Surg. Res. Comm., 1990, Vol. 9, pp. 227-233
Reprints available directly from the publisher
Photocopying permitted by license only

MODIFICATIONS INDUCED BY CYCLOSPORIN A ON ISCHEMIC LIVER REGENERATION

I. GARCÍA-ALONSO, V. PORTUGAL, I. ITURBURU, I.L. de TEJADA and J. MÉNDEZ*

Laboratory of Experimental Surgery, Faculty of Medicine (Univ. of the Basque Country) 48940-LE, Spain

(Accepted for publication 25 April, 1990)

Cyclosporin A (CsA) induces the synthesis of DNA in normal hepatocytes and also improves liver regeneration following partial hepatectomy. This study was designed to determine whether these properties of CsA might be useful to improve hepatic regeneration in ischemic liver. Ischemia was established in rats by clamping the hepatic hilum, the superior mesenteric artery and the celiac axis for 15 min while liver regeneration was induced by a 70% hepatectomy. CsA was administered intraperitoneally (20 mg/Kg) 24 h and 4 h prior to the operation. Hepatocytic DNA was measured 24 h after partial hepatectomy and the mean percentage of regenerating hepatocytes (MPRH) and the regenerative gradient (RG) were calculated. Liver ischemia decreased MPRH (5.71 vs. 21.51) but did not affect GR (1.61 vs. 1.48). CsA partially restored MPRH (12.19 vs. 21.51) and increased GR (2.29 vs. 1.48). Possible explanations are discussed regarding these interesting findings. We conclude that CsA exerts regenerative effects on hepatocytes probably by means of lymphocytic regulation.

KEY WORDS: Cyclosporin, hepatectomy, liver regeneration, DNA, ischemia.

INTRODUCTION

Liver transplantation is now widely accepted as an established surgical therapy. However, shortage of pediatric donor grafts and difficulty in finding a liver of the appropriate size remain as serious obstacles precisely in those cases with better perspectives for success. A possible solution, already being tested, is to graft one part of the liver from an adult donor. Studies have been reported concerning the major complications of partial liver transplantation (PLT), the surgical techniques dealing with bleeding from the transected surface of the liver, and the adequate preservation of the graft after a longer period of warm ischemia.[1,2]

Little attention is paid to the importance of liver regeneration in PLT, as the grafted organ must grow in order to restore a proper balance between host body weight and liver mass.[3] On the other hand, ischemia is known to depress DNA replication in hepatocytes. Therefore, the discovery of drugs or other therapeutic agents which minimize or reverse such an effect, or simply improve normal liver regeneration, would be a valuable adjunct in PLT. A previous study from our laboratory showed that CsA induces the synthesis of DNA in normal hepatocytes and also enhances the synthesis of DNA induced in hepatocytes by partial hepatectomy.[4]

In this study, the ability of CsA to improve liver regeneration following short periods of hepatic ischemia was assessed.

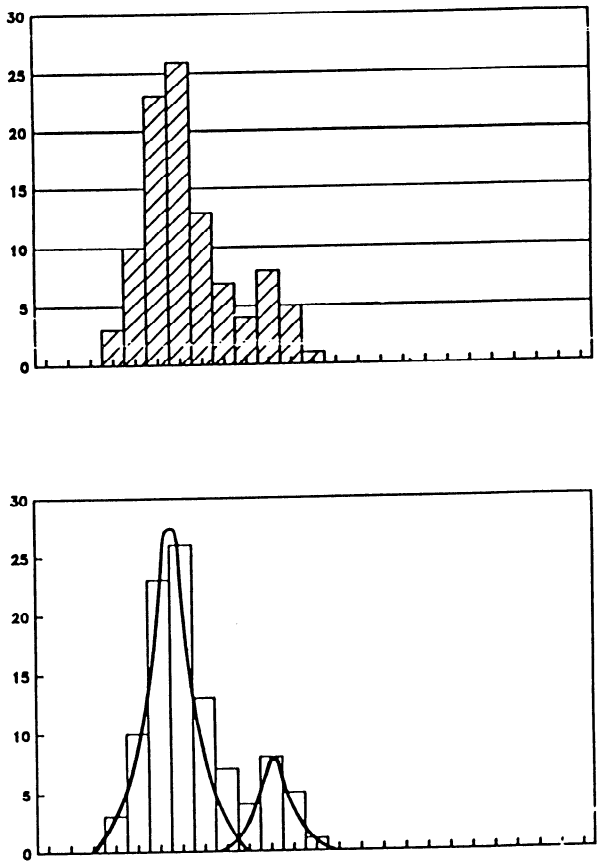

FIGURE 1. Frequency histogram of hepatocytic-DNA content from a regenerating liver. The two peaks are clearly distinguishable: the left one represents normal "resting" cells while the right peak corresponds to "regenerating" hepatocytes. Gaussian curves as calculated by a personal computer have been superimposed.

RESULTS

Liver ischemia ranging from 5 to 20 min was well tolerated. Nevertheless, when prolonged to 25 min it was followed by a 60% mortality rate within the first 24 h after operation. In 70% hepatectomized animals, the mortality rate jumped to 60% and 80% after 20 and 25 min, respectively (Table I). It was noteworthy that all deaths occurred in less than 24 h after operation. In order to avoid mortality an ischemic period of 15 min was selected as the most suitable for our experiment.

DNA quantification (Table II) showed that hepatic ischemia significantly decreased M.P.R.H. ($p < 0.001$), while the Regenerative Gradient was not modified (Table III).

When CsA was administered intraperitoneally prior to ischemia induction, M.P.R.H. (Table II) was clearly increased ($p < 0.05$), though it did not reach normal values ($p < 0.05$). On the other hand, CsA also enhanced the Regenrative Gradient (Table III) when compared with control regenerating livers ($p < 0.01$).

METHODS

Animals

Male Sprague-Dawley rats (Stabulary of the Univ. of the Basque Country, Spain) weighing 200–220 g. were used. The animals received an ordinary pellet diet (Panlab S.L., A-40, Spain) and water ad libitum prior to the experiments.

Surgical Procedure

Surgery was performed between 9:00 a.m. and 11:00 a.m. to standardize for natural diurnal rhythms. The abdomen was opended by means of a midline incision under ether anesthesia. Ischemia was induced by occluding the hepatic hilum, the superior mesenteric artery and the celiac axis with small vascular clamps (Yasargil-clip, Aesculap) for 10, 15, 20 and 25 min. When performed, 70% hepatectomy was carried out just after inducing the ischemia. The abdomen was closed and the rats were allowed to awake. Following different time intervals of ischemia, the abdomen was reopened and the vascular clamps were removed. The restablishment of the blood flow was appreciated by the recoloration of the liver and the gut. Animals still alive 7 days after the operation were considered permanent survivors.

Experimental Series

Liver regeneration was studied in three groups of ten animals. In control rats a 70% hepatectomy was performed. The second group included 15 min ischemic-70% hepatectomized rats. The third set of animals was similar to the second, but rats were treated with CsA (20 mg/Kg i.p.) the day before and two hours prior to operation. CsA was dissolved in 95% ethanol and diluted with Intralipid 10% (Kavi-Vitrum) to 20 mg/ml.

DNA Quantification

Rats were sacrificed 24 hours after operation and a fragment of liver was quickly removed and embedded in paraffin. DNA quantification was carried out by means of microspectrocytophotometric techniques on 5 μm histological sections stained in Schiff Reagent (Feulgen-Rossenbeck Reaction). From these data frequency histograms were calculated for each animal (Figure 1). Using a modification of Bartels' method,[5] histograms were resolved into two gaussian curves the left one corresponding to static cells and the other to regenerating hepatocytes. This method allowed us to assess the "Mean Percentage of Regenerating Hepatocytes" (MPRH) and the relation between mean DNA values in static cells and mean DNA values in regenerating hepatocytes ("Regenerative Gradient").

Statistical Analysis

Comparison of regenerative parameters was determined using the Rank Sum Test, and "p" value below 0.05 was regarded as significant.

TABLE I
Survival rates following different hepatic ischemic periods.

Animals	Length of ischemia (mins)	Survival Rate	
		Ischemia	Is + Hep
5	10	100	100
5	15	100	100
5	20	100	40
5	25	40	20

Is + Hep = Ischemia and hepatectomy

TABLE II
Percentage of hepotocytes entering phase "S" after different surgical procedures

	Mean	S.D.
70% Hepatectomy	21.51	10.05
Ischemia + Hepatectomy	5.71	4.44
CsA + Ischemia + Hep.	12.19	4.29

TABLE III
Regenerative Gradient after different surgical procedures.

	Mean	S.D.
70% Hepatectomy	1.613	0.142
Ischemia + Hepatectomy	1.482	0.867
CsA + Ischemia + Hep.	2.299	0.353

DISCUSSION

Portal drainage interruption is scarcely tolerated in the rat: even short periods are responsible for general disorders as well as intestinal wall damage. The most common solution employed to avoid this complication in experimental surgical procedures is to perform a portocaval shunt which is later closed once the ischemic period[6] has ended. However, this is only feasable in large animals (cats, dogs) while the shunt must remain open when used in rats adding one more variable to the physiopathology under study. This is why an alternative "derivative" technique is used in rats. First reported by Asakawa *et al.*,[7] while clamping the pedicules of the main hepatic lobules the afferent flow through one small lobule is maintained which is subsequently excised before removing the clamps. However, when studying hepatic regeneration the existence of a portion of the normal liver during the ischemic phase may interfere with the experiment. To avoid this problem we have completely abolished the splachnic flow by occluding the celiac axis and the superior mesenteric artery during liver ischemia. Though brief periods of portal hypertension have great repercussions on the organism, intestinal ischemia of short duration (15 min) is perfectly tolerated. In fact it has been reported that ischemic periods longer than 60 min are needed to induce a minimum mortality rate.[8]

The parameters analyzed in this experience provide information about two different aspects of DNA synthesis. MPRH is a good index of the "intensity" of the regenerative response, as it is directly related to the number of hepatocytes undergoing DNA replication. Usually, the stronger the regenerative stimulus, the larger the number of regenerating hepatocytes, as may be seen after 40% and 70% hepatectomies.[4,9] Nevertheless, the intensity of the stimulus and the amplitude of the response do not follow a linear relation but an assymtotic-like one. Therefore, beyond a certain limit (about 80% hepatectomy) MPRH increments are nearly inappreciable or do not exist at all.[10,11] A diminution of this index should then imply an interference with the stimulus (or its mechanism of action) or an impairment of the cells' ability to respond to such a stimulus.

The regenerative gradient is an accurate index to gauge the speed of liver regeneration, which is firmly related to the intensity of the stimulus. DNA replication in regenerating liver takes place in a syncronic fashion which is manifested by successive "peaks" in DNA synthesis.[12] For instance, the first peak following a 70% hepatectomy in the rat appears 24 h after operation,[13] while it is delayed up to 40 h after a 40% hepatectomy.[14] To estimate this "rate" and compare it between different series without making sequencial studies to determine the cronology of the peaks we used the RG. Once the mean DNA content of the regenerating hepatocytes (at a fixed time) is calculated one can assume that the nearer it is to a 4C value the more rapid will be the regenerative response.

In this study, MPRH ("intensity") was found to be decreased by ischemia, while RG ("velocity") was not affected. This indicates that fewer hepatocytes entered phase S, but those which did synthesized DNA at a normal rate. This is in agreement with a non-uniform pattern of ischemic damage in liver cells: those located far from portal and arterial vessels suffer the effects of flow interruption first, while periportal hepatocytes maintain fairly good activity. Centrolobulillar cells should renounce their specific functions focusing their scarce energetic reserves to essential functions.[15] If oxygen supply is restored these cells will be able to resume their highly specialized metabolic functions or -in this case- enter phase S. In fact, 15 min of ischemia are insufficient to kill hepatocytes,[16] and they recover rapidly from anoxia.[17] From this point of view it is completely logical that after brief periods of ischemia the number of hepatocytes responding to regenerative stimuli (MPRH) diminishes while their DNA-synthesis rate is maintained.

Modifications of factors which induce[18] and/or regulate[19,20] liver regeneration could also be involved. It is well known that some of these factors are of splachnic origin.[21] It's possible that mesenteric ischemia interferes with the production or subsequent delivery of these factors. Liver regeneration would thus be depressed. Hydroelectrolitic inbalance is ordinarily associated with ischemic events which could also be responsible for a certain impairment of the normal regenerative response.[22]

All of these possible explanations are transitory and thus, after a certain lapse of time, stronger regenerative peaks would be expected. It is noteworthy that other investigators have reported that no significant difference in liver weight is found between ischemic and normal livers 7 days following partial hepatectomy in dogs.[23]

Results obtained after CsA-treatment are of great interest. This drug improved MPRH, though without attaining normal values. In addition, despite the ischemic aggression, it also enhanced the RG. In other words, CsA increased both the number of regenerating hepatocytes and the rate of DNA synthesis. These results agree with those previously reported by us on CsA and the normal regenerating liver.[14,24] Other

investigators have experienced similar results although they were not statistically significant.[25] It has also been reported that CsA induces hepatic regeneration in otherwise normal livers.[14,24] The accumulation of two regenerative stimuli could explain the increase of response both in number and rate.

Another possible explanation is to be found in previous reports concerning the protective effects of CsA on intestinal mucosa during ischemic-reperfusion injury.[26] Thus CsA, decreasing the aggressiveness of ischemia, increases the number of "responsive" hepatocytes.

It's important to bear in mind that more and more evidence is being gathered relating lymphocytes and liver regeneration.[27-29] Taking into account the specific actions of CsA over lymphocytes it should not surprise us that this drug modifies liver regeneration.

References

1. Broelsch, C.E., Emond, J.C., Thistlethwaite, J.R., Rouch, D.A., Whitington, P.F. and Lichtor, J.L. (1988) Liver transplantation with reduced-size donor organs. *Transplantation*, **45**, 519–523.
2. Tokunaga, Y., Zaima, M., Tanaka, K., *et al.* (1989) Orthotopic partial liver transplantation in dogs can be performed without cold perfusion of the donor liver. *European Surgical Research*, **21**, 137–144.
3. Kam, I., Lynch, S., Svanas, G., *et al.* (1987) Evidence that host size determines liver size: Studies in dogs receiving orthotopic liver transplants. *Hepatology*, **7(2)**, 362–366.
4. Garcia-Alonso, I., López de Tejada, I., Iturburu, I. and Méndez, J. (1988) Modificaciones en la síntesis de DNA provocadas por la cirlosporina A sobre la regeneración hepática inducida quirúrgicamente. *Cirugía Española*, **63(5)**, 683–688.
5. Bartels, P.H. (1979) Numerical evaluation of cytologic data. I. Description of profiles. *Analytical Quantitative Cytology*, **4**, 20–28.
6. Romani, F., Vertemati, M., Frangi, M., Monti, R., Codeghini, A. and Belli, L. (1988) Effect of superoxide dismutase on liver ischemia-reperfusion injury in the rat: a biochemical monitoring. *European Surgical Research*, **20**, 335–340.
7. Asakawa, H., Jeppsson, B., Mack, P., Hultberg, B., Hägerstrand, I. and Bengmark, S. (1989) Acute ischemic liver failure in the rat: a reproducible model not requiring portal decompresion. *European Surgical Research*, **21**, 42–48.
8. Garcia-Alonso, I., Ortiz, J., Basáñez, A., Portugal, V. and Méndez, J. ("In press") Cuantificación de lesiones en el síndrome de revascularización intestinal de la rata: método morfométrico. *Gastrenterología y Hapatología.*
9. Bucher, N.L.R. and Swaffield, M.N. (1964) The rate of incorporation of labeled thymidine into deoxyribonucleic acid of regenerating rat liver in relation to the amount of liver excised. *Cancer Research*, **24**, 1611–1625.
10. Kahn, D., Hickman, R., Terblanche, J. and Von Sommoggy, S.T. (1988) Partial hepatectomy and liver regeneration in pigs-The response to different resection sizes. *Journal of Surgical Research*, **45**, 176–180.
11. Zieve, L. and Anderson, R.W. (1985) Course of hepatic regeneration after 80–90 percent resection of normal rat liver. Comparison with 67–78 percent hepatectomy. *Gastroenterology*, **86(5)**, 1348.
12. Molina, L.M., De Diego, J.A., Simon, P. et al. (1982) Incorporation de timidina tritiada al DNA nuclear en el higado de rata en regeneracion. *revista Española de Enfermedades del Aparato Digestivo*, **62(4)**, 273–278.
13. Barbiroli, B. and Potter, Van R. (1971) DNA synthesis and interaction between controlled feeding schedules and partial hepatectomy in rats. *Science*, **172**, 738–741.
14. Garcia-Alonso, I., Méndez, J. and Barberá, E. (1989) Cyclosporin A modifies liver regeneration following partial hepatectomy. *Surgical Research Communications*, **6**, 43–49.
15. Noguchi, M., Tanaka, A., Taki, I., Shimahara, Y., Kamiyama, Y. and Ozawa, K. (1987) Acute responses of blood ketone body ratio following devascularization and revascularization of rabbit liver. *European Surgical Research*, **19**, 290–297.
16. Wang, W.Y., Taki, Y., Morimoto, T., Nishihira, T., Yokoo, N., Jikko, A., Nishikawa, K., Tanaka, J., Kamiyama, Y. and Ozawa, K. (1988) Effects of partial isquemia and reflow on mitochondrial metabolism in rat liver. *European Surgical Research*, **20**, 181–189.

17. O'Donohoe, M.K., Blake, A., Waldrom, R.P., Dervan, P. and Fitzpatrick, J.M. (1988) Pathophysiological sequelae of hepatic artery ligation: an experimental study". *European Surgical Research*, **20**, 330–334.
18. Francavilla, A., Ove, P., Polimeno, L., Coetzee, M., Makowka, L., Barone, M., Van Thief, D.H. and Starlz, T.E. (1988) Regulation of liver size and regeneration: importance in liver transplantation. *Transplantation Proceedings*, **XX(1)Supl.1**, 494–497.
19. Baker, A.L. (1986) Hepatotrophic factors: basic concepts and clinical implications. *Acta Medica Scandinavica*, **703**, 201–208.
20. Cruise, J.L., Knechtle, S.J., Bollinger, R.R., Kuhn, C. and Michalopoulos, G. (1987) Alphal-adrenergic effects and liver regeneration. *Hepatology*, **7(6)**, 1189–1194.
21. Rozga, J., Jeppsson, B. and Bengmark, S. (1988) The effect of pancreatic and intestinal venous blood on hepatic atrophy and compensatory hyperplasia in the rat. *Acta Physiologica Poloniae*, **39(5–6)**, 460–474.
22. Alexander, R.W., Saydjari, R., MacLellan, D.G., Townsend, C.M. and Thompson, J.C. (1988) Calmodulin antagonist trifluoperazine inhibits polyamine biosynthesis and liver regeneration. *British Journal of Surgery*, **75**, 1160–1162.
23. Mackenzie, R.J., Furnival, C.M., O'Keane, M.A.O. and Blumgart, L.H. (1975) The effect of hepatic ischaemia on liver function and the restoration of liver mass after 70 per cent partial hepatectomy in the dog. *British Journal of Surgery*, **62**, 431–437.
24. García-Alonso, I., Méndez, J. and Barberá-Guillem, E. (1988) Changes in succinate deshydrogenase zonation following cyclosporin-treatment in normal and regenerating rat liver. *Cellular and Molecular Biology*, **34(6)**, 605–614.
25. Yang, I.K., Salvini, P., Auxilia, F. and Calne, R.Y. (1988) Effect of CsA on hepatocyte proliferation after partial hepatectomy in rats: Comparison with standard immpnosuppresive agents. *American Journal of Surgery*, **155**, 245–249.
26. Ortiz, J.,García-Alonso, I., Basáñez, A., Apecechea, A., Iturburu, I. and Méndez, J. (1988) Efectos de la ciclosporina A, alopurinol y radiación corporal total sobre la isquemia aguda intestinal experimental. *Archivos de la Facultad de Medicina de Zaragoza*, **28(3)**, 178–179.
27. Sobotka, L., Simek, J., Dvoráckova, I. and Nouza, K. (1980) Influence of antithymocytic serum on the regenerating activity of the liver after partial hepatectomy in mice. *Czechoslovakian Medicine*, **3(4)**, 271–279.
28. Yasunda, K., Muranyi, M. and Lie, T.S. (1986) Immunological aspects of hepatic regeneration. *European Surgical Research*, **18(S1)**, 105.
29. Lie, T.S., Preiainger, H.K., Yoshimura, S. and Hong, G.S. (1988) Suppressor cell activity in hepatic regeneration. *European Surgical Research*, **20(S1)**, 167–168.