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Shinichi Ueno · Takashi Aikou · Gen Tanabe
Yasuyuki Kobayashi · Masahiro Hamanoue
Shinji Mitsue · Kouichi Kawaida · Toshikazu Nakamura

Exogenous hepatocyte growth factor markedly stimulates liver regeneration following portal branch ligation in dogs

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Abstract Portal branch ligation (PBL) or embolization prior to extensive hepatectomy has been employed to increase the functional reserve of the remaining liver. This study investigated the effects of human recombinant hepatocyte growth factor (rh-HGF) on liver regeneration following PBL in dogs. Beagle dogs were subjected to PBL and were divided into two groups, a control group ($n = 11$) without rh-HGF and a treated group ($n = 12$) receiving postoperative rh-HGF at 250 ng/kg via the portal vein. Dogs were killed 72 h or 14 days following PBL. We studied the changes in serum HGF level, DNA synthesis of the liver, hepatocyte size, liver weight, and liver function tests. In the HGF group, the ratio of whole liver weight to body weight increased significantly, and both ligated and nonligated lobes showed marked increases in weight. The nonligated lobes in the HGF group showed significant increases in both DNA synthesis and hepatocyte size. Moreover, ligated lobes in the HGF group showed an increase in DNA synthesis without hypertrophy compared with the control group. Administration of rh-HGF did not significantly affect liver function tests. Ligation of the portal branch supplying the portion of liver to be resected, coupled with the administration of rh-HGF, is a useful strategy to increase hepatic reserve in advance of major hepatectomy.

Key words HGF · Liver regeneration · Portal branch ligation

S. Ueno, (✉) · T. Aikou · G. Tanabe · Y. Kobayashi.
M. Hamanoue · S. Mitsue · K. Kawaida
First department of surgery, Kagoshima University School of medicine, 8-35-1 Sakuragaoka, Kagoshima 890, Japan
Fax 81–992–65–7426

T. Nakamura
Division of Biochemistry, Biomedical Research Center, Osaka University Medical School, Osaka, Japan

Introduction

The postoperative course of patients undergoing hepatectomy depends largely on the functional reserve of the remaining liver. If liver function falls below a defined critical level, there is little likelihood of recovery. One solution to this problem would be to enlarge the remaining liver segments. Portal branch ligation (PBL) or embolization prior to surgery has been relatively successful, and is considered a useful approach when the resected portion of the liver is very large [13, 21]. In some cases, however, these procedures do not result in the remaining liver attaining an adequate hypertrophy.

An alternative approach has been suggested following the discovery of a factor in the blood following hepatectomy, hepatocyte growth factor (HGF), which stimulates hepatic proliferation [1, 22, 25, 27, 28]. Recently, Fujiwara et al. [8] have reported that exogenous HGF triggers and promotes liver cell growth in normal and partially hepatectomized rats, and recombinant human HGF (rh-HGF) is even more potent than human HGF in this action. In the present study, we investigated the effect of exogenously administered rh-HGF following PBL on liver regeneration in dogs.

Materials and methods

Materials

Recombinant human HGF was purified from the conditioned medium of CHO cells transfected with the expression vector containing full-size human HGF cDNA as described previously [29, 31].

Experimental model

A group of 23 adult male beagle dogs weighing 8 to 11 kg were used. Following a 12-h fast, the animals were anesthetized with intravenous sodium pentobarbital (15 mg/kg) after initial sedation with

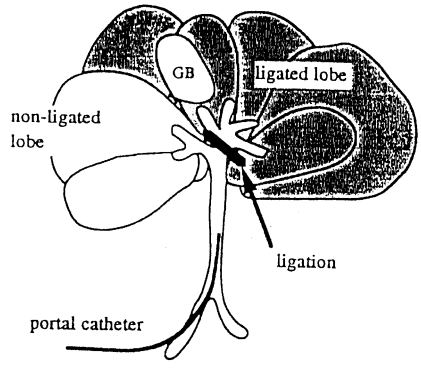

Fig. 1 Diagram of portal branch ligation

ketamine (50 mg). Under controlled ventilation with room air via an endotracheal tube, the abdomen was opened with a midline incision, and the portal vein was cannulated through a small ileocolic vein for administration of rh-HGF. Following isolation of the right and left main portal branches, the left branch was ligated (Fig. 1). A continuous intravenous infusion of 5% glucose solution was administered through the external jugular vein intraoperatively and for 6 h postoperatively. Oral fluids and a standard diet were provided thereafter. An antibiotic (cefaloridine 500 mg/day) was given intravenously for 2 days following PBL. The experimental animals were then divided into two groups, the HGF group and the control group. For the HGF group, a bolus infusion of rh-HGF (250 ng/kg; Toyobo, Osaka, Japan) was administered four times, at 0.5, 12, 24 and 48 h following PBL, through the intraportal catheter which had been tunneled through the abdominal wall to the back of the dogs. Normal saline was injected using the same time schedule into the control dogs.

Measurement of serum human HGF levels

Five dogs from each group were studied to estimate the change in serum HGF concentrations and liver cell growth following PBL. Peripheral venous blood samples were obtained 2, 10, 60, and 720 min following the initial administration of rh-HGF or normal saline. Serum HGF concentration was measured using a sensitive enzyme-linked immunosorbent assay kit (Otsuka Assay Laboratories, Tokushima, Japan) described by Tsubouchi et al. [35].

Measurement of liver cell growth

The dogs were given 50 mg/kg of 5-bromodeoxyuridine (BrdU; Sigma Chemical Co., St. Louis, Mo.) intravenously 72 h after PBL. After a further 90 min, while the dogs were under sodium pentobarbital anesthesia, specimens were taken from the ligated and nonligated lobes of the liver and fixed in 10% normal buffered formalin. The dogs were then killed with an intravenous bolus injection of potassium chloride, and the patency of the ligation and correct positioning of the catheter tip were confirmed.

The liver tissues were embedded in paraffin and cut into 4–μm sections. Following deparaffinization, the samples were treated with 2 *N* HCl to denature the DNA. Immunohistochemical detection of BrdU was performed by the routine ABC method using anti-BrdU monoclonal antibody (Becton Dickinson, Mountain View, Calif.,) as the primary antibody. Neighboring sections were stained with hematoxylin-eosin. To determine the BrdU labeling index (LI), the number of BrdU-positive nuclei per 1000 random parenchymal cells was recorded in several fields under the light microscopic examination. The ratio of BrdU-labeled cells to total cells was taken as the LI.

The size of individual hepatocytes (the index of hypertrophy or atrophy) was determined by tracing out at least 500 midzonal liver cells as projected on standard thickness paper, then cutting out and weighing the individual silhouettes. This method has been shown to be acceptable for determining hepatocyte size and has been validated by planimetry and in the study of unicellular organisms where the size estimation was compared with direct determination [10, 32].

Changes in liver function tests

Six control and seven HGF dogs were studied to determine changes in liver function tests and liver weights over 14 days following PBL. Blood samples for serum were obtained 1, 3 and 14 days following PBL, and aminotransferase, alkaline phosphatase, and total bilirubin were determined with an automatic analyzer (Hitachi, Model 705; Tokyo, Japan). The normotest time was measured using an assay kit (Hepaplastintest; Eisai Pharmaceutical Co., Tokyo, Japan). Indocyanine green (ICG, 0.5 mg/kg) was given intravenously, and serum ICG concentrations (prior to and 5, 10 and 15 min following injection) were measured using a spectrophotometer (wavelength 805 nm, Uvidec-40; Japan Spectroscopic Co., Tokyo, Japan). The disappearance rate of ICG (KICG) was determined to evaluate hepatic functional reserve using a method described previously [18, 30].

Measurement of liver weight

The dogs were killed 14 days following PBL by the method described above, and the livers were removed and weighed. The ratio of whole wet liver weight to body weight was calculated, and the weight ratio of each ligated or nonligated lobe to the total liver was determined. The changes in weight of the ligated and nonligated lobes were calculated by a previously described method [26].

Statistical evaluation

Results are expressed as means $\pm$ SD. One-way ANOVA and Student's t-test were used where appropriate to compare differences between ligated and nonligated lobes and between groups of dogs. Significance was defined as $P < 0.05$.

Results

Serum HGF concentratations

HGF was not detected in the serum of the control group at any time. In the HGF group, HGF was detected 2 min following an initial administration of rh-HGF at 0.28 ± 0.1 ng/ml. However, concentrations were never detected in the serum thereafter.

Changes in BrdU labeling index and cell size

Table 1 shows the BrdU LI and the size of individual hepatocytes 72 h following PBL. The mean LIs of the ligated and nonligated lobes in the control group were $0.2 \pm 0.1\%$ and $1.5 \pm 0.4\%$, respectively, and in the

HGF group were 0.9 ± 0.5% and 2.7 ± 0.7%, respectively. There was a significant increase in the HGF group LI in both the ligated and nonligated lobes. The cell size of the ligated lobes was not significantly different between the two groups. By contrast, the cell size of the nonligated lobes in both groups was significantly larger than the ligated lobes, and there was a significant increase in the HGF group over the control group.

Changes in liver function tests

Liver function tests prior to and 1, 3, and 14 days following PBL are summarized in Table 2. No significant difference was observed between the two groups. Hepatic functional reserve (KICG) 14 days following PBL in the HGF group was better than the control group. Although the p-value for KICG was 0.05, we did not consider the difference to be statistically significant because the value was inflated due to a multiple comparison.

Changes in liver weight

Each dog had lost body weight by 14 days following PBL, but no significant difference was found between the HGF and control groups. Figure 2a shows the ratio of wet liver weight to body weight in both groups 14 days following PBL. A significant increase was noted in the HGF group (4.6 ± 0.4%) compared with the control group (3.5 ± 0.3%). However, the relative weight ratios of both ligated (control 61.8 ± 4.4%, HGF 62.5 ± 3.7%) and nonligated (control 38.2 ± 4.4%, HGF 37.5 ± 3.7%) lobes in each group were not significantly different as shown in Fig. 2b. Changes in the ratio of hepatic lobe weight to body weight 14 days following PBL are shown in Fig. 3. The ligated lobes decreased in weight (− 15.0 ± 6.4%), whereas the nonligated lobes increased in weight (28.5 ± 18.3%) in the control group. Both ligated and nonligated lobes markedly increased in weight (ligated 10.5 ± 12.3%, nonligated 57.7 ± 16.6%) in the HGF group.

Table 1 BrdU labeling inedx and cell size 72 following portal branch ligation. values are means ± SD

		Labeling index (%)		Cell size (arbitrary units)	
Group	*n*	Ligated	Nonligated	Ligated	Nonligated
Control	5	0.2 ± 0.1	1.5 ± 0.4[a]	16.2 ± 2.6	22.4 ± 2.6[c]
HGF	5	0.9 ± 0.5[b]	2.7 ± 0.7[a,b]	16.8 ± 2.0	28.9 ± 2.0[c,d]

[a] $P < 0.02$ vs ligated
[b] $P < 0.03$ vs control
[c] $P < 0.01$ vs ligated
[d] $P < 0.01$ vs control

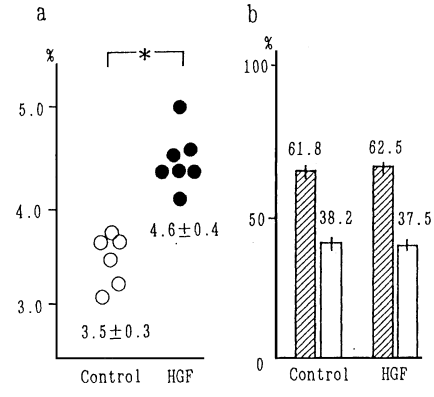

Fig. 2 a, b Ratio of whole wet liver weight to body weight (**a**) and relative weight ratios of ligated and nonligated lobes to total liver weight (**b**) 14 days following portal branch ligation in control ($n = 6$) and HGF ($n = 7$) groups (*hatched bar* ligated lobes, *open bars* nonligated lobes) *$P < 0.01$

Table 2 Liver function tests prior to and following portal branch ligation. values are means ± SD (*C* control group ($n = 6$), *H* HGF group ($n = 7$), *TB* total bilirubin, *ALT* alanine aminiotransferase, *AIP* alkaline phosphatase, *KICG* clearance ratio of indocyanine green)

			Day after surgery		
	Group	Before surgery	1	3	14
TB (mg/dl)	C	0.2	0.2 ± 0.4	0.2 ± 0.1	0.2
	H	0.2	0.2 ± 0.1	0.2 ± 0.1	0.2
ALT (u/l)	C	27.7 ± 7.1	231 ± 127	105 ± 86	28 ± 6
	H	24.5 ± 8.9	159 ± 157	110 ± 59	23 ± 9
ALP (KAU)	C	4.2 ± 0.2	17.0 ± 4.7	18.0 ± 4.0	18.7 ± 6.2
	H	5.1 ± 0.1	18.3 ± 5.7	16.2 ± 6.3	13.5 ± 1.1
Normotest (%)	C	150	130 ± 23	150	150
	H	150	128 ± 22	150	150
KICG	C	0.10 ± 0.02			0.11 ± 0.01
	H	0.10 ± 0.02			0.15 ± 0.02*

*$P < 0.05$ vs control

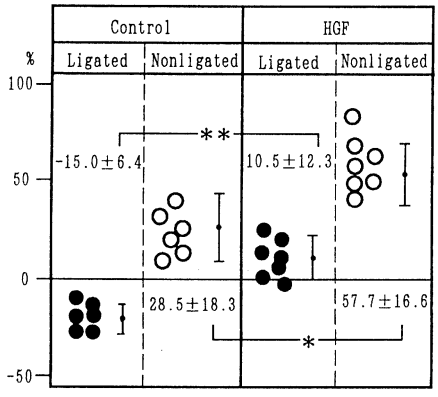

Fig. 3 Changes in the ratio of hepatic lobe weight to body weight 14 days following portal branch ligation (ligated left four lobes, nonligated. right two lobes). The lobe weight ratio was determined from the following equation: {(postop. hepatic lobe weight/body weight) ÷ (normal hepatic lobe weight/body weight) − 1} × 100 (%). $*P < 0.03$, $**P < 0.01$

Discussion

HGF is a potent growth factor for hepatocytes in primary culture [27, 28]. Elevated HGF activity has been observed in various liver injuries [2, 9, 17, 19, 27] and may trigger liver regeneration [8]. Direct injection of HGF into the portal vein leads to DNA synthesis in dog livers and adult rat livers [7, 20]. Previous studies in this laboratory have shown that intravenously injected rh-HGF markedly enhances renal regeneration following acute renal injury and prevents renal dysfunction caused by $HgCl_2$ or cisplatin [16]

Based on these studies, the present investigation evaluated the effects of the administration of exogenous rh-HGF on liver regeneration following PBL. Our results indicate that dog liver regeneration and hypertrophy are promoted by exogenous administration of rh-HGF, resulting specifically in a significant increase in whole wet liver weight 14 days following PBL. The procedure could be applied clinically as an adjunctive procedure prior to hepatic lobectomy or extended lobectomy. The results of the liver function tests, the lack of a significant difference in enzyme release between the two groups and the greater increase in the KICG value in the HGF group than in the control group also support the clinical use.

Liver regeneration was also observed in the ligated lobes of the HGF group. In this study, rh-HGF was given directly via the portal vein to avoid any influence on other organs since a variety of biologic activities of HGF have been demonstrated by other investigators [14, 15, 23, 33]. Liver regeneration without hypertrophy seen in the ligated lobe of the HGF group might be explained by an early phase elevation of circulating rh-HGF and a continuous deficiency of additional factors (e.g. insulin) in the portal blood. The one elevation of serum HGF in the treated group was probably due to exogenous rh-HGF, and the dose of rh-HGF would appear to have exceeded the capacity of the nonligated lobes to clear it. HGF was not detected in blood samples from the control group in the present study, but Tsubouchi et al. [35] have shown that serum HGF concentrations as low as 0.2 ng/ml are detectable in normal human blood samples. The combined amount of recombinant and native HGF might have been enough for hepatocyte proliferation in the ligated lobes of the HGF group. Alternatively, following the report of Francavilla et al. [7] that insulin also provokes significant increases in hepatocyte proliferation and was the only hormone that prevented hepatocyte atrophy following portacaval shunt, it may be that the unenlarged cell size in the ligated lobes of the HGF group could have been due to a lack of high levels of insulin, which are present only in portal and not systemic blood.

Our results provide the justification for a clinical trial of rh-HGF following PBL or pericutaneous transhepatic portal embolization (PTPE) [21]. Although these procedures often are performed in patients with malignant tumors, it has been reported recently that HGF has antiproliferative activity against tumor cells. Tajima et al. [34] have demonstrated that HGF has potent suppressive effects on various tumor cell lines (e.g. KB, B6/F1 and HepG2), and Higashio et al. [11] have shown that high concentrations of HGF inhibit growth or are cytotoxic to some carcinoma and sarcoma cell lines. These observations would favor the clinical use of exogenous HGF. It is also known that HGF promotes cell proliferation of another hepatoblastoma cell line (HuH-6 clone-5) [24]. Furthermore, Bottaro et al. [3] have shown that c-*met* protooncogene is a high-affinity receptor for HGF. Since c-*met* gene expression is found in many types of cells [4, 5], carcinogenic and/or transforming activity through an autocrine or a paracrine mechanism of the HGF–c-*met* system has become a subject of interest. In a report on these issues, Higuchi et al. [12] have reported that the expression of the c-*met* gene in mesenchymal derived cells which produce HGF may lead to the development of tumors. This may result from the continuous stimulation of c-*met* tyrosine kinase through an autocrine mechanism. More data, in particular the relationship between HGF and carcinogenesis, is therefore required before the clinical use of rh-HGF with PBL or PTPE in advance of major hepatectomy is attempted.

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