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Effect of FK 506 and Antiproliferative Agents for Heart and Liver Xenotransplantation From Hamster to Rat

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XENOTRANSPLANTATION requires the suppression of vigorous humoral as well as cellular immune responses. FK 506, which has been used successfully for clinical allotransplantation of a variety of organs,[1] interferes with the signal transduction pathway in T cells. For hamster to rat xenotransplantation in this study, FK 506 was combined with antiproliferative drugs, which inhibit DNA synthesis required for expansion of both T- and B-cell clones.

MATERIALS AND METHODS

Animals and Operation

Inbred male Lewis rats (LEW, RT1[l]) and Golden Syrian hamsters were used as recipients and donors, respectively. Heterotopic abdominal heart transplantation was performed by the method of Ono and Lindsey.[2] Liver transplantation was performed according to Kamada's method.[3] Rejection was diagnosed by the cessation of the heart graft beat or by the liver recipient death, followed by the histopathological examination.

Immunosuppressive Agents

Intramuscular FK 506 (Fujisawa Pharmaceutical Co, Osaka, Japan) was given as a baseline immunosuppression. The dose for heart xenograft recipients was 2.0 mg/kg/d on days 0 to 5, followed by 1.0 mg/kg/d on days 6 to 30. Liver xenograft recipients were given 1.0 mg/kg/d on days 0 to 30. After 30 days out to day 100, both liver and heart recipients received FK 506 at a dose of 0.5 mg/kg every other day.

Antiproliferative drugs combined with FK 506 were Brequinar (BQR; Du Pont Medical Products, Wilmington, Del), RS-61443 (RS; Syntex Inc), Mizoribine (Asahi Chemical Industry Co, Ltd), azathioprine, cyclophosphamide, and methotrexate. These drugs were prepared daily and administered orally by daily gastric installation. The antimacrophage agent deoxyspergualine (DSPG; Bristol-Meyers Squibb Pharmaceutical Research Institute) was administered intramuscularly.

Antibody Analysis

Antibody analysis was with the complement-fixing lymphocytotoxic antibody assay described by Terasaki.[4] The titer was defined as the highest serum dilution at which more than 50% of the hamster lymphocytes were lysed. For indirect immunofluorescence studies, frozen hamster liver sections were incubated with sera obtained from hamster xenograft recipients, followed by goat anti-rat immunoglobulin G (IgG) or IgM to detect the heterospecific antibodies.

RESULTS

Heart Xenograft Survival

Untreated xenografts were rejected in 3 days. FK 506 alone prolonged the survival by 1 day. BQR, RS, mizoribine, methotrexate, and DSPG were more effective as monotherapy, but the prolongation of xenograft survival was limited to 4 to 15 days with nearly toxic doses. Cyclophosphamide alone consistently prolonged the heart graft survival for more than 30 days with daily doses of 10 or 15 mg/kg.

When baseline FK 506 treatment was combined with a short course (9 to 30 days) of antiproliferative drugs, xenograft survival was enhanced in all drug combinations. Enhancement of survival was dramatic with cyclophosphamide, BQR, and RS, with routine survival beyond 100 days for almost all grafts. Mizoribine, methotrexate, and DSPG were moderately effective as adjuvant agents, but azathioprine in toxic dose allowed only a slight prolongation of graft survival when used with FK 506.

Liver Xenograft Survival

Untreated animals died within 8 days. Used as monotherapy, FK 506 increased the median survival to 34.5 days. When used alone, the antiproliferative drugs slightly prolonged the median survival, which was 9 days with 7.5 mg/kg of cyclophosphamide and 19 days with 3.0 mg/kg BQR.

Animal survival was remarkably enhanced by combining these drugs with baseline FK 506. Cyclophosphamide, BQR, or RS for 10 to 14 days permitted long-term survival (>100 days) for 80% to 90% of the animals under continuous FK 506. Success rate was reduced to 15% to 40% when antiproliferative drugs were continued for 30 days or more.

Anti-Hamster Antibodies

After heart grafting, the increase of lymphocytotoxic antibodies, which reached 256-fold to 512-fold in untreated animals on postoperative day 3, was suppressed in animals treated with induction therapy with antiproliferative drugs (BQR, RS, or cyclophosphamide for 30 days) combined with FK 506. Cytotoxic antibody titer after liver xenotransplantation was 10 times higher than after heart grafting, and this increase was only partially inhibited in animals with combined treatment. However, indirect im-

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munofluorescence IgM deposition was lighter and less blood vessel directed in combined treatment.

DISCUSSION

A variety of treatment strategies have been tried to treat hamster to rat xenotransplantation. Total lymphoid irradiation combined with cyclosporine A (CyA)[5] or DSPG,[6] and CyA combined with cobra venom factor[7] have been partially effective to treat hamster to rat cardiac transplantation. As shown in this study, combination of FK 506 and antiproliferative drugs, which inhibit cell proliferation through the inhibition of key enzymes for de novo purine and pyrimidine nucleotide biosynthesis, was effective to break down the antibody barrier to xenotransplantation. Among antiproliferative drugs used with FK 506 in this study, BQR, RS, or cyclophosphamide routinely permitted successful heart and liver xenotransplantation. Once the antibody barrier had been broken down, antiproliferative drugs were no longer necessary, and FK 506 alone was sufficient to maintain the xenograft function. These results are clinically applicable to prevent xenograft rejection in humans. After completing these studies, it was learned that Hasan et al[8] had demonstrated a pronounced prolongation of heart xenograft survival with cyclophosphamide–CyA therapy.

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