

## The Effects of FK 506, Cyclosporine, and Rapamycin on Liver Growth In Vitro and In Vivo

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**I**N ADDITION to immunosuppression, complex and for the most part similar metabolic effects are caused by cyclosporine (CyA) and FK 506, even though these drugs are chemically unrelated and have different cytosolic binding sites (called immunophilins) (Table 1). One of the striking nonimmunological effects of CyA and FK 506 is hepatocyte proliferation, which can be demonstrated in the *in vivo* models shown in Table 2,<sup>1-6</sup> but not in hepatocyte cultures.<sup>7</sup>

In this report, we will emphasize some *in vivo* and *in vitro* experiments with another drug, rapamycin (RPM), which is chemically related to FK 506 and has the same binding site (called FK-binding protein or FKBP).<sup>8,9</sup> It was found that RPM has hepatocyte growth effects opposite to CyA and FK 506.

### PARTIAL HEPATECTOMY EXPERIMENTS

Makowka et al<sup>1</sup> first demonstrated that 4 days of treatment with CyA at an oral dose of 10 mg/kg per day increases hepatocyte proliferation in rats then submitted to 70% partial hepatectomy (PH). The same stimulatory effect was found with 4 days of FK 506 given intramuscularly at the dose of 1 mg/kg (Fig 1).<sup>4</sup> The effect was liver specific (Fig 2) in that FK 506 administration did not alter the regeneration response of remaining small intestine after 40% resection, or that of the remaining kidney after unilateral nephrectomy.

At first, it was suspected that the foregoing results reflected immune modulation of regeneration which had been perturbed (augmented) by T-cell-specific immunosuppression. This hypothesis was tested by repeating the experiments in T-cell-deficient nude rats.<sup>10</sup> The results were the same as normal animals (Fig 3) and suggested that the augmentation of regeneration was by a nonimmunologic mechanism.

In contrast to the augmentation of liver regeneration caused by 4 days of pretreatment with CyA or FK 506, the administration of rapamycin in the 70% PH rat model

Table 1. Effects of CyA and FK 506

Immunological Effects	Nonimmunological Effects
Inhibition of T-lymphocyte proliferation and activation	Neurotoxicity
	Nephrotoxicity
	Diabetes
	Hair growth
	Hypercholesterolemia
	Modulation of hepatic size and proliferation

Table 2. Methods

In vitro:	Primary hepatocyte cultures. <sup>7,14</sup>
In vivo:	Seventy percent partial hepatectomy (PH) in rats (pre-operative administration of the drugs [for 4 days] followed by 70% PH + another administration. Sacrifice 24 hours later). <sup>1-4</sup>
	Portacaval shunt (PCS) in dogs (sacrifice after a 4-day continuous infusion of the drug in the left portal vein). <sup>5,6,12,13</sup>

induces a strong and dose-related inhibition of hepatocyte proliferation (Fig 4).<sup>11</sup> RPM also inhibited DNA synthesis in the remaining small intestine after 40% resection, and in the remaining kidney after unilateral nephrectomy (Fig 2).<sup>11</sup>

### THE ECK FISTULA

The other *in vivo* model was in dogs, who underwent a functional end-to-side portacaval shunt (PCS) (Table 2). For 4 days after the shunt, drugs were continuously infused through an external pump into the detached left branch of the portal vein (Fig 5).<sup>12</sup> Hepatocyte size and organelle structure were quantitated and proliferation was estimated by nuclear thymidine incorporation (classical autoradiography). These parameters were compared in the left (treated) versus the right (untreated) lobes. Both CyA (not shown) and FK 506 (Table 3) greatly increased the rate of hepatocyte replication.<sup>5,6</sup> They also prevented the acute hepatocyte atrophy and disruption of organelles which are caused by portacaval shunt.<sup>5,6</sup> In addition, it was demonstrated that infusion of recombinant FKBP in drug-free Eck fistula animals also had these same "hepatotrophic" effects of proliferation and maintenance of hepatocyte size and organelle integrity (Table 4).<sup>13</sup>

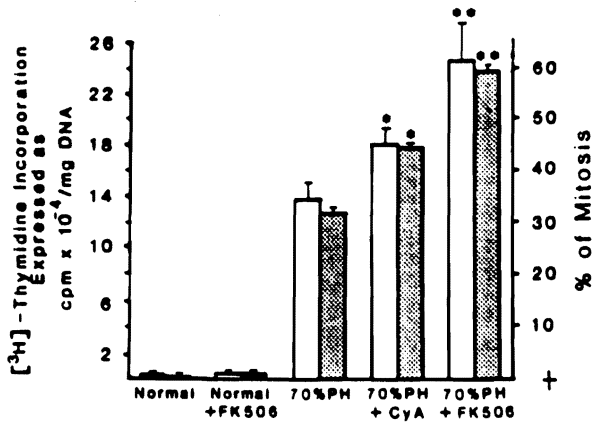
RPM infused into the left portal branch had none of the

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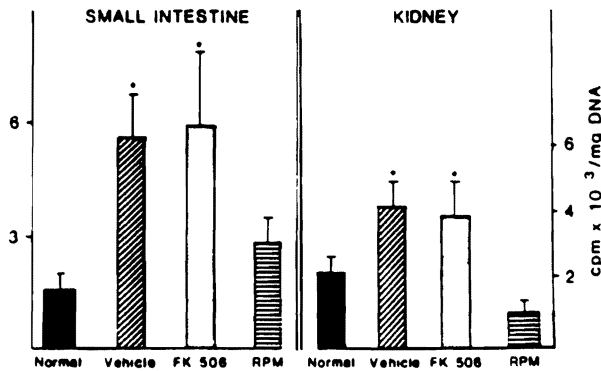
**Fig 1.** [<sup>3</sup>H]thymidine incorporation and percentage of mitosis in normal and 70% hepatectomized rats treated or not treated with FK 506 and CyA. The animals were given oral CyA and intramuscular FK 506 at 10 mg/kg and 1 mg/kg body weight, respectively, or vehicle, for 3 days before surgery and again just after completing hepatic resection. The values are the means from at least 15 rats ± SD. \*Significantly different from 70% hepatectomized rats ( $P < .05$ ). \*\*Significantly different from 70% hepatectomized rats ( $P < .01$ ) and CyA-treated 70% hepatectomized rats ( $P < .05$ ).

stimulatory and antiatrophic effects of CyA, FK 506, and recombinant FKBP.

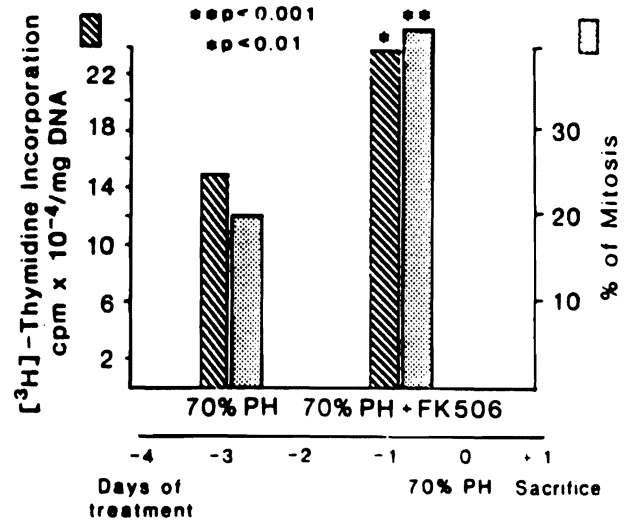
**HEPATOCTYTE CULTURE**

It was previously shown that CyA and FK 506 did not alter hepatocyte proliferation at doses in the medium up to 400 ng/mL. The lack of effect was in standard hepatocyte culture or in culture enriched with 10 ng/mL epidermal growth factor.<sup>7</sup>

In contrast, RPM inhibited hepatocyte proliferation with



**Fig 2.** [<sup>3</sup>H]thymidine incorporation in normal and nephrectomized (right) and in the normal and 40% small intestine resection (left) rats treated or not treated with RPM or FK 506. The animals received an injection of vehicle or drug solution as described in Fig 1. The values reported are the means from 5 rats ± SD. \*Significantly different from thymidine incorporation in kidneys from untreated, operated animals ( $P < .05$ ).

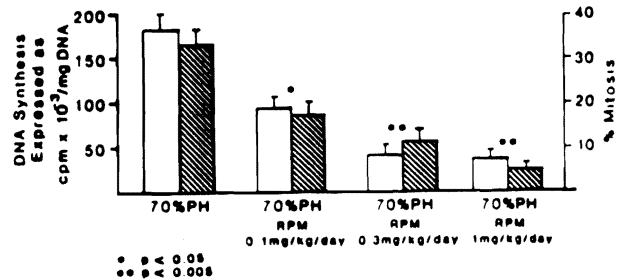


**Fig 3.** [<sup>3</sup>H]thymidine incorporation and percentage of labeled hepatocyte nuclei 24 hours after 70% hepatectomy in nude (NIH-RNU) rats treated (right) or not treated (left) with FK 506. The animals received an injection of vehicle or drug solution as described in Fig 1. Mean ± SEM (\* $P < .05$  or \*\* $P < .01$  versus untreated animals). Ten animals in each group.

both of the foregoing culture media conditions (Fig 6) with a dose/effect relationship (doses and other data given in ref. 14).

**RPM EFFECT ON GENE EXPRESSION**

The mRNA of the growth inhibitory factor TGF- $\beta$ , was determined in the liver of normal, nonoperated rats treated for 1, 2, 3, or 4 days with 0.3 mg/kg per day RPM, and in the liver fragment after 70% PH in rats pretreated before PH for 4 days with the same dose of RPM.<sup>14</sup> In both conditions, unexpected results were obtained with a striking decrease of the TGF- $\beta$  mRNA (Fig 7). The specificity



**Fig 4.** [<sup>3</sup>H]thymidine incorporation and percentage of mitosis in 70% hepatectomized rats not treated or treated with RPM at different doses. Control and immunosuppressive drug-treated animals were given intramuscular injections of vehicle and RPM, respectively, for 3 days before surgery, and again just after completing hepatic resection. The values represented by the bars are the means from at least 10 rats ± SD. \*Significantly different from 70% hepatectomized untreated rats ( $P < .05$ ). \*\*Significantly different from 70% hepatectomized untreated rats ( $P < .01$ ).

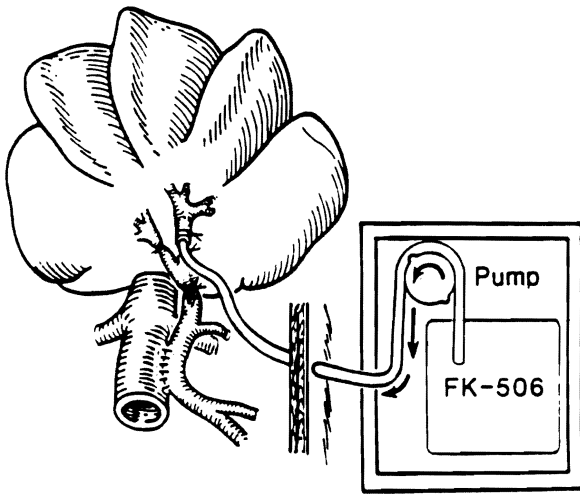


Fig 5. Surgical model of Eck-Fistula (portacaval shunt) with external continuous infusion of FK 506 on other drugs.<sup>12</sup>

of this effect was demonstrated by the complete preservation of albumin mRNA and glyceraldehyde-3-phosphate (gap) mRNA which are nongrowth genes.

These results and other evidence<sup>14</sup> have shown the intrinsically noncytotoxic nature of the RPM effect, and its specificity to at least one growth control gene.

DISCUSSION

This body of work shows that CyA and FK 506 have powerful growth promotional and trophic (hepatotropic) effects on intact livers. These qualities of CyA and FK 506 cannot be demonstrated in hepatocyte culture. They contrast sharply with the growth effects of RPM, which are antiproliferative and easily demonstrated in cultured hepatocytes. The discovery that RPM has an opposite effect of FK 506 is particularly interesting, because these two drugs are related chemically, and have the same cytosolic binding sites (FKBP).

Schreiber and Crabtree have reported at this symposium that these drugs (particularly FK 506) are "prodrugs" which may be inert by themselves, but which form biologically active complexes with their binding proteins. By this hypothesis, the complexes then act immunologically

Table 3. Hepatocyte Proliferation Activity After FK 506 Infusion Into the Left Portal Vein of Eck-Fistula Dogs\*

Group	FK 506 Infusion Rate (mg/kg per day)	Percent Labeled Hepatocytes		
		Right Lobe	Left Lobe	P Value
1	0 (controls)	4.42	4.45	NS†
2	0.01	4.77	5.43	.0247
3	0.1	5.14	5.98	.0888
4	1	5.37	9.13	.0510

\*Data from ref. 12.  
†NS = nonsignificant.

Table 4. Effect of Recombinant FKBP Infusion in Left Portal Branch for 4 Days After Canine Eck Fistula\*

Group	FKBP Dose (ng/kg per day)	Percent Labeled Hepatocytes		
		Right Lobe	Left Lobe	P Value
1	0	3.9	3.9	NS
2	200	4.0	5.0	NS
3	500	3.9	5.8	.05
4	750	4.0	6.7	.01
5	1000	4.1	8.2	.005

\*Data from ref. 13.  
NS = nonsignificant.

through another (target) molecule tentatively identified by Schreiber as calcineurin phosphatase.

Further speculation about mechanisms is not warranted at this time. However, the possibility is a real one that the alterations in signal transduction that are thought to be the molecular basis for the immunologic actions of CyA, FK 506, and RPM may also subserve the growth effects of

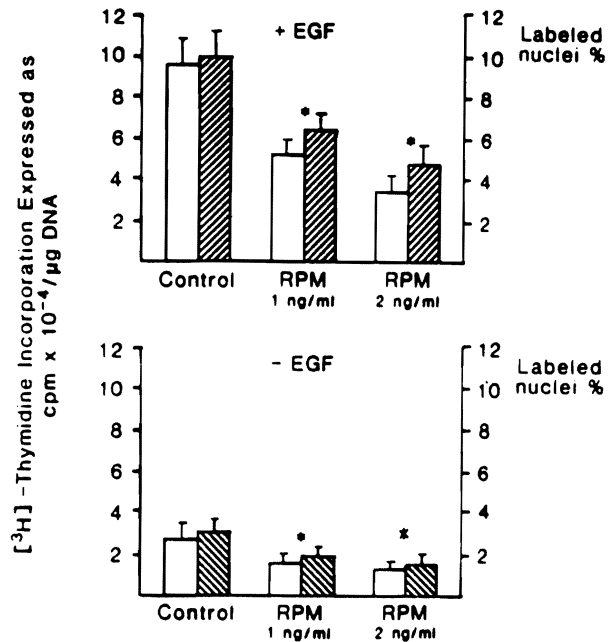
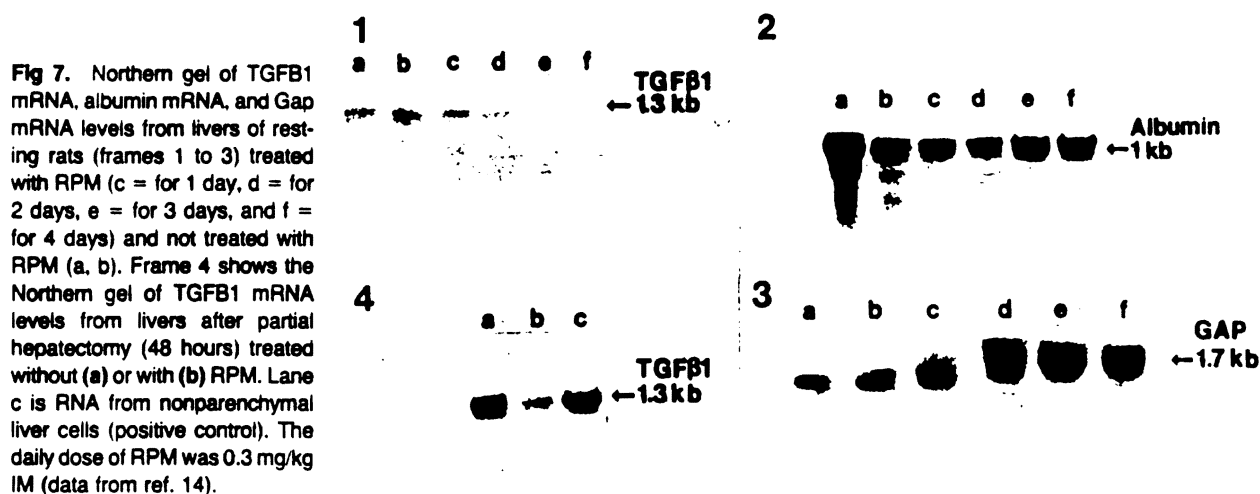


Fig 6. Effect of different doses of RPM on DNA synthesis in hepatocytes cultured in the presence (upper) or not (lower) of epidermal growth factor (EGF). Hepatocytes were isolated and plated at a density of  $1.5 \times 10^5$  cell/dish in 1.5 mL of minimum essential medium (MEM) + 5% fetal calf serum + insulin ( $10^{-7}$  mol/L), and maintained at 37°C in a 5% CO<sub>2</sub> atmosphere. Following a 3-hour attachment period, the medium was substituted with 1.5 mL of fresh MEM and insulin with or without EGF (10 mg/mL). DNA content, [<sup>3</sup>H]thymidine incorporation, and labeled nuclei were determined as described.<sup>7,14</sup> When added to the incubation medium, RPM was used at concentrations as reported in the figure, which did not affect hepatocyte viability (data not shown). The values reported represent the means of 10 experiments ± SD.



**Fig 7.** Northern gel of TGFβ1 mRNA, albumin mRNA, and Gap mRNA levels from livers of resting rats (frames 1 to 3) treated with RPM (c = for 1 day, d = for 2 days, e = for 3 days, and f = for 4 days) and not treated with RPM (a, b). Frame 4 shows the Northern gel of TGFβ1 mRNA levels from livers after partial hepatectomy (48 hours) treated without (a) or with (b) RPM. Lane c is RNA from nonparenchymal liver cells (positive control). The daily dose of RPM was 0.3 mg/kg IM (data from ref. 14).

these drugs by similar alteration of signal transduction and consequent specific changes of gene expression.

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