Protamine enhances the proliferative activity of hepatocyte growth factor in rats

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1Faculty of Pharmaceutical Sciences, University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113; 2Bioproducts Industry Company, Ltd., Tomigaya, Shibuya-ku, Tokyo 151; and 3Biomedical Research Center, Osaka University School of Medicine, Suita, Osaka 565, Japan

Liu, Ke-Xin, Yukio Kato, Tai-Ichi Kaku, Kunio Matsumoto, Toshikazu Nakamura, and Yuichi Sugiyama. Protamine enhances the proliferative activity of hepatocyte growth factor in rats. Am. J. Physiol. 274 (Gastrointest. Liver Physiol. 37): G21–G28, 1998.—The effect of protamine on the proliferative activity of hepatocyte growth factor (HGF) was examined in α-naphthyl naphthol tetraethylbenzenesulfonate–iodoacetate–intoxicated rats. Protamine pre-injection increased the hepatocyte labeling index induced by HGF four- to fivefold. A similar effect was also observed in partially hepanetomized rats. Because a cell surface heparin-like substance can bind to HGF and protamine has an affinity for heparin, protamine may affect HGF pharmacokinetics. In fact, protamine injection caused a transient increase in plasma HGF concentrations after administration of HGF and, in vitro, protamine eluted HGF prebound to heparin-Sepharose. Protamine also reduced the plasma clearance of HGF and increased 2.5-fold the exposure of hepatocytes to HGF in vivo. The enhancing effect of protamine on the mitogenic response of hepatocytes to HGF was also observed in vitro (~2-fold after protamine pretreatment compared with HGF alone), suggesting that the enhancing effect of protamine on HGF-induced liver regeneration results from dual effects exerted by protamine 1) lowering the overall elimination of HGF and 2) directly stimulating hepatocyte mitosis induced by HGF.

liver regeneration; liver function; drug delivery system

HEPATOCELLULAR GROWTH FACTOR (HGF) is a heterodimeric protein with a molecular mass of 82–85 kDa (18). HGF stimulates proliferation of a variety of epithelial cells, including hepatocytes (6, 8, 16, 20). Its gene expression is increased not only when there is hepatic damage, such as with partial hepatectomy (25, 31) and carbon tetrachloride poisoning (10), but also after renal (5) and pulmonary injury (20). In such cases, HGF levels in circulating plasma are increased, and therefore HGF is believed to be a hepatotrophic, renotrophic, and pulmotrophic factor (5, 20, 31).

HGF is a basic polypeptide and one of the heparin-binding proteins (2, 19). HGF can bind to heparan sulfate expressed on the surface of ubiquitous cells and in the extracellular matrix (15, 32). Mutational deletion of its NH2-terminal hairpin loop or second kringle domain reduces the affinity of HGF for heparin, suggesting that these structures are the heparin-binding domains on the HGF molecule (17). An oligosaccharide moiety in heparan sulfate required for binding to HGF has also been identified and is different from that required for binding to basic fibroblast growth factor, another heparin-binding protein (26). Low concentrations (<0.1–10 μg/ml) of sulfated oligosaccharides of sufficient length (6 glucose units) induce dimerization of HGF and also increase its mitogenic effect on cultured rat hepatocytes (24). This effect may result from stabilization of the HGF dimer, which stimulates dimerization of the HGF receptor on the cell surface (24).

HGF markedly accelerates regeneration of damaged organs in animals with hepatic and renal failure (6). However, a large dose (>100 μg/kg) is usually required to exert such a pharmacological effect (4). This may be one of the stumbling blocks for the clinical application of HGF. We have been trying to identify the way in which HGF is cleared from the circulation (11–14), and we have previously suggested that both receptor-mediated endocytosis and another low-affinity uptake system, probably mediated by a cell surface heparin-like substance in the liver, are involved in the systemic clearance of HGF (11–14). When HGF is premixed with heparin and then given intravenously, its plasma clearance is reduced (8, 14). Thus a heparin-HGF complex such as this one may be used to increase the plasma residence time of HGF. Such an inhibitory effect of heparin on HGF clearance possibly results from occupation of the heparin-binding domain on the HGF molecule by heparin, which results in a reduction in HGF binding and subsequent internalization through a cell surface heparin-like substance (8, 11–14). However, high concentrations (>100 μg/ml) of heparin reduce the mitogenic activity of HGF (8). In addition, heparin has anti-coagulant activity. Therefore, further studies need to be performed to develop a reliable and efficient drug delivery system (DDS) for HGF.

Protamine is a basic protein with an affinity for heparin, and it has been used clinically to neutralize any excessive pharmacological effect exerted by heparin. The molecular mass of protamine is usually ~4 kDa, and more than one-half of its amino acid sequence consists of arginine. If protamine can bind to a cell surface heparin-like substance and inhibit the binding of HGF to this substance, it may be that it can be used as another type of DDS to increase the HGF plasma residence time. Hence, in the present study, we examined the effect of protamine on both the proliferative activity and pharmacokinetics of HGF in rats.

MATERIALS AND METHODS

Animals. Male Wistar rats weighing 250 g (Nisseizai, Tokyo, Japan) were used and treated humanely. Studies were carried out in accordance with the Declaration of Helsinki and the Guide for the Care and Use of Laboratory Animals [DHEW Publication No. (NIH) 85–23, Revised 1985, Office of Science and Health Reports, DRR/NIH, Bethesda, MD 20205]
as adopted and promulgated by the National Institutes of Health. α-Naphthylisothiocyanate (ANIT) dissolved in olive oil was injected intraperitoneally at a dose of 50 mg/kg body wt. While rats were under ether anesthesia, partial (30%) hepatectomy was performed by removing the left lateral lobe of the liver through a subxyphoid incision.

Materials. Protamine sulfate was obtained from salmon roe from Wako Pure Chemical (Osaka, Japan), ANIT and 3,3′-diaminobenzidine were from Sigma (St. Louis, MO), and 125I-deoxyuridine was from New England Nuclear (Boston, MA). Human recombinant HGF was purified from a culture medium of C127 cells transfected with plasmid containing human HGF cDNA (18). Epidermal growth factor (EGF) was supplied by Wako Pure Chemical (Hiroshima, Japan).

HGF injection. HGF dissolved in saline was administered through the penile vein 30 min before and 8, 22, 32, 46, 56, 70, 80, 94, 104, and 118 h after ANIT treatment or 8, 22, 32, and 46 h after partial hepatectomy. Rats were killed 12, 24, 48, 72, 96, and 120 h after ANIT treatment or 48 h after 30% partial hepatectomy.

Protamine injection. Under light ether anesthesia, protamine dissolved in saline was administered through the penile vein 10 min before HGF or EGF injection.

Measurement of labeling index. One hour before rats were killed, 5-bromo-2′-deoxyuridine dissolved in normal saline was injected intraperitoneally (100 mg/kg body wt). One hour after injection, the rats were exsanguinated via the abdominal artery, under light ether anesthesia. The liver was then removed and fixed in 10% buffered Formalin for 24 h. The fixed samples were embedded in paraffin, and the paraffin sections (4 µm) were mounted on glass slides. After deparaffinization of the liver sections, endogenous peroxidase was inactivated in 0.3% hydrogen peroxide in absolute methanol, and nuclei incorporating 5-bromo-2′-deoxyuridine were stained with the use of a cell proliferation kit (Amersham, Arlington Heights, IL). The labeling index of hepatocytes was determined by counting more than 500 nuclei in photographs of three randomly selected fields under light microscopy.

Determination of bilirubin concentration and activity of liver cytosolic enzymes in serum. The total bilirubin concentration and the activity of liver-specific cytosolic enzymes such as glutamic-pyruvic transaminase (GPT), lactate dehydrogenase (LD), alkaline phosphatase (ALP), and γ-glutamyltranspeptidase (γ-GTP) in rat serum obtained 48 h after ANIT treatment were determined using the appropriate assay kits (Wako Pure Chemical).

Pharmacokinetic analysis of HGF in ANIT-treated rats. Under light ether anesthesia, protamine (0 or 1.6 mg/kg) was administered to rats through the penile vein 24 h after ANIT treatment. Ten minutes after the protamine injection, HGF (300 µg/kg body wt) dissolved in saline was also given through the penile vein. Plasma was collected from the external jugular vein, and the HGF concentration was determined using an enzyme-linked immunoassay (EIA) kit (Institute of Immunology, Tochigi, Japan).

The plasma concentration (Cp)-time profiles of HGF after intravenous administration were fitted to the following two-exponential equation by a nonlinear iterative least-squares method (11)

$$C_p = A \exp(-\alpha t) + B \exp(-\beta t)$$

where α and β are the apparent rate constants, A and B are the corresponding time 0 intercepts, and t is time. The input data were weighted as the reciprocal of the square of the observed values, and the algorithm used for the fitting was the damping Gauss-Newton method.

The area under the plasma concentration-time curve (AUC) and area under the moment curve (AUMC) were calculated as

$$AUC = A/\alpha + B/\beta$$

$$AUMC = A/\alpha^2 + B/\beta^2$$

The plasma clearance (CLplasma), distribution volume of the central compartment (V1), and steady-state distribution volume (Vdss) were calculated from Eqs. 4, 5, and 6, respectively

$$CL_{plasma} = \text{dose/AUC}$$

$$V_i = \text{dose/(A + B)}$$

$$V_{dss} = \text{doseAUMC/AUC}^2$$

Effect of protamine injection on the plasma elimination of HGF. Under light ether anesthesia, 1 µg/kg HGF was injected through the penile vein of normal rats. At indicated times, blood was withdrawn through the left external jugular vein. At 3.5 min, 250 µL saline containing protamine (0–20 mg/kg) were also injected through the penile vein, and blood samples were collected. The plasma concentration of HGF was determined by EIA.

Assay for DNA synthesis in primary cultured rat hepatocytes. Parenchymal hepatocytes were plated at a density of 1.25 x 10^5 cells/1.88 cm² and cultured for 24 h. The fixed samples were embedded in paraffin, and the paraffin sections (4 µm) were mounted on glass slides. After deparaffinization of the liver sections, endogenous peroxidase was inactivated in 0.3% hydrogen peroxide in absolute methanol, and nuclei incorporating 5-bromo-2′-deoxyuridine were stained with the use of a cell proliferation kit (Amersham, Arlington Heights, IL). The labeling index of hepatocytes was determined by counting more than 500 nuclei in photographs of three randomly selected fields under light microscopy.

RESULTS

Statistical analysis. Statistical analysis was performed by Student’s t-test to identify significant differences between various treatment groups.

RESULTS

Effect of protamine on liver regeneration induced by HGF in rats with liver damage in vivo. Protamine (0 or 1.6 mg/kg body wt) was injected intravenously into ANIT-toxintoxicated rats 10 min before administration of HGF (300 µg/kg), and hepatocyte labeling indexes were determined at specified times after ANIT intoxication (Fig. 1). The labeling indexes after the administration of protamine before HGF injection were 0.95 ± 0.08, 1.37 ± 0.33, 4.63 ± 1.01, 3.87 ± 0.45, 5.02 ± 1.08, and 1.32 ± 0.32% (means ± SE; n = 6) at 12, 24, 48, 72, 96, and 120 h after ANIT treatment, respectively (Fig. 1). These values were respectively 1.2, 2.4, 4.9, 3.8, 2.1, and 1.4 times the value after the injection of HGF alone.
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Fig. 1. Time profiles of DNA synthesis in hepatocytes of \textalpha-naphthylisothiocyanate (ANIT)-intoxicated rats treated with hepatocyte growth factor (HGF) alone or protamine before HGF injection. ANIT-intoxicated rats were treated with HGF (300 µg/kg) alone (C), protamine (1.6 mg/kg) before injection of HGF (P), or protamine alone (\(\text{H} + \text{P}\)), and the labeling index in hepatocytes was determined at designated times after ANIT intoxication. Data are means ± SE of 3–6 rats. *P < 0.05, **P < 0.01, significantly different from HGF alone.

Fig. 2. Effect of protamine on liver regeneration induced by HGF in ANIT-intoxicated (A) and partially hepatectomized (B) rats. ANIT-intoxicated rats were treated with HGF (300 µg/kg) alone (H) or with various doses of protamine before HGF injection (H + P), or protamine (1.6 mg/kg) alone (P), and the labeling index in hepatocytes was determined 48 h after ANIT intoxication (Fig. 2A). Protamine alone could not stimulate liver regeneration in ANIT-intoxicated rats (Fig. 2A). The labeling index increased in a protamine dose-dependent manner when the dose of protamine was increased from 0 to 1.6 mg/kg, and the peak value was reached at 1.6 mg/kg protamine (Fig. 2A). When the dose of protamine was further increased to over 1.6 mg/kg, a dose-dependent reduction in the labeling index was observed (Fig. 2A). The effect of protamine on liver regeneration induced by HGF fell to almost the control level when the dose of protamine was 6.4 mg/kg (Fig. 2A). An enhancing effect of protamine on liver regeneration induced by HGF was also found in partially (30%) hepatectomized rats (Fig. 2B). The protamine dose dependence in the labeling indexes was similar to that in ANIT-intoxicated rats (Fig. 2). When the dose of protamine was increased, the peak value of the labeling index occurred at a protamine dose of 1.6 mg/kg (Fig. 2B).

Effect of protamine on bilirubin concentration and activity of liver cytosolic enzymes in ANIT-intoxicated rats. To examine whether protamine promotes the repair of liver function induced by HGF in ANIT-intoxicated rats, we determined the change in total bilirubin concentration and activity of liver cytosolic enzymes such as GPT, LAP, and ALP, and \(\gamma\)-GTP in serum from rats after administration of HGF alone or protamine before HGF injection (Fig. 3). Protamine alone did not reduce the total bilirubin concentration or the activity of liver cytosolic enzymes in serum caused by ANIT administration was significantly countered by injection of HGF (300 µg/kg) alone (Fig. 3). When protamine at a dose of 0.8 or 1.6 mg/kg was administered before HGF injection, the serum level of \(\gamma\)-GTP was significantly lower than that after injection of HGF alone (Fig. 3). Protamine slightly enhanced the reduction produced by HGF in bilirubin concentration, GPT, and LAP, although this effect was not significant (Fig. 3).

Effect of protamine on HGF clearance from the circulation in ANIT-intoxicated rats. To examine whether protamine reduces the clearance of HGF from the circulation, plasma concentration-time profiles of HGF in ANIT-intoxicated rats were determined after intravenous administration of HGF alone or HGF following protamine treatment (Fig. 4). The elimination of HGF from plasma after injection of HGF following protamine treatment was slower compared with that after administration of HGF alone (Fig. 4). The AUC after administration of HGF following protamine injection fell 39.5%, 34.7%, and 19.1%, respectively, of the values after administration of HGF without protamine treatment (Table 1).
To examine whether the stimulant effect of protamine on liver regeneration induced by HGF can be attributed to the increase in HGF AUC produced by protamine preinjection, the hepatocyte labeling index was plotted against AUC (Fig. 5). The labeling index at 300 µg/kg HGF following 1.6 mg/kg protamine treatment was 5.23 ± 0.99%, which was much higher than that after administration of HGF alone at a dose of 500 µg/kg (0.530 ± 0.104%) (Fig. 5), although in both cases the AUC had almost the same value (Fig. 5).

Effect of protamine on DNA synthesis rate induced by HGF and EGF in primary cultured rat hepatocytes. To examine the direct effect of protamine on hepatocytes, we examined the effect of protamine on DNA synthesis in primary cultured hepatocytes in the presence of HGF (Fig. 6, A and B). When the protamine concentration was increased to 12.5 µg/ml, no significant change in the DNA synthesis rate induced by HGF was observed in hepatocytes cultured for 3 and 24 h (Fig. 6, A and B). When the protamine concentration in the medium was increased to 25 µg/ml, DNA synthesis in hepatocytes cultured for 24 h was increased approximately twofold compared with that in the presence of HGF alone (Fig. 6B). The DNA synthesis rate in hepatocytes in the presence of any concentration of HGF was inhibited almost completely when the protamine concentration in the medium was 200 µg/ml (Fig. 6, A and B). To examine whether the enhancing effect of protamine is specific to HGF, we performed the same experiment with EGF (Fig. 6C). In the presence of 6–25 µg/ml protamine, DNA synthesis was increased approximately two- to threefold compared with DNA synthesis in the presence of EGF alone (Fig. 6C). When the concentration of protamine was increased to 50 and 200 µg/ml, DNA synthesis of hepatocytes was inhibited (Fig. 6C).

Table 1. Comparison of the pharmacokinetic parameters of HGF in ANIT-intoxicated rats after intravenous administration of HGF alone and protamine before HGF injection

<table>
<thead>
<tr>
<th>Condition</th>
<th>AUC, µg·min⁻¹·ml⁻¹</th>
<th>CLplasma, ml·min⁻¹·kg⁻¹</th>
<th>V₁, ml/kg</th>
<th>Vdss, ml/kg</th>
<th>MRT, min⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>HGF alone (300 µg/kg)</td>
<td>9.98 ± 0.28</td>
<td>32.1 ± 0.9</td>
<td>131 ± 4</td>
<td>1.10 × 10³ ± 0.17 × 10³</td>
<td>1.08 × 10³ ± 0.11 × 10³</td>
</tr>
<tr>
<td>HGF + protamine (1.6 mg/kg)</td>
<td>24.7 ± 3.2</td>
<td>127 ± 1.4</td>
<td>45.3 ± 6.5</td>
<td>389 ± 72</td>
<td>2.36 × 10³ ± 1.49 × 10³</td>
</tr>
</tbody>
</table>

Data are means ± SE. HGF, hepatocyte growth factor; ANIT, α-naphthyl isothiocyanate; AUC, area under the plasma concentration-time curve; CLplasma, plasma clearance; V₁, distribution volume of the central compartment; Vdss, steady-state distribution volume; MRT, mean residence time.
Protamine causes a transient increase in the plasma concentration profile of HGF after intravenous administration of HGF. To support the hypothesis that protamine competes with HGF for binding to a heparin-like substance in vivo, we studied the effect of protamine injection on the plasma concentration-time profile of HGF in normal rats (Fig. 7). After intravenous administration of HGF (1 µg/kg), plasma HGF concentrations fell rapidly (Fig. 7). After various doses of protamine (0.48–20 mg/kg) were injected, the plasma concentrations of HGF increased immediately in a protamine dose-dependent manner (Fig. 7). However, this protamine dose dependence differed from that for the enhancing effect on the labeling index (Fig. 2) and reached a maximum at 20 mg/kg protamine (Fig. 7).

Protamine elutes HGF prebound to heparin-Sepharose in a column chromatography experiment. HGF bound to heparin in a heparin affinity column could not be washed off by PBS but was easily eluted with 2 M sodium chloride (Fig. 8A). The recovery of HGF from the heparin column was 85.7% (Fig. 8A). To further support the hypothesis of competition for the binding of HGF to heparin by protamine, we added protamine (20 mg/ml) to the heparin affinity column prebound with HGF (Fig. 8B). The HGF bound to the column was eluted by addition of protamine, and the recovery of HGF was 84.4% (Fig. 8B). After elution with protamine, only a small amount of HGF was further eluted by 2 M sodium chloride (Fig. 8B). In this analysis we confirmed that the determination of HGF...
by EIA was not influenced by 20 mg/ml protamine (data not shown).

**DISCUSSION**

In the present study, we found that, when administered before injection of HGF, protamine enhances HGF-induced liver regeneration (Fig. 1). Such an enhancing effect of protamine was found in both ANIT-intoxicated rats and partially hepatectomized rats (Fig. 2), and the protamine dose dependence in the hepatocyte labeling indexes was almost identical in both cases (Fig. 2), suggesting that this effect may be general for a number of liver diseases. Protamine also significantly further reduces $\gamma$-GTP (Fig. 3) at 300 µg/kg HGF, and at 50 µg/kg HGF, bilirubin concentration and the activity of all cytosolic marker enzymes examined were significantly reduced by preinjection of protamine, compared with those with HGF alone (data not shown). Thus the effect of protamine is also observed in the repair of liver function. The dosage of protamine in clinical situations is 10–15 mg for the neutralization of 1,000 U heparin (29). Because the regular clinical single dose of heparin is 100 U/kg iv, 1.0–1.5 mg/kg protamine is usually used as an antidote for heparin. In the present study, we required 1.6 mg/kg protamine to observe its maximum enhancing effect on liver regeneration (Fig. 2). Thus this dose of protamine is very similar to the clinical dose and therefore may also be used in clinical situations. The dose of protamine should be strictly controlled, since a higher dose of protamine reduces the mitogenic response to HGF (Figs. 2A and 6), probably because of its cytotoxic effect.

The $CL_{\text{plasma}}$ of HGF was reduced by preadministration of protamine (Fig. 4 and Table 1). We believe that the likely mechanism involves inhibition of the nonspecific clearance of HGF by protamine. HGF has two binding sites on epithelial cell surfaces: 1) the HGF receptor, a specific binding site, and 2) a heparin-like substance, which has a lower affinity for HGF (2, 5). In previous studies, we suggested that one of the major clearance mechanisms for HGF is its nonspecific uptake in the liver, which is probably mediated by a heparin-like substance (16–19). Given that protamine has a high affinity for heparin (7) and can elute HGF molecules prebound to heparin-Sepharose (Fig. 8), a transient increase in plasma HGF after intravenous administration of protamine (Fig. 7) may reflect the transfer of HGF molecules bound to the heparin-like substance on cell surfaces and/or the extracellular matrix of various tissues into the circulating plasma after protamine injection. Thus protamine and HGF bind to the same region of the heparin-like substance or, at least, to a similar location so that each compound can affect the binding of the other.

There are two possible mechanisms for the enhancing effect of protamine on HGF-induced liver regeneration in vivo: one is the increase in HGF AUC, which results from inhibition of the nonspecific uptake of HGF by protamine (Fig. 4), and the other is a direct stimulatory effect on the mitogenic response of hepatocytes to HGF (Fig. 6B). Protamine increases HGF AUC 2.5-fold (Table 1), whereas the increase in liver regeneration, assessed as the area under the time course of the labeling index after ANIT intoxication, was approximately fivefold (Fig. 1). Therefore, the enhancing effect of protamine on HGF-induced liver regeneration can be partially explained by increased exposure of hepatocytes to HGF. As shown in Fig. 5, HGF at a dose of 300 µg/kg plus protamine has a markedly higher labeling index than HGF alone at 500 µg/kg but has an AUC nearly identical to that of HGF alone at 500 µg/kg. The data shown in Fig. 5 provide clear evidence against a direct relationship between HGF availability (as expressed by the AUC) and liver regeneration (as expressed by the labeling index). Thus the mechanism of the effect of protamine on HGF-induced liver regeneration is not principally related to its inhibitory effect on HGF clearance. The difference in the protamine dose dependence between the labeling index (Fig. 2) and plasma disappearance of HGF (Fig. 7), where maximum effect can be observed at 1.6 and 20 mg/kg protamine, respectively, also supports that the enhanced liver regeneration cannot be fully explained by such an indirect effect. In fact, the DNA synthesis in hepatocytes in primary culture induced by HGF was increased approximately twofold through the direct stimulatory effect of protamine (Fig. 6B). Therefore, the direct effect of protamine on hepatocytes may be considered one of the more rational mechanisms for the beneficial effects of protamine on the labeling index in vivo.
The effect of protamine on several cytokine receptors has been investigated (23, 28). Lokeshwar et al. (14a) reported that protamine induced an increase in the number of EGF receptors by activating cryptic or inactive receptors to become functionally active in Swiss 3T3 cells and human epidermoid carcinoma A431. Protamine also increases EGF-induced phosphorylation of the EGF receptor. In the present study, we also found that protamine enhances EGF-induced DNA synthesis in hepatocytes in vitro (Fig. 5C). This indicates that the direct enhancement effect of protamine on hepatocyte DNA synthesis is not specific to HGF. Sacks and McDonald (23) have also reported that protamine enhanced the insulin-induced autophosphorylation activity of insulin receptors. Like EGF and insulin receptors, HGF receptors are also transmembrane protein tyrosine kinase receptors (27). The diverse biological actions of HGF are a result of signaling through this receptor (22, 30). According to current thinking, HGF activates its corresponding PTK receptors by inducing receptor dimerization and autophosphorylation as a first step in an intracellular signaling cascade (3). Therefore, such an interaction of protamine with the HGF receptor or its signal transduction cascade may occur, resulting in the increase in DNA synthesis.

The suppressive effect of protamine on bilirubin concentration and the activity of cytosolic enzymes, except γ-GTP, was not significant (Fig. 3). Because the suppressive effect of HGF alone on bilirubin concentration and the activity of cytosolic enzymes could not be further increased even when the dose of HGF was raised to 710–1,000 µg/kg (data not shown), we believe that 300 µg/kg HGF exerts an almost maximal effect in suppressing bilirubin concentration and the activity of these cytosolic enzymes in serum in ANIT-intoxicated rats.

In conclusion, we find that protamine enhances HGF-induced liver regeneration in vivo. Such an effect of protamine can be explained by its dual effects, 1) a direct stimulatory effect on hepatocyte DNA synthesis and 2) an indirect stimulatory effect on HGF clearance, which results in increased exposure to HGF.

We are very grateful to Drs. Kohji Tanaka in Development Research Laboratories, Dainippon Pharmaceutical Co., Ltd., and Satoru Inagaki in Development Research Laboratories, Banyu Pharmaceutical Co., Ltd., for kindly advising us how to determine the labeling index in hepatocytes in rats.

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.

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