JOURNAL OF SURGICAL RESEARCH 61, 527–530 (1996)
ARTICLE NO. 0158

Hepatotrophic Effect of Folinic Acid in Rats

VICENTE PORTUGAL, PH.D., IGNACIO GARCÍA-ALONSO, PH.D., AND JAIME MÉNDEZ, PH.D.

Laboratory of Experimental Surgery, Department of Surgery, Faculty of Medicine, University of Basque Country, Leioa, Spain

Submitted for publication June 2, 1995

Hepatic ischemia hinders the proliferative response of hepatocytes, necessary to restore the liver/body ratio after liver resection/transplantation. Folinic acid administered during the ischemic period following 70% hepatectomy plus 15 min of normothermic liver ischemia has restored the regenerative response to the levels of normoperfused livers. This unexpected finding has guided us to design the present study in order to find out whether the folinic acid is an hepatotrophic substance or not. Sprague–Dawley rats submitted to partial (40 or 70%) hepatectomies were used. Saline (2 cc) or folinic acid (2.5 mg/kg) have been administered i.v. Forty-eight hours after hepatectomy the hepatocyte's DNA content has been assessed by means of a cytophotometric technique, and the percentage of regenerating hepatocytes (PRH) has been calculated. Folinic acid administration has significantly increased the PRH in both resting (5.1 vs 1.2) and regenerating livers (70% hepatectomy) (22.2 vs 41) when compared with nontreated groups. Folinic acid administration after liver ischemia plus hepatectomy has shown similar results, corroborating our previous study. Although its mechanisms of augmentation of liver regeneration remain unclear and further studies are required, folinic acid seems to be a promising therapeutic tool in liver surgery.

INTRODUCTION

Since Pringle described his maneuver in 1908 [1], liver ischemia has been commonly used during those surgical procedures that benefit from a bloodless surgical field. Nowadays, the main indications are: hepatic injury, partial hepatectomies, metastasectomies, nontumoral hepatic resections, and liver transplantation [2]. In all these situations, liver regeneration is necessary to restore the liver/body ratio.

However, in previous studies we proved that normothermic liver ischemia impairs the proliferation of hepatocytes [3]. In order to reduce this injury (ischemic injury), we administered some antioxidant drugs, such as superoxide dismutase (SOD) (a scavenger of oxygen free radicals), allopurinol, and folinic acid (inhibitors of xanthine oxidase) [4]. In our model, the administration of SOD and folinic acid improved hepatic regeneration following 70% hepatectomy and 15 min of normothermic liver ischemia. But it was quite more striking to find out that prereperfusion treatment with folinic acid completely reverted the deleterious effect of ischemia. To elucidate this unexpected finding, two possible mechanisms were discussed: the ability of folic acid and folate analogues to inhibit the xanthine oxidase [5, 6] or an unsuspected hepatotrophic capacity.

We have designed the present research in order to find out whether the folinic acid is an hepatotrophic substance or not.

MATERIAL AND METHODS

Male Sprague–Dawley rats weighting 250 g were used. The animals received an ordinary pellet diet (A04-Panlab) and water *ad libitum* prior to and after the experiments. Surgery was performed between 9 and 11 AM to standardize for natural diurnal rhythms. All the experiments have been carried out following the *Spanish National Guide for Animal Care* in experimentation and other scientific purposes (RD 223/88).

The folinic acid has been administered through the left femoral vein, just prior to liver resection, by means of a continuous perfusion pump at doses of 2.5 mg/kg [7] (Lederfolin, Lederle) diluted into 2 cc of saline. In ischemic animals the drug was administered 10 min prior to reperfusion.

Seventy or forty percent hepatectomies were carried out, under ether anesthesia, following Higgins' method (just prior to reperfusion in ischemic animals). When performed, liver ischemia was induced by occluding the hepatic hilum with a small vascular clamp for 15 min; the mesenteric artery and the celiac trunk were clamped too, to avoid splachnic congestion [8]. Once the ischemic period was finished, the abdomen was reopened and the vascular clamps were removed. The restoration of the blood flow was appreciated by the coloration of the liver and gut.

Liver regeneration was studied in eight groups of 10 animals randomly assigned by quantifying the nuclear DNA content of hepatocytes (Table 1).

TABLE 1

Experimental Series

1. Control
2. Folinic acid admon. (2.5 mg/kg)
3. 70% Hepatectomy
4. 70% Hepatectomy + folinic acid
5. Hepatic ischemia + 70% hepatectomy
6. Hepatic ischemia + 70% hepatectomy + folinic acid
7. 40% Hepatectomy
8. 40% Hepatectomy + folinic acid

0022-4804/96 $18.00

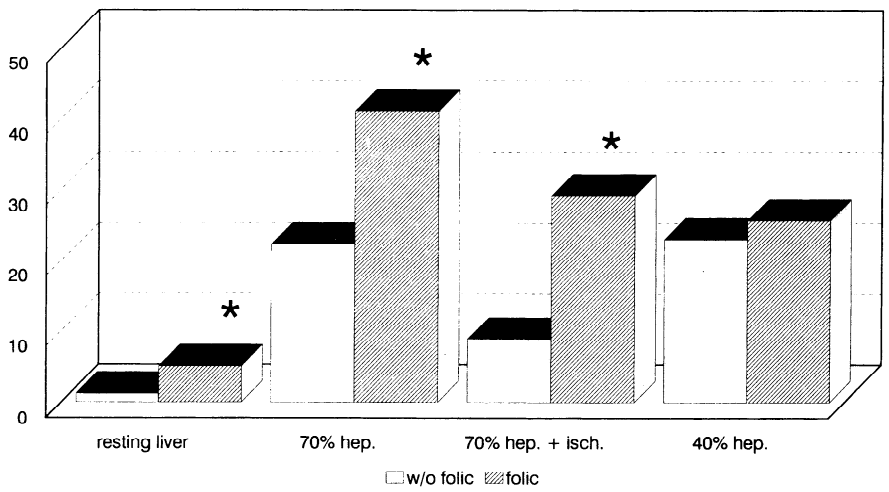

FIG. 1. Effect of folinic acid (striped bars) upon percentage of regenerating hepatocytes. It significantly increases PRH in both resting and following 70% hepatectomy, (*$P < 0.05$ when compared with nontreated groups).

DNA quantification. Rats were sacrificed 24 hr after surgery and a fragment of liver was quickly removed and embedded in paraffin. On 5 μm of histological sections stained in Schiff reagent, DNA was quantified in 100 hepatocytic nuclei by means of a microspectrocytophotometer (VMS-0.5, Carl Zeiss, Obercochen) (λ = 560 nm). Then, frequency histograms were calculated for each animal, where DNA was expressed in arbitrary units. Using a modification of Bartel's method [9], histograms were resolved into Gaussian curves, the left one corresponding to static cells and the others to regenerating hepatocytes. This method allowed us to assess the percentage of regenerating hepatocytes (PRH) of each animal, which fairly represents the intensity of the regenerative response in a certain cellular population [10, 11]. In the same way the regenerative gradient (RG) of each animal was obtained from the ratio: DNA content of regenerating hepatocytes to DNA content of static ones. This new parameter represents the chronology of the regenerative process and it tries to express the differences observed between curves with the same PRH [3].

Statistical analysis. Given that the distribution of the results was not normal, the comparison between the different experimental series was carried out with a nonparametric test: Rank Sum Test. Those differences with a $P < 0.05$ were considered significant.

RESULTS

Folinic acid has induced hepatic regeneration per se (Fig. 1). It has increased the percentage of regenerating hepatocytes (PRH = 5.07, SD = 4.7; $P < 0.005$ vs controls) (Table 2) and simultaneously, it has induced a lower RG (RG = 1.76, SD = 0.14; $P < 0.005$ vs controls) (Fig. 2, Table 3).

On normoperfused livers, folinic acid has also increased the PRH following 70% hepatectomy (22.29, SD = 9.7 vs 41.03, SD = 17.4; $P < 0.005$). However, in this case the RG has not shown any significant change.

When we have associated a lower regenerative stimulus—that is 40% instead of 70% hepatectomy—folinic acid has not modified the number of regenerating hepatocytes or the regenerative rhythm.

Regarding our studies on ischemic livers, folinic acid administration has increased the PRH following normothermic ischemia plus 70% hepatectomy (8.85, SD = 7.3 vs 29.10, SD = 11.28; $P < 0.005$). But once again, it has not modified the RG (1.74 vs 1.95, $P = 0.12$).

DISCUSSION

The widespread practice of liver surgery has increased the interest on the ischemia-reperfusion syndrome. The correct knowledge of the lesions it induces and of its pathogenic mechanisms will allow designing specific treatments, which in turn will improve postsurgical liver function [12]. On the other hand, the shortage of pediatric donors has impelled partial liver trans-

TABLE 2

Percentage of Regenerating Hepatocytes

Experimental series	Mean	S.D.	Median	Minimum	Maximum
Control	1.211	1.167	0.759	00.00	3.885
Folic acid admon.	5.071	4.721	3.967	0.756	16.00
70% Hepatectomy	22.29	9.698	20.82	10.66	35.51
70% Hepatectomy + folic acid	41.03	17.43	37.01	13.41	71.31
Hepatic ischemia + 70% hepatectomy	8.852	7.385	7.560	00.00	33.29
Hepatic ischemia + 70% hepatectomy + folic acid	29.10	11.28	27.89	13.84	47.91
40% Hepatectomy	23.00	26.11	13.00	2.339	69.58
40% Hepatectomy + folic acid	25.65	17.20	22.84	7.738	50.35

TABLE 3

Regenerative Gradient

Experimental series	Mean	S.D.	Median	Minimum	Maximum
Control	2.161	0.2412	2.097	1.892	2.660
Folic acid admon.	1.756	0.1408	1.758	1.522	1.928
70% Hepatectomy	1.607	0.1254	1.624	1.389	1.773
70% Hepatectomy + folic acid	1.562	0.0879	1.580	1.364	1.676
Hepatic ischemia + 70% hepatectomy	1.739	0.3326	1.761	1.302	2.244
Hepatic ischemia + 70% hepatectomy + folic acid	1.955	0.2344	1.890	1.602	2.378
40% Hepatectomy	1.754	0.3642	1.660	1.351	2.420
40% Hepatectomy + folic acid	1.700	0.1863	1.677	1.397	2.044

plantation [13, 14]; and so, it happens to be a yet more important aim to control liver regeneration, as it is the only way to adapt body and liver sizes after transplantation [15].

The results obtained in this study allow us to consider the folinic acid as an "hepatotrophic" substance, as it has increased the PRH both in resting and proliferating livers. This drug behaves quite similarly to cyclosporine [16] and FK-506 [17], which have been considered hepatotrophic also.

What this experiment has failed to clarify is the mechanism through which the folinic acid exerts its hepatotrophic activity. As we have not found any references regarding this specific point, there are no grounds to establish any solid hypothesis. However, we may propose some comments.

It is well-known that folic acid plays a central role in different steps of DNA synthesis [18]: its congener 5,10-methylenetetrahydrofolate donates the methylene group necessary for the synthesis of thymidilate from deoxyuridylate (an extremely important reaction in DNA synthesis), and the participation of derivates of folic acid is also required in two steps of the synthesis of purine nucleotides. On the other hand, hepatic parenchima plays the major role in the folate's metabolism (store, bile excretion, and enterohepatic cycle).

After partial hepatectomy, the stores of folic acid diminish while DNA synthesis—and therefore, folic requirements—increases rapidly. In this way, perhaps the availability of the folate compounds could limit DNA synthesis, both its velocity and intensity. Just the opposite situation has already been established for hemolityc anemias, in which a high rate of cell turnover may induce a deficiency of folate [19].

This hypothesis could explain the hepatotroffic effect of folinic acid administration following 70% hepatectomy, but not on resting livers. Why and how the folinic acid does act as an hepatotrophic stimulus in this last condition, is unclear. Perhaps, the transmethylating ability of folinic acid could induce activating changes in the biochemical structure of the target molecules implicated in DNA-synthesis. Other possible suggestions include structural similarities with other hepatotrophic drugs, and actuation over a common second messenger.

Anyway, taking into account the unexpensiveness and lack of toxic effects of folinic acid [20, 21], it seems worthy to further study a few more questions previous to clinical trials. First of all, the best dose should be found, trying to correlate plasma levels and hepatotrophic effect. On the other hand, the lack of effect following 40% hepatectomy should also be investigated,

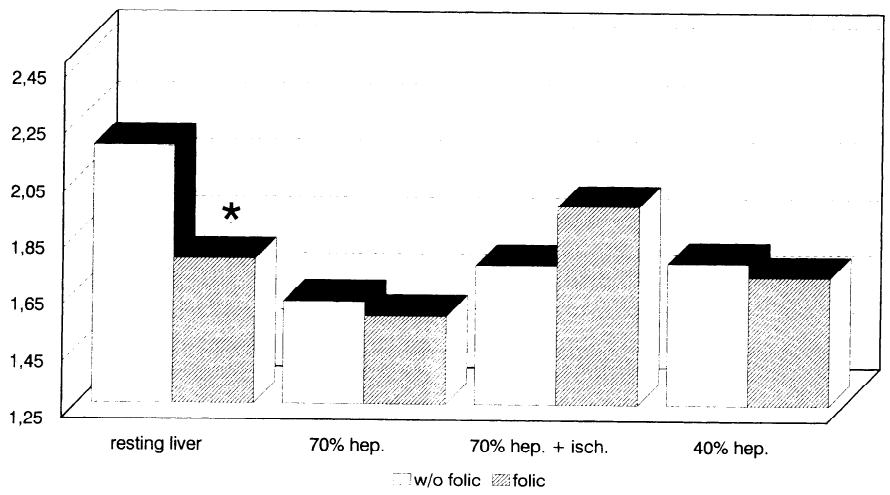

FIG. 2. Effect of folinic acid (striped bars) upon RG. It significantly diminishes RG in resting livers. It does not modify RG in hepatectomized ones (*$P < 0.05$ when compared with nontreated groups).

looking for better results at different intervals (30, 40, 48 h posthepatectomy).

In short, folinic acid combines antioxidant plus hepatotrophic properties, which make it a promising tool in liver surgery including ischemia and parenchymal resection.

REFERENCES

1. Foster, J. H. History of liver surgery. *Arch. Surg.* **126**(3): 381–389, 1991.
2. Kemeny, M. M. Hepatic resection: When, what kind and for which patients. *J. Surg. Oncol.* **2**(Suppl.): 54–58, 1991.
3. García-Alonso, I., Portugal, V., Iturburu, I., L. de Tejada, I., and Méndez, J. Modifications induced by cyclosporin A on ischemic liver regeneration. *Surg. Res. Commun.* **9:** 227–233, 1990.
4. Portugal, V., García-Alonso, I., Barceló, P., and Méndez, J. Effect of allopurinol, folinic acid, SOD and Ciclosporine A on ischemic liver regeneration. *Eur. Surg. Res.* **27:** 69–76, 1995.
5. Granger, D. N., McCord, J. M., Parks, D. A., and Hollwarth, M. E. Xanthine oxidase inhibitors attenuate ischemic induced vascular permeability changes in the rat intestine. *Gastroenterology* **90:** 80–84, 1986.
6. Lewis, A. S., Murphy, L., McAlla, C., Fleary, M., and Purcell, S. Inhibition of mammalian xanthine oxidase by folate compounds and amethopterin. (Folic acid is a potent inhibitor of xantine oxidase). *J. Biol. Chem.* **259:** 12–15, 1984.
7. Bilbao, J., García-Alonso, I., Portugal, V., Barceló, P., Apecechea, A., and Méndez, J. Efficacy of antioxidant therapy (folic acid and alphatocopherol) in intestinal lesions produced by reperfusion. *Cirugia Española* **51**(4): 262–265, 1992.
8. Yamamoto, S., Nitta, N., Ozaki, N., Morimoto, T., Kamiyama, Y., Yamaoka, Y., Okamoue, T., and Ozawa, K. Deleterios effect of splanchnic congestion on hepatic energy metabolism following repeated portal tiad cross-clamping in dogs. *Circ. Shock* **26:** 193–201, 1988.
9. Bartel, P. H. Numerical evaluation of the cytological data. I. Description of profiles. *Anal. Quan. Cytol.* **4:** 20–28, 1979.
10. Bötger, T., Störkel, S., Stökle, M., Wahl, W., Jugenheimer, M., Effenberger-Kim, O., Vinh, T., and Junginger, T. DNA image cytometry. A prognostic tool in squamous cell carcinoma of the esophagus? *Cancer* **67**(9): 2290–2294, 1991.
11. Herman, C. J. Cytometric DNA analysis in the management of cancer. Clinical and laboratory considerations. *Cancer* **69**(6): 1553–1556, 1992.
12. Blankestein, J. D., and Terpstra, O. Liver preservation: The past and the future. *Hepatology* **13**(6): 1235–1250, 1991.
13. Badger, I. L., Czerniak, A., Beath, S., Tisone, G., Deakin, M., Sherlock, D. J., Kelly, D. A., McMaster, P., and Buckels, J. A. C. Hepatic transplantation in children using reduced size allografts. *Br. J. Surg.* **79:** 47–49, 1992.
14. Houssin, D., Soubrane, O., Boillot, O., Dousset, B., Ozier, Y., Devictor, D., Bernard, O., and Chapius, Y. Orthotopic liver transplantation with a reduced size graft: An ideal compromise in pediatrics. *Surgery* **111:** 532–542, 1992.
15. Francavilla, A., Ove, P., Polimeno, L., Coetzee, M., Makowka, L., Barone, M., Van Thiel, D. H., and Starzl, T. E. Regulation of liver size and regeneration: Importance in liver transplantation. *Transplant. Proc.* **20**(Suppl. 1): 494–497, 1988.
16. García-Alonso, I., Méndez, J., and Barberá, E. Cyclosporin modifies liver regeneration following partial hepatectomy. *Surg. Res. Commun.* **6:** 43–49, 1989.
17. Starzl, T. E., Porter, K. A., Mazzaferro, V., Todo, S., Fung, J., and Francavilla, A. Hepatotrophic effects of FK506 in dogs. *Transplantation* **51:** 67–70, 1991.
18. Hillman, R. S. Hematopoietic agents: growth factors, minerals and vitamins. In A. Goodman Gilman, T. W. Rall, A. S. Nies, and P. Taylor, (Eds.), *The Pharmacological Basis of Therapeutics (8th Ed.).* New York: Pergamon Press, 1990. Pp. 1294–1306.
19. Lindenbaum, J. Folic acid requirement in situations of increased need. In *Folic Acid: Proceedings of a Workshop on Human Folate Requirements,* 1975. Washington D.C.: National Academy of Sciences, 1977. Pp. 256–276.
20. Graf, W., Weiber, S., Glimelius, B., Jiborn, H., Pahlman, L., and Zederfeldt, B. Influence of 5-fluorouracil and folinic acid on colonic healing: An experimental study in the rat. *Br. J. Surg.* **79:** 825–828, 1992.
21. MRC Vitamin Study Research Group. Prevention of neural tube defects: Results of the Medical Research Council Vitamin study. *Lancet* **338:** 131–137, 1991.