive binding before combination studies are attempted.

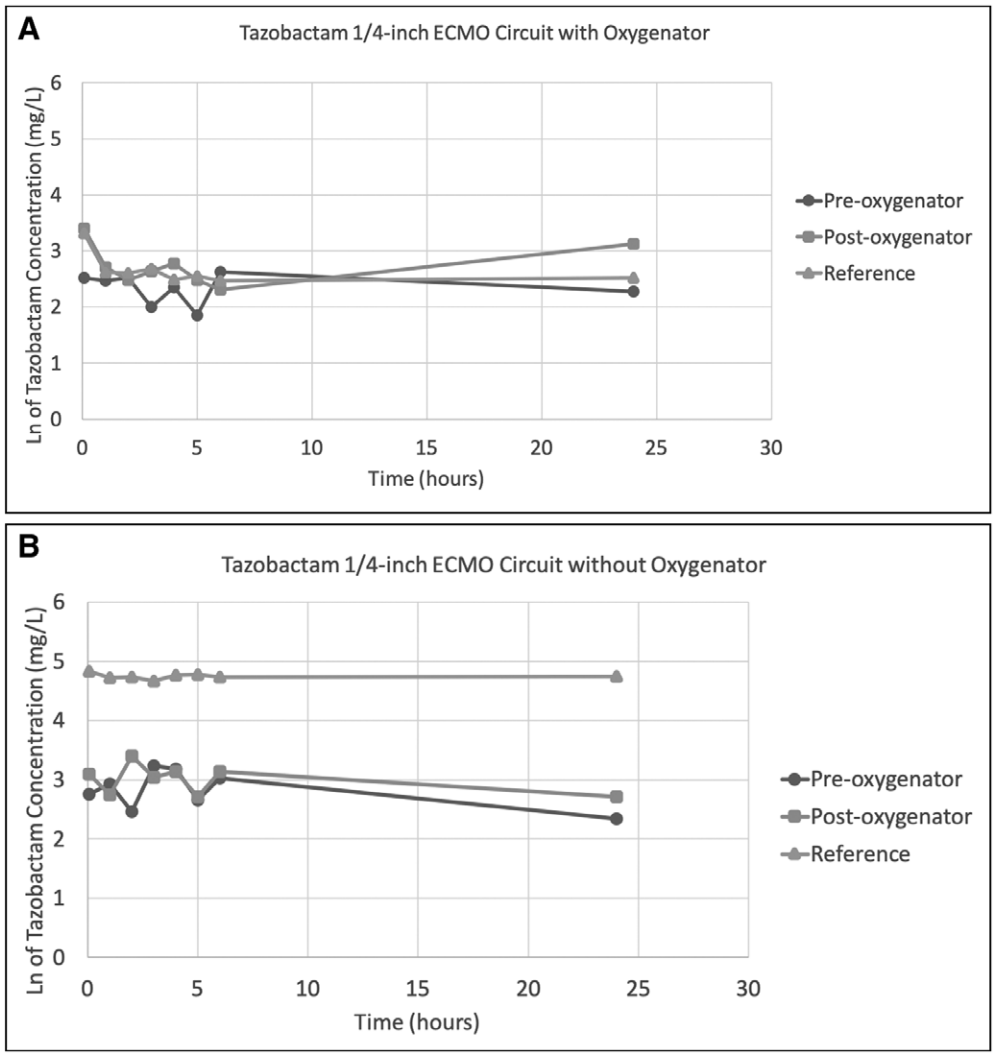

Figure 3. Ceftolozane concentration versus time graphs. **A**, Graph of the natural logarithm of preoxygenator, postoxygenator, and reference tazobactam concentrations versus time in a 1/4-inch extracorporeal membrane oxygenation (ECMO) circuit with a Quadrox-i pediatric oxygenator. Actual reference sample values are 10^3. **B**, Graph of the natural logarithm of preoxygenator, postoxygenator, and reference tazobactam concentrations versus time in a 1/4-inch ECMO circuit without a Quadrox-i pediatric oxygenator. Actual reference sample values are 10^3.

Lipophilicity and protein binding have also been implicated to alter drug concentrations within an ECMO circuit (16). The octanol/water partition coefficient (logP) is a measure of the relative lipophilicity of a given drug. A higher, more positive logP value indicates increasing lipophilicity. Earlier investigations suggested higher lipophilicity correlated with a greater likelihood of drug sequestration when compared with hydrophilic drugs due to the higher solubility of lipophilic compounds in the organic components of the ECMO circuit (6, 16). The extent of drug-protein binding can also impact drug sequestration (16). ß-lactams are considered hydrophilic compounds with varying degrees of protein binding and one investigation showed minimal impact on meropenem and piperacillin/tazobactam (TZP) as a result of the ECMO circuit which is consistent with their low logP, –0.69 and 0.67, and protein binding estimates, 2% and 30%, respectively (19, 20). Surprisingly, ampicillin has similar logP and protein binding estimates

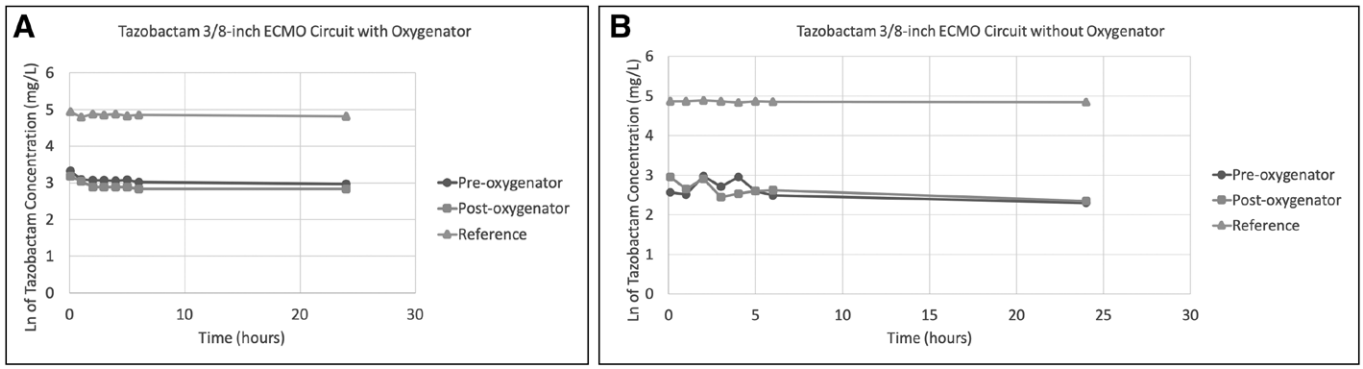

Figure 4. Ceftolozane concentration versus time graphs. **A**, Graph of the natural logarithm of preoxygenator, postoxygenator, and reference tazobactam concentrations versus time in a 3/8-inch extracorporeal membrane oxygenation (ECMO) circuit with an oxygenator in series. Actual reference sample values are 10^3. **B**, Graph of the natural logarithm of preoxygenator, postoxygenator, and reference tazobactam concentrations versus time in a 3/8-inch ECMO circuit without an oxygenator in series. Actual reference sample values are 10^3.

TABLE 1. Percent Ceftolozane and Tazobactam Drug Loss by Extracorporeal Membrane Oxygenation Circuit Size and Presence of an Oxygenator

Circuit Size	Oxygenator in Series	Drug	% Drug Loss at 24 hr Preoxygenator	% Drug Loss at 24 hr Postoxygenator
1/4-inch[a]	Yes	Ceftolozane	92.5	91.9
1/4-inch	No	Ceftolozane	30	19.4
3/8-inch[b]	Yes	Ceftolozane	85	86.1
3/8-inch	No	Ceftolozane	25.4	27.3
1/4-inch	Yes	Tazobactam	22	24.8
1/4-inch	No	Tazobactam	34.2	31.7
3/8-inch	Yes	Tazobactam	29.5	29.2
3/8-inch	No	Tazobactam	23.1	26.7

[a]$p < 0.01$ for the comparison of drug loss between the 1/4-inch circuit with and without an oxygenator.

[b]$p < 0.01$ for the comparison of drug loss between the 3/8-inch circuit with and without an oxygenator.

(0.67 and 15–28%, respectively) as TZP but showed a range of 15% loss in a blood primed circuit and 71% loss in a crystalloid primed circuit (13). Ceftolozane has low plasma protein binding (~16–20%) and is predominantly excreted unchanged in the urine (≥ 95%) and about 30% of tazobactam is bound to plasma protein with greater than or equal to 80% excreted unchanged in the urine (1). The log *p* values for ceftolozane and tazobactam range from –8.7 to –1.2 and from –1.8 to –1.4, respectively, and as such the expected drug loss within an ECMO circuit would be minimal (20). However, in this investigation with a blood primed circuit and Quadrox-i oxygenators, there was substantial ceftolozane loss when the oxygenator was in series and significant loss when the oxygenator was removed suggesting significant ceftolozane loss due to the oxygenator and 20% to 30% resulting from the other ECMO circuitry. Considering CT is now being used clinically for the management of infections, it is imperative to understand the implications ECMO circuitry can have on the alterations of CT since the consequence of inadequate concentrations would be treatment failure and presumably increased morbidity and mortality.

As described previously with similar work, there are several limitations of this investigation (7, 8). First, this was an observational study with a small sample size. Second, a crystalloid primed circuit was not evaluated, but this was intentional in an effort to simulate the effects of blood within the ECMO circuit as would be seen in clinical practice. Third, a single dose of CT was given and the effects of repeated dosing could not be evaluated. Fourth, since a single dose of CT was given, the effects of circuit age and potential saturation of CT within the ECMO circuit could not be evaluated. Fifth, patient factors such as metabolism and elimination could not be evaluated. Despite these limitations, this investigation provides interesting insight into the effects of ECMO circuitry, specifically the oxygenator, on ceftolozane alterations and can be used to guide future experiments with CT and other antimicrobials.

CONCLUSIONS

This ex vivo investigation demonstrated substantial ceftolozane loss within an ECMO circuit with an oxygenator in series with both sizes of the Quadrox-i oxygenator at 24 hours and significant ceftolozane loss in the absence of an oxygenator. Tazobactam loss was similar regardless of the presence of an oxygenator. Further evaluations with multiple dose in vitro and in vivo investigations are needed before specific drug dosing recommendations can be made for clinical application with ECMO.

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