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HEPATOTROPIC PROPERTIES IN DOGS OF HUMAN FKBP, THE BINDING PROTEIN
FOR FK506 AND RAPAMYCIN¹

Cyclosporine and FK506 have been shown with two experimental models to influence liver structure, function, and the capacity for regeneration. Both drugs augment the liver regeneration in rats that is the normal response to partial hepatectomy (1). In addition, the increase in hepatocyte replication that is caused by portacaval shunt in dogs is more than doubled by intrahepatic infusion of these drugs via the tied-off central portal vein. Also, the atrophy and organelle disruption caused by portacaval shunt is prevented (2, 3). Immune modulation of lymphocytes and NK cells as an explanation for these so-called "hepatotropic" effects has been ruled out by direct experimentation in nude rats (4).

The foregoing findings, as well as observations of manifold metabolic effects in patients treated either with FK506 or with cyclosporine, have led to a hypothesis that these drugs modify signal transduction in a variety of cells, not limited to those of the immune system (3, 5). Consistent with the hypothesis is the recent demonstration that FK506 and cyclosporine inhibit signaling processes in the mast cell at concentrations similar to those used to block signaling pathways in T cells (6; also Schreiber SL, et al., unpublished observations). Central to the signal transduction hypothesis was the discovery that the cytosolic receptors for FK506 and rapamycin (FK506-binding protein, FKBP)* and cyclosporine (cyclophilin) are distinct proteins that exhibit peptidyl-prolyl cis trans isomerase (rotamase) activity (7, 8). Rotamases have been shown to facilitate protein folding (9, 10) and to catalyze the interconversion of

rotamers of peptidyl-prolyl bonds in vitro (11, 12). However, the inhibition of this enzymatic activity is not related to the action of these drugs in the T cell (13) or the mast cell (Schreiber SL, Hohman RJ, et al., unpublished observations). Instead, these proteins are envisioned as members of a class of small molecular weight cytoplasmic proteins (collectively called immunophilins) that modulate a wide variety of calcium-dependent signal transduction pathways by forming complexes with immunosuppressive drugs (13, 14). The potential importance and pleiotropism of the immunophilin network is emphasized by the ubiquitous distribution of cyclophilin as detected by immunocytochemical techniques in the cytoplasm of virtually all cells in the body, and in the nuclei of some (15, 16).

How the immunophilins are activated by immunosuppressive agents is not known, nor is it known if modulation of the immunophilin's activity requires binding of an exogenous (drug) or endogenous ligand. With the cloning and overexpression of FKBP in bacteria (17), it became possible to test the intrinsic activity of FKBP, free of drug. Briefly, with the use of the expression cassette polymerase chain reaction (18), the proper sequences were added to the FKBP cDNA that allow for its overexpression in *E. coli*. Following a simple purification protocol (17), recombinant human FKBP was demonstrated to be pure by SDS-PAGE analysis.

The recombinant FKBP was dissolved in normal saline modified by the addition of 5 mM ammonium acetate and 5 mg/L bovine serum albumin (to prevent adhesion to the plastic tubing). After performing completely diverting portacaval shunt, an infusion catheter was inserted into the tied-off left portal vein for pump-driven constant infusion of the FKBP over the next 4 days. At the end of 4 days, the animals were injected with 0.2 mCi/kg intravenous ³H-thymidine (New Eng-

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* Abbreviation: FKBP, FK506-binding protein.

TABLE 1. Effect of cloned FKBP infusion in left portal branch for 4 days after canine ECK fistula

Animal No.	Drug dose (/kg/day)		Hepatocyte (size of units)		Labelled nuclei (per 1000 hepatocytes)	
			Left	Right	Left	Right
1	—	—	0.0812	0.0764	3.9	4.0
2	—	—	0.0999	0.1007	4.1	4.0
3	—	—	0.0899	0.0979	4.0	4.1
4	Insulin	0.4 units	0.1497	0.0924	10.2	4.2
5	FKBP	200 ng	0.1149	0.1003	5.0	4.0
6	FKBP	500 ng	0.1174	0.1011	5.8	3.8
7	FKBP	500 ng	0.1181	0.1032	6.7	4.2
8	FKBP	1000 ng	0.1298	0.1053	8.2	4.1
Controls (n = 4)	—	—	0.1005±0.007	0.0980±0.020	3.98±0.06	3.91±0.06

land Nuclear, Boston) and killed 2 hr later. Specimens were obtained for comparison of the hepatocytes in the left (infused) and right (not infused) liver lobes using previously described morphometric and autoradiographic techniques (3, 19). Autoradiography was carried out with Kodak NTB2 liquid emulsion with an exposure time of at least 30 days. The number of replicating hepatocytes as an index of hepatocyte regeneration was determined by counting the number of ³H-thymidine-labeled nuclei per 1000 hepatocytes. The size of individual hepatocytes (index of hypertrophy or atrophy) was determined by tracing out at least 500 midzonal liver cells projected on standard-thickness paper, cutting out the individual silhouettes, and weighing each. This method has been shown to be accurate for determining hepatocyte cell size and has been validated by planimetry and by studies of unicellular organisms, the size of which has been determined directly. An insulin infusion, which we use to verify the integrity of the test system (2, 19), prevented the usual hepatocyte atrophy and increased the hepatocyte proliferation in the directly infused lobes only (Table 1).

Because it has been assumed that immunophilins are activated by binding with a drug or other ligands, it was suspected when these experiments were planned that the cloned FKBP would be inert. Instead, a substantial dose-related hepatotrophic response was caused (Table 1), with partial prevention of the hepatocyte atrophy and augmentation of hepatocyte proliferation in the directly infused lobes only. This is the first demonstration of a physiologic action of an unbound immunophilin, and one with important implications. One possibility for resolving the discrepancy that a cytosolic protein has a physiologic activity outside of the cell is that there may be a secretory variant of the FKBP family that has hepatotrophic effects. Thus, one could argue that the extracellular concentration of this putative hormone has been increased by infusing a closely related protein into the liver. An alternative possibility is that the recombinant FKBP bound with a natural circulating ligand, and that the complex activated regulatory growth factors, possibly including cytokines.

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REFERENCES

1. Francavilla A, Barone M, Todo S, Zeng Q, Porter KA, Starzl TE. Augmentation of rat liver regeneration by FK 506 compared with cyclosporine. *Lancet* 1989; 1: 1248.
2. Mazzaferro V, Porter KA, Scotti-Foglieni CL, et al. The hepatotrophic influence of cyclosporine. *Surgery* 1990; 107: 533.
3. Starzl TE, Porter KA, Mazzaferro V, Todo S, Fung J, Francavilla A. Hepatotrophic effects of FK 506 in dogs. *Transplantation* 1991; 51: 67.
4. Francavilla A, Barone M, Van Den Brink MRM, et al. Further studies on the stimulatory effect of FK 506 on liver regeneration by using the NHI-RNU nude rat model. *Hepatology* 1990; 12: 942.
5. Starzl TE, Fung JJ. *Contempo 90: transplantation*. *JAMA* 1990; 263: 2686.
6. Hultsch T, Rodriguez JL, Kaliner MA, Hohman RJ. Cyclosporin A inhibits degranulation of rat basophilic leukemia cells and human basophils. *J Immunol* 1990; 144: 2659.
7. Harding MW, Galat A, Uehling DE, Schreiber SL. A receptor for the immunosuppressant FK506 is a cis-trans peptidyl-prolyl isomerase. *Nature* 1989; 341: 758.
8. Siekierka JJ, Hung SHY, Poe M, Lin CS, Sigal NH. A cytosolic binding protein for the immunosuppressant FK 506 has peptidyl-prolyl isomerase activity but is distinct from cyclophilin. *Nature* 1989; 341: 755.
9. Fischer G, Bang J. The refolding of urea-denatured ribonuclease A is catalyzed by peptidyl-prolyl cis-trans isomerase. *Biochim Biophys Acta* 1984; 828: 39.
10. Tropschug M, Wachter E, Mayer S, Schonbrunner ER, Schmid FX. Isolation and sequence of an FK506-binding protein from *N. crassa* which catalyses protein folding. *Nature* 1990; 346: 674.
11. Fischer G, Wittmann-Liebold B, Kiefhaber T, Schmid FX, Lang K. Cyclophilin and peptidyl-prolyl cis-trans isomerase are probably identical proteins. *Nature* 1990; 337: 476.

12. Albers MW, Walsh CT, Schreiber SL. Substrate specificity for the human rotamase FKBP: a view of FK 506 and rapamycin as leucine-(twisted amide)-proline mimics. *J Org Chem* 1990; 55: 4984.
13. Bierer BE, Mattila PS, Standaert RF, et al. Two distinct signal transmission pathways in T lymphocytes are inhibited by complexes formed between an immunophilin and either FK 506 or rapamycin. *Proc Natl Acad Sci USA* 1990; 87: 9231.
14. Schreiber SL. Chemistry and biology of the immunophilins and their immunosuppressive ligands. *Science* 1991; 251: 283.
15. Marks WH, Harding MW, Handshumacher R, Marks C, Lorber MI. Cyclophilin, the cyclosporine receptor: immunochemical distribution in normal mammalian tissues. *Transplantation*
16. Ryffel B, Woerly G, Greiner B, Haendler B, Mihatsch MJ, Foxwell BMJ. Distribution of the cyclosporine binding protein cyclophilin in human tissues. *Immunology* 1991; 72: 399.
17. Standaert RF, Galat A, Verdine GL, Schreiber SL. Molecular cloning and overexpression of the human FK506-binding protein FKBP. *Nature* 1990; 346: 671.
18. MacFerrin KD, Terranova MT, Schreiber SL, Verdine GL. Overproduction and dissection of proteins by the expression cassette polymerase chain reaction. *Proc Natl Acad Sci USA* 1990; 87: 1937.
19. Starzl TE, Watanabe K, Porter KA, Putnam CW. Effects of insulin, glucagon, and insulin/glucagon infusions on liver morphology and cell division after complete portacaval shunt in dogs. *Lancet* 1976; 1: 821.

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