

1246

Acute Hemolytic Anemia in Liver and Bone Marrow Transplant Patients Under FK 506 Therapy

K.M. Abu-Elmagd, O. Bronsther, M. Kobayashi, A. Yagihashi, Y. Iwaki, J. Fung, M. Alessiani, F. Bontempo, and T. Starzl

ACUTE hemolytic anemia has been observed in several occasions among different allograft recipients treated with cyclosporine (CyA).¹⁻⁸ The etiology of red blood cell (RBC) destruction was either ABO and/or Rhesus (Rh) D blood group incompatibility or drug-related immune injury. With FK 506 therapy, acute hemolysis was noticed in eight patients. The drug was given as a primary immunosuppressant in five patients and as a rescue therapy in three.

MATERIALS AND METHODS

Patient Population

Eight out of 1,400 patients who received FK 506 at the University of Pittsburgh developed acute hemolysis that required frequent blood transfusion. Seven patients were liver transplant recipients and one was treated for chronic graft vs host disease (GVHD) after an HLA identical bone marrow transplant. FK 506 was given as primary immunosuppressant therapy in five patients. The rescue group (n = 3) received CyA, azathioprine, and steroids before the FK 506 treatment. Of the eight patients, five were men and three were women with a mean age of 39 ± 14 (range 12 to 65). All patients received ABO identical grafts. Only one Rh-positive liver recipient received a Rh-negative graft (Table 1). In three of the seven liver recipients, direct T-lymphocyte cytotoxic cross-matches were strongly positive with 100% plasma renin activity (PRA).

A posttransplant lymphoproliferative disorder (LPD) was documented in three liver transplant recipients at the time of hemolysis. In one case, the diagnosis was made before the start of FK 506 therapy with involvement of the cervical, thoracic, and abdominal lymph nodes. In the other two patients, the LPD involved the liver and the diagnosis was made in the graft after retransplantation. Also, cytomegalovirus (CMV) infection was detected at the time of hemolysis in three other liver recipients with isolation of the virus from more than one organ. These three patients were treated with DHPG.

At the time of hemolysis, the 12-hour trough plasma levels of FK 506 were within the therapeutic range in all cases (0.5 to 2.7 ng/mL). All patients were receiving prophylactic doses of Mycostatin, Bactrim, acyclovir, and pepcid. In two patients, different antimicrobial agents were given because of life-threatening infections. The patient with GVHD was receiving amoxicillin for treatment of chronic sinusitis. Two of the patients received a 3-day course of OKT3 for the treatment of persistent rejection.

Hemolytic Studies

Blood samples were drawn daily for routine hematology, biochemistry, serology, and FK 506 plasma levels. The serologic tests consisted of RBC antibody screening and a complement-dependent RBC cytotoxic test evaluating the in vitro effect of FK 506 (Table 2). All patients were thoroughly evaluated for bacteriologic evidences of systemic or local sepsis. RBC life span and splenic sequestration studies were performed in three patients and bone marrow biopsies were done in four.

Complement-Dependent RBCs Cytotoxic Test

RBCs from five healthy individuals with O Rh(+ve) blood type were tested using serial dilutions of anti-H reagent and different

From the Departments of Surgery (K.M.A.-E., O.B., J.F., M.A., T.S.), Medicine (F.B.), and Immunology (M.K., A.Y., Y.I.), University Health Center of Pittsburgh, University of Pittsburgh, and the Veterans Administration Medical Center, Pittsburgh, Pennsylvania.

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Address reprint requests to Thomas E. Starzl, MD, PhD, Department of Surgery, 3601 Fifth Ave, 5/C Falk Clinic, Pittsburgh, PA 15213.

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Table 1. Demographics of the Patients

| Patient No. | Age/Sex | Diagnosis | Transplant | Crossmatch | FK 506 Therapy | Blood Type | |
|-------------|---------|------------------------|-----------------------|------------|----------------|------------|-----------|
| | | | | | | Donor | Recipient |
| 1 | 43/F | Sclerosing cholangitis | OLT | -ve | Primary | A(+ve) | A(+ve) |
| 2 | 65/F | Hemochromatosis | OLT | +ve | Primary | A(+ve) | A(+ve) |
| 3 | 33/M | Alcoholic cirrhosis | OLT | +ve | Primary | O(+ve) | O(+ve) |
| 4 | 40/M | Hepatic cirrhosis | OLT | +ve | Rescue | O(+ve) | O(+ve) |
| 5 | 45/M | Hepatic cirrhosis | OLT | -ve | Primary | A(-ve) | A(+ve) |
| 6 | 46/M | Hepatic cirrhosis | OLT | -ve | Primary | AB(+ve) | AB(+ve) |
| 7 | 12/M | Biliary atresia | OLT | ND | Rescue | B(+ve) | B(+ve) |
| 8 | 29/F | Acute myeloid leukemia | Identical bone marrow | -ve | Rescue | A(+ve) | A(+ve) |

Abbreviations: OLT, orthotopic liver transplantation; ND, not done.

Table 2. The Results of Complement-Dependent Hemolysis Test Against Autologous and Allogenic RBCs Under Various Concentrations of FK 506

| Patient no./ Serum RBC | FK 506 Concentration (ng/mL) | | |
|---------------------------|------------------------------|----------|----------|
| | 0 | 10 | 20 |
| Patient 2 | — | — | — |
| A(+ve)* | — | — | — |
| Patient 4 | — | — | Positive |
| O(+ve)* | — | — | Positive |
| Patient 5 | ND | ND | ND |
| A(+ve)* | — | — | — |
| Patient 8 | — | Positive | Positive |
| A(+ve)* | — | — | Positive |

Abbreviation: ND, not done.

* Identical with the patient blood type.

concentrations of FK 506 (10 to 20 ng/mL). The RBCs and serum of four patients were also tested for evidence of *in vitro* hemolysis. The procedure was performed as follows: blood was collected from five healthy O Rh(+ve) individuals into glass tubes using heparin as the anticoagulant and RBCs were separated by differential centrifugation. After being resuspended in Hank's balanced salt solution (HBSS) and washed two times, RBCs were then stored in Alsever's solution at 40°C. Stored RBCs were washed and resuspended in HBSS with an adjusted count of $1.0 \times 10^4/\mu\text{L}$. The RBCs were then incubated with 0, 10, and 20 ng/mL of FK 506 at 37°C for 24 hours. One microliter from each set of the RBC suspension was then added to 1 μL of anti-H using twofold serial dilutions. After incubation of all wells at room temperature for 45 minutes, 2 μL of rabbit complement adequately diluted with HBSS were added and then incubated for another 45 minutes. The end point for the anti-H was determined when more than 50% of RBCs per well were lysed.

Using the same technical steps, the RBCs of four patients and four ABO identical healthy individuals were incubated with the patient and control serums instead of the anti-H reagent. Positive results were considered when more than 50% of the RBCs per well showed evidence of hemolysis.

RESULTS

Signs of Increased RBC Destruction

In the absence of blood loss, a persistent fall in the hemoglobin concentration and a significant drop in the hematocrit (Hct) of more than eight points were observed in all of the patients with a mean Hct value of 31 ± 2 before the onset of hemolysis and 18 ± 4 at the time of diagnosis. The mean (\pm SD) onset of hemolysis was 35 ± 24 days after initiation of FK 506 therapy. All patients required RBC transfusion: four were transfused with 5 to 15 U, three with 21 to 27 U, and one received 42 U. Total serum bilirubin was high in all patients with no evidence of graft rejection in the liver recipients. The level of unconjugated bilirubin was increased in 63% of the patients ($n = 5$).

At time of hemolysis, peripheral blood smears showed classic changes of immune hemolysis with spherocytes and polychromasia in all patients. Thrombocytopenia was concomitant in six patients. The direct and indirect anti-

globulin tests were positive in the Rh-positive transplant patient who received the Rh-negative graft.

The radioactive RBC scans showed short life spans of the erythrocytes with rapid uptake by the spleen. The bone marrow biopsies showed erythroid predominance and active megakaryocyte production.

In vitro Effect of FK 506

FK 506 increased the sensitivity of erythrocytes in a complement-dependent hemolysis test. Reciprocal titers of 20 to 80 were obtained when O Rh(+ve) erythrocytes from five healthy individuals were tested against an anti-H reagent without FK 506. A one to twofold increase in the reciprocal hemolytic titers was observed when erythrocytes were treated with 10 ng/mL of FK 506. The treatment with 20 ng/mL increased the titers to a range of 1:160 to 1:640.

Consequently, a hemolysis test was carried out using the patient's own sera and own erythrocytes. Hemolysis in the presence of FK 506 was observed in two patients at 10 ng/mL and 20 ng/mL, respectively. Alloreactive erythrocytes were also hemolysed in these two patients.

Survival and Control of Hemolysis

Hemolysis was successfully controlled in five out of the seven liver recipients and in the patient with GVHD. One of these five responders died following retransplantation without any evidence of recurrent hemolysis. In the remaining two cases, destruction of the RBCs continued and both patients died of disseminated lymphoproliferative disease.

Augmented steroid therapy stopped the hemolytic process in one of the liver recipients. Splenectomy was performed in six patients with a satisfactory response in only three of them. Donor B lymphocytes were detected in three of the surgically removed spleens. In the Rh-positive liver recipient who received the Rh-negative graft, the hemolytic process was completely controlled by replacement of the graft. In the GVHD patient, hemolysis resolved within 5 days after discontinuation of the amoxicillin and reduction of the FK 506 dose. All survivors ($n = 5$) are currently receiving FK 506 with no evidence of hemolysis.

DISCUSSION

Recent pharmacologic studies have demonstrated that FK 506 binds with high affinity to the erythrocyte membranes (unpublished data). The current *in vitro* study showed an increased susceptibility of normal O Rh(+ve) RBCs to the hemolytic effect of the anti-H reagent in the presence of FK 506 with a concentration of 10 to 20 ng/mL (Fig 1). Such *in vitro* concentrations are equivalent to the practical therapeutic levels since the *in vivo* FK 506 concentrations are usually 8 to 10 times higher in the whole blood compared with the plasma. The exact mechanism of such adverse effects is unknown, however sensitization of the

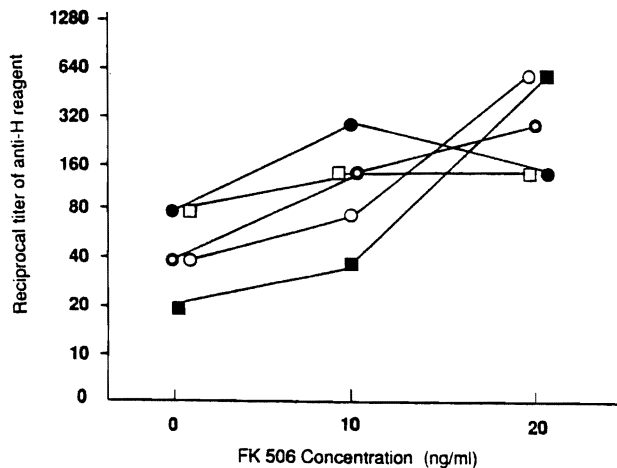


Fig 1. The effect of FK 506 on complement-dependent hemolysis.

RBCs by forming an FK 506-erythrocyte complex should be considered.

The complicated clinical course of these morbid patients precludes the ability to establish a single etiology for these hemolytic episodes. While FK 506 may predispose or initiate hemolysis, multiple other potential factors were documented in seven patients. These included CMV infection ($n = 3$), LPD ($n = 3$), donor B-lymphocyte population of recipient spleen ($n = 3$), Rh nonidentical graft ($n = 1$), and antibiotic sensitivity ($n = 1$).

The specific mechanism of autoimmunization in most of these patients remained unclear. However, in patients with CMV infection, various mechanisms have been postulated including absorption of immune complexes and complement, cross-reacting antigen, and a true autoimmune state with possible loss of tolerance secondary to the infectious organism.⁹⁻¹¹ With the LPDs, the possible role of Epstein-Barr virus (EBV) infection and/or other unknown immunologic mechanisms may be incriminated. The detection of passenger B lymphocytes in the spleen of the Rh-positive patient who received the Rh-negative graft highlighted the possible role of the donor B lymphocytes in producing anti-Rh antibodies.

With the drug-related immunologic injury of erythrocytes, it is not always possible to be certain that the cited drug is unequivocally involved in the pathogenesis of the hemolytic anemia. In this study, the serum of two patients

induced *in vitro* hemolysis of both patient and ABO identical RBCs in the presence of FK 506 with a concentration of 10 to 20 ng/mL. One of these two cases showed a positive Coombs test and the other was receiving amoxicillin treatment. The cessation of the hemolytic process both clinically and in the *in vitro* studies after replacement of the graft in the first and discontinuation of the antibiotic therapy in the second underscores the possible role of different serum factors in triggering the immune injury of the erythrocytes.

Various therapeutic modalities were employed in the management of the eight patients with satisfactory clinical and hematologic response in six. Steroid therapy was effective in one patient and splenectomy was curative in about 50% of the cases. Replacement of the liver graft was mandatory in the Rh nonidentical recipient and discontinuation of amoxicillin was effective in the GVHD patient. FK 506 therapy was continued in most of these morbid cases and all of the survivors are still receiving the proper dose of FK 506 without any evidence of RBC injury.

In conclusion, the hemolytic phenomena observed in patients receiving FK 506 seem to be triggered by different etiologic factors. The current *in vitro* results and the high affinity binding of FK 506 to the erythrocytic membrane may point out the potential role of FK 506 in inducing and/or promoting RBC destruction particularly in patients with acquired antierythrocyte antibodies.

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