In Vitro Immunosuppressive Effects of FK506 in Combination With Other Drugs

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THE IN VITRO immunosuppressive activity of the newly described drug FK506 (FK) is several hundredfold greater than that of cyclosporine A (CsA).[1,2] The strong inhibitory effect of FK can be demonstrated in primary mixed lymphocyte culture (MLC) and in secondary proliferation of alloreactive T cells generated from mixed lymphocyte response (MLR) cultures or propagated from organ transplant biopsy specimens.[1-3] The inhibitory effect of FK on lymphocyte proliferation appears to be mediated by blocking of interleukin 2 (IL2) release.[1-3]

Our in vitro studies showed a synergism between the immune suppressive effects of FK and CsA[3] on both primary and secondary T lymphocyte activation. In addition, effective prolongation of graft survival in dogs and rats was achieved by using combination therapy with low doses of FK, CsA, and steroids.[4,5]

We describe here the in vitro immunosuppressive effects on human MLR proliferation of combinations of low doses of FK, CsA, and azathioprine (Aza). These studies may have clinical application in that nontoxic low doses of drug combinations may provide effective immunosuppressive treatment.

MATERIALS AND METHODS

MLC

Human peripheral blood lymphocytes were isolated from heparinized blood by the Ficoll-Hypaque gradient method. Unidirectional MLR cultures were set up with 10^5 responder and 10^5 irradiated (2,000 R) stimulator cells in a volume of 200 μL tissue culture medium supplemented with 10% human serum for 6 days. Proliferation was assessed by the degree of ^{3}H-thymidine incorporation during the final 20 hours of incubation.[6]

Drug Sources

FK was kindly supplied by Fujisawa Pharmaceutical Co, Osaka, Japan, as a crystalline powder. It was dissolved in methanol and kept at −4°C. Aza was purchased from Sigma Chemical Co, St Louis, and was dissolved in a minimal amount of dilute NaOH. CsA was obtained from Sandoz (Hanover, NJ) and was dissolved in ethanol.

Dose Effect of FK, CsA, and Aza on Lymphocyte Proliferation

The immunosuppressive effect of FK, CsA, and Aza on MLR reactivity was measured at different concentrations of drugs ranging from 0.01 to 1,000 ng/mL. The results were expressed as percent inhibition by using the following formula: percent inhibition = [1 − (cpm with drugs/cpm without drugs)] × 100.

RESULTS

Effect of Low Doses of FK and CsA on MLR

We have previously shown that FK induced a significant inhibition of the MLR and that this effect was observed at concentrations of drug that were 500-fold lower than those of CsA.[2,3] Combinations of low doses of FK and CsA are illustrated in Fig 1. It may be seen that a significant inhibition of 60% was achieved by adding to the lymphocyte culture FK and CsA at concentrations of 0.05 and 10 ng/mL, respectively. On the other hand, at these low concentrations neither drug was effective. Our data also suggest that the synergistic effect between CsA and FK is primarily dependent on the concentration of the latter. For instance, the addition of 0.05 ng/mL of FK to lymphocyte cultures in the presence of various concentrations of CsA (1 to 10 ng/

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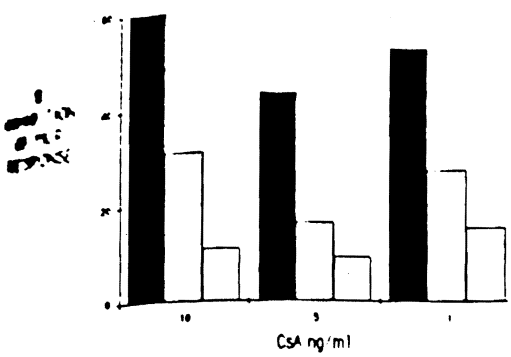

Fig 1. Effect of low doses of FK and CsA on MLR. Inhibitions of MLR in the presence of different concentrations of FK (0.05 and 0.025 ng/mL) were 2% and 0%, respectively. (■), CsA + FK 0.05 ng/ml; (□), CsA + FK 0.025 ng/ml; (□), CsA.

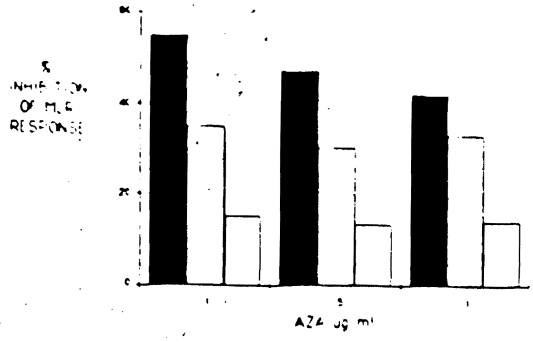

Fig 2. Effect of low doses of FK and Aza on MLR. Inhibitions of MLR in the presence of different concentrations of FK (0.05 and 0.01 ng/mL) were 7% and 4%, respectively. (■), Aza + FK 0.05 ng/ml; (□), Aza + FK 0.01 ng/ml; (□), Aza.

mL) induced a significant inhibition of MLR (43% to 61%). On the other hand, a twofold reduction of the FK dose (0.025 ng/mL) added to the same concentrations of CsA (1 to 10 ng/mL) greatly diminished the synergistic inhibitory effect of these drug combinations.

Effect of Low Doses of FK and Aza on MLR

Significant inhibition of the MLR (>50%) was demonstrated in the presence of FK at concentrations that were at least 10^6-fold lower than those of Aza (data not shown). The inhibitory effect of the combination of low doses of FK and Aza was tested on primary MLR proliferation. The two doses of FK (0.05 and 0.025 ng/mL) that alone showed no inhibition of MLR were added to lymphocyte cultures that contained Aza (1 to 0.1 μg/mL). Maximum inhibitory effect was achieved by combining FK with Aza at concentration of 0.05 ng/mL and 1 μg/mL, respectively (Fig 2). The synergism between FK and Aza was mostly affected by the concentration of FK in the mixture, as previously demonstrated for the FK-CsA combination (Fig 2).

These findings suggest that certain low concentrations of FK and CsA or FK and Aza have similar synergistic inhibitory effects on primary T lymphocyte activation as measured by the MLR test.

DISCUSSION

These results extend our original observations that low doses of FK and CsA exhibit a synergistic immunosuppressive effect on lymphocyte proliferation.[3] This synergism was observed not only between FK and CsA but also in the combination of low doses of FK and Aza.

Significant synergism was detected in the presence of the maximum FK concentration that showed no or minimal immunosuppressive effect on lymphocyte proliferation. The same pattern was observed for both combinations, FK-CsA and FK-Aza, which suggests that the presence of a certain amount of FK was essential in achieving a significant synergistic effect.

The effectiveness of any drug therapy must consider the side effects that seem to be associated with higher concentrations of the drug. A clear advantage of using combination of low doses of drugs, FK-CsA or FK-Aza to prolong transplant survival is that there would be a reduction of these complications. Recently it was shown that combination therapy with low doses of FK, CsA, and steroids may effectively induce prolongation of cardiac allografts in rats and kidney allografts in dogs.[7]

The mechanism of synergism between FK and other drugs is not well understood. CsA

and Aza exhibit their immunosuppressive effects on lymphocyte proliferation through different mechanisms. The inhibitory effect of CsA is mediated by inhibition of IL2 release, whereas Aza interferes with DNA synthesis of all dividing cells. However, both drugs show a similar synergistic effect when used in low doses in combination with FK. Recent studies have shown that cellular uptake of ^{3}H-CsA is enhanced when peripheral blood lymphocytes are incubated in a medium containing FK.[8] These findings may indicate that the synergism between FK and CsA might be related to lymphocyte membrane binding of these drugs. Studies are currently initiated to investigate the influence of FK on the uptake of other drugs such as Aza and steroids.

Furthermore, the development of a sensitive test to quantitate the amount of FK on the membrane v that bound intracellularly will enable us to obtain a better understanding of the mechanism of synergism between FK and other drugs.

REFERENCES

1. Ochiai T, Nakajima K, Kagala M, et al: Transplant Proc 19:1284, 1987

2. Zeevi A, Duquesnoy RJ, Eiras G, et al: Surg Res Commun 1:315, 1987

3. Zeevi A, Duquesnoy RJ, Eiras G, et al: Transplant Proc 19:40 (suppl 6), 1987

4. Lee P, Murase N, Todo S, et al: Surg Res Commun 1:325, 1987

5. Todo S, Makowka L, Starzl T: Surg Res Commun 1:330, 1987

6. Zeevi A, Fung J, Zerbe TR, et al: Transplantation 41:620, 1986

7. Todo S, Lee P, Demetris J, et al: Transplant Proc 19:57 (suppl 6), 1987

8. Sanghvi A, Warty V, Zeevi A, et al: Transplant Proc 19:45 (suppl 6), 1987