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INHIBITORY EFFECTS OF FK506 IN COMBINATION WITH CsA ON  
HUMAN LYMPHOCYTE ALLOPROLIFERATIVE RESPONSES<sup>1,2</sup>

G. Eiras\*, A. Zeevi\*, S. Todo\*\*, L. Makowka\*\*,  
T.E. Starzl\*\* and R.J. Duquesnoy\*

From the Departments of Pathology\* and Surgery\*\*  
University of Pittsburgh, PA

~~Immunobiology~~  
~~IT, PRESS~~

FOOTNOTES

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2. Address correspondence to:

Adriana Zeevi, Ph.D.

University of Pittsburgh

Division of Clinical Immunopathology

Room 5711, One Children's Place

Pittsburgh, PA 15213-3417

FK506 (FK) is a novel immunosuppressive drug isolated from *Streptomyces tsukubaensis*. Its in vitro effect on lymphocyte proliferation is several hundred fold greater than that of Cyclosporine A (CsA). Studies were conducted on the inhibitory effect of combinations of FK and CsA on lymphocyte proliferation in vitro. The secondary proliferative response (PLT) of alloreactive T cells was significantly inhibited by a combination of 0.06 ng/ml of FK and 1 ng/ml CsA. At these low concentrations, these drugs were ineffective. The synergistic effect between FK and CsA was also demonstrated through sequential exposure of alloreactive lymphocytes to FK followed by CsA. Pretreatment of IL-2 expanded alloreactive cells with FK yielded cell cultures whose PLT activity was considerably more sensitive to low doses of CsA than control cells or CsA pretreated cells. These findings provide evidence for a synergism between the immunosuppressive effects of FK and CsA. The combination of low dose FK plus CsA immunosuppression may benefit organ transplant outcome.

Recent studies have described a new, potent immunosuppressive drug FK 506 which induces considerable prolongation of allograft survival (1,2,3). FK is isolated from *Streptomyces tsukubaensis* in Osaka, Japan. Its molecular structure resembles the macrolide family with a molecular weight of 822 daltons (4). The immunosuppressive effect of FK can be demonstrated in primary mixed lymphocyte reaction (MLR), and in secondary proliferation of alloreactive T cells generated from MLR or propagated from organ transplant biopsies (5,6).

Studies on lymphocyte proliferation in primary MLR have previously shown that inhibition was achieved with doses of FK that were several hundred fold lower than those of CsA (5,6). An example of the difference between FK and CsA is illustrated in Table 1. Significant inhibition (>70%) of the alloreactive response, measured in primed lymphocyte test (PLT), of a liver biopsy propagated lymphocyte culture was achieved with a low dose of 0.25 ng/ml FK. On the contrary, a 200 fold higher concentration of CsA was necessary to obtain the same level of inhibition. In vivo studies have shown a similarly potent effect of FK on the prolongation of transplant survival in experimental animals (7-9).

Polypharmaceutical therapy is currently widely used in organ transplantation to avoid side effects associated with high doses of a single agent. Studies have been conducted to determine in vitro synergism between FK and CsA. Our first approach was to add to lymphocyte cultures low doses of these drug combinations. As shown in Figure 1, the combination of 0.06 ng/ml FK and 10 ng/ml of CsA induced a significant inhibition (40%) of the PLT reactivity of liver biopsy grown cells. On the other hand neither drug was effective at these low doses. In addition, a 10 fold lower dose of CsA (1 ng/ml) 0.06 ng/ml of FK induced a significant PLT inhibition indicating that FK acts synergistically with a relatively wide dose range of CsA (Figure 1). In contrast, a two-fold reduction of the FK dose (to 0.03 ng/ml) added to 10 ng/ml of CsA greatly diminished the synergistic inhibitory effect between these drugs (10,11).

The second approach to study the synergistic effect between these two drugs was through sequential exposure of alloreactive lymphocytes to FK followed by CsA. Because FK and CsA have no effect on IL2 induced proliferation of activated T cells (10), experiments were set up whereby heart transplant biopsy grown lymphocytes were expanded in IL2 in the presence of various concentrations of FK (0.1 ug/ml and 0.01 ug/ml) or CsA (10 ug/ml). After four days in culture, these pretreated cells showed similar PLT reactivity and IL-2 responsiveness as untreated control cultures. On the other hand, FK pretreated cultures showed increased CsA sensitivity when tested in PLT (Table 2). For instance, the PLT response of alloreactive T cells pretreated with 0.1 ng/ml FK was inhibited by 78% with a low dose of 0.05 ng/ml of CsA. On the other hand, pretreatment with 10 ng/ml of CsA did not effect the CsA sensitivity of a subsequent PLT (Table 2).

These findings of a synergism between FK and CsA have an important clinical application in that low doses of these drug combinations may have less adverse side effect but are still effective in increasing transplant survival. Indeed, Todo and his colleagues, using the experimental models of heterotopic heart transplantation in rats and renal allografts in dogs, have recently demonstrated that the most efficient immunosuppressive protocols are combinations of low doses of FK with subtherapeutical doses of CsA and prednisone (7).

The mechanism of the synergism between FK and CsA is unknown. Preliminary observations at our institution have shown

that FK enhances CsA uptake by peripheral blood lymphocytes (12). It is possible that the synergism between FK and CsA might be related to lymphocyte membrane binding of these drugs.

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FIGURE 1

Synergism between the immunosuppressive effects of FK506 and CsA. The donor-specific PLT reactivity of liver biopsy propagated lymphocyte culture was tested in the presence of combinations of low doses of FK (0.06 ng/ml) and CsA (10 ng/ml or 1 ng/ml).