Item S1. Supplementary methods and results.

METHODS

This prospective, open-label, single-centre, pharmacokinetic study of cefazolin was conducted at University Health Network-Toronto General Hospital in Toronto, Ontario, a hospital that manages 100 NHD patients. NHD patients were recruited, consented and sampled in March 2013. The study was approved by the University Health Network Research Ethics Board and written informed consent was completed by all participants.

Subjects

Adult patients, at least 18 years of age, from the NHD Outpatient Clinic were eligible for inclusion if they met the following criteria: non-infected (afebrile, lack of constitutional symptoms, no leukocytosis), and on NHD for at least 6 months. Patients were excluded if they had any clinical signs or symptoms of active infection, elevated white blood cell count within 1 week of the study, or treatment with any antibiotic within 2 weeks of the study, serum hemoglobin less than 100g/L within 1 week of the study, stated or documented allergy to penicillins and/or cephalosporins, pregnancy, or breastfeeding females.

Dialysis Procedure and Drug Dosing

On day 1, after 8 hours of NHD at the patient’s residence, cefazolin 2g intravenous (IV) infusion over 30 minutes was self-administered in the morning. On day 2, after 6 hours of hemodialysis at the clinic using the same blood and dialysate flow rates as in NHD, the second dose of cefazolin 2g IV was given by registered nurses (Figure S1). These NHD patients would normally undergo NHD 5-7 times per week. Patients received NHD using a conventional hemodialysis platform, Bellco Formula Home Care System (Mirandola, Italy), with high-flux
synthetic polyethersulfone membrane dialyzer, Xenium 170 (Baxter, Canada). Blood and
dialysate flow rates were both 300ml/min for all HD sessions.

Sample Collection

All blood and dialysate collections were conducted in the NHD Outpatient Clinic. On day
two, seven blood samples and three dialysate samples were collected (Figure S1). Blood samples
were drawn immediately prior to HD, and at 60, 180 (mid-dialysis), and 360 minutes (end of
HD). After HD and completion of the second 30 minute-cefazolin infusion, blood samples were
drawn immediately, and again at 30 and 60 minutes post-infusion. The three dialysate samples in
duplicates were drawn at the start of HD and at 180 and 360 minutes during HD on day 2.

Blood samples (5ml) were collected from the “arterial” side of the hemodialysis circuit
into BD Vacutainers (6ml collection tubes, additives and anti-coagulant free), and stored in a
refrigerator at 2-8°C. All samples were centrifuged within 24 hours of collection at 3000rpm for
ten minutes at 5°C (Beckman Coulter Allegra 6R Centrifuge) and serum was transferred to
cryogenic vials for storage at -80°C until analysis. Dialysate samples (10ml) were collected from
the HD machine via a luer lock port using two 10ml luer lock syringes (5mls per syringe) and
frozen at -80°C until time of analysis.

Serum and dialysate cefazolin concentrations were determined using high performance
liquid chromatography (HPLC) analysis at an external lab, Sunnybrook Health Sciences Centre
Quality Lab. A one-compartment pharmacokinetic model with intravenous (IV) infusion
administration and first-order elimination were tested to fit the data. Median and interquartile
ranges (IQR) were determined for the pharmacokinetic parameters.

Pharmacokinetic Calculations

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Elimination rate constants were calculated from the slopes of the log-normal distributions of cefazolin concentrations over time off HD ($K_{\text{interdialysis}}$) and during HD ($K_{\text{intradialysis}}$). $K_{\text{interdialysis}}$ was calculated from the peak cefazolin levels ($C_{\text{peak}}$) at 1-hour post-infusion of cefazolin on day 2 to the lowest known concentration pre-HD ($C_{\text{pre-HD}}$), inferred from pre-HD concentration on day 2. The cefazolin concentration at end of HD on day 2 ($C_{\text{end-HD}}$) was included into the clearance equation to account for accumulation:

$$Cl_{\text{intradialysis}} = \frac{dose \text{ of infusion}}{C_{\text{peak}} - C_{\text{end-HD}}} \times \left(1 - e^{-K_{\text{intradialysis}} \times \text{time of infusion}}\right)$$

Half-life off HD ($t_{1/2 \text{ interdialysis}}$) and during HD ($t_{1/2 \text{ intradialysis}}$) was determined using $t_{1/2} = \frac{\ln(2)}{K}$, using the appropriate corresponding $K$ value. Volume of distribution ($V_d$) was calculated as $V_d = \frac{Cl_{\text{intradialysis}}}{K_{\text{intradialysis}}}$ and adjusted by body weight. $V_d$ was assumed to be constant during the entire study period. With $V_d$, clearance during hemodialysis was calculated by

$$Cl_{\text{interdialysis}} = V_d \times K_{\text{interdialysis}}.$$

Observed blood cefazolin concentration versus time curve was created using Microsoft Excel® 2010 to illustrate the median concentrations of cefazolin during the study period for the 15 patients. The first post-infusion cefazolin concentration on day 1 was extrapolated based on the following equation: $C = C_{\text{pre-HD}} \times e^{-K_{\text{interdialysis}} \times t}$, where $t$ is the time between $C$ and $C_{\text{pre-HD}}$.

**Drug Removal Calculations**

Percentage of drug removed from the blood (extraction ratio) was determined for the 6 and 8 hour HD by:
Removal_B = \frac{C_{preHD} - C_{endHD}}{C_{preHD}} \times 100

To extrapolate for an 8 hour HD session, concentration at the end of HD was calculated by
\[ C_{endHD} = C_{preHD} e^{-K_{intradialysis} \times 8} \]

In blood, the initial cefazolin amount at the start of HD was calculated by
\[ \text{Amount}_{pre-HD} = C_{pre-HD} \times V_d. \]
The amount of drug removed from blood was calculated by
\[ \text{Amount}_{B-removed} = \text{Amount}_{pre-HD} \times \text{Removed}_B. \]

In dialysate, the amount of cefazolin retrieved after a 6 hour HD was measured using area under the curve (AUC_{dialysate}) via the trapezoidal rule in the following equation:
\[ \text{Amount}_D = \text{AUC}_{dialysate} \times \text{dialysate flow}. \]
To estimate the amount of drug removed from an 8 hour HD, dialysate concentration at 8 hrs was extrapolated using
\[ C_{8hr} = C_o e^{-K_D \times 8 \text{ hours}}, \]
where K_D was the slope of the log-normal distribution of cefazolin concentrations found in the dialysate during HD and C_o is the initial concentration gathered immediately when HD started. Again AUC_{dialysate} was calculated via the trapezoidal rule and incorporated into the Amount_D equation above to calculate amount of drug removed at 8 hours.

**Pharmacokinetic Modeling**

In practice, a cefazolin 2g IV loading dose is followed by 1 g IV after each NHD session. Normal practices were modeled by incorporating the median and IQR pharmacokinetic parameters obtained from this study into the following equations. Peak concentrations were calculated using the IV infusion equation,
\[ C_{\text{peak}} = \frac{\text{dose/time of infusion}}{K_{intradialysis} \times V_d \text{ per kg} \times \text{weight}} \times (1 - e^{-K_{erndialysis} \times \text{time of infusion}}). \]
Concentrations pre-HD were calculated by
\[ C_{\text{pre-HD}} = C_{\text{peak}} \times e^{-K_{intradialysis} \times \text{interdialysis time}}. \]
Concentrations during HD
were calculated by $C_n = C_{pre-HD} e^{-k_{intradialysis} \times t_n}$, where $t$ is time at 2, 4, 6, and 8 hours of HD. Accumulation from each dose was also considered by adding $C_8$ to the $C_{peak}$ for the following dose. Six consecutive doses were modeled to ensure steady-state concentrations were achieved. Concentration at 50% or 80% of the 24-hour dosing interval was calculated using standard equation, $C = C_0 e^{-k \times t}$. Three different dosing regimens were performed in this model for an 8 hour HD since 8 hours is the typical duration for NHD patients (infusion time was 30 minutes, regardless of dose):

1) Loading dose 1g IV, followed by 1g IV doses after each NHD
2) Loading dose 2g IV, followed by 1g IV doses after each NHD
3) Loading dose 2g IV, followed by 2g IV doses after each NHD

Improved efficacy for time-dependent antimicrobials, such as cefazolin, is best achieved when observed drug concentrations are 4-6 times above the minimum inhibitory concentration (MIC) of the bacteria for at least 40-60% of the dosing interval.\textsuperscript{1-3} To guide our selection of a dosing recommendation, dosing regimens were considered if the blood concentrations were above the pharmacodynamic target of 6 times the MIC of 8mg/L for 50% of the dosing interval. To be conservative, this target concentration for 80% of the dosing interval was also examined as greater exposure may be needed under certain clinical circumstances.

**Safety evaluations**

All reported adverse events (AE) were recorded by investigators from onset of cefazolin administration to 14 days after the dose. Description of the AE as well as the severity and causal relationship to the study drug was documented.

**Statistical Analysis**
For patient demographics, descriptive analyses were used to determine frequencies, means and standard deviations, and where appropriate, medians and IQR. Pharmacokinetic parameters or variables were reported with median and interquartile range to account for any outliers. All statistical analyses were performed using SAS version 9.2; (SAS Institute Inc, www.sas.com).

RESULTS

Fifteen patients were enrolled and had complete data for analysis. Patient demographics and clinical characteristics are listed in Table S1. Most patients were male, Caucasian, with an average age of 46 ±7.8 years and median body weight of 71kg (IQR: 63-81kg). All patients were anuric. Cefazolin was well tolerated by all patients and none reported any adverse effects.

During HD, the median clearance was 1.65L/hr (IQR: 1.36-2.19L/hr) and half-life was 3.44hrs (IQR: 2.93-4.36 hrs). The calculated volume of distribution was 0.13 L/kg (IQR: 0.079-0.16L/kg).

Figure S2 illustrates the median observed cefazolin concentrations versus time for blood samples obtained during the study period. The observed concentration data was fit to a 1-compartment pharmacokinetic model. In general, cefazolin blood concentrations decreased from median of 159.8 mg/L (IQR: 125.3 – 198.5 mg/L) at the beginning of HD to 48.8mg/L (IQR: 35.7 – 58.6mg/L) at the end of HD after the first dose of cefazolin 2g IV. The median percentage of cefazolin removed from blood in a 6-hour HD session was 74.3%, which translates to a median amount of 906.4mg (IQR: 727.2 – 1203.8mg) of cefazolin removed. About 67% of this amount was recovered in the dialysate fluid, which suggests 33% is cleared from blood through non-renal methods. When extrapolating the results to an 8-hour NHD session, a median cefazolin
amount of 1015.4mg (IQR: 823.7-1375.9mg) was removed from blood.

Model-predicted cefazolin concentrations for 6 consecutive doses, each given after 8-hour NHD, for a 70-kg individual are shown in Table S2. Each dosing interval was 24 hours. The three dosing regimens ([1] 1g load followed by 1 g IV after each NHD, [2] 2g load followed by 1g IV after each NHD, and [3] 2g load followed by 2g IV after each NHD) showed that steady state cefazolin concentrations were reached near the 3rd consecutive dose. All regimens successfully achieved the pharmacodynamic target serum concentrations of 6 times above the MIC breakpoint of 8mg/L for Staphylococcus species4 (48mg/L) for at least 50% of the dosing interval (Figure S3). With respect to achieving the target concentration for 80% of the dosing interval, only the third regimen (2g load followed by 2 g IV after each NHD) is predicted to be successful in the majority of patients (≥75%). However, when examining the concentrations attained at 80% of the dosing interval, the importance of a 2g load became apparent. In the first dosing regimen (without a 2g load), 50% of the population did not achieve the target of 48mg/L for the first two doses; however, the other two regimens (loaded with 2g) achieved the target. Although 50% of the population in the second dosage regimen (2g load followed by 1g IV after each NHD) did not achieve the target concentration of 48mg/L at 80% of the dosing interval, the majority (75% of the population) achieved target concentrations for at least 70% of the dosing interval. In the third dosing regimen, 75% of the population achieved target concentrations for at least 83% of the dosing interval.

Works Cited

1. Sinnollareddy MG, Roberts MS, Lipman J, Roberts JA. Beta-lactam pharmacokinetics and pharmacodynamics in critically ill patients and strategies for dose optimization: A structured

