THE EFFECTS OF A COBRA VENOM FACTOR AND ETHYL PALMITATE ON THE PROLONGATION OF SURVIVAL OF HETEROLOGOUS ERYTHROCYTES

The objective of these experiments was to explore the possibility of preventing the rapid destruction and removal from circulation of human erythrocytes transfused into rats. Significant prolongation of heterologous erythrocyte survival could pave the way for development of a system that might be useful in studies of blood preservation. There also is the possibility that certain aspects of these studies could provide the basis for modification of complement dependent hemolytic disorders in man.

Erythrocytes in heterologous serum in vitro may undergo rapid lysis due to the presence of complement-binding “natural” antibodies in serum. The lytic process probably involves sequential activation of all nine components of complement. The extremely short intravascular survival of heterologous erythrocytes is the result not only of hemolysis due to complement-binding antibody but also of the rapid removal of the sensitized erythrocytes by the cells of the reticuloendothelial system (RES), especially those of the liver and spleen.

In order to prolong the survival of human erythrocytes in the rat, the third component of complement, C3, which is essential for hemolysis, was inhibited through the administration of a cobra venom factor (CVF). This factor also is a striking inhibitor of phagocytosis, but has no effect on agglutination of red cells by immune or “natural” antibody. Further improvement in erythrocyte survival was achieved when CVF was combined with either surgical splenectomy or pretreatment with ethyl palmitate (EP) which both blocks the RES and produces functional splenic ablation (medical splenectomy), but has no known effect on hemolytic complement.

MATERIALS AND METHODS

The CVF from venom of Naja naja was isolated as a non-toxic 6.6 S protein by chromatographic techniques already described for a similar, if not...
identical, protein in venom of *Naja haje*. In brief, crude venom was applied to DEAE cellulose, pH 7.5, at low ionic strength. One major toxin, a hemolysin, and CVF were absorbed, while two other major toxins passed into the effluent. Upon elution of the DEAE column, the hemolysin separated when the salt concentration reached 0.02 ionic strength while the anticomplementary factor did not appear until ionic strength 0.12 was attained. Further purification to give a preparation showing only one band on disc electrophoresis was achieved by chromatography, first on CM-cellulose, pH 5, and then by passage through Sephadex G-200. The CVF eluted from the CM column when the salt concentration was raised to about 0.10 ionic strength. This protein was non-toxic when large doses were injected into mice, did not lyse erythrocytes in the presence of lecithin, did not kill several types of cells in tissue cultures, but was strongly anticomplementary at high dilutions when mixed with either guinea pig or human serum. The final preparation contained about 0.12 mg. protein per ml.

Approximately 15 ml. of whole human blood (ACD anticoagulated Group O, D positive) was incubated for 30 minutes at room temperature with approximately 300 µc of *51*Cr (sodium chromate). The red cells then were washed twice with saline and the hematocrit was adjusted to about 27%. Recipient animals were male Sprague Dawley rats weighing from 150 to 250 g. These were divided into six groups (4 to 15 animals in each group) as follows: Group 1—untreated controls, Group 2—surgical splenectomy 5 to 7 days prior to transfusion, Group 3—intravenous injection of 1 ml. of CVF (0.12 mg/ml.) from 1 to 10 minutes prior to transfusion, Group 4—single intravenous injection of EP (3 g/kg.) 48 hours prior to transfusion, Group 5—surgically splenectomized rats given 1 ml. of CVF from 1 to 10 minutes prior to transfusion, and Group 6—pretreated with both EP (same as Group 4) and CVF (same as Group 3) prior to transfusion. The transfusions consisted of 0.1 ml. of the *51*Cr tagged human blood per 35 grams of body weight per rat administered through the dorsal vein of the penis. Blood samples from the tail for radioassay were collected at intervals ranging from 2 to 30 min. in 20 µl glass pipettes. These samples then were counted in a well-type scintillation counter with the counting error for each sample at less than 2%. Sample counts usually ranged from 3 to 10 times background. Net counts were calculated as percent of the 2 minute post-injection sample. *51*Cr human erythrocyte T ½ survival in minutes was determined from the 50% intercept of the best straight line plot through all points on semilog paper.
RESULTS

The mean erythrocyte T½ survival for groups 1 and 2 were 6.5 and 6.4 minutes, respectively, without significant differences between the groups (Table 1). Mean erythrocyte T½ survival was significantly improved over the control group to 52 minutes in the Group 3 rats which had received only a single injection of CVF. Human erythrocyte survival in Group 4 (EP) and Group 5 (splenectomy and CVF) rats was greatly prolonged to significant T½ values of 133 and 149 min., respectively. Improvement was greatest in Group 6 (EP and CVF) rats where the mean erythrocyte T½ was 292 min. This represents an increase in mean erythrocyte T½ survival over controls by a factor of about 45. The best erythrocyte T½ survival was 380 minutes which is about a 60-fold improvement in heterologous erythrocyte T½ survival.

DISCUSSION

These observations demonstrate the effectiveness of the sequential blocking of certain factors responsible for the in vivo destruction of incompatible heterologous erythrocytes. The precise mechanisms through which erythrocyte survival is prolonged is uncertain, but information concerning the tissue effects of EP and CVF provides the basis for some reasonable speculation. Presumably, the combination of intravascular hemolysis and RES removal of heavily damaged erythrocytes was of such magnitude that splenectomy alone had no effect. CVF-induced reduction in erythrocyte surface damage and intravascular hemolysis, however, resulted in significant improvement in erythrocyte survival. EP alone induced sufficient RES blockade and

| Group no. | Experimental conditions | Human red cell T½ (min.) in each rat | Mean T½ (min.) for group ± SD | P |
|-----------|-------------------------|-------------------------------------|-----------------------------|---|
| 1         | Control                 | 13, 4, 7, 8, 3, 6, 5                | 6.5 ± 2.6                   | <.5 |
| 2         | Splenectomy             | 7, 6, 4, 8, 3, 6, 5                | 6.4 ± 2.2                   | >.5 |
| 3         | CVF*                    | 50, 55, 42, 59                     | 51.5 ± 7.3                  | <.05 |
| 4         | EP**                    | 222, 122, 108, 118, 94             | 132.8 ± 51                 | <.01 |
| 5         | Splenectomy and CVF     | 45, 250, 60, 230, 130              | 148.7 ± 86                 | <.01 |
| 6         | EP and CVF              | 350, 224, 380, 288, 216            | 291.6 ± 55                 | <.001 |

* The cobra venom factor (CVF) administered was .12 mg/rat.
** The dose of ethyl palmitate (EP) was 3 gm/kg.
splenic ablation to moderately impede the removal of erythrocytes that were heavily damaged through complement antibody binding. The favorable effect of splenectomy when combined with CVF probably represented the elimination of preferential splenic trapping of protein-coated erythrocytes that were only slightly damaged. The combination of RES blockade, splenic destruction and C3 inactivation through the use of EP and CVF produced the best results [Group 6 mean erythrocyte T ½ survival was significantly better than the mean for Group 5 (P = .01).]

It seems likely that future results will be improved through use of multiple blocking agents combined with techniques designed to inhibit agglutination and phagocytosis in animals with greater erythrocyte compatibility and more complete suppression of C3 or other components of complement that are essential for hemolysis. The C3 activity of guinea pigs is depressed for periods of from 3 to 5 days following a single injection of CVF. The possibility of developing a practical system that might permit survival of either heterologous or incompatible homologous erythrocytes for periods of several days or more does not seem unreasonable. Inhibition of autoimmune hemolytic mechanisms with CVF is an additional area of considerable promise.

SUMMARY

A fortyfold enhancement of human erythrocyte T ½ survival in the rat was achieved through the combination of RES blockade, chemically induced splenic ablation and inactivation of the third component of complement following injection of ethyl palmitate and a factor isolated from cobra venom.

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