Liver Regeneration and Tumor Growth in the Rat after Partial Hepatectomy

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ABSTRACT: Tumor growth of Yoshida sarcoma implanted in the remnant liver was studied in rats subjected to a hepatectomy. After 70 percent hepatectomy, the liver progressively regenerated and the total liver weight was reverted to by 10 days after the operation. Concomitantly with liver regeneration, tumor growth in the remnant liver was stimulated significantly, compared with that in the sham-operated liver. Incorporation of tritiated thymidine into tumor cells in the remnant liver was strikingly high and progressive, while that in the sham-operated liver was low and retained. Mitomycin C given to the hepatectomized rats was more effective against the tumor in the remnant liver than in the sham-operated liver. We conclude from this study that cancer cell proliferation in the remnant liver can be accelerated by the process of liver regeneration.

KEY WORDS: liver regeneration, mitomycin C, Yoshida sarcoma, hepatectomy

INTRODUCTION

Major hepatectomy has to be considered as a possible treatment for primary cancer of the liver or for liver metastases since this approach is at present the only hope for a cure. Recently, we treated two patients who succumbed to a rapid growth of residual tumor shortly after liver resection, one for primary hepatoma, the other for metastatic cancer of the rectum. After partial hepatectomy the liver rapidly regenerates, reaching the weight of the normal liver by 4 to 6 weeks in humans and 1 to 2 weeks in the rat. Liver carcinogenesis is reportedly enhanced by treatment with carcinogens plus partial hepatectomy.[1-3] We attempted to clarify whether or not growth of an unresected tumor in the remnant liver would be enhanced. For this we observed the growth and incorporation of a radioactive precursor of tumor cells implanted into the remnant liver of partially hepatectomized rats.

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MATERIALS AND METHODS

Rats

Adult Donryu rats weighing between 150–250g were housed in metal cages and fed a pellet diet and water, *ad libitum*. After operation, 500mg of cephalosporine were dissolved in 100ml of the drinking water given these rats and replenishments were provided daily.

Hepatectomy

The rats were anesthetized with an intraperitoneal injection of 15mg of pentobarbital and the livers were exposed through a

midline abdominal incision. Both the median lobe and left lateral lobe were excised according to the technique of Higgins and Anderson.[4] With this procedure, 70 ± 3.5 percent of the total liver (determined in 30 rats) was excised, leaving the right lateral, small caudate, and spigelian lobes. The excised livers were immediately weighed. The sham operation consisted of an abdominal incision and gentle palpation of the liver. One day after the operation, water and the usual diet was provided and no special postoperative care was given.

Wet weight of liver

Two groups of 25 hepatectomized and 25 sham-operated rats were used. Under ether anesthesia, 4 rats in each group were exsanguinated at 2 to 5 day intervals during the experiment. The remaining livers were immediately removed with scissors and weighed.

Tumor implantation

A solid tumor of Yoshida sarcoma was cut into the small pieces and two pieces weighing approximately 10mg were implanted into the parenchyma of the right lateral lobe with a trocar, immediately after the hepatectomy. To position the tumor and to stop bleeding, the liver was pressed slightly with the fingers of the operator and then a drop of Aron Alpha-A (alkyl α-cyanoacrylate monomer, Sankyo, Tokyo, Japan) was applied at the injected point. Tumor implantation was performed in two groups of rats, one hepatectomized, the other sham-operated. Tumors of rats which died within 14 days and those from survivors killed on day 14 were removed and weighed.

Injection of mitomycin C

Effect of mitomycin C was compared between the hepatectomized and sham-operated groups. Mitomycin C was given intravenously through a tail vein in a daily dose of 0.4mg per kg body weight after 0, 1 and 2 days of tumor implantation.

Tritiated thymidine autoradiography[5]

In two groups of 18 hepatectomized and 18 sham-operated rats, the tumor was implanted into the right lateral lobe of liver. Thymidine [6-^{3}H] (^{3}H-TdR) (specific activity, 21.5 Ci/mmole, New England Nuclear, Boston, Mass., U.S.A.) in a dose of 3 mCi per kg body weight was administered intraperitoneally and the rats were killed one hour later. At different times, 1, 2, 3, 4, 5 and 10 days after tumor implantation, 3 animals of each group were killed and sections of both the parenchyma of the liver and the periphery of the tumor were fixed in 10 percent buffered formalin and then coated with Sakura NRM2 stripping film.[5] The numbers of labeled hepatic of labeled tumor cell nuclei were counted in randomly selected fields with 3 readings and expressed as the number of ^{3}H-labeled nuclei per 1000 nuclei.

Statistical methods

Results were expressed as mean ± SD. Student's t-test was used for statistical analysis. A P-value of less than 0.05 was considered significant.

Results

Of 25 hepatectomized animals, 2 died on the first and 1 on the 11th postoperative day, the mortality rate being 12 percent. No animals died after sham operation. Wet weight of the remnant liver was increased repidly and the total liver weight was recovered by 10 days, as shown in Fig. 1. Mean weight of tumors in the

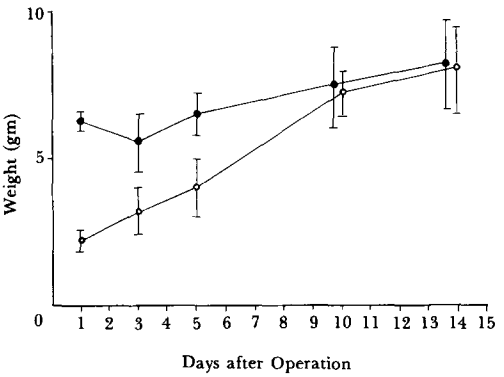

Fig. 1. Wet weight of the liver. Partially hepatectomized and sham-operated rats were killed at various intervals. Each point represents the mean wet weight of the liver from 3 hepatectomized (○) and 3 sham-operated (●) rats with SD.

Table 1. Effect of Partial Hepatectomy on the Growth of Tumor Implanted into the Liver

Manipulation	No. of Rats	Body Weight (gm)	Weight of Resected Liver (gm)	Tumor Weight* (gm)	P
Sham Operation	15	203.7±64.24**	/	0.5±0.96	<0.01
Partial Hepatectomy	15	209.2±77.09	3.9±0.92	1.7±1.23	

*Killed on the 14th day after tumor implantation
**mean±SD

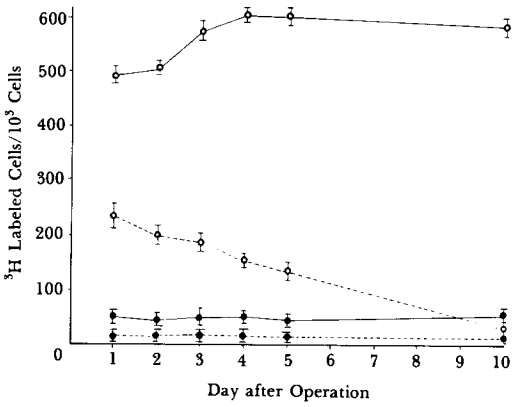

Fig. 2. ^{3}H-TdR incorporation of tumor and hepatic cells in the remnant and sham-operated livers. Each point represents the mean count of 9 readings with SD: tomor cells in the remnant liver (—○—), hepatic cells in the remnant liver (--○--), tumor cells in the sham-operated liver (—●—), hepatic cells in the sham-operated liver (--●--).

remnant liver was about 3 times that in the sham-operated liver, as shown in Table 1. The difference between the two groups was statistically significant ($p<0.01$). Fig. 2 shows the results of ^{3}H-TdR incorporation into tumor cells and hepatic cells in hepatectomized and sham-operated rats. ^{3}H-TdR incorporation into tumor cells in the remnant liver was exclusively high, increased gradually and reached a peak at 4 days after hepatectomy, while that into the tumor cells in the sham-operated liver was low and retained. As to hepatic cells, ^{3}H-TdR incorporation into hepatic cells in the remnant liver was high by 1 day after hepatectomy, then decreased gradually and returned to normal ranges by 10 days after hepatectomy. There was a certain extent of incorporation into the hepatic cells in the sham-operated liver.

Mitomycin C was more effective against tumor in the remnant liver than in the sham-operated liver, as shown in Table 2. Here, there was no statistical significance.

Table 2. Effect of Mitomycin C on Tumor Growth in Hepatectomized and Sham Operated Rats

Manipulation	No. of Rats	Body Weight (gm)	Weight of Resected Liver (gm)	Tumor Weight* (gm)	P
Partial Hepatectomy	13	139.9±9.42**	3.0±0.33	2.1±2.58	N.S.
Partial Hepatectomy + Mitomycin C	10	138.0±8.49	3.3±0.59	1.1±1.00	
Sham Operation	9	130.2±8.62	/	0.7±0.65	N.S.
Sham Operation + Mitomycin C	9	131.4±9.42	/	0.6±0.67	

*Killed on the 14th day after tumor implantation
**mean±SD

Discussion

A partial hepatectomy had a strong promoting effect on tumor cells implanted into the remnant liver of rats. Growth of the tumor in the remnant liver increased markedly, compared with that in the sham-operated liver. In the autoradiographic study, ^{3}H-TdR incorporation of the tumor in the remnant liver was extremely high, thereby indicating an accelerated DNA synthesis.[6,7] Liver regeneration involving initiation, growth and differentiation after hepatectomy is controlled by blood-borne factors[8-12] and when the liver is completely restored to the preoperative level, these factors are no longer operative.[13]

In the present study, the remnant liver returned to total liver weight and ^{3}H-TdR incorporation of the hepatic cells also decreased to normal ranges at 10 days after hepatectomy. Initiation of cell division in the hepatic and tumor cells will be synchronous. Thereafter growth of hepatic cells is controlled, however, that of tumor cells is uncontrolled and progressive. A marked progression of tumor growth occurred only in the regenerating liver thereby suggesting that hepatotrophic factors produced by the regenerating process of hepatic cells probably have a promoting effect on tumor cells growing in the liver.[7] This phenomenon resembles the neoplastic transformation in hepatic cells under special circumstances of damage and repair of liver parenchyma.[1-3]

As to the promoters that initiate hepatic regeneration, Levi and Zeppa[9] reported in a closed loop system that the remnant liver was not only the target of a humoral agent but also the source of its production. This would explain the extensive tumor growth only in the regenerating liver.

In clinical situations, great care should be directed to possible acceleration of the remaining tumor and/or to an undetectable tumor. Here, adjuvant chemotherapy is mandatory. Chemotherapy with mitomycin C was more effective against rapid dividing tumor cells in the remnant liver than against slow growing tumor cells in the sham-operated liver. Drugs that impair hepatic regeneration should be prescribed with care.[14-16]

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References

1. Craddock VM, Frei JV. Induction of liver cell adenomata in the rat by a single treatment with N-methyl-N-nitrosourea given at various times after partial hepatectomy. Brit J Cancer 1974; 30: 503–511.
2. Solt DB, Cayama E, Tsuda H, Enomoto K, Lee G, Farber E. Promotion of liver cancer development by brief exposure to dietary 2-acethylaminofluorene plus partial hepatectomy or carbon tetrachloride. Cancer Res 1983; 43: 188–191.
3. Ying TS, Enomoto K, Sarma DSR, Farber E. Effects of delays in the cell cycle on the induction of preneoplastic lesions in rat liver with 1, 2-dimethylhydrazine. Cancer Res 1982; 42: 876–880.
4. Higgins GM, Anderson RM. Experimental pathology of the liver. Arch Path 1931; 12: 186–202.
5. MacDonald RA, Mallory GK. Autoradiography using tritiated thymidine. Lab Invest 1958; 8: 1547–1562.
6. Rajewsky MF. Changes in DNA synthesis and cell proliferation during hepatocarcinogenesis by diethylnitrosamine. Europ J Cancer 1967; 3: 335–342.
7. Makowska L, Falk RE, Falk JA, Teodorczyk-injeyan J, Venturi D, Rotstein LE, Falf W, Langer B, Blendis LM, Phillips J. The effect of liver cytosol on hepatic regeneration and tumor growth. Cancer 1983; 51: 2181–2190.
8. Bucher NLR. Experimental aspects of hepatic regeneration. New Eng J Med 1967; 277: 686–696, 738–746.
9. Levi JU, Zeppa R. Source of the humoral factor that initiates hepatic regeneration. Ann Surg 1971; 174: 346–370.
10. Bocker FF. Humoral aspects of liver regeneration. In: LoBue J and Gordon AS, eds. Humoral control of growth and regeneration. New York: Academic Press, 1973; 249–256.
11. Leffert H, Alexander NM, Faloona G, Rubalcava B, Unger R. Specific endocrine and hormonal receptor changes associated with liver regeneration in adult rats. Proc Nat Acad Sci U S A 1975; 72: 4033–4036.
12. Costrini NV, Beck R. Epidermal growth factor-urogastrone receptors in normal human liver and primary hepatoma. Cancer 1983; 51: 2191–2196.
13. Grisham JW. Morphologic study of deoxyribonucleic acid synthesis and cell proliferation in regenerating rat liver: Autoradiography with thymidine-^{3}H. Cancer Res 1962; 22: 842–849.
14. Gavosto F, Pileri A. The effect of administration of

6-mercaptopurine on nucleic acids and alkaline phosphatase of regenerating liver. Cancer 1958; 11: 222–225.

15. Gonzales EM, Krejczy K, Malt RA. Modification of nucleic acid synthesis in regenerating liver by azathiopurine. Surgery 1970; 68: 254–259.
16. Nagasue N, Kobayashi M, Iwaki A, Yukaya H, Kanashima R, Inokuchi K. Effect of 5-fluorouracil on liver regeneration and metabolism after partial hepatectomy in the rat. Cancer 1978; 41: 435–443.