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HEPATOTROPHIC EFFECTS OF FK506 IN DOGS[1]

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Portacaval shunt (Eck fistula) in dogs causes hepatocyte atrophy and organelle disruption, as well as tripling of hepatocyte mitoses. After submitting dogs to this procedure, FK506 was infused into the tied-off left portal vein. The size, anatomic quality, and replication of hepatocytes were enhanced in the portion of liver infused with FK506, with a significant spillover effect in the noninfused portion. These hepatotrophic qualities of FK506 may explain part of FK506's efficacy for the treatment of chronic liver rejection. Also, the observations support a trial with this drug for the treatment of autoimmune liver diseases because, in addition to turning off the immunologic genesis of such disorders, repair and regeneration of the damaged liver may be augmented. Finally, these hepatrophic qualities are part of an emerging spectrum of biologic effects caused by drugs that may modulate the enzyme *cis-trans* peptidyl-prolyl isomerase (PPIase), the principal constituent of the cytosolic binding sites of FK506, repamycin, cyclosporine, and presumably other immunosuppressive drugs as yet undiscovered.

The "first pass" effect on the liver of orally administered drugs brought to the liver in high concentration via the portal vein has been difficult to assess. We have developed a model by which the direct hepatic action of substances such as insulin (*1*) and growth factors (*2*) can be studied in dogs. The agent under investigation is infused into the tied-off left main portal branch after portacaval shunt (Eck fistula) (*1*). The effects on the infused left hepatic lobes supplied by this branch can be determined and compared with those in the uninfused right hepatic lobes.

FK506, a new orally administered immunosuppressive drug of the macrolide class, has been shown to stop both acute and chronic liver allograft rejection in humans more reliably and completely than has been possible before (*3*). In some of these patients, the recovery of the livers, even years after transplantation, was so surprising that we suspected FK506 of having liver-restoring (hepatotrophic) qualities in addition to its ability to interrupt ongoing immunologic damage. In the present study, pronounced hepatotrophic consequences of FK506 have been documented with the canine Eck fistula model. The findings could explain some of the benefits observed in rejecting liver grafts "rescued" with FK506, and they could have therapeutic implications as well for the treatment of many hepatic diseases not involving transplantation. In addition, the findings contribute to a better understanding of the full spectrum of effects caused by drugs like FK506, rapamycin, and cyclosporine, which bind to cytosolic proteins that contain *cis-trans* peptidyl-prolyl isomerase (PPIase)* and that affect the action of this enzyme.

MATERIALS AND METHODS

Twenty adult female beagle dogs underwent Eck fistula. After performing a large side to side portacaval shunt, the left and right portal vein branches were ligated. A small infusion catheter was tied into the left branch and led through the abdominal wall and subcutaneously to a battery-charged infusion pump (Fig. 1) that was incorporated into a nonrestraining body cast. A constant infusion was started of the control or test fluids at the volume of 20–30 ml/day. Oral fluids and diet were allowed ad libitum. Four days later, the animals were administered 0.2 mCi/kg (CH_3-^3H)-thymidine with specific activity of 80–90 Ci/mmol (New England Nuclear, Boston). Two hours later, the dogs were anesthetized and killed.

Specimens were taken from 2 of the right hepatic lobes and 2 of the left lobes, fixed in 10% buffered formalin, and stained using standard hematoxylin-eosin staining techniques. Autoradiography was carried out using Ilford K5 nuclear track liquid emulsion and an exposure time of at least 30 days. The number of mitoses, 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) was determined by tracing out a large number of midzonal liver cells projected on standard-thickness paper, cutting out the individual silhouettes, and weighing each (*4*). 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 have been determined directly. In normal, unaltered dogs, about 1.6±0.4 mitosis per 1000 hepatocytes are present in the liver, and midzonal hepatocytes are about 0.17±0.01 size-units (*2*). The exceptional reproducibility of these values and the small standard deviations in experiments performed as long as 15 years apart make it easy to identify changes caused by operations such as Eck fistula, drugs, or other experimental variables.

For studies of the organelles, small cubes of each hepatic sample were taken for electron microscopy. The tissue was postfixed in glutaraldehyde followed by osmic acid. After embedding in Polarbed 812 resin, ultrathin sections were cut, stained with lead citrate, and examined in a Phillip's 300 electron microscope. Measurements of the organelles were made on electron micrographs by Loud's method (*5*).

Results are expressed as mean ±SD. The Student's *t* test was employed in individual experimental groups to compare differences between right and left lobes or between groups. A *P* value less than 0.05 was considered to be significant.

RESULTS

Infusion of the drug vehicle did not affect the hepatocyte atrophy typical of the Eck fistula liver or change the low-grade

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* Abbreviations: HSS, hepatic stimulatory substance; PPIase, *cis-trans* peptidyl-prolyl isomerase.

hyperplasia (Table 1, vehicle controls). However, when FK506 was infused into the left portal vein, atrophy of the left lobar hepatocytes was prevented in proportion to the dose, and the rate of mitoses was increased. These changes were significantly greater in the directly infused lobes at all doses, but even the noninfused lobes were significantly protected compared with the vehicle controls at the high FK506 doses (Table 1).

Comparison of the ultrastructure of the left and right lobar hepatocytes showed that the hepatocytes exposed to infused FK506 were almost normal even at the smallest doses (Tables 2 and 3). The amount of rough endoplasmic reticulum was restored relative to controls; in addition, dilatation and disruption of the cisternae were minimal. The number of microbodies, lysosomes, and small lipid-containing vacuoles were near normal levels in the FK506-infused lobes. The mitochondria in these lobes were neither enlarged (Table 3). nor abnormal.

The changes in the hepatocytes in the right lobes did not differ greatly from those seen in the controls at low doses of FK506. However, at the 1 mg/kg/day dose, there was better preservation of the RER in the right lobes compared with right lobar hepatocytes in the vehicle controls (Table 2), and reduced lipid accumulation ($P<001$). At this high dose, the right lobar hepatocytes also had reduced microbodies ($P=.07$) and lysozomes ($P<05$).

There was no histopathologic evidence of drug toxicity in the lobes infused with FK506. Both with light and electron microscopy, glycogen deposits were normal compared with the deglycogenated contralateral lobes.

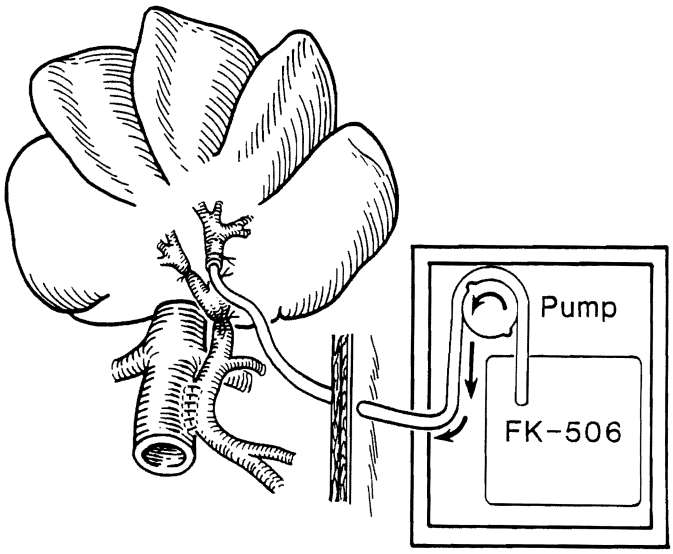

FIGURE 1. The experimental method.

DISCUSSION

Our experimental preparation should be useful as a surrogate model to test the effect on the liver of drugs given orally and absorbed into the splanchnic venous system. The principle of the model is to create the abnormal state caused by depriving the liver of its portal venous inflow, and to see what happens to the hepatic lobes supplied by the left portal vein when a test substance is directly infused into this vessel. If an agent has demonstrable effects on the portion of the liver that is infused, these can be compared with changes in the noninfused hepatic fragment that serves in each experiment as a control. From this comparison, assessment can be made not only of a drug's affect upon liver structure but also of its first-pass clearance. With insulin (*1*) and a cytosolic hepatic stimulatory substance (HSS) (*2*), the so called hepatotrophic effects of hyperplasia, hypertrophy, and maintenance of normal organelle structure that have been construed to signify hepatocyte health are found almost exclusively in the infused lobes. Some agents, such as glucagon, which have a powerful metabolic action on the liver have no hepatotrophic qualities that are demonstrable with this model (*1*). Thus, the fact that FK506 is hepatotrophic is seen as an unusual endowment.

Although these hepatotrophic effects were similar to those caused by insulin and HSS (*1*, *2*), FK506 had a somewhat greater spillover effect into the uninfused lobes. The unbalanced effect on the infused versus the noninfused liver lobes suggests that most of the hepatrotrophic mechanisms are taking place within the liver, rather than systemically. It is thought that FK506 has no direct effect on isolated hepatocytes in culture (*6*). FK506 is metabolized exclusively by the liver (*7*). To the extent that its hepatrotrophic effects were unilateral, implying a principal first-pass removal, the possibility could be raised that FK506's immunosuppression might be relatively liver-specific, and consequently that much larger oral doses will be required for the successful transplantation of extrahepatic organs. A formal study of this latter question has not been made either with FK506, or with cyclosporine, which has been shown with the same Eck fistula model to have very similar characteristics (*8*).

More interesting than such dose considerations is the possible beneficial effect of FK506 on hepatic repair. FK506 promotes hypertrophy and hyperplasia, the ingredients of regeneration, and it has been shown by direct experimentation in rats to augment regeneration after partial hepatectomy (*9*). These effects are shared with cyclosporine (*8–12*), which has a

TABLE 1. Hepatocyte size and Autoradiographic labeling

FK506 (mg/kg/day)	n[a]	Hepatocytes Size Units						Labeled Hepatocytes/1000 Hepatocytes					
		Left	*P* left vs. right	Right	Left	*P* vs. vehicle	Right	Left	*P* left vs. right	Right	Left	*P* vs. vehicle	Right
Vehicle	6	.114±.007	0.4355	.112±.011				4.45±.26	0.8429	4.42±.24			
0.01	12	.111±.008	0.0004	.100±.007	0.5782		0.1860	5.43±.35	0.0167	4.77±.60	0.0044		0.2477
0.1	12	.137±.008	0.0000	.113±.006	0.0065		0.8351	5.98±.43	0.0001	5.19±.38	0.0005		0.0086
1.0	10	.162±.012	0.0009	.134±.006	0.0004		0.0503	9.13±.89	0.0015	5.37±.78	0.0001		0.0510

[a] In each experiment, samples were taken from 2 different right lobes and 2 different left lobes; n represents both samples, and the number of animals is $\frac{n}{2}$.

TABLE 2. Area (μM^2) of endoplasmic reticulum (ER) per average midzonal hepatocyte

FK506 (mg/kg/day)	n[a]	Rough ER						Smooth ER					
		Left	P left vs. right	Right	Left	P vs. vehicle	Right	Left	P left vs. right	Right	Left	P vs. vehicle	Right
Vehicle	3	13874±1221	0.9672	13882±975				26416±3787	0.7996	26680±2202			
0.01	6	26332±1483	0.0001	18697±1010	0.0000		0.0019	18433±535	0.0000	23337±902	0.0661		0.1092
0.1	6	29889±1117	0.0001	20325±975	0.0001		0.0007	18688±498	0.0018	22747±1305	0.0703		0.0725
1.0	5	32091±1225	0.0002	20839±1612	0.0000		0.0003	17876±642	0.0024	23154±193	0.0574		0.0858

[a] One sample from a right lobe and one from a left lobe were studied; n = number of animals.

TABLE 3. Organelle volume (μM^3) in average midzonal hepatocytes

FK506 (mg/kg/day)	n[a]	Hepatocytes—mitochondria			Hepatocytes—microbodies			Hepatocytes—lysosomes			Hepatocytes—lipids		
		Left	P left vs. right	Right	Left	P left vs. right	Right	Left	P left vs. right	Right	Left	P left vs. right	Right
Vehicle	3	1786.3±506.4	0.7683	1657.6±164.5	207.0±22.6	0.0288	188.6±18.5	229.0±62.7	0.6660	208.0±29.1	94.0±16.7	0.6883	98.0±8.5
0.01	6	1233.7±222.4	0.1903	1429.5±331.2	99.2±11.8	0.0080	173.8±39.6	62.3±15.1	0.0017	162.6±48.9	23.3±8.8	0.0012	81.0±15.1
0.1	6	1288.5±236.2	0.2529	1439.3±115.4	86.3±11.5	0.0025	172.5±34.2	59.6±15.6	0.0000	187.6±14.8	22.8±6.7	0.0011	83.8±19.7
1.0	5	1284.8±178.9	0.1996	1440.6±318.7	94.2±10.4	0.1111	136.6±41.3	48.8±10.9	0.0002	160.0±23.8	15.6±2.8	0.0044	47.8±11.3

[a] One sample from a right lobe and one from a left lobe were studied; n = number of animals.

completely different molecular structure. The experiments underscore the possibility of using FK506 for the treatment of liver diseases, especially if there is an autoimmune component. The remarkable ability of FK506 to halt and reverse chronic rejection of liver allografts (*3*) may be a direct analogy to what can be achieved using this drug for nontransplant autoimmune disorders. There might even be the prospect of promoting hepatic healing in the absence of an immunologic pathogenesis.

The implications of these experiments, together with recent advances in enzyme cheimstry, go far beyond the narrow issues that were examined in the present study. Cyclosporine and FK506 act by inhibiting the synthesis and expression of interleukin 2 and other cytokines (*13, 14*). Because both drugs are hepatotrophic as well as immunosuppressive, it was suggested that they might modulate hepatocyte growth control immunologically (*6, 9*). However, other explanations are more plausible in light of recent information about the cytosolic binding sites of cyclosporine (cyclophilin) and FK506. While distinct, these binding sites have as a common constituent the enzyme *cis-trans* peptidyl-prolyl isomerase (*15, 16*). This ubiquitous enzyme, which has been highly conserved throughout evaluation, catalyzes the isomerization of proline peptide bonds in oligopeptides and accelerates rate limiting steps in the folding of several proteins during their synthesis (*17, 18*). However, the physiologic significance of PPIase was a mystery until one year ago when it was found to be the principal constituent of cyclophilin (*19, 20*) and later of the FK506 binding site (*15, 16*). Rapamycin another T cell–specific immunosuppressive agent, has the same binding site as FK506 (*15, 16*).

However, PPIase is found in many tissues, not just in lymphocytes, having first been discovered in the pig kidney (*17*). Pharmacologic inhibition of PPIase in heterogenous binding sites could explain why the nonimmunologic actions of cyclosporine and FK506 tend to alter the same clinical end-points although the mainfestations may differ quantitatively and may be in opposite directions (*21*). These include nephrotoxicity (less with FK506 than cyclosporine), tendency to diabetes (the same with both drugs), hair growth (thinning may occur with FK506), cholesterol regulation (minimal with FK506), uric acid regulation (the same or less with FK506), and neurotoxicity. The neurotoxicity usually is not serious with either cyclosporine or FK506, but it is remarkably similar with both drugs and includes tremors, paresthesias, headaches, increased sensitivity to light, mood changes, and insomnia.

A clue about the reason for the neurologic manifestations has come from studies of mutant fruit flies (*Drosophila*) the heads of which contain an abnormally low amount of cyclophilinlike receptors as well as photoreceptors that are rhodopsin-deficient (*18*). On the basis of this genetic evidence, the authors suggested that correct folding and stability of the rhodopsin in photoreceptors cannot occur in the absence of the PPIase-rich cyclophilin receptors.

Our hypothesis is that the foregoing array of clinical manifestations, the hepatotrophic affects described in the present communication, and the immunosuppression of both FK506 and cyclosporine all reflect the pharmacologically altered role of PPIase in various intracellular signal transduction processes. By working back from observations of both desired and undesired effects of FK506, cyclosporine, and other such agents, an understanding could be facilitated of the role in basic biology of what must be a heterogenous family of cytosolic binding sites which have in common PPIase activity. If the hepatotrophic influence of agents like FK506 and cyclosporine is not by a direct effect on the hepatocytes (*6*), it probably reflects the composite effect on altered receptors and second signals that remain to be delineated.

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