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INHIBITION OF LIVER, KIDNEY, AND INTESTINE REGENERATION BY RAPAMYCIN[1,2]

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For a decade, liver transplant recipients have been treated with cyclosporine, a drug with modest hepatotoxicity (1). Concern that CsA might inhibit hepatic regeneration or the ability of the transplanted liver to adjust its size to that of the recipient prompted studies by Makowka et al. (2) and others (3–5), which showed that regeneration actually was enhanced. A newer unrelated immunosuppressive agent, FK506, has the same properties (5). In addition, these 2 drugs have other actions that are collectively called hepatotrophic. The increase in hepatocyte replication that is caused by portacaval shunt in dogs is more than doubled by intrahepatic infusion of either of these drugs via the tied-off central portal vein, and the expected atrophy and organelle disruption is prevented (6,7). Direct experimentation in nude rats has ruled out immune modulation of lymphocytes and NK cells as an explanation (8).

These observations have contributed to a hypothesis that CsA and FK506 modify signal transduction in a variety of cells, not limited to those of the immune system (7,9,10), and including signaling pathways associated with growth control. If this were true, it would explain the remarkable spectrum of adverse as well as desired effects of these agents. Important elements in this hypothesis included 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 (11,12) and that these small-molecular-weight cytoplasmic proteins are in virtually all cells in the body, as well as in the nuclei of some (13,14).

How these 3 drugs (cyclosporine, FK506, and rapamycin) block or modify signaling pathways (15) is not understood. Rotamases have been shown to facilitate protein folding (16,17) and catalyze the interconversion of rotamers of peptidylprolyl bonds in vitro (18,19), but the inhibition of this enzymatic activity is not related to the action of these drugs on T lymphocytes (20). The fact that signal transduction in T-lymphocytes is blocked in different ways by FK506 and rapamycin (20,21) is of great interest, particularly because these 2 drugs are chemically related and have the same binding site (FKBP). Consequently, the hepatotrophic qualities of rapamycin were determined with the same dog and rat test models as had been used previously to test CsA, FK506 (2–7), and recombinant FKBP (22). In addition, the influence of rapamycin

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[2]The rapamycin was a gift from Dr. Joseph Y. Chang of the WyethAyerst Research Company, Princeton, New Jersey.

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on renal and intestinal regeneration was determined. The results with rapamycin were profoundly different from those observed previously with the other 2 drugs.

The dogs underwent a completely diverting portacaval shunt. Immediately afterwards, an infusion catheter was inserted into the tied-off left portal vein for pump-driven constant infusion of the rapamycin over the next 4 days in the doses shown in Table 1. At the end of 4 days, the animals were injected with 0.2 MCi/kg intravenous ^{3}H thymidine (New England 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 (6,7). There was no effect (Table 1) on the hepatocyte atrophy and the increased hepatocyte renewal that are characteristic after Eck fistula and that are prevented both by cyclosporine (6) and by FK506 (7).

For the regeneration experiments, fasted adult male inbred Fisher 344 rats weighing 180–200 g (Hilltop Lab Animals Inc., Scottsdale, P A) were assigned to groups, given water and food for 4 days, and treated daily with vehicle or rapamycin. On the fourth day, between 0900 and 1030, groups 2–5 underwent 70% hepatectomy under light ether anesthesia (5). Rats of groups 7 and 8 had right nephrectomy (2), and those in group 10 and 11 had a 40% resection of small intestine excluding the jejunum. Twenty-four hr after the operations or control period, 50 MCi-^{3}H-thymidine was administered by intraperitoneal injection. The rats were killed 2 hr later by guillotine. Hepatic DNA synthesis and liver mitosis, kidney DNA synthesis and small bowel DNA synthesis were performed as described previously in cyclosporine and FK506 experiments (5,23).

Hepatic regeneration was significantly inhibited by RPM with a dose relationship so that at 1.0 mg/kg the normal response was virtually eliminated (Table 2). Regeneration of the kidney and intestine was significantly inhibited with the intermediate dose of 0.3 mg/kg/day (Table 3).

These results add to the significance of the previous demonstration that rapamycin affects T-lymphocytes differently than FK506 (20,21) and lend support to the view of rapamycin as an inhibitor of growth factor receptor-associated signaling pathways. Instead of promoting liver regeneration and having the other hepatotrophic qualities common to FK506 and cyclosporine, rapamycin was antihepatotrophic. In rats submitted to hepatectomy, it inhibited instead of augmenting liver regeneration and in dogs submitted to Eck fistula it did not prevent the hepatocyte atrophy and organelle disruption that are caused by this operation but prevented by cyclosporine(6) and FK506 (7). The observations in rats constitute the first physiologic evidence that the immunophilin network that is thought to be pleiotropic in its metabolic, immunologic, and growth control actions may be involved in countervailing (opposite effects) regulatory processes. Regeneration of the kidney and intestine was also retarded by RPM administration, indicating that the growth-inhibitory effect of RPM is not liver-specific.

This suggests that rapamycin, a profound immunosuppressant, also should be examined as an antitumor agent. The implications are obvious in general and in transplantation in particular. Efforts to treat primary hepatic malignancies by total hepatectomy and liver transplantation have been plagued by a high rate of tumor recurrence. There might be a possibility of treating these patients or others whose extirpative procedures have been done for the reason of malignancy (24) with a drug that could prevent both rejection and tumor cell replication. Striking antineoplastic properties of rapamycin against a variety of mouse tumors (25) and against human tumors transplanted into nude mice (26,27) have been described.

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Table 1

Hepatocyte size and mitosis in dogs after ECK fistula with continuous intraportal infusion of rapamycin

Group	No. dogs	Rapamycin (mg/kg/day)	Mitosis (No. labels hepatocytes per 1000 hepatocytes)[a]		*P*	Cell size (units)[a]		*P*
			Right lobe	Left lobe		Right lobe	Left lobe	
1	5	0	4.2±0.33	4.4±0.2		0.1043±0.018	0.1102±0.016	
2	2	0.1	4.2±0.4	4.1±0.5	NS	0.1046±0.01	0.1062±0.08	NS
3	2	0.5	3.9±0.2	3.9±0.01	NS	0.1018±0.009	0.1018±0.01	NS

[a]Mean ± SD.

Table 2

Effect of preoperative intramuscular rapamycin for 4 days on rat liver regeneration

Group	No. rats	Dose/day (mg/kg)	Hepatectomy	DNA synthesis (×10^3 c.p.m./MG DNA)	% Hepatocytes in mitosis	*P*
1	5	—	—	3.5±0.4	1.7±0.1	—
2	10	—	70%	185±13.0	33.4±3.20	—
3	10	0.1	70%	92±8.5	16.8±4.2	<0.05 vs. 2
4	10	0.3	70%	40±6.2	11.2±3.3	<0.005 vs. 2
5	10	1.0	70%	3.5±3.5	4.8±0.5	<0.005 vs. 2

Table 3

[^{3}H]-thymidine incorporation in kidney and small intestine from normal, nephrectomized, or small intestine–resected rats treated or not treated for 4 preoperative days with rapamycin (0.3 mg/kg/day i.m.)

Group	n	Treatment	Surgery	DNA synthesis (×10^3/c.p.m./mgDNA)	*P*
6	5	Vehicle	No	2.4±0.2	—
7	5	Vehicle	Unilateral nephrectomy	3.8±2.3	—
8	5	RPM	Unilateral nephrectomy	0.5±0.01	<0.0005 vs. 7
9	5	Vehicle	No	17.2±2.3	
10	5	Vehicle	40% Small-intestine resection	62.3±10.4	
11	5	RPM	40% Small-intestine resection	29.3±5.1	<0.05 vs. 10