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Regulating factors of liver regeneration after hepatectomy

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Abstract. The factors regulating liver regeneration were studied by measuring changes in the liver volume and serum hepatocyte growth factor (HGF) levels after hepatectomy. Changes in the liver volumes were studied in 68 hepatectomized patients, including (A) hepatoma patients who had chronic hepatitis or liver cirrhosis ($n = 44$) and (B) metastatic liver cancer patients who had normal liver parenchyma ($n = 24$). The hepatic volume increased by 13.8% of the remnant hepatic volume in group A and by 49.1% in group B. The examined factors included the percentage of resected liver volume (%RLV) and the results of laboratory tests. Regression analysis showed that in group A, both %RLV ($\beta = 0.46$) and the serum total bilirubin (T-Bil) level ($\beta = -0.33$) correlated significantly with the extent of liver regeneration and that in group B, only %RLV ($\beta = 0.78$) correlated significantly with the regeneration. Serum HGF levels after hepatectomy were studied in 21 hepatectomized patients, including 11 hepatoma patients and 10 patients with some types of metastatic liver cancer. Serum HGF levels increased significantly after surgery in all 21 patients. Regression analysis, however, showed that the change in HGF was related to liver cirrhosis ($\beta = 0.46$) and to the maximal postoperative T-Bil level ($\beta = 0.51$) but not to the extent of liver regeneration after hepatectomy. These results suggest that liver regeneration is regulated primarily by factors relating to the percentage of the resected liver parenchyma and that serum HGF levels do not directly relate to liver regeneration after surgery.

Introduction

Liver regeneration in clinical surgery is complicated because hepatectomy is very often indicated for hepatoma patients who have chronic hepatitis or liver cirrhosis. The mechanisms of liver regeneration, however, have been studied mostly in experimental models, and many substances have been shown to stimulate hepatocyte DNA synthesis [1, 10, 11, 14, 20]. Among them, hepatocyte growth factor (HGF) has been nominated as one of the most promising substances in controlling regeneration of the liver [9, 13, 15, 26]. The physiological roles of HGF remain to be elucidated by studies on endogenous HGF in relation to liver regeneration in patients undergoing hepatic resection.

The aims of the present study were twofold: first, to quantitatively determine the effects of chronic hepatitis or liver cirrhosis on liver regeneration, and second, to determine whether increases in serum HGF correlate directly with liver regeneration after hepatectomy.

Patients and methods

Accuracy of estimation of liver volume using CT scans. The liver volume was estimated by a modification of the method of Heymsfield et al. [7] using computed tomography (CT) scans (Imatron-100 C; San Francisco, USA) of cross-sections of the liver at intervals of 6 mm. Volumes estimated from the resected areas on the CT scans were compared with weights of the corresponding surgical specimens obtained from 41 typical hepatectomies in which areas of the liver to be resected could be easily and accurately determined from CT scans.

Analysis of liver regeneration using clinical materials. This phase of the study was carried out by reviewing the protocols and CT scans of patients with hepatoma or some types of metastatic liver cancer who underwent hepatic resection. Patients who had severe surgical complications after surgery or who had tumor recurrence within 6 postoperative months were excluded from the study. The patients consisted of (A) 44 patients with hepatoma and (B) 24 patients with a metastatic liver cancer. In group A, 30 patients had liver cirrhosis of Miyake's type B and 14 had chronic hepatitis. There were 31 men and 11 women (mean age, 64.6 years). In group B, all the patients had a normal liver parenchyma. There were 19 men and 5 women (mean age, 59.1 years). The diagnoses were confirmed by histopathological examination.

The percentage of resected liver volume (%RLV) and the percentage of increase in the remnant liver volume at 6 months after surgery (%ILV) were calculated according to the following equations:

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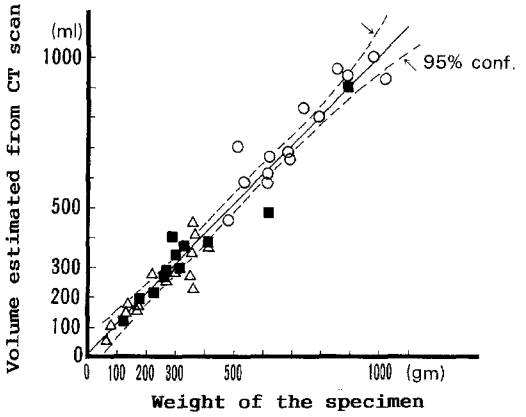

Fig. 1. Correlation between volumes estimated from the resected areas on CT scans and weights of surgical specimen in typical hepatectomies. ○, Right hepatic lobectomy (n = 14); △, left hepatic lobectomy (n = 12); ■, left lateral hepatic segmentectomy (n = 15)

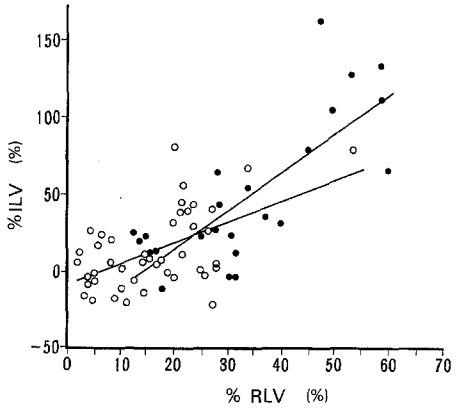

Fig. 2. Correlation between %RLV and %ILV in patients with hepatoma and metastatic liver cancer. ○, Hepatoma (n = 24); ●, metastatic liver cancer (n = 44). Formulas for the regression line were Y = 2.46X – 33.9 (R^2 = 0.609) for hepatoma and Y = 1.35X – 8.2 for metastatic liver cancer, where Y = %ILV and X = %RLV

%RLV = 100 × A/B; and %ILV = 100 × [C-(B-A)]/[B-A], where A is the total resected liver volume minus the resected tumor weight, B is the total liver volume before hepatectomy minus the tumor weight, and C is the liver volume at 6 months after hepatectomy. The %ILV was considered to represent the extent of liver regeneration after hepatectomy.

Routine laboratory tests were performed on all of the patients before surgery, on days 1, 3, 5, and 7 after surgery, and at least once a week thereafter until day 28.

Serum HGF levels before and after hepatectomy. This phase of the study was carried out on (C) 11 hepatoma patients and (D) 10 patients with a metastatic liver cancer. In group C, eight patients had liver cirrhosis of Miyake's type B and the other three had chronic hepatitis. There were six men and five women (mean age, 61.5 years). In group D, the liver parenchyma was normal in all the patients. There were eight men and two women (mean age, 55.4 years). The %ILV was calculated at 3 postoperative months for groups C and D.

Assay of serum HGF level. Sera were obtained before surgery and on days 1, 3, 5 7, 14, 21, and 28 after surgery. The sera were stored at –80°C until the HGF assay. Serum HGF was measured using an enzyme-linked immunosorbent assay kit (Otsuka Assay Laboratories; Tokushima, Japan) as previously reported [23].

Statistical analysis. Student's t-test was used to compare two samples. Multiple linear regression analysis (step-up and step-down method) [18] using a microcomputer-based program of least-squares regression was performed to find independent relationships between variables and postoperative serum HGF levels or %ILV.

The variables used for analysis of the postoperative serum HGF levels were age, sex, %RLV, %ILV, presence of liver cirrhosis, indocyanine green retention rate at 15 min, and results of routine laboratory tests.

Table 1. %RLV and %ILV in patients with hepatoma and metastatic liver cancer

Group	Sex (n)	Age (years)	%RLV	%ILV
Hepatoma	M31, F13	64.6± 8.5	13.8±25.5	16.4±10.4
Metastatic liver cancer	M19, F 5	59.1±10.1	33.7±15.1	49.1±47.6
				Mean ± SD

Abbreviations: %RLV, percentage of resected liver volume; %ILV, percentage of increase in the remnant liver volume

Results

Accuracy of estimation of liver volume using CT scans

There was a highly significant correlation (R^2 = 0.94, P <0.001) between the volumes estimated from the resected areas on CT scans and the weights of the corresponding surgical specimen in patients undergoing a typical hepatectomy. The regression line between them was Y = 0.995X + 15.6, where Y is the volume on the CT scan and X is the weight of the surgical specimen (Fig. 1).

Analysis of liver regeneration using clinical materials

In group A, %RLV ranged from 2.0% to 53.6%, with a mean (±SD) value of 13.8%±25.5%. The %ILV ranged from –21.2% to 79.8%, with a mean (±SD) value of 16.4%±10.4%. In group B, %RLV ranged from 12.7% to 60.1%, with a mean value of 33.7%±15.1%. The %ILV ranged from –11.5% to 162.0%, with a mean value of 49.1%±47.6%. Both %RLV (P <0.01) and %ILV (P <0.05) were significantly larger in group B than in group A (Table 1). Figure 2 shows the regression lines between %RLV and %ILV in group A (R^2 = 0.30) and group B (R^2 = 0.61). The line for group B was steeper than that for group A. Multiple regression analysis for group A showed that %ILV was significantly and independently related to both %RLV and postoperative serum total bilirubin (T-Bil) levels (Table 2). The squared multiple r was 0.406, and the contributions of %RLV and the T-Bil level were 25.4 and 15.2, respectively. In group B, only %ILV related significantly and independently to %RLV. The contribution of %RLV was 60.9.

Serum HGF levels before and after hepatectomy

Serum HGF levels were elevated after hepatectomy and reached a maximum within 7 days after surgery in all the patients. In group C, the mean preoperative value (±SD) of

Table 2. Multiple regression analysis for factors of hepatic regeneration after hepatectomy

Factor	Standard partial regression coefficient	Contribution	*P*
Hepatoma (n = 44)[a]			
%RLV	0.462	25.4	0.001
T-Bil	–0.334	15.2	0.01
Metastatic liver cancer (n = 24)[b]			
%RLV	0.781	60.9	<0.001

[a] Multiple r = 0.637, R^2 = 0.406, overall P <0.001
[b] Multiple r = 0.772, P <0.001
Abbreviations: %RLV, percentage of resected liver volume; T-Bil, total bilirubin level

Table 3. Multiple regression analysis for maximal serum HGF level in patients after hepatectomy[a]

Factor	Standard partial regression coefficient	*P*
Liver cirrhosis	0.457	0.006
Maximal value of		
total bilirubin	0.505	0.001
ALT	0.358	0.029
WBC	0.533	0.001

[a] Multiple r = 0.826, R^2 = 0.682, overall P = 0.001
Abbreviations: ALT, alanine aminotransferase; WBC, white blood corpuscle count

HGF was 0.32±0.12 ng/ml, and the mean maximal value was 0.85±0.51 ng/ml. In group D, those values were 0.27±0.12 ng/ml and 0.63±0.20 ng/ml, respectively (Fig. 3). The increases in serum HGF levels were significant (P <0.01) in both groups C and D. There was no significant correlation, however, between maximal HGF levels and %ILV in either group C (R^2 = 0.003) or group D (R^2 = 0.026). Multiple regression analysis showed that postoperative maximal serum HGF levels were significantly and independently related to liver cirrhosis, postoperative maximal serum bilirubin, and peripheral white blood cell count (Table 3). The squared multiple r was 0.682.

Discussion

Our method for estimation of the liver volume was shown to be accurate enough to study the change in liver volume in patients undergoing hepatectomy.

Regeneration of the liver after hepatectomy has been reported to be regulated by many factors, including the percentage of resected liver volume [3], insulin or insulin-like growth factor [5, 19], humoral factors in the portal blood flow [4, 16], the vagus nerve [24], and many of the growth factors in the serum [1, 5, 14]. In this study, liver regeneration was poorer in the hepatoma patients, and this was explained in two ways: by the smaller percentage of resected liver volume and by a lower capacity for regeneration in the hepatoma patients. The effects of the

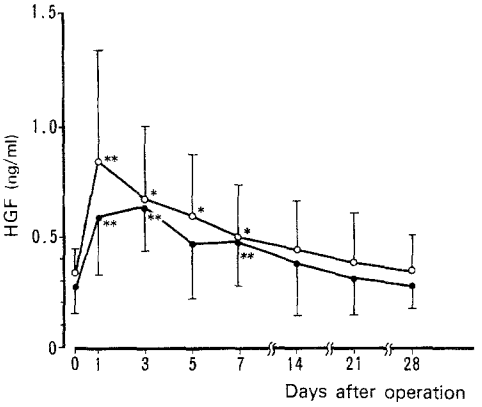

Fig. 3. Serum HGF levels before and after hepatectomy in patients with hepatoma and metastatic liver cancer. ○, Hepatoma (n = 11); ●, metastatic liver cancer (n = 10). * P <0.05, ** P <0.01 vs day 0

vagus nerve and the humoral substances were probably negligible in this study because the operative procedures did not include either lymph node dissections, which destroy nerve networks to the liver, or changes in the portal blood flow.

Our finding that liver regeneration is primarily regulated by factors relating to the percentage of resected liver volume is consistent with the report of Fausto [3]. Multiple regression analysis in the present study showed that 60.7% of liver regeneration in the normal liver is explained by the factors relating to %RLV. In the cases of hepatoma, the contribution of %RLV decreased to 0.407, and the serum total bilirubin level showed a negative contribution. These findings probably reflect the adverse effects of liver damage exerted by chronic liver diseases on liver regeneration.

Maximal serum HGF levels did not correlate directly with liver regeneration in this study, but they did correlate with factors suggesting liver damage and necrosis or surgical inflammation as reported by Tomiya et al. [22]. This result was also considered to indicate the sources of HGF release afte hepatectomy. Indeed, HGF has been shown to be produced by endothelial cells of the lung through a humoral mediator after 70% hepatectomy in rats [25], and serum HGF levels increase significantly after surgerical procedures other than hepatectomy [22]. The increase in serum HGF levels can also be explained by the decrease in the plasma clearance of HGF by the liver after hepatectomy [12]. Some reports suggest the possibility of HGF as an initiator [15] of liver regeneration as follows: (1) HGF seems to act as a trigger for liver cell proliferation [13, 26] and also to be a potent mitogen for mature hepatocytes in in vitro studies on experimental animals [8, 10, 11] as well as humans [20], (2) serum HGF levels after hepatic resection have increased up to the minimal range [17] at which hepatocyte DNA synthesis can be stimulated in primary culture [6], and (3) exogenous HGF has facilitated liver regeneration in experimental models [5, 9]. In this study, maximal serum HGF levels after hepatectomy reached the lower margin of the reported range within which hepatocyte mitosis can be stimulated [6]. Further investigations will be necessary to elucidate the relationship between the findings of our clinical study and the experimental work concerning the role of HGF in liver regeneration [15].

Other than HGF, the role of co-mitogens [2, 5, 14, 21] should also be studied in the extremely complex process of human liver regeneration.

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