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AUGMENTATION OF RAT LIVER REGENERATION BY FK 506 COMPARED WITH CYCLOSPORIN

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Summary

The immunosuppressive drug, FK 506, increased the regeneration response that follows 40% and 70% hepatectomy in rats. The effect was similar to that obtained with cyclosporin.

INTRODUCTION

Azathioprine and adrenocortical steroids, which were the most widely used maintenance immunosuppressive agents until the introduction of cyclosporin, depress the regeneration response after partial hepatectomy.^{1,2} In contrast, cyclosporin augments hepatic regeneration in rats^{3–5} and in dogs⁶ with an Eck fistula, which allows hepatocyte replication and hypertrophy to be studied separately.⁷ In dogs with an Eck fistula cyclosporin has the hepatotrophic (liver supporting) properties that have been associated with anabolic hormones such as insulin and growth factors.^{7,8} However, it is unknown whether the hepatotrophic qualities of cyclosporin are the result of its immunosuppressive action. To clarify this issue, we have studied the effect on rat liver regeneration of FK 506, an immunosuppressive macrolide obtained from cultures of *Streptomyces tsukubaensis*.⁹ FK 506 is more potent than cyclosporin on a molar basis.

METHODS

Adult male inbred Fisher 344 rats weighing 180–200 g were purchased from Hilltop Lab Animals Inc (Scottsdale, Pennsylvania). The animals were given standard rat laboratory diet and water ad libitum in a temperature and light controlled room (light 0730–1930). The rats were assigned to groups and treated for 4 days as controls or with cyclosporin or FK 506 (table I). On the fourth day, between 0900 and 1030, the rats in groups 5–10 had a standard 40% or 70% hepatectomy under light ether anaesthesia. Animals in groups 3 and 4 had sham operations in which the liver was manipulated at laparotomy. Food and drink were allowed immediately. Parenteral fluid and electrolyte support were not required.

24 h after the hepatectomies, 185×10^4 Bq ³H-thymidine was administered to all rats by intraperitoneal injection. The rats, including groups 1 and 2, were killed 2 h later by guillotine. Extraction and purification of hepatic DNA were done with the method of Ove et al¹⁰ and DNA content was measured with calf thymus DNA (Sigma) as standard.¹¹ Specimens from each

liver were prepared for histological examination with haematoxylineosin and the proportion of hepatocytes in mitosis was counted.

All results are means and SE.

RESULTS

As expected DNA synthesis and the proportion of hepatocytes in mitosis were increased in rats with a 40% or 70% hepatectomy that were not given cyclosporin or FK 506 (groups 5, 7, and 8; table II). After pretreatment for 4 days before hepatectomy with intramuscular FK 506 (groups 2, 4, and 10) or oral cyclosporin (group 9), regeneration was significantly augmented compared with controls. The effect was greater with FK 506 than with cyclosporin (group 10 compared with group 9). FK 506 did not increase resting hepatocyte mitosis or DNA synthesis. These indices were slightly increased in rats submitted to sham operation. When FK 506 was added to the sham operation group, hepatocyte mitosis and DNA synthesis were further and significantly increased.

DISCUSSION

We found that the proliferative component of rat liver regeneration was augmented by FK 506, as has been demonstrated previously for cyclosporin.³⁻⁵ FK 506 1 mg/kg intramuscularly was more effective than cyclosporine 10 mg/kg orally. However, the different doses and routes of administration make this comparison inconclusive.

The mechanism of this effect is unknown. Possibly these two agents, which have different structures, have effects on growth control that are related to their immunosuppressive action. That is, the events of regeneration may be modulated immunologically, which has been hinted at previously.¹²⁻¹⁴ For example, DNA synthesis increases in lymphoid tissues after hepatectomy,¹⁴ the serum of rats after partial hepatectomy can stimulate lymphoid proliferation,¹⁴ and splenectomy enhances regeneration.¹⁵ In contrast regeneration is depressed after non-specific immunosuppression with azathioprine¹ and steroids.² The augmentation of regeneration by cyclosporin is well established.^{3-5,13}

Both FK 506 and cyclosporin specifically inhibit the T-cell response,^{9,16} which could explain why both these agents promote increased regeneration. The dominant effect of specific components of the immune system could be to restrict cellular growth in general, not just that involved in liver regeneration. A "braking" effect of the immune system may be dependent on T-cell function and apply to normal as well as to abnormal or replicating cells.

How does the regenerating liver know when to stop regrowth at the proper size and time? Endogenous inhibitory factors or hormones are possible mediators.^{7,8} Research on immunosuppression for transplantation and investigations of hepatic regeneration could lead to a better understanding of the interaction between growth control and immune function. Non-immune mechanisms may be involved. Both cyclosporin and FK 506 inhibit interleukin 2 production and binding.^{9,16} By inhibition of this second signal, both drugs could interrupt the secretion of liver regulatory factors not directly connected with the immune system.

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TABLE I

Regimens

Group	Route	Cyclosporin (mg/kg)	FK 506 (mg/kg)	Vehicle *	Hepatectomy
1 (n=5)	IM	..	..	Saline	..
2 (n=5)	IM	..	1	Saline	..
3 (n=10)	IM	..	..	Saline	Sham
4 (n=10)	IM	..	1	Saline	Sham
5 (n=8)	IM	..	..	Saline	40%
6 (n=8)	IM	..	1	Saline	40%
7 (n=20)	PO	..	..	Olive oil	70%
8 (n=20)	IM	..	..	Saline	70%
9 (n=15)	PO	10	..	Olive oil	70%
10 (n=15)	IM	..	1	Saline	70%

* 250 µl saline or 200 µl olive oil. IM = intramuscular, PO = oral.

TABLE II

Effect of Cyclosporin and FK 506 on Rat Liver Regeneration (Mean, SE)

Group	³ H-thymidine incorporation (× 10 ³ cpm/mg DNA)	Proportion of hepatocytes in mitosis (%)
1	3.3 (0.4)	1.6 (0.1)
2	3.2 (0.3)	1.7 (0.1)
3	4.9 (0.5)	6.8 (0.6) [¶]
4	10.5 (0.8) [*]	9.5 (0.5) ^{//}
5	12.5 (1.3)	..
6	32.4 (8.2) [‡]	..
7	138.1 (13.1)	31.0 (2.0)
8	130.0 (9.2)	29.0 (2.8)
9	179.0 (14.0) [‡]	44.0 (2.1) [‡]
10	242.0 (28.0) [§]	59.0 (3.0) [§]

Student's t test:

* p < 0.005 vs groups 1, 2, and 3.

[‡] p < 0.001 vs group 5.

[‡] p < 0.05 vs group 7.

[§] p < 0.001 vs group 8.

[¶] p < 0.01 vs groups 1 and 2.

// p < 0.05 vs groups 1 and 2.