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FK 506 Has no Short-Term Effects on Endogenous or Exogenous Myeloid Reconstitution in Irradiated Mice

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IN this study, we address the question of whether or not FK 506 adversely affects the ability of myeloid progenitors in the bone marrow (BM) to regenerate and produce blood cells during the early phases of endogenous recovery after BM depletion or exogenous recovery after BM ablation and BM transplantation (BMT). This is an important question for several reasons. First, FK 506 is being tested as a means to combat graft-versus-host disease (GVHD) in allogeneic BMT patients. Since it is known that T-cell depletion of the BM before transplantation increases the likelihood that the BM will fail to engraft[1] and, furthermore, that FK 506 decreases T-cell activity,[2,3] including the production of certain cytokines,[4-6] it is possible that the loss of T-cell activities could alter hematopoietic recovery or engraftment.

Since survival after BMT depends on minimizing the period of neutropenia and thrombocytopenia caused by BM ablation, and since regeneration of blood cells depends on previous regeneration of precursors and cellularity in the BM, we decided to survey the effects of FK 506 at selected times and doses on early regeneration after whole body irradiation (WBI) or WBI plus BMT. The mouse was used as a model system because there are a variety of well-characterized assays available for hematopoietic recovery in mice. Repopulation of BM precursors, such as spleen colony forming cells, normally precedes and is necessary for the subsequent recovery of BM cellularity which, in turn, is responsible for recovery of blood cell counts. For endogenous recovery, the time of repopulation depends on the radiation dose and, for exogenous recovery, it depends on the BM cell dose. Based on previous studies,[7-16] times of recovery, radiation doses, and the BM cell dose used were selected to survey the potential of FK 506 to adversely affect hematopoietic recovery.

MATERIALS AND METHODS

The mice were female F1 (C57BL/6J × DBA/2J), purchased from Jackson Laboratories (Bar Harbor, Me) and used between 3 and 8 months of age. Except where noted, there were 10 mice per group. All mice were given food and acidified (pH 2.4) water ad libitum.

WBI was administered at 97 R/min using a Cesium 137γ source (J.L. Shepherd, Mark 1 Model 68, San Fernando, Calif). Groups of mice were given either 3 mg/kg pure FK 506 (11.1 mg/kg of powder) or the equivalent amount of FK 506 diluent. Both were administered subcutaneously each day from the day of irradiation to the day of assay. An additional control group was not injected. FK 506 was provided by Fujisawa Pharmaceutical Co (Osaka, Japan). FK 506 with carrier solvent HCO-60 (chremophor EL) and D-mannitol was diluted in normal saline. The groups given "diluent" were given at an equivalent concentration of HCO-60 and D-mannitol in the same volume (0.1 mL/g mouse) in normal saline.

Cell counts were performed with a Coulter Model ZBI electronic particle counter (Coulter Electronics, Marietta, Ga). Cell collection, cell injection, and analyses of endogenous spleen colony forming units (E-CFU-S) and exogenous CFU-S were performed as described previously.[17]

Data values from the noninjected and diluent-injected control groups were averaged, and standard errors were calculated to reflect both within and between group variations. The two-way Student's t test was applied to detect significant effects of FK 506. Groups with $P \leq .05$ were considered significantly different.

RESULTS AND DISCUSSION

For endogenous repopulation studies, three groups of mice were given 550 R. The FK 506 or diluent was administered for 10 days. On day 9, hematocrit (HCT) and white blood cell counts (WBC) were done. At 10 days, the animals were killed and marrow cellularity and E-CFU-S determined. There was no significant effect of FK 506 on any of these parameters (Table 1).

Exogenous BM recovery was measured 8 days after lethal irradiation and injection of 5×10^6 BM cells to each recipient; there were 5 mice per group. There was no significant effect of FK 506 on HCT, WBC, or BM cellularity (Table 2). Recovery of exogenous precursors in these primary BMT recipients was evaluated by pooling the BM cells from the entire group and transplanting that number of cells equivalent to 1/15 of the humeral cell count

Table 1. Endogenous Recovery After Sublethal Whole Body Irradiation (550 R)

	Blood		Bone Marrow,	
Treatment Group	HCT	WBC ($\times 10^5$/mL)	Cells/Humerus (Millions)	Spleen, E-CFU-S
FK 506 (n = 10)	43 ± 0.3	17 ± 1.5	8 ± 0.5	6 ± 1.0
Control (n = 20)	32 ± 0.4	17 ± 1.5	7 ± 0.4	5 ± 1.1

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Supported by the Claude Worthington Benedum Foundation and Research Grant No. DK29961 from the National Institutes of Health, Bethesda, Maryland.

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0041-1345/91/$3.00/+0

Table 2. Exogenous Recovery 8 Days After Transplantation of 5 × 10^6 Bone Marrow Cells Into Lethally Irradiated Mice (950 R)

Treatment Group	Blood		Bone Marrow, Cells/Humerus (Millions)
	HCT	WBC ($\times 10^5$/mL)	
FK 506 (n = 5)	45 ± 0.6	60 ± 11.2	7 ± 0.5
Control (n = 10)	46 ± 0.5	88 ± 16.0	8 ± 0.5

into irradiated recipients. The 1/15 humerus value was chosen from previous data and, in preliminary experiments with small numbers of mice, gives countable numbers of CFU-S_{12}.[14–16] There were 10 secondary recipients and 10 additional control mice given only the irradiation in each group (Table 3). After 12 days, HCT, WBC, and CFU-S_{12} were assayed in the secondary recipients (Table 3). There was no affect of prior FK 506 treatment on these parameters as assayed in secondary recipients. Since 1/15 of a humerus produced 15 ± 0.9 spleen colonies and a seeding fraction for CFU-S_{12} of about 10%,[18] the spleen colony forming cells at this time point can be estimated to be 2225 per humerus in the FK 506-treated group. This is on the rising portion of the recovery curve for these cells, and any differences in recovery should have been detectable.

Table 3. Hematopoietic Repopulation of Secondary Recipients of 1/15 Humerus Harvested 8 Days After Transplantation of 5 × 10^6 Bone Marrow Cells Into Primary Irradiated Recipients

Donor Group	Blood		Spleen, CFU-S_{12}
	HCT	WBC ($\times 10^5$/mL)	
FK 506	35 ± 0.5	9 ± 0.8	15 ± 0.9
Control	34 ± 1.0	18 ± 7.5	18 ± 2.0
No donor	30 ± 0.6	3 ± 0.4	3 ± 1.0

There were 10 recipients of cells from FK 506-treated donors, 20 recipients of cells from control groups, and 10 mice given 950 R only and no donor cells.

Thus, on the basis of these investigations, there were no detected hematopoietic effects of FK 506 in mice when administered for 8 to 10 days during the early phase of recovery from sublethal irradiation or lethal irradiation followed by BMT. Longer exposure times, higher doses, or different routes of administration of FK 506 in an animal model may reveal effects not observed in the present study.

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