Reprinted from transplantation
Volume 51, Number 4, April 1991

EFFECTS OF IN VIVO TREATMENT WITH FK506 ON NATURAL KILLER CELLS IN RATS[1]

FK506, like cyclosporine, diminishes IL-2 receptor expression and inhibits IL-2 production by T lymphocytes (*1*). IL-2 plays a crucial role in the generation and function of the large granular lymphocytes, also called natural killer cells, which are cytolytic in vitro against various tumor cells, some microorganisms, and virally infected cells (*2*). Because posttransplantation neoplasia and viral sepsis are common complications after transplantation under immunosuppression, we investigated the effects of a 4–14-day course of 1.0 mg/kg/day intramuscular FK506 on NK cell function in F344 rats. This dose has been shown to prevent allograft rejection in MHC- and non-MHC–incompatible donor-recipient strain combinations (*3*). Animals were sacrificed after 4, 7, or 14 days of treatment, and fresh mononuclear spleen cells were isolated after centrifugation on Ficoll-Hypaque gradients and passage over nylon-wool columns to remove monocytes/macrophages and B cells. NK activity of the cell isolates was determined by lysis of NK-sensitive YAC-1 tumor targets in a 4-hr ^{51}Cr release assay. Antibody-dependent cytotoxicity, which is another measure of NK activity, was measured by lysis of NK-resistant P815 tumor targets in the presence of rat anti-P815 serum. No differences were found in NK and ADCC activity after 4 and 7 days of treatment with FK506 (Table 1). After 14 days, there was a slight increase in NK (17–19%) and ADCC (22%) activity. Because FK506 suppresses in vitro mixed lymphocyte reaction responses (*1*), we also performed these NK assays on fresh spleen cells obtained from untreated rats and exposed in vitro to 0.06–250 ng concentrations of FK506. There was no effect of FK506 on fresh splenic NK cell activity over this wide dose range (Fig. 1).

The results of flow cytometry studies on splenic cell isolates were congruent with the NK assay results. The lack of effect of FK506 on NK cell activity correlated with the expression of NK-associated cell surface markers. No differences were noted in the expression of CD2 (present on NK cells and T cells) and 3.2.3. (NK cell–specific) after 4, 7, and 14 days of treatment (Table 2, data of day 14 not shown). The number of LGL in cytospin preparations was not changed after 4 days of FK506 treatment and only slightly reduced after 7 days. Asialo-GM1–positive cells (which include NK cells, subpopulations of macrophages, and subsets of T cells) were increased from 25±7 (% positive cells ±SD) on day 4 to 36±1 on day 7; all values were within normal range.

The effect of FK506 on T lymphocytes in the same rats also was investigated in order to verify that the dosage regimen was therapeutic. This was a positive control for the IL-2-dependent T cell mechanism. Fresh mononuclear spleen cells from the control and FK506-treated animals were cultured for 3 days at a concentration of 1×10^5 cells/well in the presence or absence of Con A (2 μg/ml) or recombinant IL-2 (10^3 units/ml) and were subsequently pulsed for 8 hr with [^{3}H]thymidine. There was no difference in response to stimulation with IL-2 between treated and control animals at days 4, 7, and 14 of FK506 treatment (Fig. 2). However, the response to Con A stimulation showed 53% (day 4), 24% (day 7), and 43% (day 14) inhibition of proliferation (Fig. 2).

[1] This work was supported by Research Grants from the Veterans Administration and by Project Grant DK 29961 from the National Institutes of Health, Bethesda, MD.

TABLE 1. NK and ADCC activities of fresh spleen cells from control and FK506-treated rats

Treatment of rats	Days of treatment	NK activity (cytotoxicity vs YAC-1)		ADCC (cytotoxicity vs P815[b])	
		100:1[a]	50:1	Without anti-P815 antiserum	With P815 antiserum
Control	4	31±1.1[c]	26±2.7	−1±0.5	46±1
FK506	4	28±1.5	22±1.7	−1.4±0.5	38±1.4
Control	7	36±3.5	27±3.5	1.5±0.9	24±1
FK506	7	38±1.9	29±3.2	3±0	26±1
Control	14	47±5	34±4.5	−0.5±1.1	41±3.5
FK506	14	56±0.3	41±0.6	0.7±0.2	50±1.1

[a] Effector/target ratio.
[b] Effector/target ratio of 50:1.
[c] Mean percentages ±SEM of cytotoxicity from 2 rats in each group.
[d] Control rats received 1 ml 0.9% saline/kg/day as a sham injection corresponding to the treatment group.

Thus, although IL-2 has also been shown to be important for the generation and function of NK cells (*2*), our experiments showed that FK506, which is known to inhibit IL-2 production and receptor expression did not suppress NK or ADCC activity with either in vitro or in vivo test systems. The lack of an inhibitory effect on NK cytotoxicity correlates well with findings in T cells, in which FK506 also does not interfere with the cytotoxic function of effector T cells.

Some information is available about the effect of other immunosuppressive agents on NK activity. While most investigators have found an inhibition of NK and ADCC activity during azathioprine therapy, there are conflicting reports about corticosteroids and CsA (*4–6*). Gui et al. (*4*) reported NK inhibition with CsA. However, Muller et al. (*6*) reported that ADCC activity was inhibited during corticosteroid therapy in human transplant recipients while NK activity was not. Combined treatment with CsA and corticosteroids in their patients suppressed both NK and ADCC activity, and even more acute

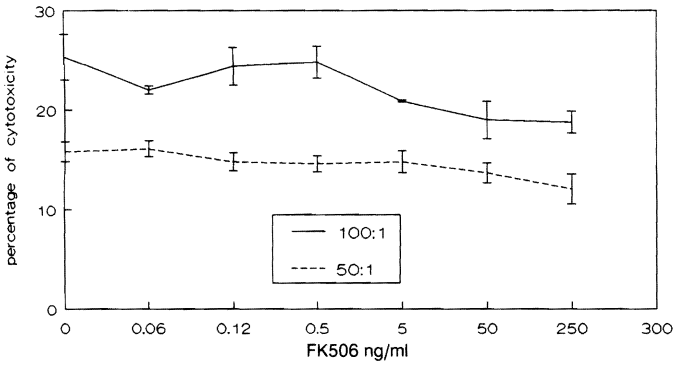

FIGURE 1. NK activity of fresh spleen cells from normal F344 rats in the presence of FK506 in varying concentrations. Data represents mean percentages ±SEM of cytotoxicity vs YAC-1. Varying concentrations of FK506, 0.06–250 ng/ml (0 = control) were added to triplicate cultures in 100:1 and 50:1 effector:target ratios.

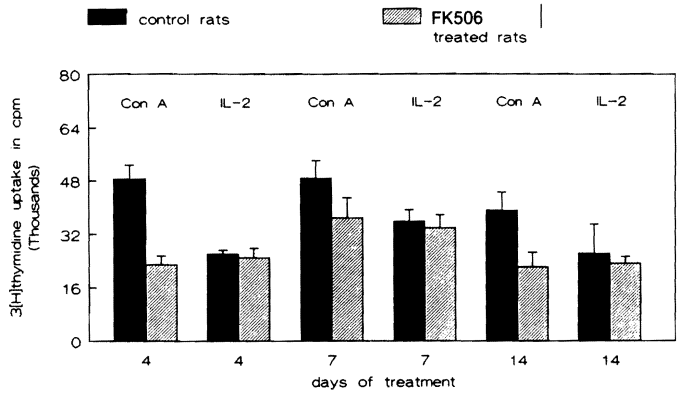

FIGURE 2. Proliferative response in vitro to Con A and IL-2 stimulation. Animals were treated for 4, 7, or 14 days with FK506 at 1.0 mg/kg/day. Controls received 1 ml normal saline/kg/day. Each column represents mean ^{3}[H]thymidine uptake in cpm ±SD from 2 rats in each control and 4 or 5 rats in each FK506-treated group.

TABLE 2. Phenotype and percentage of LGL in fresh spleen cells from control and FK506-treated rats on days 4 and 7[a]

Surface markers	(CD equivalent)	Percentage of positive spleen cells			
		From 4 days		From 7 days	
		Control	FK506-treated	Control	FK506-treated
OX-6	MHC class II	9	11±2[b]	14	12±1
OX-8	CD8	43	44±4	41	49±10
OX-19	CD5	80	84±1	70	83±5
OX-34	CD2	88	89±1	83	89±6
OX-39	IL-2 receptor	7	5±1	5	4±3
OX-41	Macrophages	3	6±1	4	4±1
W3/25	CD4	41	46±3	38	38±1
R73	TCR-alpha/beta	74	79±2	67	76±4
1F4	CD3	73	84±2	83	81±0
ASGM1	NK cells, subpopulations of macrophages, and CTLs	17	25±7	28	36±0.8
3.2.3.	NK cells	12	13±0	14	16±5
LGL		4.5±0.7[c]	4.25±1.2	5.5±3.5	3.8±0.8

[a] Rats were treated for 4 or 7 days with 1 mg/kg/day of FK506 or normal saline. Nylon wool–nonadherent spleen cells were analyzed for the expression of cell surface markers by flow cytometric analysis and for the percentage of LGL in Giemsa-stained cytospin preparations.
[b] Mean percentage ±SD from two rats in each group.
[c] Mean percentage ±SD from two rats in each control group and four rats in each FK506-treated group.

inhibition was found when azathioprine was added as a third agent. Such discrepancies may reflect differences in assays, patient populations, and extent and type of diseases or species studied.

In conclusion, we have shown that NK and ADCC function of spleen cells after 4 and 7 days of in vivo treatment with FK506 was not affected, whereas a slight increase (19% for NK and 22% for ADCC activity) was noted on day 14 compared with control animals. The number of NK cells, as determined by surface marker phenotype and by morphological analysis of the number of LGL, was not different compared with spleen cells from control rats. These findings indicate that FK506 has little or no effect on NK cell numbers or function in the rat.

PETER M. MARKUS[1,2]
MARCEL R. M. VAN DEN BRINK[3]
BRENDA A. LUCHS
JOHN J. FUNG
THOMAS E. STARZL
JOHN C. HISERODT[3]

[1] Peter Markus is a recipient of a Deutsche Forschungsgemeinschaft Research Fellowship.

[2] Address correspondence to: Peter Markus, M.D., Department of Surgery, Transplant Laboratories, University of Pittsburgh, 8200 Blood Bank Building, 203 DeSoto Street, Pittsburgh, PA 15261.

[3] Department of Pathology.

Departments of Surgery and Pathology
Pittsburgh Cancer Institute
University of Pittsburgh
Pittsburgh, Pennsylvania 15261

REFERENCES

1. Kino T, Hatanaka H, Miyata S, et al. FK506: a novel immunosuppressant isolated from streptomyces: II. Immunosuppressive effect of FK506 in vitro. J Antibiot (Tokyo) 1987; 40: 1256.
2. Trinchieri G. Biology of natural killer cells. Adv Immunol 1989; 47: 187.
3. Murase N, Todo S, Lee PH, et al. Heterotopic heart transplantation in the rat under FK506 alone or with cyclosporine. Transplant Proc 1987; 19(suppl 6): 71.
4. Gui XE, Rinaldo CR Jr, Ho M. Natural killer cell activity in renal transplant recipients receiving cyclosporine. Infect Immun 1983; 41: 965.
5. Verschluis DJ, Bijma AM, Vaessen LM, Weimar W. Changes in immunological parameters after conversion from cyclosporine A to azathioprine in renal transplant recipients. Int J Immunopharmacol 1989; 11: 157.
6. Muller C, Schernthaner G, Kovarik J, Kalinowska W, Zielinski CC. Natural killer cell activity and antibody dependent cellular cytotoxicity in patients under various immunosuppressive regimens. Clin Immunol Immunopathol 1987; 44: 12.

Received 29 May 1990.
Accepted 8 August 1990.