Correlation Between Bioassayed Plasma Levels of FK 506 and Lymphocyte Growth From Liver Transplant Biopsies With Histological Evidence of Rejection

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FK 506, a novel immunosuppressive agent, was first used to reverse rejection in liver recipients who had failed to respond to conventional therapy.[1] The successful outcome of the initial study has led to clinical trials of FK 506 as the primary immunosuppressive agent for liver allografts and other organ transplants.[2-4] Ongoing studies include dosage protocols for optimal immunosuppression without significant side effects. Monitoring plasma levels of FK 506 in the transplant patient is performed using an enzyme-linked immunosorbent assay (ELISA) in the presence of anti-FK 506 monoclonal antibody.[5] FK 506 exhibits strong immunosuppressive effect on in vitro models of T-cell activation including mixed lymphocyte reaction and secondary proliferative responses (PLT) of alloreactive T cells.[6,7] Recently we have developed another method to monitor plasma levels of FK 506, a bioassay based on the inhibition of the PLT response of an alloreactive T-cell clone.[8] Our results indicate that the levels of active FK 506 determined by the bioassay are consistently lower than those measured by ELISA.

Histologic examination of needle core biopsies differentiate acute cellular rejection from other causes of liver allograft dysfunction. The major histologic signs of hepatic allograft rejection are: a predominantly mononuclear portal infiltrate and evidence of bile duct damage.[9] Immunophenotypic analysis of this inflammatory infiltrate reveals the presence of CD4+ and CD8+ T cells, macrophages, monocytes, neutrophils, eosinophils, and B cells.[10] Graft infiltrating lymphocytes can be propagated in vitro in the presence of tissue culture media supplemented with recombinant Interleukin 2 (rIL2).[11] In vitro propagation of lymphocytes from transplant biopsies has increased our understanding of the immunological events in rejection. We have shown that in cyclosporine (CyA)-treated patients increased lymphocyte growth occurs in biopsies with proven histologic rejection.[12] In the present study we investigated the frequency of lymphocyte growth from liver allograft biopsies in FK 506-treated patients. We also examined the relationship between lymphocyte growth from biopsies with different histologic diagnoses and the plasma levels of FK 506 as determined by ELISA and bioassay.

MATERIALS AND METHODS

Patient Population

Routine percutaneous liver biopsies were performed on hepatic allograft recipients routinely 10 days posttransplant (protocol biopsy) and when indicated by signs of hepatic dysfunction. One hundred thirty-seven biopsies from 92 patients were studied. All patients were maintained on baseline immunosuppression with FK 506 with low doses of steroids.[1] Histologic diagnosis of acute cellular rejection was based on criteria previously reported.[9]

Generation of Lymphocyte Cultures From Liver Biopsies

Lymphocyte cultures propagated from liver biopsies were obtained using previously published methodologies.[11,12] In brief, a small portion of each biopsy was divided into fragments and cultured in the presence of tissue culture medium containing rIL2 (20 U/mL, Sandoz Pharmaceuticals, Basel, Switzerland). The cultures were observed on an inverted phase microscope for cellular outgrowth at 10–14 days.

Drug Source

FK 506 was supplied in crystalline form by Fugisawa Pharmaceutical Corp (Osaka, Japan). A stock solution of 100 μg/mL was prepared in methanol and kept at −4°C.

Determination of FK 506 Levels in Plasma

Five milliliters of heparinized blood were collected from each liver transplant recipient at the time of the biopsy during the first 2 months following transplantation. All blood samples were incubated at 37°C for 1 hour prior to separation by centrifugation. The plasma was analyzed for FK 506 trough levels using an indirect ELISA[5] and a bioassay.[8] In the bioassay the plasma concentration of FK 506 was calculated from a standard inhibition curve. The calculations were made using a monolinear sigmoidal model curve fitting program (Statistical Analysis System, release 5.18, SAS Institutes, Cary, NC). The IC50 was determined from a standard curve and is the concentration of FK 506 (ng mL) that caused 50% inhibition of the PLT response of an alloreactive T-cell line. The ID50 was determined from the plasma inhibition curve, and is the dilution factor at which patient plasma induced 50% inhibition of the PLT response of the same alloreactive T-cell line used to determine the IC50. From these values, the plasma concentration of FK 506 was calculated as IC50 × ID50.

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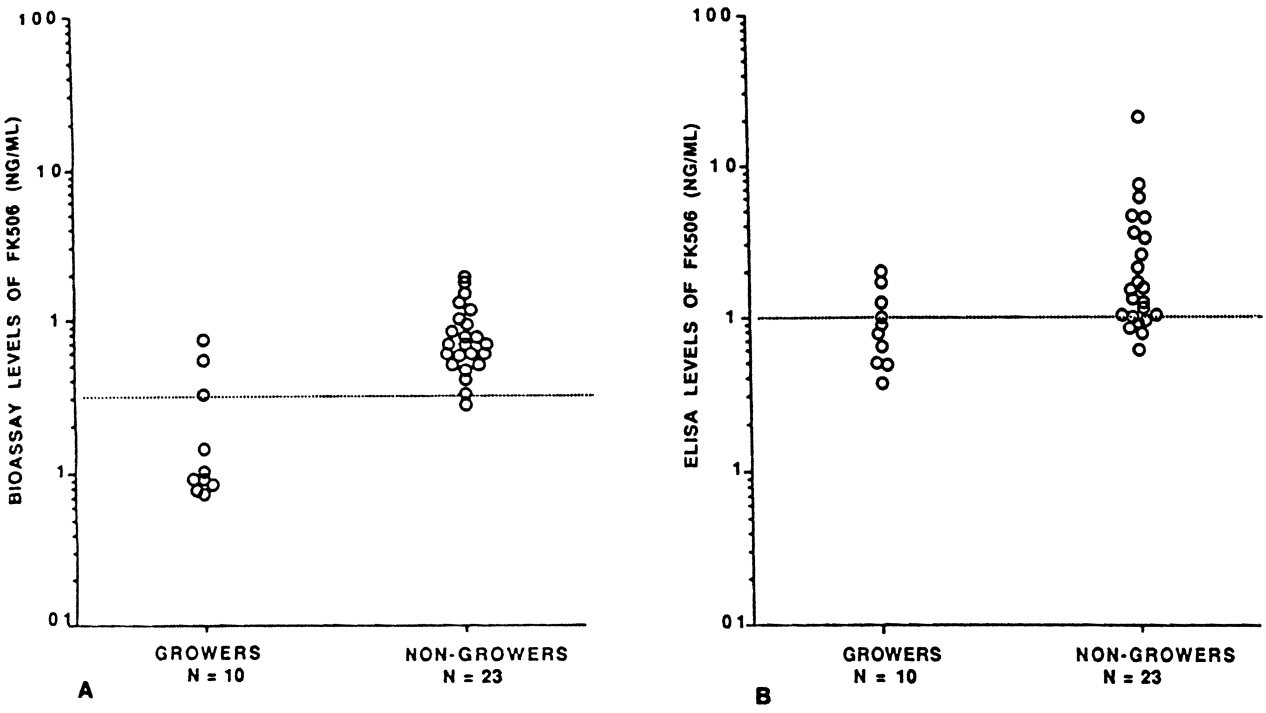

Fig 1. Correlation between biopsy growth and FK 506 plasma levels as determined by **(A)** bioassay and **(B)** ELISA.

RESULTS

Propagation of Lymphocyte Cultures From Liver Allograft Biopsies

Thirty-three percent (22 of 66) of biopsies with rejection yielded lymphocyte cultures, significantly higher (P = .001; chi-square test) than the 7% (5 of 71) growth obtained from biopsies without rejection. The latter biopsies had diagnoses of ischemic injury, cholestasis, viral infection, or nonspecific changes. These results suggest that propagation of lymphocyte cultures from liver allografts is associated with histologic evidence of rejection.

Relationship Between Lymphocyte Growth and Plasma Levels of FK 506

FK 506 levels were determined in 33 plasma samples from 24 patients at the time of liver allograft biopsy. Ten of 33 biopsies yielded lymphocyte cultures. Nine of the 10 cultures were from biopsies with histologic evidence of rejection (Fig 1). One lymphocyte culture was propagated from a biopsy with ischemic injury. In contrast, only 6 of 23 biopsies that failed to grow had histologic changes compatible with an early process of cellular rejection, and the rest of the "non-growers" (17 of 23) were protocol biopsies with no evidence of rejection.

Bioassay FK 506 levels for 10 "growers" were significantly lower than for 23 "non-growers" (P = .002; Mann-Whitney U test) (Fig 1). Using a cutoff point of 0.3 ng/mL, we observed that 21 of 23 non-growers had FK 506 concentration above this level, whereas 8 of 10 of the growers were equal or lower ($P < .001$ by chi-square test). In contrast, differences between growers and non-growers were considerably less for FK 506 levels measured by ELISA (P = .01; Mann-Whitney U test). However, no cutoff level could be established in ELISA-determined FK 506 levels to differentiate between grower and non-grower status. These data suggest that lymphocyte growth from histologically diagnosed rejection positive biopsies is associated with low levels of bioactive FK 506.

DISCUSSION

This study was designed to assess the relationship between in vitro propagation of lymphocytes from liver allograft biopsies and plasma levels of FK 506. A highly significant correlation was found between low levels of biologically active FK 506 (<0.3 ng/mL) and the ability of liver allograft biopsies to propagate lymphocyte cultures. Most of the biopsies that grew lymphocytes exhibited histopathologic changes compatible with acute cellular rejection. In contrast, ELISA measurements of FK 506 levels were less discriminatory between the "growers" and the "non-growers." The levels of FK 506 determined in ELISA were consistently higher than those measured by bioassay.[8] Considering the likelihood that FK 506 will be metabolized after administration, it seems possible that ELISA detects both biologically active drug and nonactive metabolites in the blood. In contrast, only the bioactive drug is detected by the bioassay.[8]

The liver is the primary site of FK 506 metabolism.[13] Jain et al have shown that hepatic dysfunction has a

profound effect on the metabolism and pharmacokinetics of FK 506.[14] In patients where the perioperative graft dysfunction did not resolve promptly, the ELISA plasma levels of FK 506 rose significantly.[15] However, the bioassay FK 506 levels in this group of patients were much lower than that expected according to the ELISA measurement (unpublished data). This lack of correlation between the two methods indicates that during severe hepatic dysfunction the metabolism of FK 506 is altered, resulting in accumulation of biologically non-active metabolites. In contrast, in patients with stable liver allograft function there was a good correlation between the two FK 506 measurements although the bioassay measured levels were consistently lower than levels determined by ELISA.[8] Studies are currently in progress to analyze the factors affecting FK 506 metabolism and their effect on bioassay FK 506 plasma levels.

REFERENCES

1. Starzl TE, Todo S, Fung J, et al: Lancet 2:1000, 1989
2. Starzl TE, Fung J, Jordan M, et al: JAMA 264:63, 1990
3. Todo S, Fung JJ, Starzl TE, et al: Ann Surg (in press), 1990
4. Armitage JM, Kormos RL, Fung JJ, et al: Transplantation (in press), 1990
5. Tamura K, Kobayashi M, Hashimoto K, et al: Transplant Proc 19(Suppl 6):23, 1987
6. Zeevi A, Duquesnoy RJ, Eiras G, et al: Transplant Proc 19(Suppl 6):40, 1987
7. Zeevi A, Duquesnoy RJ, Eiras G, et al: Transplant Proc 20:220, 1988
8. Zeevi A, Eiras G, Burckart G, et al: Transplant Proc 22(Suppl 1):56, 1990
9. Demetris AJ, Jaffe R, Starzl TE, et al: Pathol Annu II:387, 1987
10. Demetris AJ, Qian S, Sun H, Fung JJ: Am J Surg Pathol 14(Suppl 1):49, 1990
11. Zeevi A, Fung JJ, Zerbe TR, et al: Transplantation 41:620, 1986
12. Saidman SL, Demetris AJ, Zeevi A, Duquesnoy RJ: Transplantation 49:107, 1990
13. Venkataramanan R, Jain A, Cadoff E, et al: Transplant Proc 22(Suppl 1):52, 1990
14. Jain AB, Venkataramanan R, Cadoff E, et al: Transplant Proc 22(Suppl 1):57, 1990
15. Starzl TE, Transplant Proc (this issue) 1990