Preliminary Communication

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 dogs[6] 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 (Scottdale, 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.

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 ($\times 10^3$ 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.

24 h after the hepatectomies, 185×10^4 Bq ^{3}H-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 al[10] and DNA content was measured with calf thymus DNA (Sigma) as standard.[11] Specimens from each liver were prepared for histological examination with haematoxylin-eosin 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 cyclosporin 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 azathioprine[1] and steroids.[2] The augmentation of regeneration by cyclosporin is well established.[3-5,13]

Both FK 506 and cyclopsorin 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 regnerating 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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Reviews of Books

Handbook of Neurological Investigations in Children

J. B. P. Stephenson and Mary D. King. London: Butterworth 1989. Pp 244. £25. ISBN 0-723612951.

MY heart sank when I removed this from *The Lancet*'s jiffy bag: why should two distinguished neurologists demean themselves by joining the investigational recipe-book business? My misgivings about this approach stem from the differences between general and specialist paediatric practice. When assessing a child or infant with fits, faints, and funny turns I (a generalist) usually adopt the pooh-pooh approach of Sir Robert Hutchison. The opposite attitude he described as wind-upper, and that is how I felt after an intellectual mauling at the hands of Dr Stephenson and Dr King, who almost persuaded me that my management of children with possible neurological disorders is cavalier if not negligent. There is a serious point here. Paediatrics is tackling medical audit seriously, with proposals for protocols for investigation of certain conditions. If the compilation of these protocols becomes the exclusive responsibility of specialists there is risk of biasing that investigation towards finding the uncommon patient with some rare condition or complication dealt with by those specialists. The penalty will be costly overinvestigation of many other children who present with a symptom that might be a feature of the recherché but usually is not. Herein lies the danger of unselective clinical use of recipe books that are less concerned with what symptoms to investigate than with how best to investigate certain symptoms: the medical audit of outpatient general medicine of childhood should be directed towards symptoms rather than conditions.

Notwithstanding these strictures the general physician will learn much from this volume. An important responsibility of the specialist is to help generalist colleagues to manage their patients better; this book amply fulfils such a requirement and thus answers my opening question. The text is split into two sections, the first dealing with tests and the second with presenting complaints and their investigations. The introduction and appendix contain salutary comments on the philosophy and value of tests and this wise thinking predominates. What questions are you trying to answer by doing this investigation? What are the chances that the test will answer it? Might there be a better alternative? The risk of spraying tests at the abnormal child is emphasised; parents may become impatient with the methodical sequential approach but it is rarely in the child's interests to cut corners and the doctor's job is to resist pressures for speedy and possibly inaccurate diagnosis. Perhaps the greatest weakness is the lack of a chapter on history-taking since skill and experience here will generally lead to economical investigation—but the authors would probably say that their brief was to discuss the management of a child who has been sifted by such a discriminating process. If so, they are to be congratulated on a well written and attractively presented book. It is not for use by the uncritical and free-ranging house physician (lest he or she bankrupt the hospital) but more for the specialist trainee or a discerning general paediatric physician faced with a challenging case and who is unable to contact the paediatric neurologist.

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