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Anakinra Removal by Continuous Renal Replacement Therapy: An Ex Vivo Analysis

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OBJECTIVES: Patients with sepsis are at significant risk for multiple organ dysfunction, including the lungs and kidneys. To manage the morbidity associated with kidney impairment, continuous renal replacement therapy (CRRT) may be required. The extent of anakinra pharmacokinetics in CRRT remains unknown. The objectives of this study were to investigate the anakinra–circuit interaction and quantify the rate of removal from plasma.

DESIGN: The anakinra–circuit interaction was evaluated using a closed-loop ex vivo CRRT circuit. CRRT was performed in three phases based on the method of solute removal: 1) hemofiltration, 2) hemodialysis, and 3) hemodiafiltration. Standard control samples of anakinra were included to assess drug degradation.

SETTING: University research laboratory.

PATIENTS: None.

INTERVENTIONS: Anakinra was administered to the CRRT circuit and serial prefilter blood samples were collected along with time-matched control and hemofiltrate samples. Each circuit was run in triplicate to assess inter-run variability. Concentrations of anakinra in each reference fluid were measured by enzyme-linked immunosorbent assay. Transmembrane filter clearance was estimated by the product of the sieving coefficient/dialysate saturation constant and circuit flow rates.

MEASUREMENTS AND MAIN RESULTS: Removal of anakinra from plasma occurred within minutes for each CRRT modality. Average drug remaining (%) in plasma following anakinra administration was lowest with hemodiafiltration (34.9%). The average sieving coefficient was 0.34, 0.37, and 0.41 for hemodiafiltration, hemofiltration, and hemodialysis, respectively. Transmembrane clearance was fairly consistent across each modality with the highest during hemodialysis (5.53 mL/min), followed by hemodiafiltration (4.99 mL/min), and hemofiltration (3.94 mL/min). Percent drug remaining within the control samples (93.1%) remained consistent across each experiment, indicating negligible degradation within the blood.

CONCLUSIONS: The results of this analysis are the first to demonstrate that large molecule therapeutic proteins such as anakinra, are removed from plasma with modern CRRT technology. Current dosing recommendations for patients with severe renal impairment may result in subtherapeutic anakinra concentrations in those receiving CRRT.

KEY WORDS: anakinra; continuous renal replacement therapy; ex vivo; pharmacokinetics

Continuous renal replacement therapy (CRRT) has become the method of choice for renal support in critically ill patients due to its preferential effects on hemodynamic stability (1, 2). Solute removal occurs differently depending on the modality of CRRT which includes: continuous venovenous hemofiltration (CVVH), continuous venovenous hemodialysis

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KEY POINTS

Question: What is the anakinra—continuous renal replacement therapy (CRRT) circuit interaction when each CRRT modality is utilized?

Findings: Using the ex vivo CRRT experimental design, anakinra was removed from plasma through each modality of CRRT. The average sieving coefficient was 0.34, 0.37, and 0.41 for hemodiafiltration, hemofiltration, and hemodialysis, respectively.

Meaning: Anakinra removal from plasma occurred with modern CRRT technology commonly used in clinical practice settings. Dose adjustments based on renal dysfunction may result in subtherapeutic dosing regimens for patients receiving CRRT support.

(CVVHD), and continuous venovenous hemodiafiltration (CVVHDF). The most important variables affecting drug pharmacokinetics during CRRT include volume of distribution (Vd), protein binding, and molecular weight (3–5). Intuitively, small molecules with a low Vd and high unbound fraction may be more susceptible to drug removal from systemic circulation when a CRRT circuit is used (3–5). In addition to drug removal via filtration, adsorption of drug to circuit materials can be a mechanism of drug removal. Physicochemical properties that may predispose drugs to adsorption include lipophilicity (expressed as logP), protein binding, and molecular charge (3, 4, 6, 7). However, predicting the pharmacokinetics of medications administered to this population is challenging as it requires a thorough understanding of the interplay between drug, circuit-specific properties, and disease-related factors (4, 8).

Anakinra, a recombinant human interleukin (IL)-1 receptor antagonist (IL-1 RA) received marketing approval from the U.S. Food and Drug Administration for indications of rheumatoid arthritis and neonatal-onset multisystem inflammatory disease (9, 10). IL-1, an endogenous proinflammatory cytokine, serves as a key contributor to the pathophysiology of hyperinflammatory conditions such as rheumatoid arthritis (11). In addition to its role in chronic inflammatory conditions, IL-1 is implicated in the cytokine cascade and contributes to the pathophysiology of cytokine release syndromes (CRSs) such as hemophagocytic lymphohistiocytosis (HLH) (12, 13). Although used off-label, anakinra has been recommended as a first-line agent for the management of HLH (14–16) or CRS due to secondary HLH syndromes (17–19).

Anakinra a large molecule (17 kDa), therapeutic protein has a small volume of distribution in healthy adults (3.32 ± 0.62 L) (20). For chronic rheumatologic treatment doses are administered subcutaneously; however, IV administration is becoming more common to offset any potential impacts on absorption because of the presence of edema in patients with acute, hyperinflammatory conditions (13). The apparent half-life of elimination is longer with subcutaneous dosing, than IV, with comparable clearance (20, 21). Renal elimination accounts for a large percentage of anakinra plasma clearance (20–22). As a result, renal impairment has a significant impact on total clearance, and adjustments of maintenance dosing regimens are recommended (20).

Prior to receiving approval for use in chronic inflammatory diseases, anakinra was studied as an intriguing treatment alternative for acute, hyperinflammatory conditions including sepsis (23–26). Release of inflammatory cytokines, such as IL-1 in response to circulating microbial toxins may result in sepsis-induced organ dysfunction, including the lungs and kidneys (27, 28). Acute kidney injury (AKI) due to sepsis complications is one of the most common causes of AKI in critically ill patients, occurring in up to 50% of adults and 20% of children admitted to an ICU (29–31). Of these, a significant portion may require renal replacement therapy (RRT) to manage the morbidity associated with AKI (1, 2).

The extent of anakinra removal by different modalities of CRRT remains largely unknown. As anakinra remains a potential treatment alternative for patients with sepsis demonstrating hyperinflammatory phenotypes (23–26, 32), many of whom may require RRT, information regarding the anakinra–CRRT circuit interaction is of the utmost importance for designing safe and effective dosing regimens. Due to the physicochemical properties of anakinra (**Table 1**), it is possible that with modern CRRT technology, significant removal from plasma may occur.

We conducted this ex vivo study to characterize the drug–circuit interaction of anakinra. We hypothesized

TABLE 1.
Anakinra Physicochemical Properties and Clearance Mechanisms

Drug	Compound Type	Molecular Weight (kDa)	Protein Binding (%)	Clearance Pathways
Anakinra	Protein	17.3 (33)	0	Renal and nonrenal (20–22)

that with the use of modernized hemofilters, and differing methods of solute removal (convection vs. diffusion), anakinra would be sufficiently removed from plasma across each CRRT modality.

MATERIALS AND METHODS

Institutional Review Board

As this study did not involve human subjects, institutional review board approval was not required.

Circuit Configuration

CRRT circuits were completed with the PRISMAX System (Baxter Healthcare, Deerfield, IL) and HF1000 filter set (Baxter Healthcare) connected with a 500-mL EXACTAMIX EVA bag (Baxter Healthcare) (**Table 2**). Each circuit configuration (**Fig. 1**) was run in triplicate to assess inter-run variability in anakinra concentrations. Upon each subsequent run, new hemofilters were used in addition to repriming the CRRT circuit with new blood volume to reduce any risk of hemofilter clotting.

Continuous Renal Replacement Therapy Circuit Setup

Each circuit was primed with a solution of 1 unit of human RBCs (adenine saline-added leukocytes reduced [~300 mL]), approximately 0.4 units of thawed human plasma frozen within 24 hours after phlebotomy (125 mL), Plasma-Lyte A crystalloid (150 mL), heparin sulfate (350 units), tomethamine (1.6 g), sodium bicarbonate (7 mEq), and human serum albumin (6.25 g). Albumin concentrations throughout experiments were targeted to achieve those similar to critically ill children (20–30 g/L). Maintenance of a physiologic pH (7.4; range, 7.08–7.63) was achieved with additional tromethamine. PrismaSate 4/2.5 dialysis solution (Baxter Healthcare) was used for preblood pump, dialysis, and replacement fluids for each circuit. Target volume of primed solution within each circuit was 500 mL. A description of the target concentrations of circuit solution components are shown in **Electronic Supplementary Material**, **Table 1** (http://links.lww.com/CCX/B281).

CRRT circuit prescriptions were as follows: 1) CVVH: blood flow rate (Q_B) 80 mL/min, prefilter replacement fluid rate (Q_{pre}) 600 mL/hr, postfilter replacement fluid rate (Q_{post}) 200 mL/hr and total effluent rate (Q_{eff}) 800 mL/hr; 2) CVVHD: Q_B 80 mL/min, dialysate fluid rate (Q_D) 800 mL/hr; and 3) CVVHDF: Q_B 80 mL/min, Q_{pre} 300 mL/hr, Q_{post} 100 mL/hr, Q_{eff} 800 mL/hr, and Q_D 400 mL/hr. Flow rates incorporated within the experiment were determined based on a hypothetical child of approximately 10 kg as per consultation from nephrologists within our institution. CRRT prescriptions for each modality are presented in Table 2. Setting the patient fluid removal rate (Q_{PFR}) to 0 L/hr for each circuit ensured a consistent volume of recirculating blood was maintained over the course of the experiment.

TABLE 2.
Continuous Renal Replacement Therapy Circuit Components

Component	Manufacturer	Model	Material
Continuous renal replacement therapy system	Baxter	PrisMax with TherMax heater	Not applicable
Hemofilter	Baxter	HF 1,000 SA: 1.1 m^2	Polyarylethersulfone hollow fibers, plasticized polyvinyl chloride tubing
Reservoir	Baxter	EXACTAMIX EVA, 500 mL	Ethylene vinyl acetate

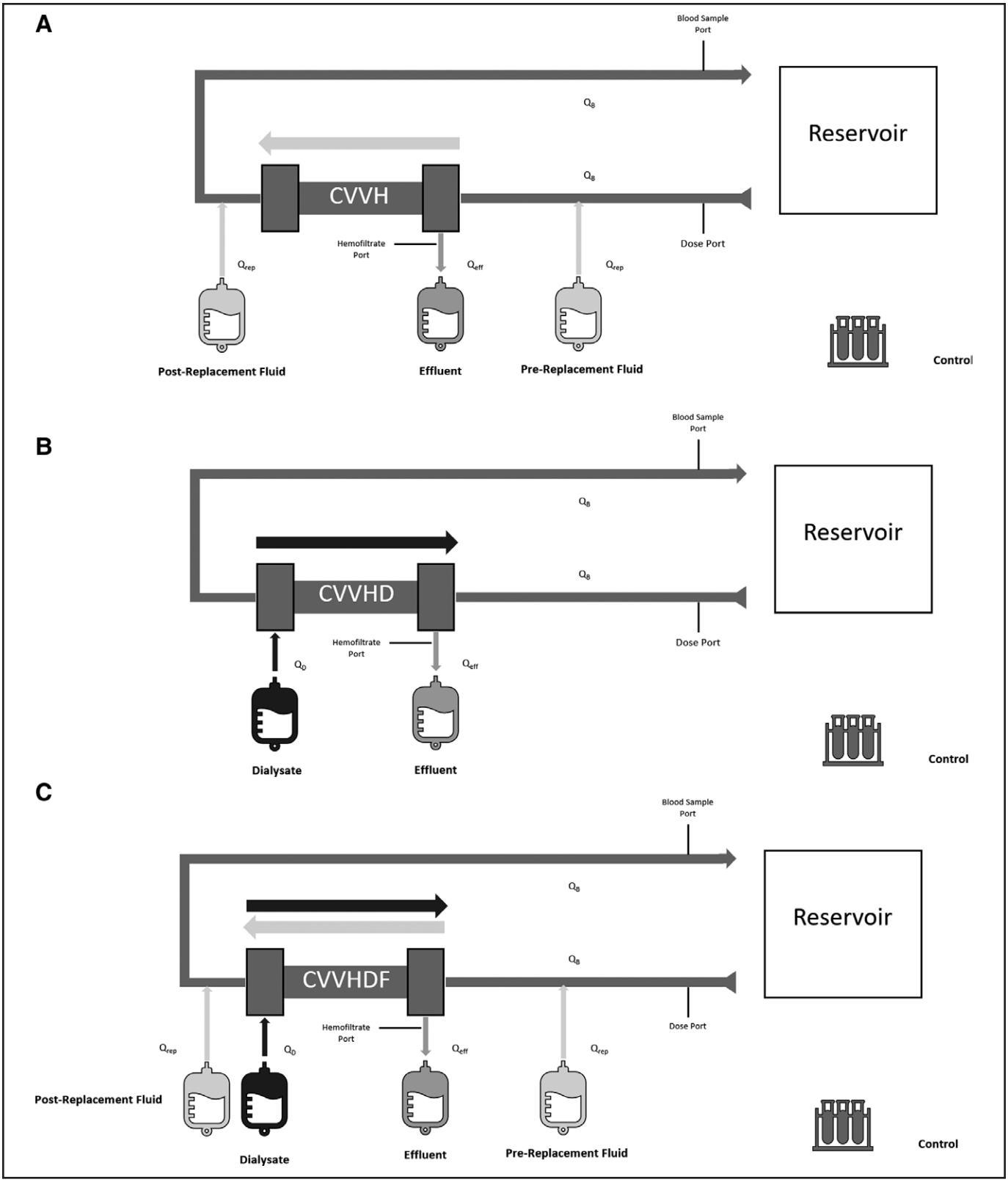

Figure 1. Illustration of the closed-loop ex vivo continuous renal replacement therapy (CRRT) circuit. Anakinra removal from plasma was determined with each modality of CRRT: **A**, continuous venovenous hemofiltration (CVVH); **B**, continuous venovenous hemodialysis (CVVHD); and **C**, continuous venovenous hemodiafiltration (CVVHDF). Standard control samples were analyzed with time-matched plasma and hemofiltrate samples. Q_B = blood flow (mL/min), Q_D = dialysate flow rate (mL/hr), Q_{eff} = total effluent flow rate (mL/hr), Q_{rep} = replacement flow rate (mL/hr).

Blood was maintained at 37°C with the use of heating pads.

Control Setup

Six standard control samples of anakinra were included to determine drug degradation throughout the experiment. A total of 50 mL of primed blood solution was drawn from the CRRT circuit after approximately 5 minutes of circulation and before administration of anakinra. The control sample was transferred into a polyvinyl chloride plastic tube and placed in a water bath maintained at 37°C.

Drug Administration and Sample Collection

Anakinra (Kineret; Swedish Orphan Biovitrum, Stockholm, Sweden) was administered (time = 0) in each CRRT circuit via an arterial port (Fig. 1). The circuit was dosed to achieve a therapeutic concentration of approximately 25 μg/mL (20). A diluted anakinra solution was administered to the control tubes at time equal to −5 minutes to mimic circuit concentrations and placed within a rotator to mix for 5 minutes. The control sample was returned to the water bath for the duration of the experiment, except for sampling. Controls were inverted gently five times before sampling.

Samples were collected at the following time points: 1, 5, 15, and 30 minutes, 1, 2, 3, and 4 hours. A hemofiltrate sample was collected at each time point from the tubing before entering the effluent bag (Fig. 1). Physiologic pH of circuit blood was analyzed before anakinra administration and each subsequent hour with an i-STAT 1 Analyzer (Flextronics Manufacturing, Singapore) and EG6+ cartridge (Abbot, Abbot Park, IL) and adjusted as needed to maintain physiologic pH. Circuit blood and control samples were centrifuged (3,000 rpm for 10 min at 4°C) immediately after collection. Plasma was pipetted and stored at −80°C. Hemofiltrate samples were stored at −80°C immediately following collection.

Drug Analysis

Anakinra concentrations were determined using a prevalidated enzyme-linked immunosorbent assay (ELISA) kit for measuring IL-1 RA (catalog number KAC1181; ThermoFisher Scientific, San Jose, CA). Calibrators were prepared in standard diluent buffer and analyzed in duplicate with every batch. The calibration curve ranged from 31.25 to 2,000 pg/mL and was fit with a four-parameter sigmoidal curve. A two-step dilution process (first into phosphate-buffered saline, then into standard diluent) was used for the samples to bring them into the working range of the ELISA kit according to the expected concentration for the sample, with dilutions ranging from 12,500- to 37,500-fold. Samples were analyzed in duplicate wells, with sample data reflecting an average of those replicates if the % coefficient of variation (% CV) between the two wells was less than 20%. Samples were repeated in a subsequent analysis of the % greater than 20%.

Percent of anakinra remaining in plasma was calculated at each time point using the following equation:

$$\text{Anakinra remaining } (\%) = \frac{C_t}{C_i} \times 100 \quad \text{Eq. 1}$$

where C_t is the concentration at time t and C_i is the concentration at time equal to 5 minutes for CRRT plasma and control samples. Additionally, the passage of anakinra across the hemofilter in each respective CRRT system was calculated using the sieving coefficient (S_C) for CVVH and the dialysate saturation constant (S_D) for CVVHD and CVVHDF. Drug passage was determined from paired hemofiltration/dialysate and plasma samples at each time point using the following equations:

$$S_C = \frac{C_H}{C_P} \quad \text{Eq. 2}$$

$$S_D = \frac{C_D}{C_P} \quad \text{Eq. 3}$$

where C_H and C_D are concentrations in hemofiltrate and dialysate, respectively, whereas C_P represents anakinra concentration in plasma.

Transmembrane clearance of anakinra was estimated for each modality using the following equations: (3, 4)

$$CL_{CVVH} = Q_f \times S_C \times CF \quad \text{Eq. 4}$$

$$CF = \frac{Q_{plasma}}{Q_{plasma} + Q_{pre}}$$

$$Q_f = (Q_{pre} + Q_{post}) + Q_{PFR}$$

$$CL_{CVVHD} = Q_D \times S_D \quad \text{Eq. 5}$$

$$CL_{CVVHDF} = Q_{Eff} \times S_D \quad \text{Eq. 6}$$

$$Q_{Eff} = (Q_{pre} + Q_{post}) + Q_{PFR} + Q_D$$

where Q_f represents the ultrafiltration rate. To account for prefilter replacement fluid dilution a correction factor (CF) was applied when estimating the transmembrane clearance via convection (CL_{CVVH}) (3, 4). In this equation, Q_{plasma} represents the circuit plasma flow as determined by $Q_b \times 1$-hematocrit. Flow rates incorporated in the transmembrane clearance equations (Eq. 4–6) are presented in **Table 3**.

Statistical Analysis

An unpaired *t* test was used to analyze the effect of CRRT on recovery of anakinra in plasma. Percent recovery of anakinra in plasma for each circuit at time equal to 4 hours was compared with the recovery in the standard control experiments at the matching time point. Data are presented as the mean with the corresponding SD.

RESULTS

A total of 144 samples were taken for measurement of anakinra concentrations in plasma and hemofiltrate. Due to incomplete mixing of the CRRT circuits, anakinra concentrations at time equal to 1 minute were dropped from the analysis and C_i was calculated based on the 5-minute sample. For CVVH circuits, one replicate indicated a potentially longer delay in mixing than subsequent runs. Consequently, percent drug remaining was calculated based on the 15-minute sample (C_i = concentration at time = 15 min) for CVVH circuits. A visual representation of the mean percent of anakinra remaining in plasma upon each CRRT circuit is shown in **Figure 2**. Percent of anakinra remaining in plasma for each individual CRRT circuit run is shown in **Electronic Supplementary Material**, **Figure 1** and **Table 4** (http://links.lww.com/CCX/B281). Pharmacokinetic variable estimates, such as sieving coefficient and transmembrane clearance, for each CRRT circuit run are reported in **Electronic Supplementary Material**, **Tables 2** and **3** (http://links.lww.com/CCX/B281).

CVVH Circuit

The average drug remaining (SD) was 79.7% (8.5) at 15 minutes and 5.86% (2.25) at 4 hours. Anakinra percent remaining in plasma was significantly different compared with the standard control at the completion of the experiment ($p \leq 0.001$). A graphical representation of the percent drug remaining in plasma and standard control is presented in **Electronic Supplementary Material, Figures 1*A*** and **2*A*** (http://links.lww.com/CCX/B281). The average sieving coefficient of anakinra over the course of the CVVH circuit analysis was 0.37 (0.12). The determined sieving coefficient was used to estimate a mean transmembrane clearance of 3.94 (0.86) mL/min (**Fig. 3**).

CVVHD Circuit

At time equal to 5 minutes the average percent of anakinra remaining was 71.2% (11.6) and 1.6% (0.6) at 4 hours. Similarly, the percent of drug remaining in plasma was significantly different compared with the standard control at 4 hours ($p \leq 0.001$). The determined dialysate saturation constant for anakinra was 0.41 (0.12), resulting in an estimated transmembrane clearance of 5.53 (1.67) mL/min (Fig. 3).

CVVHDF Circuit

Loss of anakinra occurred within minutes with hemodiafiltration, resulting in an average percent remaining in plasma at 5 minutes of 34.9% (8.8) and 1.2% (1.1) at

TABLE 3.
Continuous Renal Replacement Therapy Circuit Setup

Continuous Renal Replacement Therapy Modality	Blood Flow (mL/min)	Patient Fluid Removal Rate (mL/hr)	Replacement Fluid Rate (mL/hr)	Dialysis Fluid Rate (mL/hr)	Total Effluent Rate (mL/hr)
Continuous venovenous hemofiltration	80	0	Prefilter: 600 Postfilter: 200	Not applicable	800
Continuous venovenous hemodialysis	80	0	NA	800	800
Continuous venovenous hemodiafiltration	80	0	Prefilter: 300 Postfilter: 100	400	800

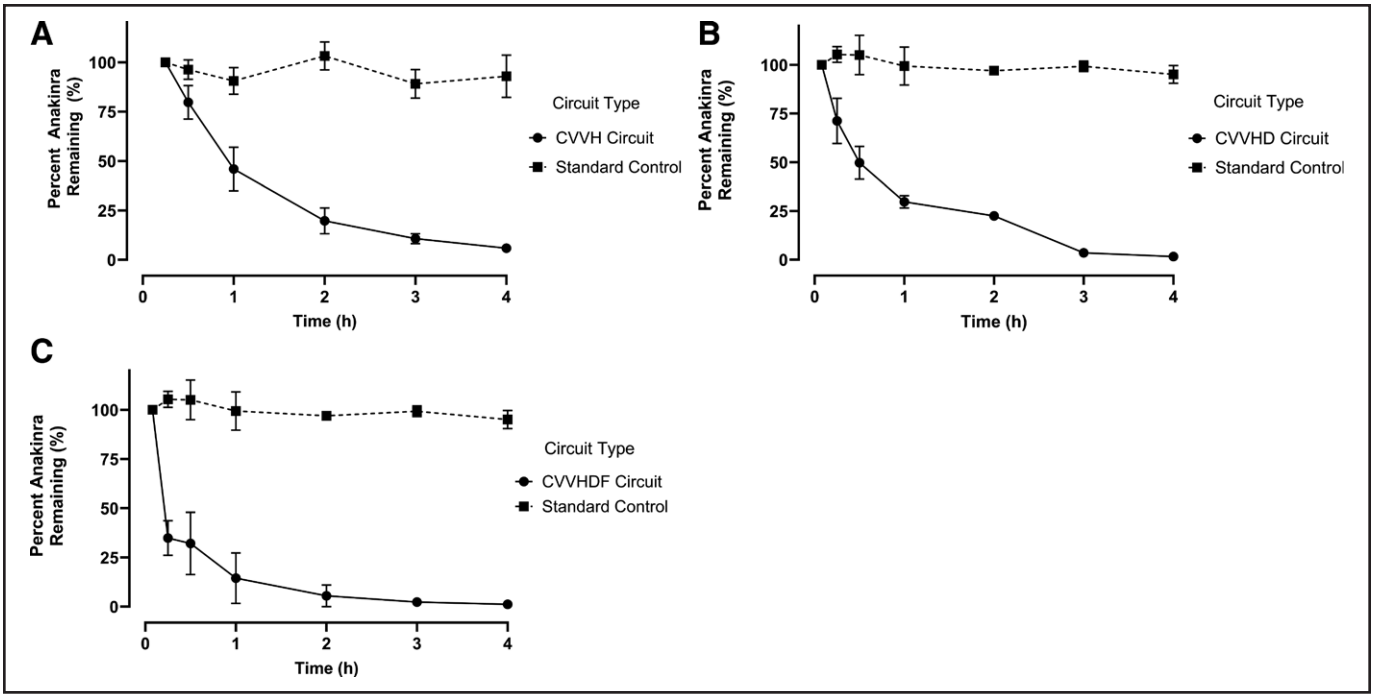

Figure 2. Average percent of anakinra removed from plasma and standard control with SD shown as *error bars*. Due to incomplete mixing of the circuit, anakinra percent remaining in plasma was determined at $t = 15$ minutes for continuous venovenous hemofiltration (CVVH) (**A**). Percent drug removed in plasma was analyzed at time equals to 5 minutes for continuous venovenous hemodialysis (CVVHD) (**B**) and continuous venovenous hemodiafiltration (CVVHDF) (**C**). Electronic Supplementary Material, Figure 1 and Table 4 (http://links.lww.com/CCX/B281) represent illustrations and raw data of the individual CRRT circuit runs over the course of the experiment.

4 hours. The percent of anakinra remaining in plasma was significantly different compared with the standard control at the end of the experiment ($p \leq 0.001$). The average dialysate saturation constant was 0.34 (0.14), corresponding to an estimated transmembrane clearance of 4.99 (1.58) mL/min (Fig. 3).

DISCUSSION

The impact of CRRT on altering the drug disposition of small molecular weight drugs has been studied extensively (3, 4, 8, 34–38); however, the effects on large molecular compounds, such as anakinra remain mostly unknown. We studied the pharmacokinetics of anakinra in a closed-loop CRRT circuit. Incorporating an ex vivo study design enabled an independent assessment of the drug–circuit interaction from many of the additional factors that may impact drug pharmacokinetics in critically ill individuals such as organ and circulatory dysfunction, drug interactions, and fluid shifts (39–41).

Ex vivo CRRT analyses provide important information for the prediction of drug clearance. Such study designs allow for testing various permutations of CRRT flow rates, membranes, and methods of solute removal that may be used in clinical practice. Additionally, the ability to include multiple sampling times, drug redosing, and analyze drug clearance without potential risks associated with in vivo studies highlights many of the advantages of ex vivo studies (42). Increasingly, clearance determined by ex vivo CRRT circuits has been shown to have a strong correlation with observed clinical data (42–44). Therefore, valuable information on drug clearance can be obtained in the absence of clinical data to assist in designing dosing regimens for those requiring CRRT.

The drug–CRRT circuit interaction may alter the pharmacokinetics in one of two ways: 1) drug clearance by the circuit, either through convection, diffusion, or a combination of each, or 2) drug adsorption to circuit components. Significant loss of anakinra occurred with each modality of CRRT, with concentrations of anakinra within hemofiltrate remaining consistent throughout (**Electronic Supplementary Material**, **Fig. 2**, http://links.lww.com/CCX/B281). The average S_C/S_D of anakinra was 0.37, 0.41, and 0.34 for CVVH, CVVHD, and CVVHDF, respectively. Transmembrane clearance was similar across each modality (Table 2),

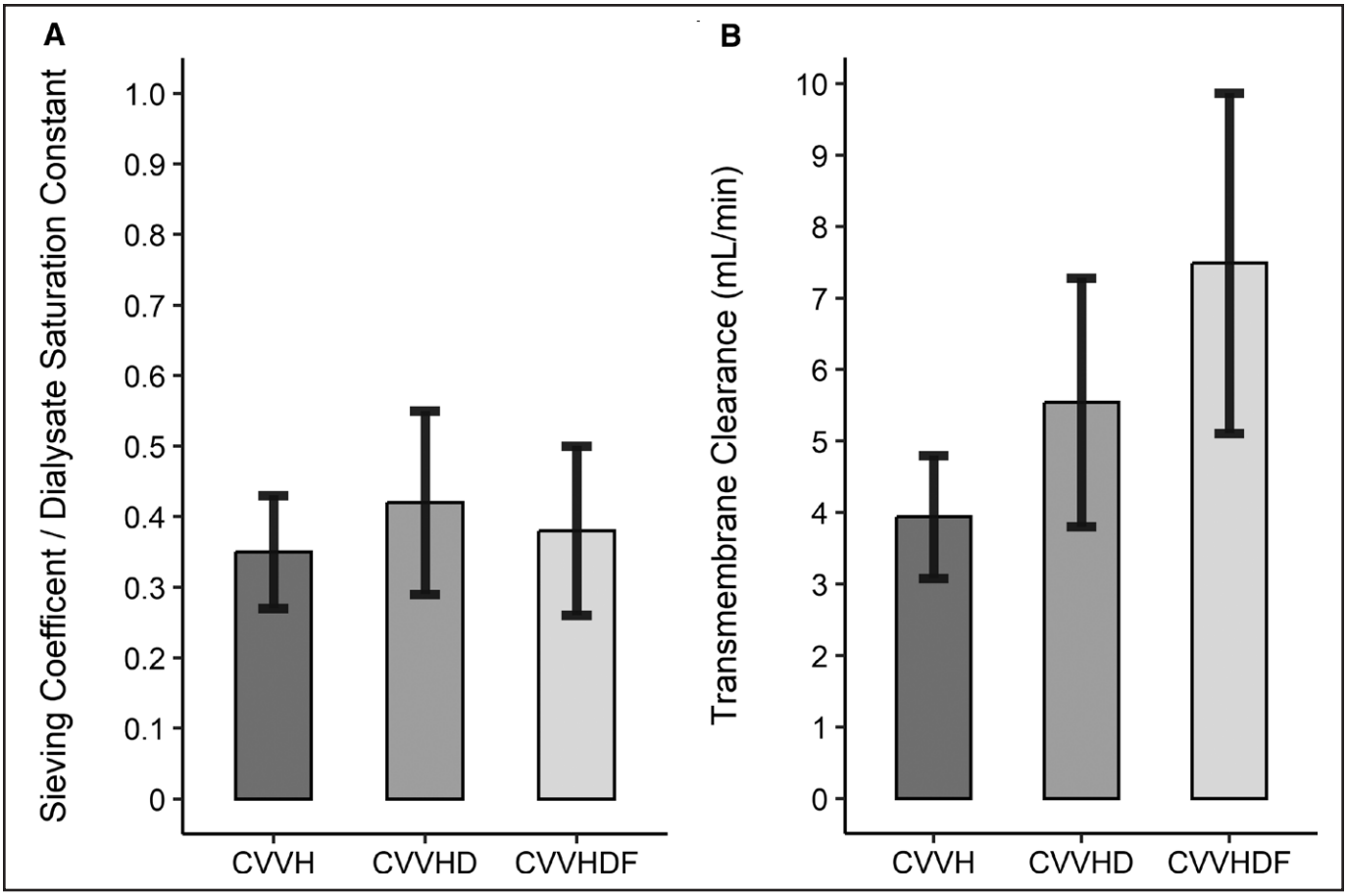

Figure 3. Graphical representation of the anakinra sieving coefficient/dialysate saturation constant (**A**) and estimated transmembrane clearance (**B**). All data presented as mean with *error bars* representing the SD. CVVH = continuous venovenous hemofiltration, CVVHD = continuous venovenous hemodialysis, CVVHDF = continuous venovenous hemodiafiltration.

indicating that molecular weight has become a less influential parameter in estimating CRRT circuit removal with modernized "high-flux" membranes.

In this experiment, it is important to understand that the percent of anakinra remaining in each circuit is dependent on the total volume of the blood pool. This experiment incorporated a CRRT prescription that was based on a hypothetical 10 kg child. Children of this weight/age range typically have a total blood volume of approximately 80 mL/kg. As the age/weight of the patient increases, the CRRT prescription flow rates applied will also increase. Such increases in flow rates will affect the estimated transmembrane clearance values. Therefore, when applying the results of the percent anakinra remaining in plasma in vivo, it is imperative that one is familiar with the CRRT prescription and the total volume of blood of the patient.

It is worth noting that transmembrane clearance was determined using the mean S_C and S_D over the course of the experiment multiplied by the corresponding flow rates. This equation may be considered less accurate compared with clearance determined by the area under the curve via noncompartmental analyses (45), as it suggests that transmembrane clearance is directly proportional to the CRRT flow rates and that S_C/S_D is static over time. However, the purpose of quantifying transmembrane clearance in this study was to compare across each CRRT modality and the values reported in Table 2 should be considered as an estimate, regardless of the method of quantification.

Although no adsorption analysis was conducted in the study, the consistently high concentrations within hemofiltration and limited loss of drug in the standard controls indicate that removal of anakinra from plasma occurs strictly by filtration. Furthermore, adsorption was not expected to occur throughout the experiment due to the physicochemical properties of anakinra and the use of nonadsorptive membranes (polyarylethersulfone [PAES]) throughout the experiment (6).

Pharmacokinetic data on the use of anakinra in patients receiving RRT is sparse. The pharmacokinetic study by Yang et al (20), was the first to describe the removal of anakinra by any modality of RRT. In this study, patients receiving conventional intermittent hemodialysis (IHD) or peritoneal dialysis were included. Removal of anakinra by dialysis was less than 2.5% of the administered dose, indicating that anakinra plasma concentrations will not be altered by dialysis (20). It was concluded that extending the dosing interval in patients with severe renal impairment or end-stage renal disease may be required to minimize anakinra toxicity (20).

The results of this experiment differ from Yang et al (20). We sought to investigate the rate of anakinra removal through each method of solute removal, hemofiltration, hemodialysis, and a combination of each. The removal of solutes via convection is molecular weight independent, as it uses a pressure gradient to remove molecules from plasma (4, 35, 42). However, the extent of anakinra removal within this study using a diffusion-based modality (CVVHD) does indicate that dialysis removes anakinra from plasma to a significant degree.

The differences in results between this study and that by Yang et al (20) may be explained by different flow settings, duration of dialysis, and most notably the use of modernized hemofilters. The molecular weight cutoff of modern CRRT circuit membranes is significantly larger than previous dialyzer membranes used in older IHD circuits. Modern "high-flux" membranes, as incorporated within this experiment are characterized by high porosity and have molecular weight cutoffs of 20–40 kDa (3, 5, 46). The use of "high-flux" hemofilters may negate the expected difference in drug removal based on method of solute removal, as they can deliver more effective hemodialysis (42). Therefore, caution should be applied when interpreting the results of the study by Yang et al (20) when administering anakinra to patients receiving CRRT with modern synthetic hemofilters. Adjusting dosing regimens by decreasing the dose or increasing the dosing interval may result in subtherapeutic dosing regimens; however, future studies evaluating the pharmacokinetics of anakinra in patients receiving CRRT are warranted.

Prior to market approval, anakinra was investigated as a treatment alternative for sepsis management due to targeting the IL-1 inflammatory cascade (23–26). Although early studies provided equivocal results, more recent studies such as the Personalized Randomized trial Of Validation and restoration of Immune Dysfunction in severE infections and Sepsis trial (32) will revisit the utility of anakinra as an efficacious agent in sepsis management. It is important to note that dosing in this population has yet to be defined, as several protocols were trialed including continuous infusions and intermittent bolus dosing (23–25, 32). However, it has been generally assumed that for severe AKI or those receiving RRT, anakinra doses should be reduced due to altered drug clearance and poor removal from RRT circuits (20). Although not confirmatory based on the ex vivo study design, the results presented suggest that clearance of anakinra via CRRT does occur. Coupled with the fact that high-dose anakinra is well tolerated, with limited side effects (23–25, 47–50), dose reduction based on altered renal function in those receiving CRRT may result in subtherapeutic concentrations. Further studies in vivo are warranted to confirm.

In addition to potential uses for sepsis management, the use of anakinra for managing CRS due to primary or secondary HLH and chimeric antigen receptor-T cell complications continues to be explored (13–19). Due to the inflammatory response triggered by cytokine release, incidence rates of AKI are high and a significant portion may require RRT (51, 52). Therefore, as anakinra continues to be investigated as a therapeutic alternative for managing complications of CRS, these results may become even more generalizable.

This study has limitations. Although incorporating different modalities of CRRT may be seen as a strength, fixed flow rates were used throughout the entirety of this experiment. Increasing flow rates may lead to greater reductions of drug from plasma for small molecules (46, 53); however, the relationship between increasing flow rates and clearance of large molecules remains unknown. Therefore, future studies applying increasing flow rates are warranted. Second, the analysis was conducted using the HF1000 (PAES) hemofilter in each circuit run. Compared with alternative synthetic hemofilters, PAES hemofilters have reduced adsorptive capacity (6, 54). Although it was hypothesized that the adsorptive potential of anakinra would be low, this study does not rule out the possibility of adsorption when differing hemofilters are applied. Careful consideration of the composition

of hemofilters is required when applying these results. Third, a single intermittent bolus dose of anakinra was administered to the CRRT circuits. The current state of the literature has investigated several dosing strategies for both management of sepsis and CRS, including continuous infusions or intermittent bolus dosing twice to three times per day. Therefore, if alternative dosing strategies such as continuous infusions are to be applied, the risk of drug accumulation may exceed that of excess drug removal. Future ex vivo study designs investigating transmembrane clearance of continuous infusion or multiple intermittent bolus dosing may be required. Finally, due to the discordance between sample concentrations and the ELISA kit concentration range, significant dilutions of samples were required. Any slight error in analysis may be magnified, increasing the variability in concentrations observed within the study. This may explain the approximately two- to three-fold higher anakinra concentrations obtained within the plasma of the CVVHDF circuit. However, the percent recovery in plasma, S_C, and estimated transmembrane clearance remained consistent between each modality, unaffecting the results.

In vitro to in vivo extrapolation (IVIVE) incorporates techniques to predict the pharmacokinetics related to absorption, distribution, metabolism, and elimination in vitro and quantifies the corresponding processes in vivo (55, 56). Information obtained from IVIVE predictive models allows for an assessment of drug pharmacokinetics and may serve as a valuable tool for clinical trial design. Similarly, quantification of drug clearance from an ex vivo CRRT circuit may allow for extrapolation to clinical practice. Recently, the use of ex vivo to in vivo extrapolation through physiologically based pharmacokinetic (PBPK) modeling with extracorporeal circuits has been informative in prospective prediction of drug pharmacokinetics in untested clinical scenarios (57, 58). Data from ex vivo studies, such as those determined within this study, can be incorporated within a PBPK model to be used to predict the drug disposition in vivo and determine optimal dosing regimens for this vulnerable population.

CONCLUSIONS

In conclusion, this study is unique in that it is the first to investigate the pharmacokinetics of anakinra in a CRRT circuit. By incorporating a closed-loop ex vivo study design, assessment of the anakinra–CRRT circuit interaction was conducted independent of many of the variables known to affect drug pharmacokinetics in the critically ill population. Throughout each CRRT modality, anakinra was significantly removed from plasma by filtration, with negligible adsorption to circuit components or degradation within the blood. The degree of loss of anakinra in the CRRT circuits suggests that administering renal dose adjustments may result in subtherapeutic concentrations when CRRT is applied. Incorporating the results into PBPK models may allow for optimal dose regimen design or aid in dose selection for future clinical trials.

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