7th CPERC
Vancouver, BC
May 31 – June 2 2016

Significant Changes in Cefazolin Protein Binding during Cardiac Surgery with Cardiopulmonary Bypass

D. Calic, R. Lillico, TM. Lakowski, C. Sayre, S. Zelenitsky

College of Pharmacy, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, Manitoba, Canada

University of Manitoba

Dr. Sheryl Zelenitsky
Professor, University of Manitoba
Winnipeg, Manitoba, Canada
s_zelenitsky@umanitoba.ca

ABSTRACT

BACKGROUND: Cefazolin is used for antimicrobial prophylaxis in cardiac surgery with cardiopulmonary bypass (CPB). Although protein-bound cefazolin is inactive, most studies measure total plasma concentrations and only estimate the free fraction (e.g. 20%). Given the significant pharmacokinetic alterations during cardiac surgery, our goal was to characterize the protein binding of cefazolin using an adapted assay for total concentrations and developing a method for measuring free concentrations.

METHODS: Blood samples were collected from 55 patients undergoing cardiac surgery with cefazolin prophylaxis. Samples were centrifuged to yield plasma (total cefazolin) and a portion was centrifuged in a Centrifree® filter to yield ultrafiltrate (free cefazolin). The stable isotope cefazolin $^{13}C_2{}^{15}N$ sodium salt was used as the internal standard and 85% acetonitrile in water with 0.1% formic acid as the mobile phase. The analysis was conducted using Shimadzu Nexara UHPLC and LCMS 8040 triple quadrupole mass spectrometer. Extensive intra- and inter-day validation was performed for total cefazolin concentrations from 4 to 100 mg/L and free concentrations from 1 to 100 mg/L.

RESULTS: A total of 135 intra-operative blood samples were analyzed. Total and free cefazolin concentrations ranged from 12.9 to 225.1 mg/L and 4.4 to 99.9 mg/L, respectively, with an average free fraction of 28.1 ± 7.6%. Initial observations were consistent with saturable protein binding at concentrations exceeding 150 mg/L. However, further analysis identified two sample populations including those drawn before (n = 52) and after (n = 83) starting the CPB pump. The protein binding was linear in both cases, however the free fraction was significantly higher in samples drawn after compared with before starting the pump (29.7 ± 8.4% vs. 25.6 ± 5.2%, p = 0.002).

CONCLUSION: The study characterizes important changes in cefazolin protein binding during cardiac surgery and highlights the limitations of utilizing literature values of free fraction to predict prophylaxis effectiveness.

BACKGROUND

- Cefazolin, a 1st generation cephalosporin is a drug of choice for antimicrobial prophylaxis during cardiac surgery with cardiopulmonary bypass (CPB)
- Free (unbound) cefazolin is pharmacologically active, but percent (%) free cefazolin has been estimated, rather than measured in most studies of cefazolin antimicrobial prophylaxis in cardiac surgery with CPB (Fellinger 2002, Caffarelli 2006, Adembri 2010)
- GOAL: To characterize the plasma protein binding of cefazolin in patients undergoing cardiac surgery with cardiopulmonary bypass (CPB)

METHODS

- Inclusion criteria: adult patients undergoing elective cardiac surgery with CPB
- Exclusion criteria: received >1 pre-op cefazolin dose, known/suspected infection or other antimicrobial use within 3 days of surgery, chronic liver disease, renal function <50 mL/min/72 kg as per calculated creatinine clearance (Clcr)
- Patients received 1 or 2 g of cefazolin prophylaxis pre-op, q4h during surgery and q8h for 48 hours after surgery, per institutional protocol
- Patient and surgery data collected from medical records
- Blood samples collected at 30 min post-infusion, prior to any intra-op dose, and within 15 min of wound closure
- Whole blood samples centrifuged at 1300 x g for 10 min to yield plasma and a portion of the plasma centrifuged again in a Centrifree® device at 2000 x g for 45 min to yield protein-free ultrafiltrate
- Total (plasma) and free (ultrafiltrate) cefazolin concentrations determined by LC-MS/MS using Shimadzu Nexara ultra-high performance liquid chromatograph and 8040 triple quadrupole mass spectrometer and an Acquity UPLC BEH C18 1.7µm column
- Stable isotope $^{13}C_2{}^{15}N$ cefazolin (Toronto Research Chemicals) was utilized as internal standard
- Mobile phase consisted of 85% acetonitrile in water with 0.1% formic acid at a flow rate of 0.4 mL/min
- Mass spectrometer run in positive ion mode with multiple reaction monitoring (MRM)
- Precursor to product ion transitions were m/z 454.60 to 322.90 for cefazolin and 458.00 to 326.00 for the internal standard
- Retention times for both cefazolin and the internal standard were 2.76 min
- Assays validated according to FDA guidelines (FDA Bioanalytical Method Validation 2001) for accuracy and precision for a linear range from 4 to 100 mg/L for total concentrations and from 1 to 100 mg/L for free concentrations
- Total, free, and % free cefazolin concentrations described and variables associated with % free cefazolin analyzed using Pearson correlation regression analysis for continuous variables (i.e., age, gender, body weight, Clcr, duration of surgery, volume of intra-operative fluids, and plasma albumin) and Student's t-test for categorical variables (i.e., gender, obesity, before or after starting CPB)

RESULTS

- Total (n = 136) and free (n = 135) cefazolin concentrations ranged from 12.9 to 225.1 mg/L and 4.4 to 99.9 mg/L, respectively
- Overall average % free cefazolin (n = 135) was 28.1 ± 7.6%
- Initial observations of % free cefazolin suggested saturable protein binding at total cefazolin concentrations exceeding 150 mg/L but further analysis identified two populations, including those drawn before the start of CPB (n = 52) and those drawn afterwards (n = 83) (Figure 2)
- Linear protein binding observed in both groups (Figure 2), but % free cefazolin was significantly higher in samples drawn after the start of CPB (29.7 ± 8.4% vs. 25.6 ± 5.2%, p = 0.002) (Figure 3)
- Albumin concentrations pre-surgery were significantly higher than post-surgery (39.4 ± 2.3 g/L vs. 31.2 ± 3.0 g/L, p < 0.001)
- Post-surgery albumin significantly associated with % free cefazolin after start of CPB (Table 2, Figure 4)
- No other significant relationships observed between examined variables and % free cefazolin during cardiac surgery, even when analysis separated samples based on timing before or after starting CPB (Table 2)

CONCLUSIONS

- ✓ Significantly higher % free cefazolin observed in samples drawn after starting CPB compared to before CPB start
- ✓ Post-surgery albumin significantly associated with % free cefazolin after starting CPB
- ✓ Other patient and surgery variables (e.g., age, gender, intra-op fluids) were not associated with % free cefazolin in cardiac surgery with CPB

Table 1: Patient and surgery characteristics

Patient Characteristics (n = 55)	
Age (years)	65 ± 10
Gender (male)	38 (69%)
Weight (kg)	90 ± 17
BMI (kg/m^2)	30.9 ± 5.3
Obese (BMI ≥ 30 kg/m^2)	27 (49%)
Clcr (mL/min/72kg)	80 ± 19
Albumin pre-surgery (g/L)	39.4 ± 2.3
Albumin post-surgery (g/L)	31.2 ± 3.0
Surgery Characteristics (n = 55)	
CABG surgery	26 (47%)
Valve surgery	14 (26%)
Mixed/Other surgery	15 (27%)
Duration of surgery (min)	258 ± 99
Intra-op fluids (mL)	3547 ± 1301

Fig 1: Total and free cefazolin concentrations

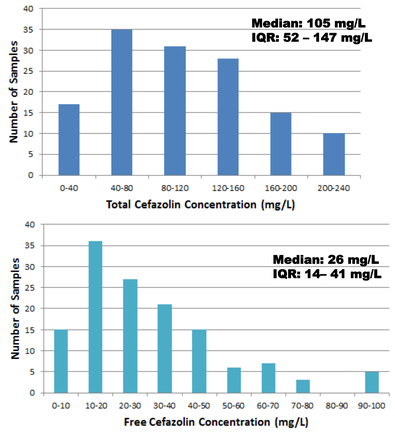


Fig 2: Relationship between free and total cefazolin concentrations overall and before and after starting CPB

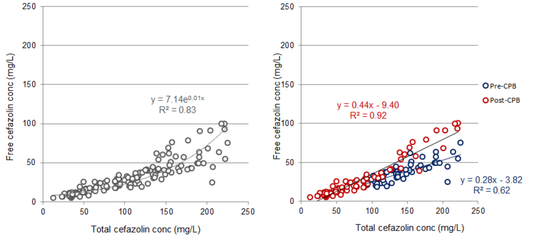


Table 2: Variables examined for association with % free cefazolin

Patient Variables	p-value
Age (years)	0.50
Gender	0.07
Weight (kg)	0.47
BMI (kg/m^2)	0.78
Obese (BMI ≥ 30 kg/m^2)	0.94
Clcr (mL/min/72kg)	0.75
Albumin pre-surgery and % free cefazolin before CPB start	0.57
Albumin post-surgery and % free cefazolin after CPB start	<0.01
Surgery Variables	
Duration of surgery (min)	0.06
Intra-op fluids (mL)	0.17

Fig 3: % free cefazolin before and after starting CPB

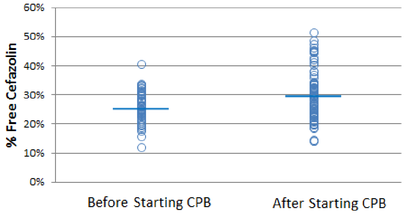
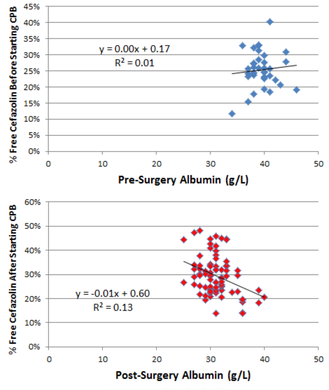


Fig 4: % free cefazolin before and after starting CPB in relation to serum albumin levels



ACKNOWLEDGEMENTS:
The authors would like to acknowledge the generous support of the AFPC Student Research Poster Award (DC), the University of Manitoba Graduate Fellowship (DC) Leslie F. Buggey Professorship (SZ) and CSHP Foundation Grant.