Assessment of the Biological Activity of Cyclosporine Metabolites Using the Human JURKAT Cell Line

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CYCLOSPORINE (CyA) is a potent immunosuppressive agent that is metabolized extensively by the cytochrome P_{450} system. The importance of the various metabolites of CyA in both immunosuppression and nephrotoxicity has been debated for several years. We have hypothesized that as the CyAs become more polar they progressively lose immunosuppressive activity but gain nephrotoxic activity.[1,2] However, this claim has been disputed by others who suggest that the metabolites have neither immunosuppressive nor nephrotoxic activities.[3] Unfortunately, the assays used to measure metabolite activity vary in sensitivity and we therefore set out to compare the immunosuppressive activities of the metabolites using a more sensitive and reproducible system.[4,5] This assay uses the mitogen-induced production of interleukin-2 (IL-2) in the human T-cell leukemia line, JURKAT 6.8, which is as sensitive to the effects of CyA as any known biologic event.

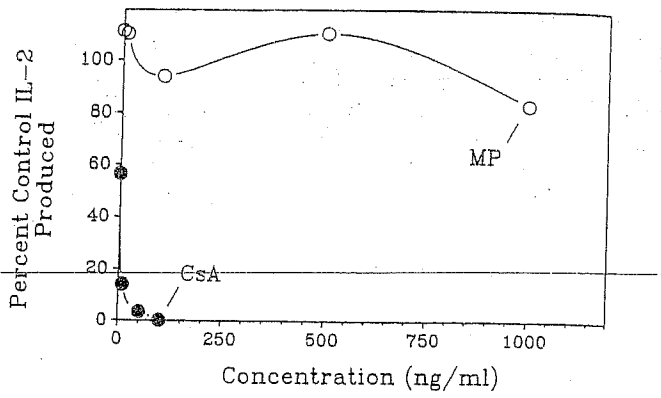

Fig 1. Effect of cyclosporine (CyA) and steroids on IL-2 production by JURKAT cells. Methylprednisolone sodium succinate (MP) was diluted in PBS and aliquoted into tissue culture plates as described for CyA.

METHODS

JURKAT 6.8 cells (provided by Dr Kendall Smith, Dartmouth College) were resuspended to 5×10^5 cells/mL in RPMI 1640 plus 10% fetal bovine serum and plated in 24-well tissue culture plates (1 mL/well). CyA metabolites were isolated from the urine of renal allograft recipients by pumping filtered urine into a reverse-phase adsorption column packed with 40 μm C-18 at a rate of 100 mL/min. The metabolites were then eluted with 100% acetonitrile and subsequently purified by preparative elution high-performance liquid chromatography (HPLC) on a 7 μm octylsilica column. The identity and purity of the isolated metabolites were confirmed by analytic HPLC and mass spectroscopy. The metabolites were reconstituted in ethanol, aliquoted into the 24-well plates, and evaporated under a laminar flow hood prior to the addition of cells. The cells were then incubated with the metabolites for 60 minutes at 37°C, and then stimulated with PHA (1:40 dilution; HA15, Wellcome Diagnostics, England) and 1nM PMA (Sigma Chemical Co, St Louis, Mo) at 37°C in 95% air/5%CO_2. Supernatants were collected 24 hours later and assayed for IL-2 activity using the murine HT-2 cell line as previously described.[6]

RESULTS

Production of IL-2 by JURKAT 6.8 cells was extremely sensitive to CyA, which acts directly on T cells, but was insensitive to methylprednisolone, which affects primarily the accessory cells (Fig 1). IL-2 production by JURKAT 6.8 cells was 10-fold more sensitive to CyA than was IL-2 production by normal human peripheral blood mononuclear cells (data not shown). We therefore used this system to assess the immunosuppressive activities of the CyA metabolites. As can be seen in Fig 2, metabolites AM1 (M17) and AM9 (M1) exhibited significant immunosuppressive activity, although they were less active than the parent compound. The IC_{50} of CyA was 7 ng/mL in this system, compared to 50 ng/mL for AM1 and 100 ng/mL for AM9. The secondary metabolite AM1c (M18) was at least 100-fold less active than the primary metabolites, and the polar metabolites AM19 (M8) and AM1A (M203-218) were essentially inactive. None of the metabolites inhibited the growth of JURKAT 6.8 cells, which is not dependent upon the presence of IL-2, at concentrations up to 1000 ng/mL (data not shown).

DISCUSSION

The data presented here clearly suggest that the most potent metabolite (M17) has approximately 10% of the immunosuppressive activity of the parent compound. Based on the concentration of this metabolite in the blood of CyA-treated patients, we might predict that AM1 would

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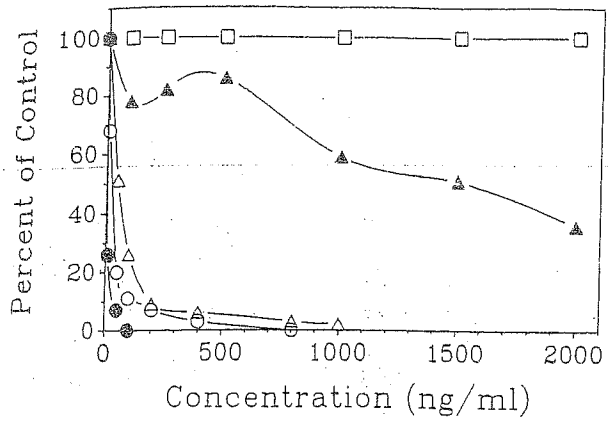

Fig 2. Effect of cyclosporine (CyA) and metabolites on IL-2 production by JURKAT cells. JURKAT 6.8 cells were treated with various concentrations of CyA (●), AM1 (○), AM9 (△), and AM1c (▲). The results for the polar metabolites AM19 and AM1A are shown by one line (□).

provide less than 20% of the overall immunosuppressive effect in vivo. However, Kunzendorf et al[7] reported that the blood of renal allograft recipients with a low frequency of rejection had twofold higher trough levels of metabolites AM1 and AM9 than the blood of patients who frequently rejected. Furthermore, Kim et al[8] reported that a pool of CyA metabolites, which contained AM1, AM9, AM1c, and other polar metabolites, inhibited the rejection of rat intestinal allografts without evidence of nephrotoxicity. This observation is particularly intriguing in light of the fact that the metabolites were cleared from the rat circulation much more rapidly than the parent compound. These observations highlight the need to evaluate the in vivo immunosuppressive effects of CyA more fully.

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