Transplantation of spleen cells in patients with hemophilia A

A report of 20 cases

Abstract It has been reported that coagulation factor VIII (F. VIII) is produced in the spleen and other organs. Transplantation of splenic whole organ and spleen cells may, therefore, be used to treat patients with hemophilia A. The donor spleen from brain-dead donors was used to prepare spleen cell suspension for transplantation. Twenty-two spleen cell transplantations were performed on 20 patients suffering from severe hemophilia A at our institutes. Two of them underwent a second infusion of spleen cells since there was no increase in plasma F. VIII activity after the first transplantation. All but two patients showed a marked clinical improvement. Increased plasma F. VIII activity was observed in 18 of 20 cases. The peak plasma F. VIII activity in these recipients rose to 10%-15% posttransplantation in 14 cases and to over 15% in 4 cases from pretransplant levels of 0%-3%. Generally, the elevation of plasma F. VIII activity could be detected 4-7 days following transplantation of spleen cells and this lasted from 22 to 58 weeks. Four patients whose peak plasma F. VIII activity was greater than 15% experienced an uneventful course after transplantation. The patients with plasma F. VIII activity over 10% showed less frequent bleeding and prolonged intervals between bleed as well as improvement in hemophilic arthropathy. Two patients who had interval hematuria before transplantation did not have any relapse for up to 2 years after infusion of the spleen cells. These results indicate that spleen cell transplantation may be a promising method for the management of patients with hemophilia A.

Key words Spleen cell transplantation, hemophilia A
Hemophilia A, spleen cell transplantation - Factor VIII, spleen cell transplantation

Introduction

Hemophilia A, also known as classical hemophilia, is an X-linked genetic disorder [9]. This disease is characterized by spontaneous hemorrhages or bleeding following minor trauma and hemorrhagic arthropathy related to the lack of, or deficiency in, coagulating factor VIII (F. VIII). Thus far, there is no ideal treatment and no cure this disease.

Several experimental studies have demonstrated that F. VIII is produced in the spleen, liver, and vascular endothelial cells [8, 9, 13]. Over the past years transplantation of splenic whole organ [2, 3, 5, 6, 12] and infusion of spleen cells [1, 7] have been attempted in order to manage the patient with hemophilia A, and rising plasma F. VIII activity has, indeed, been reported in patients after both. This paper presents the results of a large group of spleen cell transplantations in hemophilic patients, and several related aspects of F. VIII synthesis are also discussed.
Twenty-two spleens from adult brain-dead donors were procured to group, a total of 22 spleen cell transplantations were performed. In consecutive transplantations of spleen cells at our institutes. Five of the 20 recipients underwent a second infusion of spleen cells since there was no increase in plasma F VIII activity after the first transplantation. These patients ranged in age from 2 to 29 years (mean age 16 years).

Fourteen of the 20 recipients required multiple fresh blood transfusions or infusions of exogenous F VIII concentrates before transplantation of the spleen cells. Three of the 20 recipients had varying levels of specific antibodies against human F VIII in their blood and they failed to respond to the strategies of fresh blood transfusion and infusion of exogenous F VIII. Mean plasma F VIII activity in all 20 patients varied from 0 % to 3 % prior to transplantation of the spleen cells.

Preparation of spleen cell suspension

Twenty-two spleens from adult brain-dead donors were procured to prepare spleen cells for transplantation. The donor spleen was immersed in a container with an ice-balanced salt solution at 4 °C. The splenic artery was catheterized and the spleen was flushed with cold Ringer’s solution supplemented with heparin until the effluent of the splenic vein became almost red cell-free. The donor spleen was then preserved with Euro-Collins solution at 4 °C until its second perfusion. Warm ischemia time of the donor spleen was no more than 10 min. The mean cold ischemia time of these donor spleens was 6 ± 4 h.

Since the spleen is a blood reservoir with rich blood sinusoids and since its perfusion is much more difficult than that of the kidney, liver, pancreas, and other organs, a second perfusion of the spleen is usually required. The donor spleen was reperfused in vitro using 300 ml albumin-anticoagulant acid citrate dextrose (AADD) solution through the splenic artery before disruption of splenic tissue. The composition of AADD solution is summarized in Table 1.

Three steps were followed to prepare spleen cells for transplantation, as previously described [4]. In brief, the splenic capsule and vessels in the splenic parenchyma were removed in a sterilized operating room. The spleen was cut into fragments 0.3 × 0.3 cm in size. The splenic fragments were weighed and they were then divided into a unit per 100 g. In the same manner, 100 g of AADD solution ice-power was considered as one unit. Two units of splenic fragments were mixed with one unit of ice-powder. The mixture was put into a sterilized homogenizer and rotated at 700 g for 3–5 min. Finally, the splenic homogenate was diluted with cooled AADD solution in an appropriate ratio after stirring.

Three filtrations of the splenic homogenate were performed. The blood transfusion net was used for the first filtration of the splenic homogenate, and a stainless steel filter net with holes 180 μm in diameter was used for the second filtration of the spleen cell suspension, another stainless steel filter net with holes 120 μm in diameter was employed for the final filtration. Afterwards the spleen cells were resuspended and preserved in AADD solution at 4 °C until transplantation. Details on how the spleen cell suspension was prepared for transplantation are given in Fig. 1.

The vitality of spleen cells was determined using trypan blue staining under phase contrast microscopy. A mean vitality of 85 % was obtained. Spleen cell suspension was abandoned when its vitality dropped below 65 %. Spleen cells were infused through a large peripheral vein, and the infusion speed of spleen cell suspension was adjusted according to the recipient’s cardiovascular status. The number of spleen cells infused in each patient ranged from 108 x 10⁷ to 380 x 10⁷ in child recipients and from 450 x 10⁷ to 1200 x 10⁷ in adult recipients. All infusions were performed in the intensive care unit (ICU). Examinations of smears for bacteria and bacterial cultures of the spleen cell suspension were done after the first transplantation in order to determine what if any, prophylactic antibiotics were needed. Two patients who failed to respond to the first transplantation of spleen cells in this group received a second infusion 2 months later.

HLA matching

There was a mean of two HLA loci mismatch between donors and recipients. Donors and recipients were ABO-compatible in 16 cases and ABO-incompatible in 4 cases.

Immunosuppressive schedule

Azathioprine (2 mg/kg per day) and cyclosporin A (CyA, 10 mg/kg per day) were used as the immunosuppressive pretreatment in recipients 5 days prior to transplantation. On the day of spleen cell transplantation, as previously described [4]. In brief, the splenic capsule and vessels in the splenic parenchyma were removed in a sterilized operating room. The spleen was cut into fragments 0.3 × 0.3 cm in size. The splenic fragments were weighed and they were then divided into a unit per 100 g. In the same manner, 100 g of AADD solution ice-power was considered as one unit. Two units of splenic fragments were mixed with one unit of ice-powder. The mixture was put into a sterilized homogenizer and rotated at 700 g for 3–5 min. Finally, the splenic homogenate was diluted with cooled AADD solution in an appropriate ratio after stirring.

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