Contribution of Parenchymal and Non-Parenchymal Liver Cells to the Clearance of Hepatocyte Growth Factor From the Circulation in Rats

Ke-Xin Liu,[1] Yukio Kato,[1] Tetsuya Terasaki,[1] Shoichi Aoki,[2] Kazuo Okumura,[2] Toshikazu Nakamura,[3] and Yuichi Sugiyama[1,4]

Received January 13, 1995; accepted June 16, 1995

Purpose. The distribution of ^{125}I-hepatocyte growth factor (HGF) to either liver parenchymal cells (PC) or non-parenchymal cells (NPC) was investigated in rats.

Methods. After injection of a trace amount of ^{125}I-HGF, the distribution of radioactivity determined by microautoradiography closely resembled that of ^{125}I-epidermal growth factor which distributes mainly to PC.

Results. The uptake clearance of ^{125}I-HGF estimated by determining the radioactivity of isolated liver cells was three times higher for PC than for NPC. This suggests that HGF distributes mainly to PC at relatively low doses. On the other hand, the uptake clearance by PC fell on coadministering an excess (80 μg/kg) of unlabeled HGF, while no change was observed for NPC, indicating that a saturable process for the hepatic handling of HGF exists only in PC where the HGF receptor is expressed.

Conclusions. At such a dose the uptake clearance was comparable for both PC and NPC showing that HGF distributes to both cell types although NPC have few HGF receptors. Since the distribution to NPC was relatively non-specific and heparin-sensitive, it may be that heparin-like substances, which are believed to exist on PC and/or the extracellular matrix, also exist on NPC.

KEY WORDS: hepatocyte growth factor; receptor-mediated endocytosis; pharmacokinetics; liver.

INTRODUCTION

Hepatocyte growth factor (HGF) highly stimulates DNA synthesis in various types of epithelial cells including liver parenchymal cells (PC) (1,2). This mitogenic activity is expressed through the specific binding of HGF to its receptor, a c-met protooncogen product (1,2). In rats, after a partial hepatectomy, the synthesis of HGF is induced in the kidney, lung, and spleen (1), and HGF acts as a mitogen for hepatocytes through the endocrine system. In addition, liver non-parenchymal cells (NPC) also synthesize HGF, which also acts through the paracrine system (3).

We have previously demonstrated that liver is the major clearance organ for HGF and that both receptor-mediated endocytosis (RME) and a non-specific uptake mechanism contribute to the hepatic disposition (4,5). The liver consists of heterologous cells including PC and NPC, and the HGF receptor exists on PC. It is still unknown which cells contribute mainly to the hepatic clearance although there would be some possibility that PC may contribute a specific portion to the hepatic clearance. HGF is considered to bind heparin-like substances on the cell-surface (6) and/or the extracellular matrix (7). Since such heparin-like proteoglycans are distributed in ubiquitous tissues (8), NPC may also possibly contribute to the clearance.

In this study, we examined the contribution of both PC and NPC to the HGF clearance using microautoradiography and a cell isolation technique.

MATERIALS AND METHODS

Materials

Human recombinant HGF was iodinated as described previously (2). Epidermal growth factor (EGF, human recombinant, Wakunaga, Osaka) and Mannosylated BSA (Man-BSA, Cosmo-bio, Tokyo) were radiolabeled as described previously (9). The specific activity of ^{125}I-HGF, ^{125}I-EGF and ^{125}I-Man-BSA was 70-160, 188 and 405 Ci/g, respectively.

Microautoradiography

Under light ether anesthesia, ^{125}I-HGF (70 μCi/kg body wt) or ^{125}I-EGF (35 μCi/kg body wt) was administered through the tail vein. Ten min after the injection, the rats were exsanguinated via the abdominal artery. The liver was then removed and fixed in 20% buffered formalin. The paraffin section (2 μm) was mounted onto a glass slide. All stage specimens were coated with photographic emulsion (NR-H2, Konica) and the exposure was carried out at 4 °C for 35 days. The specimens were stained with hematoxylin and photographed under a magnification of 150.

Determination of the Radioactivity in the Isolated Liver Cells After the Administration of Iodinated Peptides

Under light ether anesthesia, an iodinated peptide (28 μCi/kg body wt) was administered through the femoral vein and trichloroacetic acid (TCA)-precipitable radioactivity in plasma was determined (4). Ten min after the administration, the preperfusion buffer was perfused through the portal vein for 10 min, followed by the collagenase-perfusion for 15 min, and the radioactivity recovered in each outflow was determined (10). Finally, the liver was excised, and both PC and NPC were isolated separately (10). Both the cell number and the radioactivity were determined to calculate the radioactivity per cell number. The yield of PC and NPC was 3-4.5 × 10^7 and 0.6-0.8 × 10^7 cells/g liver, respectively. The viability assessed by trypan blue exclusion was more than 90% and 85% for PC and NPC, respectively. To estimate the radioac-

[1] Faculty of Pharmaceutical Sciences, University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113, Japan.
[2] Pharmaceuticals Research Center, Toyobo Co., Ltd., Otsu, Shiga 520-02, Japan.
[3] Biomedical Research Center, Osaka University School of Medicine, Suita, Osaka 565, Japan.
[4] To whom correspondence should be addressed.

Abbreviations: HGF, hepatocyte growth factor; EGF, epidermal growth factor; RME, receptor-mediated endocytosis; PC, parenchymal; NPC, non-parenchymal; BSA, bovine serum albumin; TCA, trichloroacetic acid.

0724-8741/95/1100-1737$07.50/0

tivity per liver weight, we cited the previous report that the number of PC and NPC is 1.25×10^8 and 0.65×10^8 cells/g liver, respectively (11).

Calculation of Uptake Clearance (CL_{uptake})

The plasma concentration (Cp) time profiles of the TCA-precipitable radioactivity were fitted to the following equation by the use of a nonlinear iterative least squares method (4):

$$Cp = A \exp(-\alpha t) + B \exp(-\beta t) \quad (1)$$

The CL_{uptake} was obtained by:

$$CL_{uptake} = X_{10}/AUC_{(0\text{-}10)} \quad (2)$$

where X_{10} is the radioactivity in either PC or NPC at 10 min, and $AUC_{(0\text{-}10)}$ was calculated using:

$$AUC_{(0\text{-}10)} = \int_0^{10} Cp\, dt = A/(1 - exp(-10\alpha)) + B/(1 - exp(-10\beta)) \quad (3)$$

RESULTS

Distribution of Radioactivity in the Liver After Intravenous Administration of ^{125}I-HGF

Ten min after the intravenous administration of a tracer amount of ^{125}I-HGF, the distribution of radioactivity in the liver was examined by microautoradiography and found to be quite similar to that after injection of ^{125}I-EGF (Fig. 1), which is known to distribute mainly to PC (12). When we isolated both cells and determined the radioactivities, 75.3% of the total liver cell-associated radioactivity was attributed to PC, while only 24.7% was attributed to NPC (Table 1). As control experiments, we examined the distribution of ^{125}I-Man-BSA, which is known to distribute mainly to NPC (13) and of ^{125}I-EGF (Table 1). After ^{125}I-EGF administration, 97.3% of total liver cell-associated radioactivity was attributed to PC, while 75.4% was attributed to NPC after ^{125}I-Man-BSA administration (Table I).

Effect of the Coadministration of an Excess of Unlabeled HGF or Heparin on the Distribution of ^{125}I-HGF

After the coadministration of an excess (80 μg/kg body wt) of unlabeled HGF with a tracer ^{125}I-HGF, the CL_{uptake} by NPC was almost comparable with that following the administration of only a tracer amount of ^{125}I-HGF (1.17 and 1.03 ml/min/kg body wt under tracer and excess dose conditions, respectively), while the CL_{uptake} by PC was remarkably lower (3.48 and 1.02 ml/min/kg body wt under tracer and excess dose conditions, respectively) (Fig. 2A,2B). After the coadministration of heparin, the CL_{uptake} by PC and

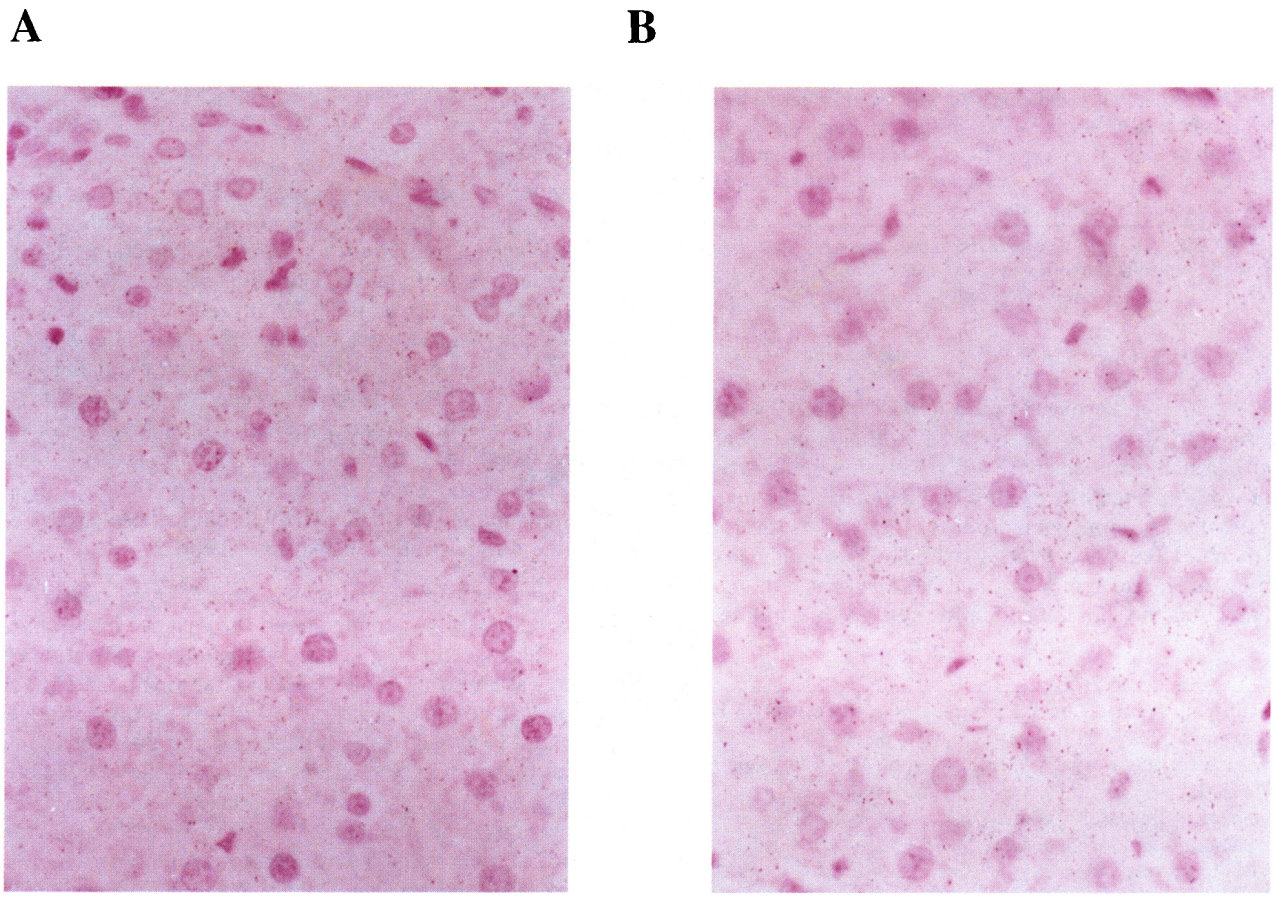

Fig. 1. Distribution of radioactivity in the liver after intravenous administration of ^{125}I-HGF or ^{125}I-EGF. Ten min after the intravenous administration of a tracer amount of ^{125}I-HGF (A) or ^{125}I-EGF (B) into rats, the liver was excised, and the distribution of radioactivity was determined by microautoradiography.

Table I. Distribution of Radioactivity to PC and NPC After the Intravenous Administration of ^{125}I-HGF, ^{125}I-EGF, or ^{125}I-Mannosylated BSA[a]

	^{125}I-HGF	^{125}I-EGF	^{125}I-Man-BSA
PC	75.3 ± 1.6	97.3 ± 1.2	24.7 ± 1.4
NPC	24.7 ± 1.6	2.68 ± 1.27	75.4 ± 1.4

[a] Ten min after the intravenous administration of each iodinated compound, both PC and NPC were isolated and counted. Each value was normalized by the total liver cell-associated radioactivity determined by the summation of the value for PC and NPC, and represents the mean ± SE of three rats.

NPC were decreased to 1.27 and 0.357 ml/min/kg body wt, respectively (Fig. 2A,2C).

Radioactivity Emergence in the Hepatic Outflow During the Pre-Perfusion and Collagenase-Perfusion for the Cell Isolation

To recover the radioactivity leaked from the liver during the cell isolation, we collected the outflow of the perfusion (Table II). During a preperfusion, both the TCA-precipitable and -soluble radioactivity recovered in the outflow were slight (1.74% and 4.51% of the dose, respectively) (Table II). Interestingly, remarkable radioactivity emerged in the outflow immediately after the start of the collagenase perfusion, and TCA-precipitable and -soluble radioactivity were 4.51% and 7.89% of the dose, respectively (Table II). The sum of the radioactivity found both in the plasma and in the liver was more than 70% of the injected dose for each dosage (Table II).

DISCUSSION

We have previously reported that both RME and a nonspecific uptake mechanism contribute to the hepatic disposition of HGF (4). However, whether PC or NPC contribute to the disposition was still unknown. After the administration of a tracer amount of ^{125}I-HGF, the distribution of radioactivity in the liver, as determined by microautoradiography, was quite similar to that after the administration of ^{125}I-EGF (Fig. 1). In addition, more than 70% of the total liver cell-associated radioactivity, which was estimated by the determination of the radioactivity in both cells isolated after the ^{125}I-HGF injection, was attributed to PC (Table I). These results suggest that HGF distributes mainly to PC under relatively low (<10 pmol/kg body wt) dose condition.

On the other hand, after the coadministration of an excess (8 nmol/kg body wt) of unlabeled HGF with ^{125}I-HGF, the CL_{uptake} of ^{125}I-HGF for PC was lower than that after the tracer ^{125}I-HGF administration, while the CL_{uptake} for NPC was comparable with that after the tracer ^{125}I-HGF administration (Fig. 2). Considering that the distribution volume of ^{125}I-HGF is approximately 50 ml/kg body wt (4), the initial plasma HGF concentration is considered to be approximately 20 nM, which is a thousand times larger than the equilibrium dissociation constant of the HGF receptor (Kd = 20-40 pM) (2). In addition, we had previously determined the plasma HGF concentration ten min after the intravenous administration of 80 μg/kg body wt of HGF by an enzyme-linked immunosorbent assay, and found that the value was approximately 530 pM (unpublished observation), which was still 10-20 times the Kd value. Therefore, under such a dose condition, almost all cell-surface receptors might be occupied by unlabeled HGF. The reason why only the CL_{uptake} for PC was reduced under such a high dose condition, was probably, the saturation of receptor binding on the PC.

The contributions of PC and NPC to the HGF clearance were almost identical under high dose conditions (Fig. 2B). This suggests that we must consider the clearance of HGF not only by PC, but also by NPC at relatively high doses. To characterize the binding site on the surface of NPC, we have to take the following information into consideration: (i) The NPC except biliary epithelial cells do not have a HGF receptor (1); (ii) The distribution of HGF to NPC is relatively non-specific, since the CL_{uptake} of ^{125}I-HGF for NPC under a high dose condition was comparable with that under a tracer condition (Fig. 2); (iii) The distribution of HGF to NPC could be reduced by the coadministration of heparin (Fig. 2). This information hints at a possibility that heparin-like substances exist also on NPC and bind to HGF. In fact, heparin-like proteoglycans are distributed in ubiquitous tissues (8). However, the identification of the binding site on NPC must be awaited.

We found that a large amount of radioactivity emerged during the collagenase perfusion after the administration of ^{125}I-HGF (Table II). Although the identification of this radioactivity must be awaited, one possibility is that this radioactivity represents HGF molecules loosely bound to an extracellular matrix. Masumoto et al. found HGF activity in

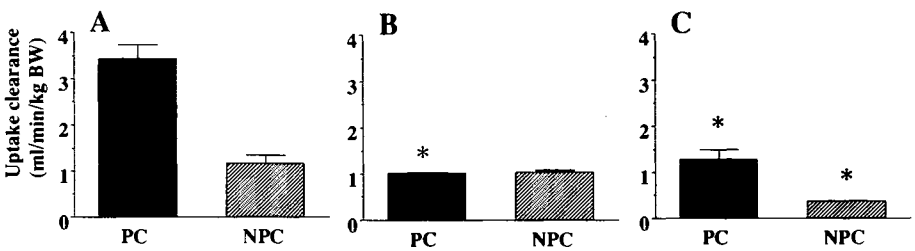

Fig. 2. CL_{uptake} of ^{125}I-HGF by PC and NPC. Ten min after the intravenous administration of the tracer ^{125}I-HGF, solely (A), or with an excess (80 μg/kg body wt) of unlabeled HGF (B) or heparin (20 mg/kg body wt) (C), both PC and NPC were isolated and counted to determine the uptake clearance calculated by Eq. (2). Each value represents the mean ± SE of three rats. *: Values are significantly different from control ($p < 0.05$).

Table II. Recovery of Radioactivity in Plasma, PC and NPC, and Hepatic Outflow During the Perfusion for the Cell Isolation[a]

	Tracer ^{125}I-HGF [% of dose]	(+) Excess HGF [% of dose]	(+) Heparin [% of dose]
[Plasma]			
TCA-precipitable	15.1 ± 1.4	26.4 ± 3.6*	43.0 ± 4.5*
-soluble	1.93 ± 0.25	2.09 ± 0.31	2.99 ± 0.56
[Pre-perfusion]			
TCA-precipitable	1.74 ± 0.22	0.725 ± 0.307*	1.16 ± 0.18
-soluble	4.51 ± 0.43	3.22 ± 0.09	3.74 ± 0.50
[Collagenase]			
TCA-precipitable	4.51 ± 0.25	3.52 ± 0.16	3.78 ± 0.29
-soluble	7.89 ± 0.46	5.63 ± 0.18	6.54 ± 0.09
[PC]	24.6 ± 1.5	13.3 ± 0.3*	14.2 ± 1.2*
[NPC]	8.21 ± 1.05	13.4 ± 0.8	4.09 ± 0.24*

[a] Ten minutes after the intravenous administration of the tracer ^{125}I-HGF, solely, or with an excess (80 μg/kg body wt) of unlabeled HGF or heparin (20 mg/kg body wt), the radioactivity in plasma was determined. After that, the preperfusion of 10 min and the following collagenase perfusion of 15 min was done, and the radioactivity which emerged in the outflow was counted. Both PC and NPC were isolated, and the distribution of radioactivity was determined. To calculate the total radioactivity in plasma, we assumed that the distribution volume was 52.3 ml/kg body wt. Each value was normalized by the injected dose and represents the mean ± SE of three rats.

* Values are significantly different from control ($p < 0.05$).

the hepatic outflow during a perfusion of a hypertonic buffer in rats (7). They speculated that such HGF activity came from the extracellular matrix (7). The radioactivity emerged in the collagenase perfusion may represent HGF molecules bound to such a matrix, although we must consider the other possibility that such radioactivity represents HGF molecules bound to its receptor or a non-specific binding site on liver cells which had been damaged by the collagenase treatment.

In conclusion: (i) The saturable portion of the hepatic clearance of HGF exists only in PC; (ii) HGF distributes not only to PC, but also to NPC, and NPC contribute especially to HGF clearance at relatively high doses of HGF. Such a distribution of HGF in NPC is non-specific and heparin-sensitive.

ACKNOWLEDGMENTS

This study was supported in part by a Grant-in-Aid for Scientific Research provided by the Ministry of Education, Science and Culture of Japan.

REFERENCES

1. K. Matsumoto and T. Nakamura. Role of HGF as a pleiotropic factor in organ regeneration. In I. D. Goldberg and E. M. Rosen (eds), *Hepatocyte growth factor-Scatter Factor (HGF-SF) and the C-Met Receptor,* Birkhauser Verlag, Basel, 1993, pp. 225-249.
2. O. Higuchi and T. Nakamura. Identification and change in the receptor for hepatocyte growth factor in rat liver after partial hepatectomy or induced hepatitis. *Biochem. Biophys. Res. Commun.* 176:599-607 (1991).
3. S. Noji, K. Tashiro, E. Koyama, T. Nohno, K. Ohyama, S. Taniguchi and T. Nakamura. Expression of hepatocyte growth factor gene in endothelial and kupffer cells of damaged rat livers, as revealed by in situ hybridization. *Biochem. Biophys. Res. Commun.* 173:42-47 (1990).
4. K. X. Liu, Y. Kato, M. Narukawa, D. C. Kim, M. Hanano, O. Higuchi, T. Nakamura, and Y. Sugiyama. The importance of the liver in the plasma clearance of hepatocyte growth factor in rats. *Am. J. Physiol.* 263 (Gastrointest. Liver Physiol. 26): G642-G649 (1992).
5. K. X. Liu, Y. Kato, M. Yamazaki, O. Higuchi, T. Nakamura, and Y. Sugiyama. Decrease in the hepatic uptake clearance of hepatocyte growth factor (HGF) in CCl_4-intoxicated rats. Hepatology 17:651-660 (1993).
6. R. Zarnegar, M. C. DeFrances, L. Oliver, and G. K. Michalopoulos. Identification and partial characterization of receptor binding sites for HGF on rat hepatocytes. *Biochem. Biophys. Res. Commun.* 173:1179-1185 (1990).
7. A. Masumoto and N. Yamamoto. Sequestration of a hepatocyte growth factor in extracellular matrix in normal adult rat liver. *Biochem. Biophys. Res. Commun.* 174:90-95 (1991).
8. E. Ruoslahti and Y. Yamaguchi. Proteoglycans as modulators of growth factor activities. *Cell* 64:867-869 (1991).
9. H. Sato, Y. Sugiyama, Y. Sawada, T. Iga, S. Sakamoto, T. Fuwa, and M. Hanano. Dynamic determination of kinetic parameters for the interaction between polypeptide hormones and cell-surface receptors in the perfused rat liver by the multiple-indicator dilution method. *Proc. Natl. Acad. Sci. USA.* 85:8355-8359 (1988).
10. S. Horiuchi, K. Takara, and Y. Marino. Characterization of a membrane-associated receptor from rat sinusoidal liver cells that binds formaldehyde-treated serum albumin. *J. Biol. Chem.* 260:475-481 (1985).
11. R. Blomhoff, H. K. Blomhoff, H. Tolleshaug, T. B. Christensen, and T. Berg. Uptake and degradation of bovine testes β-galactosidase by parenchymal and nonparenchymal rat liver cells. *Int. J. Biochem.* 17:1321-1328 (1985).
12. R. J. S. Hilaire, G. T. Hardek, and A. L. Jones. Hepatic sequestration and biliary secretion of epidermal growth factor: Evidence for a high-capacity uptake system. *Proc. Natl. Acad. Sci. USA.* 80:3791-3801 (1983).
13. M. Nishikawa, Y. Ohtsubo, J. Ohno, T. Fujita, Y. Koyama, F. Yamashita, M. Hashida, and H. Sezaki. Pharmacokinetics of receptor-mediated hepatic uptake of glycosylated albumin in mice. *Int. J. Pharm.* 85:75-85 (1992).