The Hemostatic Imbalance of Plasma-Exchange Transfusion

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Plasma exchange has been proposed as a treatment for multiple disorders. Three patients with amyotrophic lateral sclerosis, who were hemostatically normal, were studied through a total of 11 4-liter exchanges. Plasma was replaced by an equal volume of 5% albumin or 5% plasma protein fraction. Serial studies revealed that immediately after the exchange transfusion, there was significant prolongation of the prothrombin, partial thromboplastin, and thrombin times with reduction of the fibrinogen and antithrombin III levels. Factors V, VII–X, IX, and X were all significantly decreased, as were the factor VIII antigen, procoagulant, and the ristocetin cofactor activities. Platelet counts were obtained before and after exchanges and revealed significant decreases. Four hours after exchange, all parameters remained abnormal except the factor IX, ristocetin cofactor, and factor VIII procoagulant activities. By 24 hr, all hemostatic parameters had returned to normal. These studies indicate that plasma-exchange transfusion with material devoid of coagulation factors results in a coagulation defect that may be of clinical significance in a hemostatically compromised patient.

Plasmapheresis was introduced as a therapeutic modality in 1914. Rapid plasma exchange became feasible with the advent of the continuous-flow centrifuge. Since its introduction in 1969, plasma-exchange transfusion has been proposed for the therapy of multiple disorders, including hypercholesterolemia, paraproteinemias, immune complex diseases, Goodpasture syndrome, myasthenia gravis, acute hepatic failure, procoagulant deficiencies, hemophilia A with a factor VIII inhibitor, and spontaneous factor VIII inhibitor.

The solutions used for exchange have primarily been fresh frozen plasma or plasma protein fraction. Transfusion with fresh frozen plasma carries with it the risk of hepatitis transmission and red cell blood incompatibility, as well as the theoretical disadvantage of utilizing a limited resource. Plasma protein fraction and albumin solutions avoid these problems. However, these substances do not contain coagulation factors and thus may result in a hemostatic imbalance.

We studied three hemostatically normal patients with amyotrophic lateral sclerosis (ALS) to determine the effects of exchange transfusion with albumin or plasma protein fraction on selected parameters of hemostasis. The studies performed were the prothrombin time (PT), activated partial thromboplastin time (APTT), and thrombin time (TT); fibrinogen and fibrin degradation products; factor
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VIII procoagulant, antigen, and ristocetin cofactor activities; factors V, VII–X, X, and IX; antithrombin III level; and platelet count.

MATERIALS AND METHODS

Three patients with ALS were entered on a trial of plasma-exchange transfusion in an attempt to improve their neurologic disorder. Transfusions were undertaken because of the possibility of an underlying immune or toxic-metabolic etiology of ALS. All patients were fully informed regarding plasma exchange and consented to the procedure. Each patient underwent 12 4-liter exchanges on an Aminco continuous-flow centrifuge (Continuous Flow Celltrifuge, American Instrument Co., Silver Spring, Md.). Rotor speed was 1600–2000 rpm and the flow rate was 60–85 ml/min. Each plasma exchange was replaced by an equal volume of 5% albumin or 5% plasma protein fraction (Plasmatein, Abbott Laboratories, South Pasadena, Calif.), containing 4 meq/liter KCl and 4.8 meq/liter calcium gluconate. Prior to and during each exchange, the patients were anticoagulated with heparin (6500–10,500 U) and Anticoagulant Citrate Dextrose, USP, Formula A (American Instrument Co.) (185–450 ml). Heparin was not neutralized at the end of the exchange.

Hemostatic parameters were studied prior to, at the end of, and 4 hr after a total of 11 exchanges. One patient was also studied at 24 hr postexchange. Platelet counts were obtained prior to and immediately after a total of 34 exchanges.

Blood for coagulation studies was collected in 1/100 volume of 40% sodium citrate. Plasma was separated after centrifugation at 1800 g at 4°C for 15 min. All coagulation studies were performed immediately, or the sample was frozen at −30°C and studies performed at a later date. The PT, PTT, TT, and chronometric fibrinogen were always performed immediately. Platelet count was performed on blood collected in EDTA. The plasma removed from the patient was refrigerated and pooled at the completion of the exchange; an aliquot was then removed and frozen after measurement of the total volume.

The PT, PTT, TT, factors V, VII–X, VIII, and IX and reptilase time were performed as previously described. Factor X was assayed by the method of Denson. An arbitrary endpoint for the PT, PTT, and TT was placed at 120 sec. Fibrinogen was determined by three methods: tyrosine determination on the fibrin clot, chromometric determination by the method of von Clauss, and immunologically by immunodiffusion. Fibrinogen degradation products were measured by tanned sheep red cell hemagglutination inhibition. Ristocetin cofactor activity was measured by the method of Allain et al. Counterimmunoelectrophoresis and crossed antigen-antibody electrophoresis of factor-VIII-related antigen were performed as previously described. Antithrombin III was determined by immunodiffusion (M Partigen Antithrombin III Kit, Behring Diagnostics, Somerville, N.J.). Platelet count was performed by phase contrast microscopy.

Dilution studies to determine the effect of heparin on coagulation factor analysis was performed by adding bovine lung sodium heparin (Upjohn Co., Kalamazoo, Mich.), which had been diluted to 1 U/ml with 0.9% NaCl (Abbott Laboratories, South Pasadena, Calif.) to normal plasma. Thrombin time and factor analyses were then performed.

Statistical analysis of the data was performed by the student two-tailed t test on a Hewlett-Packard Model 10 Calculator (Hewlett-Packard Co., Loveland, Colo.).

RESULTS

With the exception of one patient, clinical and laboratory evaluation of hemostasis was normal prior to the onset of plasma-exchange transfusion therapy and before each individual exchange. The patient who was studied over a 24-hr period had a mild decrease in fibrinogen and antithrombin III, which persisted throughout the observation period.

The effect of exchange on the PT, PTT, and TT is shown in Fig. 1. These values were significantly prolonged both immediately (p < 0.001) and 4 hr after exchange (PT, PTT p < 0.001, TT p < 0.02) when compared with initial values. However, at 4 hr, the PTT and TT were within the normal range. By 24 hr, all values were normal (p > 0.05). When prolonged, the TT was corrected by toluidine blue, and
Fig. 1. The mean of the PT, PTT, and TT before, immediately after, and 4 hr after plasma exchange. The hatched area represents the normal range. The brackets represent ± 1 SEM.

Fibrinogen concentration was significantly reduced by plasma exchange (p < 0.001) as seen in Fig. 2. Fibrinogen, determined by the chronometric (p < 0.005), fibrin tyrosine content (p < 0.001), and immunologic methods (p < 0.001),
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Fig. 3. The mean values of factor VIII procoagulant, antigen, and ristocetin cofactor activities before, immediately after, 4 hr after, and 24 hr after plasma-exchange transfusion. The brackets represent ±1 SEM. The solid line represents values from all 3 patients, while the broken line represents only those obtained in patient A. The normal range for factor VIII procoagulant is 62–155, for antigen is 50–150, and for ristocetin is 50–156.

remained abnormal for 4 hr. The total of fibrinogen cleared during a single exchange ranged from 3.9 g to 10.2 g. Increased fibrin degradation products could not be detected in any patient.

All other coagulation factors were normal prior to plasma exchange and were significantly decreased immediately after the exchange. Factor VIII procoagulant (p < 0.001), ristocetin cofactor (p < 0.001), and factor-VIII-related antigen (p < 0.001) were significantly depressed immediately postexchange (Fig. 3). By 4 hr, the factor VIII procoagulant and ristocetin cofactor activities were no longer significantly decreased. In contrast, the factor-VIII-related antigen remained significantly depressed at 4 hr (p < 0.01) (Fig. 3). Twenty-four hours after exchange, no significant differences could be demonstrated (p > 0.05). Crossed antigen–antibody electrophoresis demonstrated no qualitative differences of factor-VIII-related antigen at any time. The pooled plasmapheresis specimen from

Fig. 4. Mean of the activities of factor V and IX before, immediately after, 4 hr after, and 24 hr after plasma-exchange transfusion. The brackets represent ±1 SEM. The solid line represents values from all 3 patients, while the broken line represents only those obtained in patient A. The normal range for factor V is 65–140 and factor IX is 65–135.
Fig. 5. The mean of the activities of factor VII–X and X before, immediately after, 4 hr after, and 24 hr after plasma-exchange transfusion. The brackets represent ±1 SEM. The solid line represents values from all 3 patients, while the broken line represents only those obtained in patient A. The normal range for factor VII–X is 71–130 and for factor X is 70–122.

each patient was devoid of factor VIII procoagulant activity, while maintaining ristocetin and antigenic activity of 54% and 50%, respectively.

Factor IX activity decreased immediately postexchange ($p < 0.02$) and returned to the preexchange level by 4 hr ($p > 0.05$) (Fig. 4). The pooled plasmapheresis specimen contained 81% factor IX activity. However, this was significantly less than the mean of the preexchange levels ($p < 0.005$).

The factor V activity decreased to 50% immediately after exchange ($p < 0.001$) and remained depressed at 4 hr. The values obtained at 24 hr were normal ($p > 0.05$) (Fig. 4).

Postexchange, the factor VII–X decreased to 33% ($p < 0.001$) and factor X to

Fig. 6. Antithrombin III, determined immunologically, before, immediately after, 4 hr after, and 24 hr after plasma-exchange transfusion. The brackets represent ±1 SEM. The solid line represents values from all 3 patients, while the broken line represents only those obtained in patient A. The hatched area represents the normal range.
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22% ($p < 0.001$) of normal. At 4 hr, these values remained significantly decreased; by 24 hr, all values were normal ($p > 0.05$) (Fig. 5).

Immunologic determination of antithrombin III revealed a 58% decrease immediately after exchange transfusion ($p < 0.001$) (Fig. 6). A 40% decrement was still noted at 4 hr ($p < 0.001$). The single patient studied over a 24-hr period had a slightly low initial value, showed similar decrements immediately postexchange and at 4 hr, and returned to his baseline value at 24 hr.

The platelet count decreased from a mean of 240,000 prior to exchange to 161,000 immediately afterwards, a 33% decrease ($p < 0.001$).

Despite all of the aforementioned hemostatic abnormalities, no instance of bleeding or thrombosis was evident during or after these 11 well studied and 25 other partially studied exchange transfusions.

DISCUSSION

Plasma-exchange transfusion may be performed rapidly and efficiently with the continuous-flow centrifuge. The therapeutic goal of this procedure is the removal, from the patient, of mediators of various disease states. However, normal constituents of plasma will also be removed. This study was performed to determine the effect of plasma-exchange transfusion on selected parameters of hemostasis when material devoid of coagulation factors is used as replacement.

A model has been proposed by Russell et al.\(^{23}\) to predict the proportion of paraprotein remaining after plasma exchange.\(^{\star}\) A 100% plasma exchange will theoretically remove 67\% of paraprotein, assuming the presence of a closed system and stability of plasma volume. The authors state that this is approximated only in some patients. Bayer et al.\(^{24}\) determined the efficiency of exchange in 6 patients with varying volumes of plasma removed and found this to be 61\%. Calculated plasma blood volume\(^{25}\) in our 3 patients ranged between 3.34 liters and 3.40 liters. Efficiency of exchange was therefore calculated to be 71\%.

Plasma-exchange transfusion significantly prolonged the PT, PTT, and TT, with abnormalities persisting for 4 hr.

Fibrinogen was efficiently depleted by plasma exchange. This is in accordance with the results of Lockwood et al.\(^{7}\) where daily exchanges performed on patients with Goodpasture syndrome resulted in fibrinogens "around 75 mg/100 ml." Defibrination may play a role in the beneficial effects of plasma exchange on the renal lesions of Goodpasture syndrome and acute glomerulonephritis with renal failure.\(^{26}\) Defibrination was transient and had returned to the baseline value when determined 24 hr after the exchange transfusion. This implies that more intensive, i.e., daily, exchanges may be required if fibrinogen removal is a therapeutic goal. Chronometric determination of fibrinogen may be a simple means of following the efficiency of exchange, since this adheres closely to the mathematical model. Since there is no evidence of fibrin(ogen)alysis, the drop in fibrinogen was due to its removal. This is supported by the recovery of large amounts of fibrinogen in the pooled plasma.

Major changes were seen in the levels of factor VIII procoagulant, antigen, and

\[ x = e^{-pt} \]  where $x$ is the proportion of original paraprotein remaining, $p$ is the proportion of plasma volume removed, and $e$ is the Napier's log base.
ristocetin cofactors at the completion of the exchanges. The procoagulant and ristocetin cofactor activities rapidly increased with no significant differences demonstrable at the 4-hr evaluation. That the antigenic activity remained abnormal may be a reflection of the heterogeneous nature of the factor VIII molecule with synthesis or retention of a subset of the molecule that has biologic activity but relatively sparse antigenic activity. No differences, however, could be demonstrated by crossed antigen–antibody electrophoresis.

The decline of factor VIII procoagulant activity after exchange is at variance with other reports in which volumes of 3278 ml and 5000 ml were exchanged. The type of centrifuge used does not appear to be the reason for this discrepancy, since an Aminco centrifuge was also utilized by Bayer et al. Graybeal and coworkers found that double-bag plasmapheresis was a satisfactory method of obtaining factor-VIII-rich plasma. The plasma recovered from our patients contained no factor VIII procoagulant activity and would, therefore, be a poor material for the production of either cryoprecipitate or factor VIII concentrate. Antigen and ristocetin cofactor were present in decreased amounts.

The smallest decrement was seen with factor IX, which decreased 26% and remained well above the hemostatic level. This is likely due to redistribution and/or rapid synthesis of the molecule and is in accordance with previous reports. That redistribution from an extravascular compartment may be the major factor accounting for the small decrease is suggested by factor IX infusion studies. Although significantly less than preexchange levels, the pooled plasma contained large amounts of factor IX and could conceivably be used as a source for this factor.

Factor V, VII–X, and X showed varying decreases immediately after the exchange, and remained abnormal at 4 hr. Antithrombin III showed similar changes. That this may be of significance is suggested by Sultan et al. who found thrombosis at a femoral vein catheter site in one patient studied. Antithrombin III levels were found to be “dramatically affected” with levels undetectable in some patients.

The 33% decrease in platelet count after plasma exchange is somewhat greater than that found by McCullough and Fortuny and Graw et al. in donors undergoing leukopheresis. Platelet count, however, remained well above thrombocytopenic levels.

In summary, the significant alterations caused by plasma-exchange transfusion with plasma protein fraction or albumin are: (1) immediately after exchange: PT, PTT, and TT are significantly prolonged; (2) immediately after exchange: the platelet count, fibrinogen, antithrombin III, factor V, VII–X, IX, X, factor VIII procoagulant, antigen, and ristocetin cofactor activities were significantly decreased; (3) all parameters studied remained abnormal at 4 hr after exchange except the factor IX, factor VIII procoagulant, and ristocetin cofactor activities. Twenty-four hours after exchange all parameters examined were normal.

These abnormalities are due to a decrease of multiple factors that result from replacement of plasma by material devoid of coagulation factors. Although no adverse effects were noted, alterations of hemostatic parameters may be of clinical significance in a hemostatically compromised patient. These changes should therefore be considered prior to the undertaking of an exchange in such a patient.
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