Plasma Clearance Rates of Coagulation Factors VIII and IX in Factor-Deficient Individuals

By Dennis A. Noe, William R. Bell, Paul M. Ness, and Jack Levin

The plasma clearance rates of factors IX and VIII were determined in patients with hemophilia A and B who had received factor replacement by prolonged, continuous infusion of factor concentrates. The clearance rates were calculated by dividing the factor infusion rates by the steadystate plasma factor activities corrected for baseline factor activities. The mean factor IX clearance rate in eight factor IX-deficient patients was 233 mL/h (range 159 to 340 mL/h). The mean normalized clearance rate was 3.4 mL/h/kg. The mean factor VIII clearance rate in eight factor VIII-deficient patients was 294 mL/h (range 229 to 361 mL/h) and the mean normalized rate was 5.0 mL/h/kg. Both factors show linear relationships between the factor infusion rates and the steadystate plasma factor activities achieved.

The disappearance of procoagulant factors IX and VIII from the plasma of factor-deficient individuals receiving replacement therapy is usually described in terms of first-order elimination rate constants or half-lives. However, both of these kinetic parameters are hybrid measures that depend as much on the volume of distribution of a factor as they do on the rate of elimination of the factor. The plasma clearance rate of a factor, on the other hand, is the primary kinetic parameter that uniquely measures the net removal of the factor from the plasma. In addition, because the plasma clearance rate is a model-independent measure, no assumptions need be made concerning the distribution of a factor in the body or its sites of metabolism or excretion. Hence, clearance rate is the logical and preferable way to quantify the disappearance from plasma of the coagulation factors because details concerning distribution, metabolism, and excretion of the factors are unknown.

There are two experimental approaches to the measurement of the plasma clearance rate of a substance. One method is to administer an intravenous (IV) bolus dose of the substances and then to construct its plasma concentration time curve. The clearance rate is calculated by dividing the dose by the area under the curve. In the other method, the substance is infused IV at a constant rate until the steady state is achieved. In this case, the plasma clearance rate equals the infusion rate divided by the steady-state plasma concentration. We have used this relationship to estimate the plasma clearance rates of factors IX and VIII in factor-deficient individuals who received factor replacement therapy in the form of prolonged, continuous infusions of factor concentrates.

MATERIALS AND METHODS

Subjects. From 1978 to 1982, many of the factor VIII- and factor IX-deficient patients admitted to The Johns Hopkins Hospital participated in a clinical study in which they received factor replacement therapy using a continuous infusion protocol. This therapeutic approach was used to achieve constant plasma factor levels rather than the varying levels that result from an intermittent dosing regimen. The patients who were treated with this protocol were identified by a search of the medical records maintained by the Division of Hematology. The pertinent demographic, clinical, and laboratory data on these patients were extracted from the individual medical records. Patients with antifactor antibodies were excluded. This study was approved by the Joint Committee on Clinical Investigation of The Johns Hopkins University School of Medicine.

Factor infusion protocol. At least one and sometimes multiple pretreatment blood specimens were collected and assayed for plasma factor activity. The patients received two to 16 bolus doses of reconstituted factor concentrate (factor IX concentrate: Konyne, Cutter Laboratories, Berkeley, Calif; factor VIII concentrate: Factorate, Armour Pharmaceutical Company, Kankakee, Ill) on an every-12-hour schedule prior to the start of continuous infusion therapy. The doses of concentrate administered were those believed to be appropriate in the respective clinical situations. The factor content of the concentrate doses were calculated from the manufacturers' stated product content times the volume of concentrate infused. Blood specimens for plasma factor activity determination were drawn ~15 minutes before and 15 to 30 minutes after each dose.

The last of the bolus doses served as a loading dose, immediately after which the continuous infusion of factor concentrate was started. The rate of infusion of concentrate was selected so that the total daily factor activity infused would be equal to that given during the intermittent bolus dose regimen. Each container of infusate was administered over 12 hours; therefore, adequate concentrate to provide one half of the total daily factor activity appropriate for the patient was prepared and placed in the infusion solution. The infusion solution was 1 L of 0.2 mol/L of NaCl, 5% dextrose solution, packaged in a polyvinyl plastic bag. The rate and constancy of administration was controlled by a volumetric displacement pump (Abbott/Shaw LifeCare IV Pump, Model II, Abbott Laboratories, North Chicago). Continuous infusion therapy lasted from 60 to 312 hours. Blood specimens were drawn 10 to 30 minutes after the start of each container of infusate and approximately ten minutes prior to the termination of each container. The specimens were obtained by venipuncture of the arm that did not receive the infusion. The infusions were discontinued when indicated clinically. Plasma samples were drawn approximately every six hours after discontinuation of replacement therapy until factor levels of <0.1 U/mL were detected. Factor activities were assayed in all the blood specimens.

Kinetic analysis. The plasma clearance rate of the factors was calculated by dividing the factor infusion rate by the corrected steadystate plasma factor activity: plasma clearance rate = infusion rate/steady-state factor activity. Steadystate factor activity was defined as the average of three to five plasma factor activities.

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measured once the factor levels had reached an apparent plateau during continuous infusion therapy. The corrected steady-state factor activity equaled the steady-state factor activity minus the baseline factor activity. The average of a patient’s pretreatment factor levels constituted the baseline activity.

Analytical methods. Routine procedures for blood collection, sample preparation, and plasma factor assays were used in our patients. Blood was collected in 5-mL samples in plastic tubes containing 0.5 mL of 0.13 mol/L of sodium citrate. Within 30 minutes of collection, the samples were centrifuged at 1,800 g for ten minutes, rendering the plasma platelet-poor. The plasma supernates were transferred to plastic tubes and immediately placed into storage at −20°C. Factor assays were performed within 72 hours. Factor VIII and factor IX activities were quantified by one-stage clotting-time bioassays using factor VIII- and factor IX-deficient plasma, respectively, as substrates. The factor deficient plasmas were obtained by plasmapheresis of patients with severe congenital factor deficiencies as determined by family and clinical histories and corroborating laboratory studies. Single clotting-time determinations for sample plasmas were compared with the clotting time of plasma concentration dose-response curves for normal, pooled plasma. Sample coagulant activities were interpolated from the control plasma curves as graphed on double logarithmic paper. Normal plasma was obtained from healthy blood bank donors with normal prothrombin and partial thromboplastin times. The plasma pools consisted of plasma from 60 to 120 donors. One unit of factor per milliter was defined as the factor activity in normal human plasma.

RESULTS

Eight factor IX-deficient patients and eight factor VIII-deficient patients were identified as having received factor replacement by continuous infusion of factor concentrate. In every case, replacement therapy was administered either to control a bleeding episode or to provide hemostatic factor levels during and after a surgical procedure. Among the factor IX-deficient patients, 3 had received 1 course of continuous infusion therapy, 3 had 2 courses, 1 had 3 courses, and 1 had 6 courses (Table 1). Among the factor VIII-deficient patients, 6 had received a single course of continuous infusion therapy, 1 had 2 courses, and 1 had 3 courses (Table 2). For 5 of the patients, some or all of the courses of therapy had been conducted during a single hospital stay.

Clearance rate. Apparent plateaus of plasma factor activity were achieved in all patients after two to five days of therapy. However, three factor IX-deficient patients and three factor VIII-deficient patients exhibited substantial day-to-day variability in their steady-state factor activities (i.e., a range of values ±25% of the mean value). The ranges in these patients, expressed as a percentage of their mean plasma activities, were ±30%, 34%, and 69% for the factor IX-deficient patients, and ±36%, 41%, and 47% for the factor VIII-deficient patients. The factor clearance rate for each patient was calculated by averaging the clearance rates determined from each of the infusions administered to the patient.

The factor IX clearance rates ranged from 159 to 340 mL/h (normalized for body weight: 1.9 to 5.8 mL/h/kg) with a mean value of 233 mL/h (3.4 mL/h/kg). The clearance rates do not correlate with body weight (r = −.17) or body surface area (r = −.13). The factor VIII clearance rates ranged from 229 to 361 mL/h (3.5 to 5.9 mL/h/kg) with a mean rate of 294 mL/h (5.0 mL/h/kg). The correlations with body weight (r = −.50) and body surface area (r = −.60) are not significant.

Dose response. The relationships of the corrected steady-state factor activities to the rates of factor infusion were studied by least-squares linear regression analysis. The results of all of the studies were analyzed. The regression equation for the dose response to factor IX is: corrected steady-state factor IX activity = 0.08 + 0.0034 × factor IX infusion rate (factor IX activity in U/mL, rate in U/h). The regression is statistically significant (r = −.81, P < .001) and, as shown in Fig 1, fits the data well (SE of the estimate = 0.10 U/mL). The analysis of variance of the regression indicates that 65% of the variation in steady-state factor IX activities is attributable to the different infusion rates. Conversely, 35% of the variation is unexplained. Separate regression analysis of the study data from patient 5 indicates that intraindividual variability accounts for >65% of the total unexplained variation. The possibility that variability in body weight accounts for some of the unexplained interindi-

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<th>Weight (kg)</th>
<th>Height (cm)</th>
<th>Surface Area (m²)</th>
<th>Infusion Rate (U/h)</th>
<th>Corrected Steady-State Factor Activity (U/mL)</th>
<th>Clearance Rate (mL/h)</th>
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Table 1. Subject Data for Factor IX-Deficient Individuals

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Table 2. Subject Data for Factor VIII-Deficient Individuals
CLEARANCE RATES OF FACTORS VIII AND IX

Fig 1. Relationship of the corrected steady-state factor IX plasma activity to the factor IX infusion rate during continuous infusion therapy. The regression line (—) and the regression line passing through the origin (—) are shown.

Fig 2. Relationship of the corrected steady-state factor VIII plasma activity to the factor VIII infusion rate during continuous infusion therapy. The regression line (—) and the regression line passing through the origin (—) are shown.

Individual variance was tested by regression of the steady-state factor activities on the individual infusion rates divided by the patient's body weight. This approach did not produce a better fit of the data ($r = 0.78$; SE of the estimate $= 0.10$ U/mL).

The regression equation describing the factor VIII dose response is: corrected steady-state factor VIII activity $= 0.06 + 0.0030 \times$ factor VIII infusion rate (factor VIII activity in U/mL, rate in U/h). This regression also achieves statistical significance ($r = 0.91, P < 0.001$) and fits the data well (SE of the estimate $= 0.07$ U/mL), as shown in Fig 2. Here, the variability in the infusion rates accounts for 76% of the variation in steady-state factor VIII activities. As with the factor IX data, normalizing the factor VIII infusion rates for body weight did not result in an improved fit of the data ($r = 0.87$, SE of the estimate $= 0.09$ U/mL).

Ordinary linear regression analysis generates equations that have nonzero intercepts. The presence of a constant term allows the equations to fit the data better but, as in this case, does not strictly adhere to the form of the physiologic model. Because neither of the constant terms in the ordinary regression lines were significantly different from zero, the data were reexamined by a linear regression analysis in which the fitted lines were forced to pass through the origin. The resulting equations are: corrected steady-state factor IX activity $= 0.0042 \times$ factor IX infusion rate (factor IX activity in U/mL, rate in U/h); and corrected steady-state factor VIII activity $= 0.0035 \times$ factor VIII infusion rate (factor VIII activity in U/mL, rate in U/h).

As shown in Figs 1 and 2, over the range of factor activities achieved in this study, these equations differ very little from those found by ordinary regression analysis. In addition, these equations are essentially identical to those which result from rearrangement of the clearance model equation (first equation) and substitution of the appropriate mean factor clearance rates: corrected steady-state factor IX activity $= 0.0043 \times$ factor IX infusion rate (factor IX activity in U/mL, rate in U/h); and corrected steady-state factor VIII activity $= 0.0034 \times$ factor VIII infusion rate (factor VIII activity in U/mL, rate in U/h).

The excellent fit of the data confirms the validity of the clearance equation as a mathematical model of factor kinetics during continuous infusion therapy.

DISCUSSION

The reliability of an estimate of the plasma clearance rate of a substance determined with the continuous infusion method depends on accurate measurement of the dose administered and the plasma concentrations achieved. The factor activities in this study were measured by experienced technicians using a standard, quality-controlled, one-stage bioassay. Optimal specimen collection and handling techniques were used and the plasma specimens were stored for <72 hours prior to being assayed. Therefore, there is no reason to suspect any systematic error in the measurement of the factor concentrations.

Because of the retrospective nature of this study, it was necessary to calculate the factor infusion rates by using the manufacturers’ stated product content of factor in the concentrates. It would have been preferable to have measurements of the factor contents of the infusates. A number of studies have found that the stated potencies of the high-purity concentrates used in these patients are accurate, however. The factor IX concentrate has also been found to contain a minimal amount of activated factor IX. The factor concentrates have also been found to be stable at least 12 hours when kept at room temperature. This is true for the factor IX concentrate in its reconstituted form, as stated in the product insert (Document 14-7620-203; rev Sept 1978), and for the factor VIII concentrate in dilute solution, the condition under which it was administered in this study.

An additional concern in the measurement of clearance rates using the continuous infusion method is that the plasma concentrations must be measured when the substance is in steady state. If they are measured when the substance is still accumulating in the body, the clearance rate will be underestimated. In this study, the factor activities two to five days after the beginning of infusion therapy were taken to represent the steady-state factor concentrations. During that interval, the activities appeared to have reached a steady-state plateau; ie, the activities were not continuing to increase, although at times there was appreciable day-to-day variability in the measured activities. In addition, by using
published descriptions of the bolus kinetics of factor IX\textsuperscript{14} and VIII,\textsuperscript{1} it can be calculated\textsuperscript{15} that factor IX will have reached at least 95\% of its steady-state value by 102 hours following the beginning of infusion therapy, and factor VIII will have reached 95\% of its steady-state value by 60 hours. Both of these times are within the sampling interval chosen on empirical grounds in this study.

Further evidence that the clearance rate calculations in this study are valid is that the estimates agree with others in the literature. The normalized estimate of the plasma clearance rate of factor VIII, 5.0 mL/h/kg, is close to the rates that can be calculated from two studies of the bolus dose kinetics of factor VIII concentrate. In six patients receiving each of three brands of concentrate, the average clearance rate was 5.5 mL/h/kg.\textsuperscript{16} In another 35 patients, each receiving one of three brands of concentrate, the average clearance rate was 3.9 mL/h/kg.\textsuperscript{11} The present estimate is also very close to the rate that can be calculated from the data in a report of the dose response of five children to continuous factor infusion therapy using cryoprecipitate and factor concentrate,\textsuperscript{13} 5.3 mL/h/kg. The plasma clearance rate of factor IX that can be derived from a study of the bolus dose kinetics of factor IX concentrate in seven patients is 5.0 mL/h/kg.\textsuperscript{18} This value compares favorably with the normalized clearance rate in the patients reported here, 3.4 mL/h/kg, although the agreement is not as close as that among the factor VIII clearance rates. In a study of the bolus dose kinetics of radiolabeled factor IX, the clearance rate of factor IX in three patients was found to be 5.3 mL/h/kg (the clearance rate in a fourth subject was much higher, 10.5 mL/h/kg).\textsuperscript{19}

\textbf{ACKNOWLEDGEMENT}

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\textbf{REFERENCES}

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