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Plasma and Tissue Pharmacokinetics of Cefazolin in an Immature Porcine Model of Pediatric Cardiac Surgery

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Abstract

Background: Surgical Site Infection (SSI) prevention for children with congenital heart disease is imperative and methods to assess and evaluate the tissue concentrations of prophylactic antibiotics are important to help maximize these efforts. The purpose of this study was to determine the plasma and tissue concentrations of standard-of-care peri-operative cefazolin dosing in an immature porcine model of cardiac surgery and cardiopulmonary bypass.

Methods: Piglets (3-5 days old) underwent either median sternotomy (MS) or cardiopulmonary bypass with deep hypothermic circulatory arrest (CPB+DHCA) and received standard of care prophylactic cefazolin for the procedures. Serial plasma and microdialysis sampling of skeletal muscle and subcutaneous tissue adjacent to the surgical site was performed. Cefazolin concentrations were measured, non-compartmental pharmacokinetic analyses were performed, and tissue penetration of cefazolin was assessed.

Results: Following the first intravenous dose, maximal cefazolin concentrations for plasma and tissue samples were similar between groups with peak tissue concentrations 15-30 minutes after administration. After the second cefazolin dose given with initiation of CPB, total plasma cefazolin concentrations remained relatively constant until the end of DHCA and then decreased while muscle and subcutaneous unbound cefazolin concentrations showed a second peak during or after rewarming. For the MS group, 60-67% of the intraoperative time showed tissue cefazolin concentrations greater than16 μg/mL while this percentage was 78-79% for the CPB+DHCA group. There was less tissue penetration of cefazolin in the group that underwent CBP+DHCA (P=0.03).

Conclusions: The cefazolin dosing used in this study achieves plasma and tissue concentrations that should be effective against methicillin-sensitive *Staphylococcus aureus* but may not be effective against some gram-negative pathogens. The timing of cefazolin administration prior to incision and a second dose given during cardiopulmonary bypass may be important factors for achieving goal tissue concentrations.

Keywords: Cefazolin; *Staphylococcus aureus;* Pediatric; *Escherichia coli*, *Enterobacter cloacae*, *Klebsiella pneumoniae*

Abbreviations:

SSI: Surgical Site Infection; DHCA: Deep Hypothermic Circulatory Arrest; PK: Pharmacokinetics; CPB: Cardiopulmonary Bypass; MSSA: Methicillin-Sensitive *Staphylococcus aureus*; MRSA: Methicillin-Resistant *Staphylococcus aureus*; MD: Microdialysis; IF: Interstitial Fluid; HPLC-MS/MS: High-Performance Liquid Chromatography and Tandem Mass Spectrometry; Cmax: Maximum Plasma Concentration; Tmax: Time to Maximum Plasma Concentration; LLQ: Lower Limit of Quantification; AUC: Area Under the Concentration–Time Curve; CL: Clearance; RR: Relative Recovery; PK-PD: Pharmacokinetic-Pharmacodynamic; fT>MIC: Minimum Inhibitory Concentration for Bacterial Growth; MS: Median Sternotomoy

Background

Children with congenital heart disease undergoing open cardiac procedures are a vulnerable population with reported surgical site infection (SSI) rates varying from 1.7 to 8.0 per 100 cases [1-5]. Multiple risk factors for development of an SSI have been identified in pediatric cardiac surgical populations, including intraoperative hypothermia and duration of surgery [1-5,6-11]. Cardiopulmonary bypass (CPB) and deep hypothermic circulatory arrest (DHCA) used during some pediatric cardiac surgeries have physiologic effects that alter drug disposition and pharmacokinetics (PK) [6,7]. In pediatric patients, these changes may be more pronounced and different than adults, given that the ratio of CPB priming volume to patient's blood

volume is large and organ function may be immature [7]. SSIs can also predispose these patients to bloodstream infections [4,12-16] and major post-operative infections following pediatric cardiac surgery have been shown to increase mortality and increase hospital length of stay [16]. Bacterial pathogens that cause SSIs described in the pediatric cardiac literature include, but are not limited to, methicillin-sensitive *Staphylococcus aureus* (MSSA), methicillin-resistant *Staphylococcus aureus* (MRSA), coagulase-negative staphylococci, *Escherichia coli*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* [1-5,8-10].

A critical component of SSI prevention in cardiac surgery is the administration of prophylactic peri-operative antibiotics. For SSI prevention, effectiveness depends on selecting the appropriate antibiotic as well as timely administration to achieve effective plasma and tissue concentrations before skin incision, during the procedure, and in the immediate postoperative period [17]. Previously published pediatric pharmacokinetic studies investigating prophylactic antibiotics with CPB have used plasma concentrations as a surrogate for tissue drug concentrations [18-21]. Porcine models have been used routinely for Pharmacokinetic (PK)/Pharmacodynamic (PD) analysis [22]. Microdialysis (MD) is a method to directly sample interstitial fluid of tissue, and permits quantification of unbound drug concentrations in tissue [23-28]. Data on tissue penetration of antimicrobials into the surgical site, or tissue adjacent to the site, may result in modification of the current prophylactic antibiotic dosing recommendations.

The purpose of this study was to determine the plasma and tissue disposition of cefazolin, a first-generation cephalosporin commonly used for peri-operative antibiotic prophylaxis, in an immature porcine model of pediatric cardiac surgery and cardiopulmonary bypass.

Methods

Animal Model:

All protocols were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania, in compliance with the National Institutes of Health guidelines. Piglets (3-5 days old, female N=4/group, 2.1-2.7 kg) underwent induction and maintenance of anesthesia with isoflurane, fentanyl, and pancuronium. A tracheostomy was performed and femoral arterial and venous catheters were placed for blood pressure monitoring and blood sampling. Piglets received a median sternotomy (MS) or median sternotomy plus cardiopulmonary bypass with deep hypothermic circulatory arrest (CPB+DHCA). Piglets in the MS alone group were maintained at 37°C for the entire experiment and were subsequently euthanized with 4M KCl. The published protocol used for CPB +DHCA included cooling to 18°C over 30 minutes, DHCA for 30 minutes followed by 1 hour of low flow CPB (20 ml/kg/min), and finally rewarming over 30 minutes to 37°C [29]. Cefazolin dosing mimicked the clinical pathways currently in place at the Children's Hospital of Philadelphia Cardiac Center.

Microdialysis method

Microdialysis (MD) utilizes the implantation of a small catheter with a semi-permeable membrane into tissue and can be used to obtain samples of interstitial fluid (IF) of the particular tissue of [26-28]. A physiological fluid is slowly perfused through the microdialysis catheter. Substances (analytes) present in the surrounding IF diffuse through the semipermeable membrane via a concentration gradient into the perfusion fluid and then the microdialysate fluid is collected and analyzed. It is a reliable, relatively low cost, and minimally invasive method used in clinical pharmacology to collect unbound drug in tissue IF [26-28].

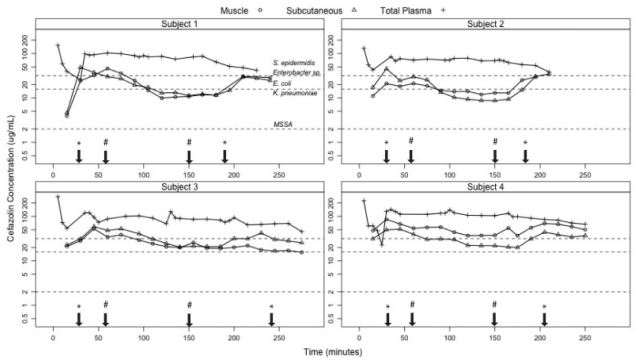

Figure 1: Semi-logarithmic concentration-time profile for total plasma, muscle, and subcutaneous tissue cefazolin concentrations over time for piglets that underwent cardiopulmonary bypass with deep hypothermic circulatory arrest (CPB+DHCA, n=4). The horizontal lines indicate various concentration targets and associated SSI pathogen(s). Vertical arrows labeled with "*" represent the initiation of CPB (30 minutes) and the end of CPB (various times) for each subject. Vertical arrows labeled with "#" represent the initiation of DHCA (60 minutes) and end of DHCA (150 minutes) for each subject. Abbreviations: µg: microgram; mL: milliliter; MSSA: methicillin sensitive *Staphylococcus aureus*; E: Escherichia; K: *Klebsiella*; S: *Staphylococcus*; sp: species.

Study Protocol

Two in vivo microdialysis (MD) catheters (CMA 20 Elite, Solva, Sweden) with a microdialysis membrane length of 10 mm and molecular weight cut-off of 20,000 Daltons were used in each subject. Under sterile conditions, one catheter was inserted under sterile conditions into the subcutaneous tissue and one catheter was inserted into the dorsal pectoralis muscle immediately adjacent to the sternum and future incision site. In vivo retrodialysis [26,28] was used to calibrate the microdialysis catheters. Briefly, cefazolin was diluted in 0.9% NaCl solution to a final concentration of either 25 or 30 µg/mL (Cefazolin Concentrationperfusate) and perfused the microdialysis catheters at a rate of 1.5 µL/min. After an equilibration time of 30 minutes, microdialysate vials were changed and a dialysate sample was collected over 30 minutes. The concentrations of cefazolin were measured in the dialysate (Cefazolin Concnentrationdialysate) to determine the relative recovery with the following equation [28]:

Relative Recovery (%)=100-(100* Cefazoli Concentrationdialysate/ Cefazolin $\text{Concentration}_{\text{perfusate}}$)

The microdialysis catheter as well as inflow and outflow tubing was flushed with 0.9% NaCl for 30 minutes and for the remainder of the experiments the microdialysate perfusion fluid was 0.9% NaCl at a flow rate of 1.5 µL/min.

After *in vivo* calibration and washout, piglets in both groups received intravenous cefazolin 25 mg/kg five minutes prior to incision.

Piglets that underwent CPB+DHCA received a second dose of 25 mg/kg of cefazolin added to the pump prime for a total dose of 50 mg/kg. At the time of this study, this antibiotic dosing protocol was standard of care for human children undergoing similar procedures at The Children's Hospital of Philadelphia. Microdialysis samples from the subcutaneous and muscle catheters were collected continuously from the initial dose of cefazolin until the animal was separated from CPB and/or sacrificed and stored at -80°C until analysis. Blood samples were collected post-oxygenator via the extracorporeal circuit into lithium-heparin tubes, centrifuged, and plasma separated with storage at -80°C until analysis.

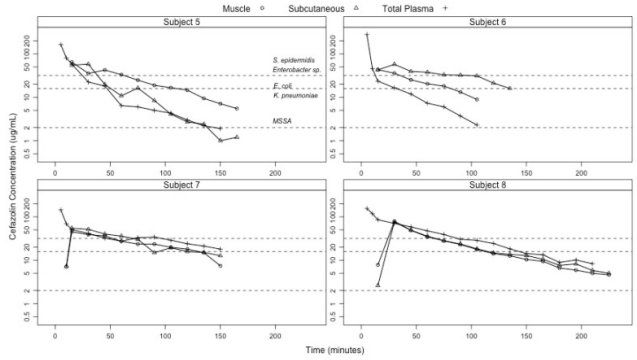

Figure 2: Semi-logarithmic concentration-time profile for total plasma, muscle, and subcutaneous tissue cefazolin concentrations over time for piglets that underwent median sternotomy (MS, n=4). The horizontal lines indicate various concentration targets and associated SSI pathogen(s). Abbreviations: µg: Microgram; mL: Milliliter; MSSA: Methicillin sensitive *Staphylococcus aureus*; E: *Escherichia*; K: *Klebsiella*; S: *Staphylococcus*; sp: Species.

Cefazolin Concentration Determination

Cefazolin concentrations in muscle and subcutaneous MD samples (unbound cefazolin concentrations) as well as plasma samples (total cefazolin concentrations) were determined utilizing a validated high-performance liquid chromatography and tandem mass spectrometry (HPLC-MS/MS) methodology at The Children's Hospital of Philadelphia.

Plasma samples were prepared by adding 25 µL of an internal standard (5 mcg/mL ampicillin in water) to 25 µL of sample followed by 500 µL of cold methanol. The sample was vortexed for 1 minute, 450 µL of water was added, vortexed a second time and then centrifuged at 4000 rpm for 15 minutes. 100 µL of the supernatant was added to a microtiter plate followed by 900 µL of water prior to analysis. Microdialysate samples were loaded onto a microtiter plate in 10 µL volumes followed by 500 µL of the internal standard (20 ng/mL Ampicillin in water) prior to analysis.

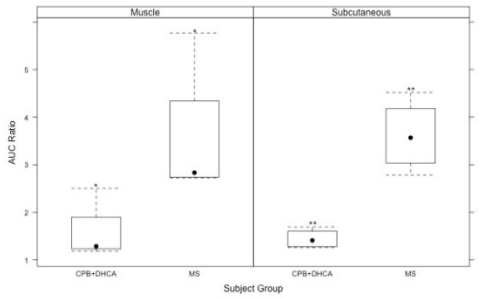

Figure 3: Box and whisker plots of median AUC ratios. Muscle AUC/total plasma AUC ratios for piglets that underwent CPB +DHCA (n=4) were significantly decreased compared to MS only (n=4) (*P=0.03). Subcutaneous AUC/total plasma AUC ratios for piglets that underwent CPB+DHCA (n=4) were significantly decreased compared to MS only (n=4) (**P=0.03).

Chromatographic separation utilized a HyPurity C18 column (50 mm × 2.1 mm × 3 µm) (Thermo Scientific, Waltham, MA, USA) with mobile phases A (5mM Ammonium acetate in water, pH 4.8) and B (5mM Ammonium acetate in 90/10 acetonitrile/water). The flow rate through the column was 0.7 mL/min with the mobile phase gradient starting at 95% Phase A, decreasing to 75% at 0.5 min, decreasing to 50% at 1 min, decreasing to 25% at 1.5 min, and increasing to 95% at 2 min until the total run time of 4 minutes was complete.

Cefazolin and ampicillin were detected using an API2000 tandem mass spectrometer (AB Sciex, Redwood City, CA). The mass spectrometer ion masses were 422.11 -> 323 (cefazolin) and 350.25 -> 106.10 (ampicillin). All assay methods were validated using best practice guidelines [30]. Two sets of quality control samples were run with each assay batch and had accuracy and precision within 15%. The lower limit of quantification (LLQ) for the plasma assay was 1 µg/mL and the LLQ for the microdialysate assay was 0.1µg/mL.

Pharmacokinetic Analysis

Pharmacokinetic analyses were performed on cefazolin concentrations based on non-compartmental methodology. Maximum plasma concentration (Cmax) and time to maximum plasma concentration (Tmax) were determined. For piglets that underwent CPB+DHCA, Cmax1 and Tmax1 were identified after the first bolus and prior to initiation of CPB while Cmax2 and Tmax2 represent the concentration and time after the second bolus was administered on CPB. Area under the concentration - time curve (AUC) for plasma samples was calculated using the linear trapezoid method. Total clearance was calculated by dividing the total dose by the total AUC. Descriptive statistics were calculated for Cmax, Tmax, AUC and clearance (CL).

	Total Plasma		Muscle		Subcutaneous	
	MS	CPB +DHCA	MS	CPB +DHCA	MS	CPB +DHCA
AUC (µg*min*mL-1)	5220.1 (4275.5-6487.1)	21844.1 (16527.4-26587.8)	3720.3 (3194.0-2121.3)	5611.4 (4188.1-5465.8)	3989.5 (3575.0,-4225.8)	6289.5 (4188.3-8408.2)

Cmax 1 (μg /mL)	159.5 (135.1-192.8)	189.1 (147.9 – 238.8)	54.8 (44.6-68.2)	26.3 (23.5-42.6)	57.7 (56.7-62.4)	47 (41.8-49.0)
Tmax 1 (min)	5 (5-5)	5 (5-5)	15 (15,-19)	30 (30-30)	30 (26-30)	30 (30-30)
Cmax 2 (μg /mL)	-	111.4 (98.3-122.5)	-	49.3 (41.8-56.8)	-	40.7 (36.7-47.8)
Tmax 2 (min)	-	5 (4-11)	-	98 (26-141)	-	95 (15-176)
Total Clearance (L/hr)	0.7* (0.6-0.8)	0.3* (0.3-0.4)	-	-	-	-

Values displayed as medians (IQR). Cmax2 and Tmax2 are not reported for the MS group as they received only one dose of cefazolin. Total clearance is only able to be calculated from plasma samples so is not reported for the microdialysis samples. Significant p values reported below were calculated using the Wilcoxon rank sum test. Abbreviations: MS : Median Sternotomy; CPB +DHCA: Cardiopulmonary Bypass with Deep Hypothermic Circulatory Arrest; AUC: Area under the Concentration-Time Curve; Cmax1: Maximum Concentration after Dose 1; Cmax2: Maximum Concentration after Dose 2; Tmax1: Time to Maximum Concentration after Dose 1; Tmax2: Time to Maximum Concentration after Dose 2; μg: Microgram; mL: Milliliter; min: Minutes; L: Liters; hr: Hour. * P = 0.03

Table 1: Pharmacokinetic parameters for cefazolin in piglets undergoing MS only (n=4) and CPB+DHCA (n=4).

For microdialysis samples, the AUC was calculated by multiplying the measured unbound cefazolin concentration corrected for relative recovery (RR) by the time interval of the microdialysis sample collection then summation of the areas for each interval. Interstitial concentrations for microdialysis samples were calculated using the following:

Interstitial Concentration=100 × (concentration$_{dialysate}$/*in vivo* relative recovery (%))

The median RR (IQR) was 33.5% (27.9-38.4%) for the subcutaneous microdialysate samples and 28.5% (20.6-42.2%) for the muscle samples, respectively.

Cefazolin penetration from plasma into tissue space was determined by calculating the ratio of tissue AUC (muscle and subcutaneous) divided by the total plasma AUC.

The pharmacokinetic-pharmacodynamic (PK-PD) factor most closely associated with cephalosporin antibacterial effectiveness is the amount of time the concentration of cephalosporin exceeds the minimum inhibitory concentration for bacterial growth (*f*T>MIC) in the bloodstream or tissue in question [30]. For this reason, total plasma concentrations and measured unbound interstitial fluid concentrations of cefazolin in skeletal muscle and subcutaneous tissues were compared to the relevant MIC90 values31 and the duration of time above target concentrations was estimated based on visual inspection of the data. Summary data are reported as medians with interquartile ranges (IQR) with non-parametric comparisons, where appropriate. Statistical analyses and plots were completed using R, Microsoft Excel, or S-PLUS. In this pilot study, eight animals completed the protocol due to budgetary considerations.

Results

Cardiopulmonary bypass

The median Cmax1 concentrations were not statistically different between the plasma, muscle, and subcutaneous samples for the CPB +DHCA piglets but the Tmax1 was longer in the muscle and subcutaneous samples (Table 1). After the initial peak, total plasma concentrations of cefazolin fell precipitously but, with the second cefazolin bolus given upon initiation of CPB, plasma concentrations again increased (Figure 1). After the second bolus, total plasma concentrations again peaked (Cmax2) quickly but not as high as Cmax1 (Table 1) and, unlike following the first dose, the plasma concentrations remained relatively constant until the end of DHCA and then showed a slow rate of decline when the piglets were separated from CPB until the end of the procedure (Figure 1). The cefazolin concentration in muscle and subcutaneous tissue showed a second peak that occurred as the piglets were being rewarmed (Subject 1) or after DHCA was complete (Subjects 2-4) (Figure 1). No statistical differences were found between Cmax1 and Cmax2 for the total plasma, muscle, or subcutaneous tissue concentrations (P=0.13 for unbound plasma, P=0.38 for muscle, and P=0.88 for subcutaneous using Wilcoxon signed rank test) (Table 1).

Median Sternotomy (MS)

After initial bolus, total plasma concentrations of cefazolin rapidly declined over time (Figure 2). Tissue concentrations of cefazolin peaked between 15 and 30 minutes and then declined over time (Figure 2). No statistical differences were found between Cmax for the MS only group and Cmax1 for the CPB+DHCA group (P=1 for unbound plasma, P=0.25 for muscle, P=0.13 for subcutaneous using the Wilcoxon rank sum test) (Table 1). The total clearance of cefazolin was decreased in the CBP+DHCA group compared to the MS only group (P=0.03) (Table 1).

Tissue Penetration

The calculated cefazolin AUC$_{total}$ ratios for both muscle/total plasma and subcutaneous/total plasma for both groups are another representation of tissue penetration of cefazolin. There was a significant difference in this ratio for both muscle and subcutaneous interstitial fluid exposure between the piglets that underwent CPB +DHCA compared to the MS only group (Figure 3).

For the CPB+DHCA group, 100% of the measured total plasma, skeletal muscle, and subcutaneous tissue cefazolin concentration time points were above 2 μg/mL. Except for the total plasma cefazolin concentrations in the CPB+DHCA group, this percentage decreased as the concentration targets increased to 16 μg/mL and 32 μg/mL, respectively (Table 2).

Discussion

The purpose of this study was to determine the plasma pharmacokinetics and tissue disposition of cefazolin when current pediatric standard of care cefazolin dosing is used in an immature porcine model of cardiac surgery and cardiopulmonary bypass.

We found that the maximal tissue concentrations of cefazolin were similar between piglets who underwent CPB+DHCA and who received twice the amount of cefazolin (total dose 50 mg/kg) compared to piglets that underwent MS alone (total dose 25 mg/kg).

Furthermore, peak cefazolin concentrations in the tissue samples were not seen until approximately 15-30 minutes after the bolus dose in both groups compared to within 5 minutes for the plasma samples.

Target	SSI Pathogen	Total Plasma		Muscle		Subcutaneous	
		MS	CPB+DHCA	MS	CPB+DHCA	MS	CPB+DHCA
2 µg/mL	MSSA	100% (97-100%)	100% (100-100%)	100% (100-100%)	100% (100-100%)	100% (96-100%)	100% (100-100%)
16 µg/mL	*E. coli K. pneumoniae*	48% (28-76%)	100% (100-100%)	67% (60-73%)	78% (55-99%)	60% (38-82%)	79% (56-100%)
32 µg/mL	*S. epidermidis* *Enterobacter sp.*	31% (15-46%)	99% (98-100%)	26% (23-29%)	18% (12-41%)	30% (19-55%)	26% (12-41%)

Each concentration target represents an estimated MIC90 of bacteria [31] potentially susceptible to cefazolin and known to cause SSIs in pediatric cardiac surgical patients. Abbreviations: MS: Median Sternotomy; CPB+DHCA: Cardiopulmonary Bypass with Deep Hypothermic Circulatory Arrest; µg: Microgram; mL: Milliliter; min: Minutes; MSSA: Methicillin Sensitive *Staphylococcus aureus*; E.: *Escherichia*; K.: *Klebsiella*; S: *Staphylococcus*; sp: Species.

Table 2: Estimated percentage of time during surgical procedure cefazolin concentrations spent above different concentration targets.

For the CPB+DHCA group, total plasma levels peaked early and dropped precipitously following the first dose, which is likely a reflection of initial cefazolin distribution. Importantly, after the second bolus dose of cefazolin was given to this group, there was a lower Cmax2 compared to Cmax1, which is likely a reflection of the increased volume of distribution seen with the initiation of CPB. In addition, after the second bolus dose, the peak tissue concentrations appeared during or after rewarming from hypothermia and close to the end of CPB. This was seen despite twice the total dose of cefazolin being given to the CPB+DHCA group and that the total clearance of cefazolin was about half of that seen in the MS only group. This decreased clearance of cefazolin resulted in relatively constant plasma concentrations of cefazolin during CPB+DHCA that then decreased again during rewarming and following separation from CPB, which was accompanied by an increase in the muscle and subcutaneous tissue concentrations of cefazolin. While microdialysis has many advantages for pharmacokinetic investigations, it must be noted that microdialysis samples lack the temporal discrimination of plasma samples. This is because drug concentrations measured in the microdialysis samples are average concentrations over the time interval collected and, therefore, cannot be analyzed as point estimates. To mitigate this effect in interpreting the data, relatively short time intervals of collection were used, microdialysis samples were paired with plasma samples where possible, and AUC comparisons were made, which inform about the overall exposure of skeletal muscle, subcutaneous tissue, and plasma to cefazolin, respectively.

The CPB+DHCA group had significantly lower AUCtotal ratios for both muscle/plasma and subcutaneous/ plasma. This suggests that the combination of hypothermia and circulatory arrest impacts the ability for cefazolin to penetrate into tissue, possibly due to alterations in perfusion. This is supported by the observation that despite the decrease in plasma concentrations after rewarming and removal from CPB, tissue concentrations increased. Alternatively, in these experiments, it is unknown how hypothermia affects the relative recovery of the microdialysis membranes.

Cephalosporins are classified as time-dependent in their activity and the time above the MIC90 is important for their effectiveness [31]. Prophylactic antibiotic coverage during surgical procedures mainly targets skin flora (mainly gram-positive organisms) but in the SSI literature for pediatric cardiac surgical populations there is a significant proportion of SSIs that are caused by gram-negative pathogens [1-5,8-10]. There are no clear guidelines on what tissue concentrations should be targeted for prophylactic antibiotics, but targeting the MIC90 of organisms (see Methods section) seems reasonable. In these experiments, the dosing of antibiotics that was tested is likely adequate to cover MSSA (MIC90 2 µg/mL) [31], in both MS and CPB+DHCA groups, and may be adequate to cover organisms with MIC90 values close to 16 µg/mL, such as *Escherichia coli* and *Klebsiella pneumoniae* [32].

Given how CPB can alter drug pharmacokinetics and how quickly the plasma and tissue concentrations of cefazolin decrease in the MS group, a second dose of cefazolin in the bypass prime or given on initiation of CPB in pediatric populations appears to be prudent. This reinforces the conclusions that our group proposed in a recently published paper using similar methodologies in a human pediatric cardiac surgical population [33], and now provides a high-fidelity large animal model from which to test dose response and schedule that will inform translational pediatric studies for optimal dosing strategies. In addition, as tissue concentrations do not peak until approximately 15-30 minutes after the first bolus dose, the timing of cefazolin administration related to the initial surgical incision may be important to ensure adequate tissue concentrations at the time of incision, a time with high risk for potential contamination of the surgical wound. The timing of the first dose is critical for prophylaxis and this study gives some clinical direction for the exact timing of the first dose of prophylactic antibiotics in an immature animals. Future translational studies can now focus on this time period in high-risk pediatric populations. This delayed time of tissue distribution has also been demonstrated in adult populations and one pediatric population with peri-operative prophylactic antibiotic administration [33-37].

Limitations of this study include a small sample size and there is no ability to make any comments on post-operative cefazolin concentrations or pharmacokinetics. We did not estimate unbound cefazolin concentrations in plasma, as there exists no good estimate of protein binding of cefazolin in a porcine model. In humans, cefazolin does demonstrate concentration-dependent protein binding, with *in vivo* binding ranging from 85% at low concentrations to 52% at high concentrations [38]. *In vivo* studies in animal models have also shown concentration-dependent protein binding as well [39-41]. For this study, when we use the total plasma concentrations of cefazolin, there is a decrease in the estimated *f*T>MIC with higher concentration targets in the MS only group (Table 2) that would only be exacerbated if protein-binding were known. This is potentially important clinically as the MS only group may be thought of as representing non-cardiac surgical procedures and warrants further investigation in other pediatric surgical populations.

Finally, the data presented here further demonstrate that CPB and DHCA significantly alter the plasma, muscle, and subcutaneous tissue pharmacokinetics of cefazolin. To date, except for one study [33] previously published pediatric pharmacokinetic studies investigating prophylactic antibiotics during CPB have used plasma concentrations as a surrogate for tissue drug concentrations [18-21], which may not provide adequate information to fully inform dosing strategies. This study provides further practical and conceptual knowledge for translation of these methods into pediatric populations so that objective data can be generated that will help guide peri-operative antibiotic dosing. One can foresee the application of minimally invasive methods and advances in pharmacokinetic-pharmacodynamic modeling that will help optimize drug delivery during extracorporeal support and various pathophysiologic states that could adapt to emerging pathogens and resistance in human children.

Conclusions

This large animal translational model provides the principles and foundations for further clinical investigation as proof of concept and safety for these high-risk pediatric patients to evaluate whether current cefazolin dosing recommendations achieve desired pharmacodynamic targets. Perhaps, even more importantly, these techniques will advance our understanding of how to individualize pharmacotherapy for patients with complex congenital heart disease undergoing surgical procedures.

References

1. Holzmann-Pazgal G, Hopkins-Broyles D, Recktenwald A, Hohrein M, Kieffer P, et al. (2008) Case-control study of pediatric cardiothoracic surgical site infections . Infect Control Hosp Epidemiol 29: 76-79.
2. Allpress AL, Rosenthal GL, Goodrich KM, Lupinetti FM, Zerr DM (2004) Risk factors for surgical site infections after pediatric cardiovascular surgery. Pediatr Infect Dis J 23: 231-234.
3. Nateghian A, Taylor G, Robinson JL (2004) Risk factors for surgical site infections following open-heart surgery in a Canadian pediatric population. Am J Infect Control 32: 397-401.
4. Mehta PA, Cunningham CK, Colella CB, Alferis G, Weiner LB (2000) Risk factors for sternal wound and other infections in pediatric cardiac surgery patients. Pediatr Infect Dis J 19: 1000-1004.
5. Sarvikivi E, Lyytikäinen O, Nieminen H, Sairanen H, Saxén H (2008) Nosocomial infections after pediatric cardiac surgery. Am J Infect Control 36: 564-569.
6. Buylaert WA, Herregods LL, Mortier EP, Bogaert MG (1989) Cardiopulmonary bypass and the pharmacokinetics of drugs. An update. Clin Pharmacokinet 17: 10-26.
7. van Saet A, de Wildt SN, Knibbe CA, Bogers AD, Stolker RJ, et al. (2013) The effect of adult and pediatric cardiopulmonary bypass on pharmacokinetic and pharmacodynamic parameters. Curr Clin Pharmacol 8: 297-318.
8. Costello JM, Graham DA, Morrow DF, Morrow J, Potter-Bynoe G, et al. (2010) Risk factors for surgical site infection after cardiac surgery in children. Ann Thorac Surg 89: 1833-1841.
9. Ben-Ami E, Levy I, Katz J, Dagan O, Shalit I (2008) Risk factors for sternal wound infection in children undergoing cardiac surgery: a case-control study. J Hosp Infect 70: 335-340.
10. Kagen J, Lautenbach E, Bilker WB, Matro J, Bell LM, et al. (2007) Risk factors for mediastinitis following median sternotomy in children. Pediatr Infect Dis J 26: 613-618.
11. McAnally HB, Cutter GR, Ruttenber AJ, Clarke D, Todd JK (2001) Hypothermia as a risk factor for pediatric cardiothoracic surgical site infection. Pediatr Infect Dis J 20: 459-462.
12. Shah SS, Lautenbach E, Long CB, Tabbutt S, Gaynor JW, et al. (2005) *Staphylococcus aureus* as a risk factor for bloodstream infection in children with postoperative mediastinitis. Pediatr Infect Dis J 24: 834-837.
13. Shah SS, Kagen J, Lautenbach E, Bilker WB, Matro J, Dominguez TE, et al. (2007) Bloodstream infections after median sternotomy at a children's hospital. J Thorac Cardiovasc Surg 133: 435-440.
14. Long CB, Shah SS, Lautenbach E, Coffin SE, Tabbutt S, et al. (2005) Postoperative mediastinitis in children: epidemiology, microbiology, and risk factors for Gram-negative pathogens. Pediatr Infect Dis J 24: 315-319.
15. Tortoriello TA, Friedman JD, McKenzie ED, Fraser CD, Feltes TF, et al. (2003) Mediastinitis after pediatric cardiac surgery: a 15-year experience at a single institution. Ann Thorac Surg 76: 1655-1660.
16. Barker GM, O'Brien SM, Welke KF, Jacobs ML, Jacobs JP, et al. (2010) Major infection after pediatric cardiac surgery: a risk estimation model. Ann Thorac Surg 89: 843-850.
17. Kirby JP, Mazuski JE (2009) Prevention of surgical site infection. Surg Clin North Am 89: 365-389, viii.
18. Hatzopoulos FK, Stile-Calligaro IL, Rodvold KA, Sullivan-Bolyai J, Del Nido P, et al. (1993) Pharmacokinetics of intravenous vancomycin in pediatric cardiopulmonary bypass surgery. Pediatr Infect Dis J 12: 300-304.
19. Haessler D, Reverdy ME, Neidecker J, Brûlé P, Ninet J, et al. (2003) Antibiotic prophylaxis with cefazolin and gentamicin in cardiac surgery for children less than ten kilograms. J Cardiothorac Vasc Anesth 17: 221-225.
20. Masuda Z, Kurosaki Y, Ishino K, Yamauchi K, Sano S (2008) Pharmacokinetic analysis of flomoxef in children undergoing cardiopulmonary bypass and modified ultrafiltration. Gen Thorac Cardiovasc Surg 56: 163-169.
21. Knoderer CA, Saft SA, Walker SG, Rodefeld MD, Turrentine MW, et al. (2011) Cefuroxime pharmacokinetics in pediatric cardiovascular surgery patients undergoing cardiopulmonary bypass. J Cardiothorac Vasc Anesth 25: 425-430.
22. Reed MD (2000) Optimal antibiotic dosing. The pharmacokinetic-pharmacodynamic interface. Postgrad Med 108: 17-24.
23. Theuretzbacher U (2007) Tissue penetration of antibacterial agents: how should this be incorporated into pharmacodynamic analyses? Curr Opin Pharmacol 7: 498-504.
24. Langer O, Müller M (2004) Methods to assess tissue-specific distribution and metabolism of drugs. Curr Drug Metab 5: 463-481.
25. Müller M, dela Peña A, Derendorf H (2004) Issues in pharmacokinetics and pharmacodynamics of anti-infective agents: distribution in tissue. Antimicrob Agents Chemother 48: 1441-1453.

26. Chaurasia CS, Müller M, Bashaw ED, Benfeldt E, Bolinder J, et al. (2007) AAPS-FDA workshop white paper: microdialysis principles, application and regulatory perspectives. Pharm Res 24: 1014–1025.
27. Brunner M, Derendorf H, Müller M (2005) Microdialysis for *in vivo* pharmacokinetic/pharmacodynamic characterization of anti-infective drugs. Curr Opin Pharmacol 5: 495-499.
28. Joukhadar C, Müller M (2005) Microdialysis: current applications in clinical pharmacokinetic studies and its potential role in the future. Clin Pharmacokinet 44: 895-913.
29. Pastuszko P, Pirzadeh A, Reade E, Kubin J, Mendoza A, Schears GJ, et al. (2009) The effect of hypothermia on neuronal viability following cardiopulmonary bypass and circulatory arrest in newborn piglets. Eur J Cardiothorac Surg 35: 577-581.
30. Guidance for Industry: Bioanalytical Method Validation. Washington, DC: U.S Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), and Center for Veterinary Medicine (CVM); 2001.
31. Craig WA (1998) Pharmacokinetic/pharmacodynamic parameters: rationale for antibacterial dosing of mice and men. Clin Infect Dis 26: 1-10.
32. Andes DR, Craig WA (2010) Chapter 22: Cephalosporins. In: Mandell GL, Bennett JE, Dolin R. Mandell, Douglas and Bennett's Principles and Practice of Infectious Diseases, (7thedn.)323-339.
33. Himebauch AS, Nicolson SC, Sisko M, Moorthy G, Fuller S, et al. (2014) Skeletal muscle and plasma concentrations of cefazolin during cardiac surgery in infants. J Thorac Cardiovasc Surg 148: 2634-2641.
34. Hutschala D, Skhirtladze K, Kinstner C, Mayer-Helm B, Müller M, et al. (2007) *In vivo* microdialysis to measure antibiotic penetration into soft tissue during cardiac surgery. Ann Thorac Surg 84: 1605-1610.
35. Barbour A, Schmidt S, Rout WR, Ben-David K, Burkhardt O, et al. (2009) Soft tissue penetration of cefuroxime determined by clinical microdialysis in morbidly obese patients undergoing abdominal surgery. Int J Antimicrob Agents 34: 231-235.
36. Douglas A, Udy AA, Wallis SC, Jarrett P, Stuart J, et al. (2011) Plasma and tissue pharmacokinetics of cefazolin in patients undergoing elective and semielective abdominal aortic aneurysm open repair. Antimicrob Agents Chemother 55: 5238-5242.
37. Andreas M, Zeitlinger M, Hoeferl M, Jaeger W, Zimpfer D, et al. (2013) Internal mammary artery harvesting influences antibiotic penetration into presternal tissue. Ann Thorac Surg 95:1323-1329.
38. Vella-Brincat JW, Begg EJ, Kirkpatrick CM, Zhang M, Chambers ST, et al. (2007) Protein binding of cefazolin is saturable *in vivo* both between and within patients. Br J Clin Pharmacol 63: 753-757.
39. Fritz PE, Hurst WJ, White WJ, Lang CM (1987) Pharmacokinetics of cefazolin in guinea pigs. Lab Anim Sci 37: 646-651.
40. Matsui H, Okuda T (1988) Penetration of cefpiramide and cefazolin into peritoneal capsular fluid in rabbits. Antimicrob Agents Chemother 32: 33-36.
41. Nadai M, Hasegawa T, Kato K, Wang L, Nabeshima T, et al. (1993) Alterations in pharmacokinetics and protein binding behavior of cefazolin in endotoxemic rats. Antimicrob Agents Chemother 37: 1781-1785.