Etanercept Clearance during an in vitro Model of Continuous Venovenous Hemofiltration

Geoffrey M. Fleming^a Noha N. Salama^{b,d} Saada K. Eid^c Kenneth R. Cooke^c
Bruce A. Mueller^b

^aDepartment of Pediatrics, Division of Critical Care, Monroe Carell Jr. Children's Hospital at Vanderbilt, Vanderbilt University, Nashville, Tenn., ^bUniversity of Michigan College of Pharmacy, Ann Arbor, Mich., and ^cDepartment of Pediatrics, Division of Hematology Oncology, Rainbow Babies and Children's Hospital and Case Western Reserve University School of Medicine, Cleveland, Ohio, USA; ^dDepartment of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Cairo University, Cairo, Egypt

Key Words

Etanercept • Continuous renal replacement therapy • Hemofiltration

Abstract

Background/Aims: Etanercept is a tumor necrosis factor- α antagonist used in inflammation-mediated conditions. Continuous venovenous hemofiltration (CVVH) has also been used in patients with inflammatory conditions. This study evaluated etanercept clearance using an in vitro CVVH model. **Methods:** Etanercept clearance was assessed in vitro in bovine blood at 1–3 mg/l final serum concentration, and urea control at 750 mg/l. CVVH was performed using polyacrylonitrile, polysulfone, and polymethylmethacrylate filters at 3 l/h ultrafiltrate and 200 ml/min blood flow rates. Transmembrane clearance was estimated using sieving coefficient calculations, and adsorptive removal rate was approximated using a mass balance calculation. **Results:** Urea sieving coefficient remained constant (1.04 ± 0.01). Ultrafiltrate etanercept concentrations were undetectable (sieving coefficient < 0.02) and transmembrane and adsorptive clearances were negligible. **Conclusion:** Etanercept is not cleared

appreciably by transmembrane or adsorptive mechanisms in CVVH using polyacrylonitrile, polysulfone, or polymethylmethacrylate hemofilters.

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Introduction

Therapeutic agents have evolved in recent decades and newer 'designer' chimeric immunomodulators are available for an expanding list of disease processes. Etanercept (Enbrel[®], Amgen Inc., Thousand Oaks, Calif., USA) is a soluble dimeric tumor necrosis factor- α (TNF- α) binding protein that is used in many clinical situations involving inflammation. Approved indications for use in adults have expanded to include rheumatoid arthritis, ankylosing spondylitis, and psoriasis [1]. In the pediatric population, ongoing trials and off-label uses include inflammatory bowel disease, juvenile rheumatoid arthritis, uveitis, and idiopathic pneumonia syndrome related to stem cell transplantation [1–6]. Other therapeutic modalities, such as continuous venovenous hemofiltration (CVVH), have been employed with success in some of the same disease

processes where etanercept is used [7]. Although population pharmacokinetic studies of subcutaneously administered etanercept exist for both healthy volunteers and adult patients with rheumatoid arthritis, there are no published data regarding clearance of etanercept during CVVH, although this modality has been documented to remove many other drugs [8–10]. Hence, literature supporting the use of etanercept and CVVH for disease processes such as idiopathic pneumonia syndrome requires an understanding of the interaction of these therapies when used concomitantly.

Etanercept is a large multimeric protein fabricated from 2 identical polypeptides consisting of the extracellular region of human TNF receptor p75 fused to the Fc portion of a human Ig, with a total molecular weight of 150 kDa. As such, this molecule is unlikely to undergo transmembrane clearance in commercially available hemofilters with molecular cut-offs of 25–30 kDa. Yet, due to larger pore size (at 100 Å), etanercept may cross into the ultrafiltrate (UF) during hemofiltration using polymethylmethacrylate (PMMA) hemofilters. In a study of chronic hemodialysis patients, PMMA filters (BK1, 6F Filtrizer®; Toray Industries, Tokyo, Japan) produced UF containing protein concentrations up to 1 g/l, including IgG at 180 kDa in size [11].

Etanercept clearance through protein adsorption to the hemofilter membrane may also be an important clearance mechanism with polyacrylonitrile (AN69) and polysulfone (PS) membranes [12]. Deposition of protein is affected by properties of the membrane, but ranges from 15–75 µg of protein per cm² for the most commonly used hemofilters worldwide [12, 13]. Protein adsorption to all types of artificial membranes is well described, occurring in the first 20 min of blood-membrane interface [14–16]. This mode of clearance has previously been documented for other proteins, such as cytokines, in both laboratory and human studies of CVVH in sepsis [17–21]. Although protein adsorption occurs with AN69 and PS membranes, PMMA membranes were designed specifically for protein adsorption to limit β₂-microglobulin accumulation and to prevent dialysis-related amyloidosis [22]. Hence, these PMMA membranes may confer additional adsorptive clearance properties as compared to AN69 and PS membranes with regards to clinically relevant proteins.

The objective of this study was to evaluate the clearance of etanercept during CVVH, as well as to evaluate the effects of time and filter type on this clearance. Two extracorporeal clearance mechanisms were evaluated for each filter type: transmembrane clearance and removal from the system by protein adsorption to the filter.

Fig. 1. In vitro CVVH model schematic. Blood is continuously circulated in the blood reservoir warmed in a water bath at 37°C. UF is returned to the blood reservoir and sampling ports are available immediately before and after the hemofilters as indicated.

Methods

An in vitro model (fig. 1) of CVVH was previously developed and validated in our laboratory and has been used to generate clearance data for trace minerals and antimicrobials that has been validated in patients [10, 23, 24]. This model utilizes 1 liter of pH-regulated bovine whole blood (Animal Technologies, Tyler, Tex., USA), anticoagulated with 3.8% sodium citrate, placed in an Erlenmeyer flask submerged in a 37°C water bath as the venous blood reservoir.

Blood Reservoir and Drug Preparation

The blood reservoir was prepared by the addition of 5,000 IU heparin and 2 g of dextrose during continuous stirring. Urea was added to the blood reservoir to achieve an approximate blood urea nitrogen concentration of 75 mg/dl. Following blood preparation, whole blood was drawn from the reservoir into 2 capillary tubes (SafeTec Clinical Products Inc., Ivyland, Pa., USA), which were then placed into a HemataStat II (Separation Technologies Inc., Valparaiso, Ind., USA) machine for hematocrit measurement. The 2 hematocrit values were averaged for the calculation of plasma volume prior to the addition of the study drug.

Etanercept was reconstituted as recommended by the manufacturer using sterile water to a final concentration of 25 mg/ml. Etanercept was added to the venous reservoir to achieve a plasma concentration of 1–3 mg/l based on a calculated serum volume [blood volume × (1 – hematocrit)]. The reservoir was stirred for 5 min prior to beginning hemofiltration.

CVVH Technique

We studied 3 filter types: AN69, (Multiflow 100®; Gambro, Lakewood, Colo., USA), PS (Optiflux 160®; Fresenius Medical Care, Homburg, Germany), and PMMA (BK-2.1F Filtrizer®;

Toray Industries, Tokyo, Japan). CVVH was performed using the Braun-Diapact® system, including standard tubing packs (Diapact® CRRt Kit; B. Braun Medical Inc., Bethlehem, Pa., USA) used in clinical practice, with a new filter and tubing for each experiment. The circuit was initially prepared by normal saline priming following the manufacturer's instructions for each of the 3 types of hemofilters under study. UF was returned to the venous line by connecting the filter replacement fluid inlet line to the outlet of the UF collection bag, thus making a closed circuit. After 5 min of continuous stirring of the blood reservoir, the system was connected to the reservoir and allowed to circulate in CVVH mode for 240 min. During CVVH, transmembrane pressure was recorded from the Diapact in serial fashion. Hemofiltration was performed with a blood flow rate of 200 ml/min with an ultrafiltration rate of 3,000 ml/h.

Data Collection

All samples were obtained from the sampling ports in the circuit, including arterial, venous, and UF. Etanercept samples were obtained using 1-ml sterile syringes at 0 (baseline), 5, 10, 15, 20, 30, 60, 90, 120, 180, and 240 min. Samples were immediately placed into 5-ml polystyrene tubes and centrifuged at 3,000 rpm for 10 min. The supernatants were stored in cryovials at -80°C until sample analysis. Identical sampling and sample processing techniques were used for blood urea nitrogen samples at 0 (background), 5, 60, 120, 180, and 240 min.

Data Analysis

Urea concentrations from each sample were analyzed by a kinetic test with the absorbance measured at $\lambda = 340$ nm with a COBAS Integra® 400 Plus (Roche Diagnostics, Indianapolis, Ind., USA). The coefficient of variation of the assay was $<10\%$ and the blood urea nitrogen lower limit of detection was 1.8 mg/dl. Etanercept concentrations were measured using cytokine enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's protocol (R&D Systems, Minneapolis, Minn., USA). Briefly, human sTNF RII was captured by specific primary antibody and detected by horseradish peroxidase detection antibody (DuoSet reagents; R&D Systems). The assay was then developed using 3,3',5,5'-tetramethylbenzidine substrate (BD Biosciences, San Jose, Calif., USA). The absorbance was measured at $\lambda = 450$ nm using a microplate reader (ELx800; BioTek, Winooski, Vt., USA). Recombinant human sTNF RII was used for the preparation of standards (DuoSet Reagents; R&D Systems). Samples and standards were run in duplicates. The coefficient of variation was estimated at $<11\%$ with a detection range of 78–2,500 pg/ml.

Extracorporeal clearance was calculated for urea and etanercept using the following equations:

$$\text{blood sieving coefficient} = (C_{\text{UF}})/(C_{\text{A}})$$

where C_{UF} = concentration of solute in ultra filtrate and C_{A} = concentration of solute obtained from blood from arterial port;

$$\text{transmembrane clearance} = (\text{SC}) \times (\text{Quf})$$

where SC = blood sieving coefficient and (Quf) = ultrafiltration rate.

The prefilter (arterial) and postfilter (venous) etanercept concentrations were normalized to the prefilter blood flow rate (Q_{b}) (200 ml/min) and the postfilter flow rate ($Q_{\text{b}} - Q_{\text{uf}} = 150$ ml/

min), to determine the amount of drug entering or exiting the filter per unit time (etanercept transfer rate), respectively. Adsorptive clearance was estimated by mass balance equation as follows: etanercept adsorptive clearance = prefilter transfer rate – (UF transfer rate + postfilter transfer rate).

Statistical Analysis

Power analysis was performed using a 1-tailed t test to detect a 20% change in drug concentration over 4 h of CVVH. Using $\alpha = 0.05$, $\beta = 0.15$, and assuming a standard deviation for drug measurement of 10%, 5 filters were required in each group. SPSS statistical software version 14.0 was used to analyze the data. A linear mixed model was used to compare etanercept transfer rates, adsorptive clearance, and sieving coefficients across time using Bonferroni for post hoc analysis. G-criterion was used for testing outlier extreme values [25]. Statistical significance was set at <0.05 .

Results

A total of 16 filters were studied, 5 each in the PS and AN69 arms of the study, and 6 in the PMMA group. The mean sieving coefficient for urea was 1.03 ± 0.01 , 1.04 ± 0.01 , and 1.05 ± 0.06 for the PS, AN69, and PMMA hemofilters, respectively, with no statistically significant difference for between and within group analysis ($p > 0.05$). Transmembrane pressure remained less than 300 mm Hg for 14 of 15 filters at all time points. The urea and transmembrane pressure data suggest the filters performed as expected during the course of the experiment. Problems were encountered at 180–240 min in maintaining UF volumes at 3,000 ml/h in the PMMA hemofilters. In these instances, the UF rate was reduced to maintain circuit patency, and ranged from 1,500 to 2,500 ml/h.

The target initial etanercept plasma concentrations set in this study were 1–3 mg/l. The initial mean etanercept concentrations were 1.15 ± 0.44 mg/l, 1.24 ± 0.47 mg/l, and 1.4 ± 0.6 mg/l for the PS, AN69, and PMMA hemofilters, respectively. Mean etanercept blood sieving coefficients were 0.001 ± 0.002 for both the PS and AN69 groups, respectively, and 0.002 ± 0.003 for the PMMA group. These data suggest negligible transmembrane clearance for etanercept by the filters under study.

Adsorptive clearance of etanercept was approximated with a mass balance equation. Since the UF content of etanercept was near zero, the arteriovenous difference becomes the approximation of protein adsorption. For the AN69 filter group, the arterial sample concentration measured at 90 min was censored because it was 2- to 3-fold higher compared to the remaining 10 sampling time points (G-criterion outlier testing), and the subsequent

Fig. 2. Effect of time on the etanercept transfer rate ($\mu\text{g}/\text{min}$) for the AN69 filter group ($n = 5$).

Fig. 3. Effect of time on the etanercept transfer rate ($\mu\text{g}/\text{min}$) for the PS filter group ($n = 5$).

Fig. 4. Effect of time on the etanercept transfer rate ($\mu\text{g}/\text{min}$) for the PMMA filter group ($n = 6$).

analysis reflects exclusion of this time point. For the AN69 membrane, there were no significant differences in etanercept concentrations between the arterial samples as a function of time, nor was there any significant difference between arterial and venous etanercept sample concentrations at each time point. These findings collectively suggest the absence of demonstrable adsorptive clearance for etanercept by AN69 filters (fig. 2).

We detected similar findings with the PS hemofilters, with the exception of the sample at 240 min where there was a significant arteriovenous difference, as well as a significantly higher arterial etanercept concentration at

240 min (considered an outlier based on G-criterion outlier testing), compared to all arterial concentrations up to 180 min ($p = 0.001$; fig. 3). Data for the PMMA hemofilter group showed significant etanercept concentration differences between the arterial samples as well as arteriovenous differences at various time points (fig. 4). However, despite these findings, the mean arterial concentration at 240 min was not significantly different from the baseline concentration, suggesting no substantial drug was adsorbed to the PMMA membrane during the study.

Discussion

In this study, we did not find substantial CVVH clearance of etanercept by either extracorporeal clearance mechanism studied. Transmembrane clearances were exceedingly small by virtue of a SC of approximately 0.001 for AN69 and PS, suggesting that hemofiltration is not a meaningful form of etanercept elimination. PMMA filters were developed for the removal of β_2 -microglobulin (11 kDa) during chronic hemodialysis, and previous work with PMMA filters suggested molecules as large as 180 kDa would pass through the membrane [11]; however, etanercept (molecular weight 150 kDa) also had a very small SC (0.002) in our study. In studies of cytokine clearance in sepsis with AN69 membranes, adsorption provided significant clearance for these proteins [26], yet adsorption of etanercept was below the level of detection for all hemofilters in our study.

Limitations of this study include our use of a bovine blood model instead of a human blood model. Theoretically, etanercept binding could be different in bovine blood versus human blood. However, the rate-limiting step to etanercept crossing the hemofilter membrane is likely its large molecular weight (150 kDa) rather than its protein binding. Also, we modeled the CVVH clearance when the plasma concentration was approximately 1–3 mg/l. Obviously, a patient's plasma concentration will decline with time; however, the SC typically remains constant in CVVH, regardless of drug plasma concentrations [10]. Additionally, the approximation of etanercept clearance via protein adsorption using mass balance is less precise than direct measurement of etanercept on used membranes. However, these limitations do not affect the primary goal of this study, which was to determine if a significant amount (20%) of etanercept is removed from the system during CVVH.

Indications for, and use of, both CVVH and etanercept are expanding, and knowledge of the interface of these 2 therapies is important. In pediatric patients with idiopathic pneumonia syndrome after stem cell transplantation, the addition of etanercept to the regimen has reduced mortality from nearly 70 to 21% [2, 5]. In this

same group, CVVH has also reduced mortality [7] as well as multiorgan failure in other pediatric patients [7, 27]. Although we report absence of substantial etanercept clearance by each of the 3 studied filter types, we feel these results are important to report. Our findings suggest that the investigated modalities (etanercept and CVVH) exert their influence independently on outcomes as we found no evidence of changes in etanercept plasma concentration during CVVH in an in vitro model. These data do not suggest that there is a need for dosage alterations with etanercept during CVVH due to extracorporeal clearance.

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