REFERENCES

1. Velchik MG, Kressel HY, Noel AW, et al. Primary aldosteronism: CT, MRI, scintigraphic correlation. *Invest Radiol* 1985;20:237–241.
2. Gross MD, Shapiro B. Scintigraphic studies in adrenal hypertension. *Semin Nucl Med* 1989;19:122–143.
3. Alterman SL, Dominguez C, Lopez-Gomez A, et al. Primary adrenocortical carcinoma causing aldosteronism. *Cancer* 1969;24:602–609.
4. Shenker Y, Gross MD, Grekin RJ, et al. The scintigraphic localization of mineralocorticoid-producing adrenal cortical carcinoma. *J Endocrinol Invest* 1986;9:115–120.
5. Weinberger MH, Grim CE, Hollifield JW, et al. Primary aldosteronism: diagnosis, localization and treatment. *Ann Intern Med* 1979;90:386–395.
6. Gross MD, Valk TW, Swanson DP, et al. The role of pharmacologic manipulation in adrenal cortical scintigraphy. *Semin Nucl Med* 1981;9:128–148.
7. Gross MD. The role of the adrenal gland in hypertension. *Urol Radiol* 1982;3:233–235.
8. Beierwaltes WH, Lieberman LM, Ansari AN, et al. Visualization of human adrenal glands in vivo by scintillation scanning. *JAMA* 1971;216:275–277.
9. Sarkar SD, Cohen EL, Beierwaltes WH, et al. A new and superior adrenal imaging agent, I-131-6β-iodomethyl-19-norcholesterol (NP-59): evaluation in humans. *J Clin Endocrinol Metab* 1977;45:353–362.
10. Francis IR, Gross MD, Shapiro B, et al. Integrated imaging of adrenal disease. *Radiology* 1992;184:1–13.
11. Ikeda DM, Francis IR, Glazer GM, et al. The detection of adrenal tumors and hyperplasia in patients with primary aldosteronism: comparison of scintigraphy, CT and MR imaging. *AJR* 1989;153:301–306.
12. Biglieri EG, Schamberlan M, Slaton PE, et al. The intercurrent hypertension of primary aldosteronism. *Circ Res* 1970;27(suppl 1):I-195–I-202.
13. Baer L, Sommers SC, Krakoff LR, et al. Pseudoprimary aldosteronism. An entity distinct from true primary aldosteronism. *Circ Res* 1970;27(suppl 1):I-203–I-216.
14. Cain JP, Tuck ML, Williams GH, et al. The regulation of aldosterone secretion in primary aldosteronism. *Am J Med* 1972;53:627–637.
15. Gross MD, Shapiro B, Grekin RJ, et al. Scintigraphic localization of adrenal lesions in primary aldosteronism. *Am J Med* 1984;77:839–844.
16. Britton KE, Shapiro B, Hawkins LA. Emission tomography for adrenal imaging. *Nucl Med Commun* 1980;1:37–40.
17. Britton KE, Shapiro B, Londres S, et al. The tomoscanner II-clinical results. In: Paras P, Eikman EA, eds. *Emission computed tomography: the single photon approach.* HHS Publication FDA 81-8177. Rockville, MD: U.S. Department of Health and Human Services; 1981:215–225.

Increased Technetium-99m-GSA Uptake per Hepatocyte in Rats with Administration of Dimethylnitrosamine or Hepatocyte Growth Factor

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Technetium-99m-diethylenetriaminepentaacetic acid-galactosyl-human serum albumin (GSA) is a new scintigraphic agent that binds specifically to asialoglycoprotein receptors on hepatocytes, and can be used to evaluate hepatic function. Asialoglycoprotein receptor is a hepatocellular membrane receptor responsible for the endocytosis of asialoglycoproteins, and the function of this receptor is affected in various disease states. The aim of this study was to investigate GSA uptake per hepatocyte in the convalescent stage from hepatic damage. **Methods:** We used rats with dimethylnitrosamine (DMN)-induced hepatic injury and rats with recombinant human hepatocyte growth factor (rhHGF) stimulation. Plasma clearance of GSA and the number of hepatocytes in whole liver were calculated. **Results:** In the DMN-treated rats, the total number of hepatocytes and GSA plasma clearance were reduced significantly at 3 wk after the final administration of DMN. However, calculated GSA uptake per individual hepatocyte was significantly greater by 53.2% than in the normal controls. The area of hepatic nucleus was also significantly greater than in the normal controls. In the rhHGF-treated rats, an increase in the total number of hepatocytes was not demonstrated on the final day of rhHGF administration (Day 4). However, calculated GSA uptake per hepatocyte was significantly greater (59%) than in the controls. **Conclusion:** Augmented GSA uptake per hepatocyte during the convalescent stage after hepatic injury suggests a cellular compensation to the decreased number of hepatocyte. This mechanism may be caused by the secretion of some hepatotropic factors such as HGF.

Key Words: technetium-99m-galactosyl-human serum albumin; asialoglycoprotein receptors; hepatocyte growth factor; dimethylnitrosamine

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Technetium-99m-diethylenetriaminepentaacetic acid-galactosyl-human serum albumin (GSA) (Nihon Medi-Physics Chemical, Nishinomiya, Japan) is a new scintigraphy agent that binds specifically to asialoglycoprotein receptors (ASGPR) (*1*) on hepatocytes, and can be used to evaluate hepatic function (*2*). Diethylenetriaminepentaacetic acid has been used to obtain stable labeling with ^{99m}Tc (*3,4*).

Asialoglycoprotein receptor is a hepatocellular membrane receptor responsible for the metabolism of serum glycoproteins. Asialoglycoprotein receptor recognizes and binds galactose-terminated glycoproteins by a second-order chemical reaction (*5,6*). After binding, the glycoproteins are transported to lysosomes, where the ligands are catabolized, and the receptor recycles to the plasma membrane (*5,6*).

It has been reported that receptor levels are reduced in galactosamine-treated rats (*7*) and patients with chronic liver disease (*8*). Similarly, decreased ligand clearance from plasma has been observed in streptozotocin-diabetic rats (*9*). These results suggested alterations in expression of the receptor, whereas the in vivo studies addressed the ASGPR activity per body or liver weight. In contrast, the issue of whether the decreased GSA uptake by the liver is associated with a reduction in the function of each cell or the total number of hepatocytes has not been examined in detail.

In vitro studies have examined asialoglycoprotein uptake activity per hepatocyte under various conditions. Although there is an abundance of evidence for decreased binding of asialoglycoprotein by isolated hepatocytes in a variety of experimental liver injuries (*10–12*), increased binding has been observed in diethylnitrosamine-treated rats (*13*).

Increased hepatocyte growth factor (HGF) secretion has been demonstrated during hepatic damage in rats (*14,15*). Human HGF, which strongly stimulates deoxyribonucleic acid (DNA) synthesis and proliferation of hepatocytes, was originally purified from the plasma of patients with hepatic failure (*16*). Its

complementary DNA (cDNA) was cloned from human placental and livers cDNA libraries (*17,18*). Recombinant human HGF was purified from the culture supernatant of Chinese hamster ovary cells transfected with the human HGF cDNA (*19*). The ability of recombinant human HGF (rhHGF) to stimulate DNA synthesis of both human and rat primary culture hepatocytes was equal to that of native HGF (*20*). Recently, Ishii et al. (*21*) discovered that protein synthesis of hepatocytes in primary culture is stimulated by rhHGF addition, and this occurs prior to the increase in the number of cells. In contrast, little is known about the effect of HGF on the GSA uptake of individual hepatocyte.

In this study, we attempted to identify any alterations in GSA uptake per individual hepatocyte in vivo. We measured GSA plasma clearance and the total number of hepatocytes both in the convalescent stage after hepatic injury and with rhHGF stimulation.

MATERIALS AND METHODS

Animals

One group of male Sprague-Dawley rats (12 wk, 340–380 g) was administered dimethylnitrosamine (DMN), as described previously (*22*): 1% DMN dissolved in saline was given intraperitoneally at 10 μl/kg body weight for 3 consecutive days each week for 3 wk. Untreated rats were included as controls.

The second group of male Sprague-Dawley rats (13 wk, 420–460 g) were treated with rhHGF. Infusion of rhHGF (Mitsubishi Chemical, Yokohama, Japan) continued over 4 days, as described previously (*21*). The rhHGF was dissolved in 10 mM phosphate buffer (ph 7.4) containing 0.7 *M* NaCl and 0.005% Tween 80 to prevent aggregation. An osmotic minipump (Alzet Model 2001, ALZA Corp., Palo Alto, CA; capacity = 200 μl), with a nominal delivery rate of 24 μl/day, was used. Rats underwent pentobarbital anesthesia. A cannula was surgically placed in the right jugular vein and the other end was connected to the minipumps that were implanted subcutaneously. The injected dose of rhHGF was 100 μg/kg/day. In the corresponding control rats, 0.9% saline was loaded into the minipumps.

All rats received care in compliance with Kansai Medical University's Institutional Animal Care and Use Committee guidelines.

Measurement of Galactosyl Human Serum Albumin Plasma Clearance

A single rapid injection of GSA (1665 μg/kg body weight) via the jugular vein was performed under pentobarbital anesthesia (25 mg/kg of body weight, intraperitoneally). Blood samples were collected at 2, 4, 6 and 8 min after the injection. In this animal model, we found that the diminution in plasma radioactivity closely followed an exponential curve. The plasma clearance was calculated with the following formula: $C(t) = C(0) \times e^{-kt}$, where C(t) = counts at time t; C(0) = counts at time 0, obtained by extrapolation from the disappearance curve; and k = the plasma disappearance rate. The injected amount of GSA times k equals the initial removal rate of GSA (μg/min).

To obtain an optimum GSA disappearance curve, which was independent of changes in hepatic blood flow, we examined the changes in GSA clearance at a variety of doses (208–6660 μg/kg body weight). A single injection of 1665 μg/kg body weight GSA was chosen as the optimum dose for this study.

In the DMN-treated rats, the initial removal rate of GSA was measured three times in each rat, both before and after the administration of DMN, as well as 3 wk after the final administration. In the rhHGF-treated rats, the initial removal rate of GSA was measured three times in each rat: pretreatment, Day 4 and 3 days after the completion of rhHGF administration.

Morphometric Analysis

The DMN-treated (n = 6) and control group (n = 6) rats were killed, and their livers were removed 3 wk after the final DMN administration, whereas this was done on Day 4 in the rhHGF-treated (n = 4) and control (n = 4) groups. Livers were fixed in 10% formalin, embedded in paraffin, cut into 5-μm-thick sections and stained with hematoxylin and eosin. The microscope view was transmitted to a television camera. Using an image analysis system (Image Processor for Analytical Pathology, Sumika Technos, Osaka, Japan) (*23*), hepatocyte nuclear size (μm^2) and hepatocyte density (number of hepatocytes/mm^2) were assessed quantitatively by measuring the hepatic cells contained in 10 parenchymal areas (total = 2.44×10^5 μm^2 in each rat). In the DMN-treated livers, the damage was diffuse, and fibrous septa could not readily be located. Assuming that the control rat liver contained 1.6×10^8 hepatocytes/g (*9,24*), the number of hepatocytes in whole liver was calculated using the following formula: number of hepatocytes in total liver of subject rat = $1.6 \times 10^8 \times$ liver weight of subject rat (g) × hepatocyte density of subject rat (number/mm^2)/mean hepatocyte density of control rats (number/mm^2).

Expression of Data

The activity of GSA uptake per hepatocyte in all treatment and control groups was calculated using the following formula: initial removal rate of GSA per hepatocyte (μg/min) = initial removal rate of GSA (μg/min)/number of hepatocytes in total liver.

Statistical Analysis

Data are expressed as the mean ± s.d. The Pearson's correlation coefficient was used to assess the correlation between the total number of hepatocytes and the initial removal rate of GSA. The paired t-test was used to compare mean values of the initial removal rate of GSA in pre- and post-treatment. The unpaired t-test was used to compare the total number of hepatocytes, the area of the nucleus and the rate of GSA uptake per hepatocyte in the DMN and rhHGF treatment and control groups. A p value of <0.05 was considered significant.

RESULTS

Effects of Dimethylnitrosamine Administration

Pre- and post-treatment comparison (n = 6) demonstrated that the GSA clearance decreased as a result of the administration of DMN. Before DMN administration, the removal rate of GSA per liver was 119.8 μg/min ± 26.2 μg/min. After DMN administration, it was 48.6 μg/min ± 18.3 μg/min. This was significantly lower ($p < 0.005$; Fig. 1). Moreover, 3 wk after the final administration of DMN, a slight tendency toward recovery was identified (Fig. 1). In the control rats (n = 6), there was a slight tendency toward decreased GSA clearance with aging, despite the absence of hepatotoxic treatment (Fig. 1).

In both normal and liver-damaged rats (n = 12), the number of hepatocytes and the initial removal rate of GSA per liver demonstrated a positive correlation ($r = 0.885$; $p < 0.0001$; Fig. 2) at 3 wk after the final administration of DMN. Morphometric examinations showed that the number of viable hepatocytes decreased in the liver removed from the treated rats 3 wk after the final administration of DMN, and residual hepatocyte enlarged (Fig. 3; Table 1). The total number of hepatocytes in the treated rats (n = 6) was $1.31 \times 10^9 \pm 0.16 \times 10^9$, and in the control group (n = 6), it was $3.02 \times 10^9 \pm 0.44 \times 10^9$. Clearly, the total number of hepatocytes in the treated rats was significantly lower than in the control rats ($p < 0.0001$; Table 1). The initial removal rate of GSA per hepatocyte was 5.76×10^{-8} μg/min ± 1.27×10^{-8} μg/min in the rats with DMN-induced liver injury (n = 6), and 3.76×10^{-8} μg/min ± 1.54 ×

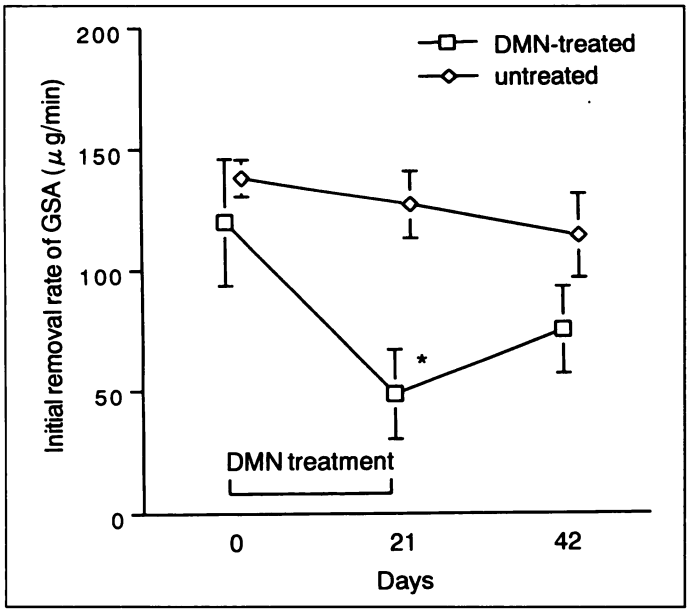

FIGURE 1. Effects of dimethylnitrosamine (DMN) administration. Values are means ± s.d. of 6 animals. DMN was given for 3 consecutive days per week for 3 wk. Galactosyl human serum albumin removal rate was measured pretreatment, on Day 21 (the final day of treatment), and on Day 42 (3 wk after the completion of DMN treatment). * $p < 0.005$ compared with the rate before DMN treatment.

10^{-8} μg/min in the control group (n = 6). This difference was statistically significant ($p < 0.05$; Table 1).

Effects of Recombinant Human Hepatocyte Growth Factor Administration

The removal rates of GSA per liver before the administration of rhHGF on the final day of rhHGF administration (Day 4) and 3 days after the termination of HGF administration were 131.6 μg/min ± 24μg/min, 197.7 μg/min ± 51.3 μg/min and 121.3 μg/min ± 15.6 μg/min, respectively (n = 6). The GSA removal rate after rhHGF administration increased significantly compared with the rate before rhHGF administration ($p < 0.05$). However, 3 days after the termination of HGF administration, the rate ultimately returned to the pre-rhHGF administration level (Fig. 4).

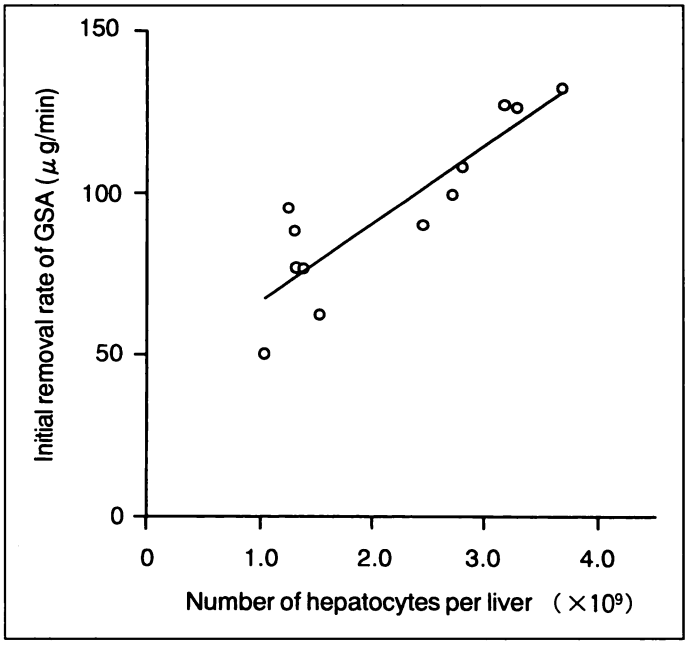

FIGURE 2. Relationship between galactosyl human serum albumin (GSA) clearance and hepatocyte number in both normal and dimethylnitrosamine-damaged rats (n = 12) at 3 wk after the final treatment. The number of hepatocytes and the initial removal rate of GSA per liver demonstrated a positive correlation ($r = 0.885$; $p < 0.0001$).

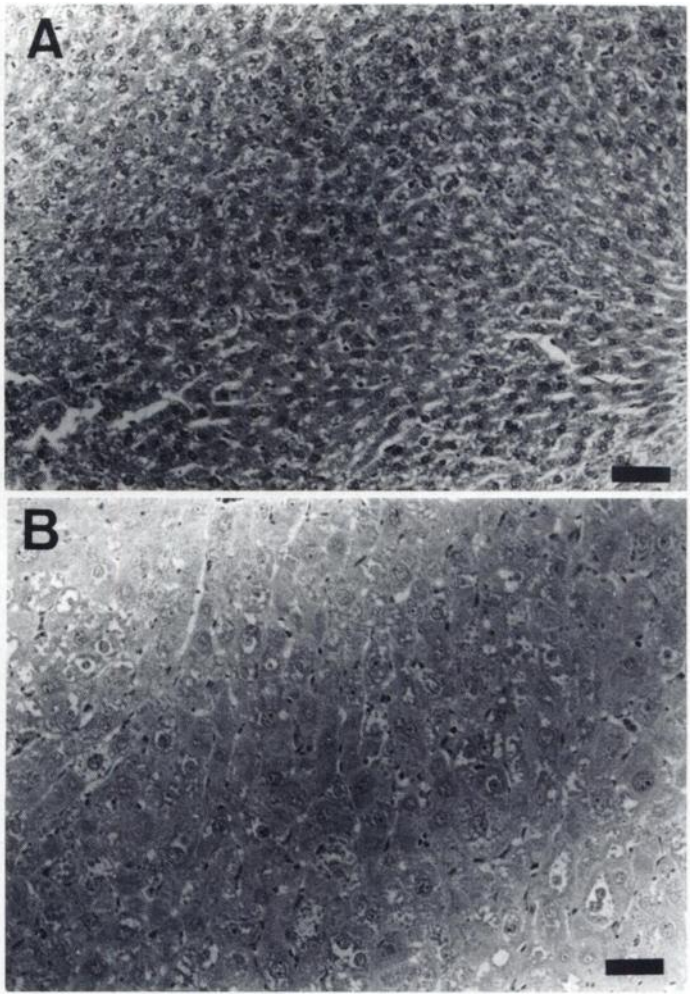

FIGURE 3. Comparison of control liver (A) and Dimethylnitrosamine (DMN)-treated liver (B) at 3 wk after the final treatment (hematoxylin and eosin; bar = 50 μm). Many hepatocytes of large size are present in DMN-treated liver.

On the final day of rhHGF administration (Day 4), the number of hepatocytes in the rhHGF-administered group (n = 4) was $2.59 \times 10^9 \pm 0.56 \times 10^9$, whereas it was $2.88 \times 10^9 \pm 0.20 \times 10^9$ in the control group (n = 4). Thus, rhHGF did not induce a substantial increase in the number of hepatocytes (Table 2). The initial removal rate of GSA per hepatocyte in the rhHGF-administered group (n = 4) was 8.81×10^{-8} μg/min ± 1.84×10^{-8} μg/min, whereas it was 5.54×10^{-8} μg/min ± 0.52×10^{-8} μg/min in the control group (n = 4). This difference was statistically significant ($p < 0.05$; Table 2).

DISCUSSION

The question of whether the decrease in GSA blood clearance associated with liver damage results from a reduction in the function of each cell or in the total number of hepatocytes had not previously been adequately explored. In this study, we observed that DMN reduced the average number of hepatocytes to 43.4% of control animals at 3 wk after the final administration of DMN. The GSA clearance also fell to 69.3% and demonstrated a linear correlation with the total number of hepatocytes. These results indicate that an impaired GSA clearance results predominantly from the decreased number of hepatocytes in the whole liver. Furthermore, this suggests that

TABLE 1
Effects of Dimethylnitrosamine (DMN) on Liver and Galactosyl Human Serum Albumin (GSA) Clearance 3 Wk After Completion of Treatment

Group	Liver weight (g)	Total number of hepatocytes ($\times 10^9$)	Area of hepatic nucleus (μm^2)	Initial removal rate of GSA per hepatocyte ($\times 10^8$ μg/min)
Untreated	19.1 ± 1.2	3.02 ± 0.44	51.8 ± 4.4	3.76 ± 1.54
DMN-treated	17.7 ± 1.5	1.31 ± 0.16*	83.4 ± 13.3†	5.76 ± 1.27‡

*$p < 0.0001$, compared with the untreated group. †$p < 0.005$, compared with the untreated group. ‡$p < 0.05$, compared with the untreated group. Values are means ± s.d.; n = 6 per group.

the decreased clearance in rats with hepatic damage (*9*) or patients with chronic liver disease (*8*) is due to a decrease in the number of hepatocytes.

When our data were expressed per hepatocyte, we found that the GSA uptake in the DMN-damaged group was greater by 53.2% than in the normal control group. Furthermore, the area of the cell nucleus in the DMN-damaged group was also greater by 61% than in the normal control group. In general, when partial hepatectomy or hepatotoxic injury occurs in rats, parenchymal cells and nuclei start enlarging, and metabolic activity also increases (*25–27*). Wanson et al. (*13*) demonstrated increased nuclear size and ligand uptake of ASGPR compared with the control group in hepatocytes isolated 6 mo after the beginning of diethylnitrosamine treatment, findings consistent with this present in vivo study. Cell or nuclear volume enlargement can be considered an expression of activated function (*28*). From these findings, we speculate that the increased GSA uptake per hepatocyte and nuclear swelling are components of a compensatory mechanism in response to the loss in cell numbers after cytotoxic injury. We also hypothesized that hepatotrophic factors such as HGF may improve the activity of ASGPR in the convalescent stage after hepatic injury.

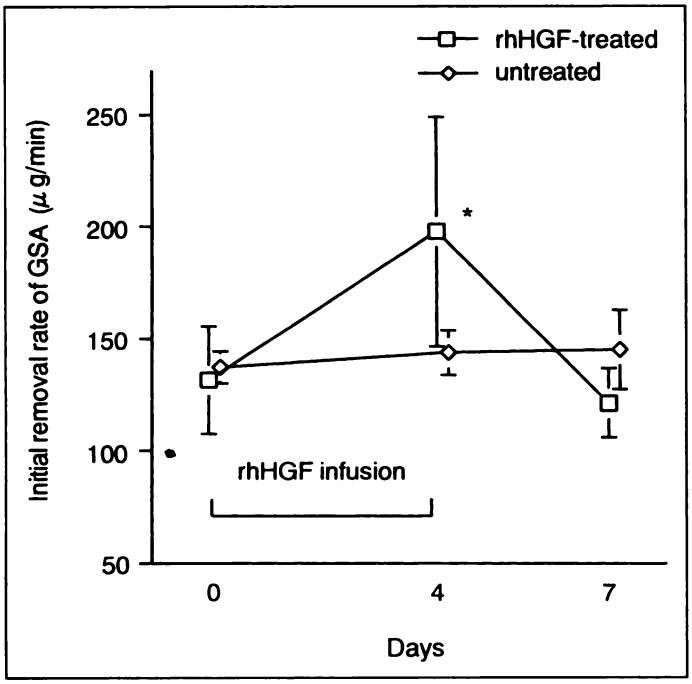

FIGURE 4. Effects of recombinant human hepatocyte growth factor (rhHGF) administration. Values are means ± s.d. of 6 animals. Recombinant HGF was administered to rats on 4 consecutive days. Galactosyl human serum albumin removal rate was measured pretreatment and on Days 4 and 7. *$p <$ 0.05, compared with the rate before rhHGF administration.

In rats given DMN (10 μl/kg body weight, intraperitoneally) 3 days per week for 3 wk, diffuse fibrosis and foci of hemorrhagic necrosis with large zones of collapse were found in all of the animals on Day 21. Hemorrhagic necrosis had decreased, but the architecture of the parenchyma remained abnormal on Day 35, with persistence of a micronodular pattern and feathery degeneration of groups of hepatocytes (*22*). From these findings, we postulated that Day 42 of our study (3 wk after the final administration of DMN) represented the convalescent stage of liver damage.

The mechanism of liver regeneration is not well-understood, but it has been clearly demonstrated that secretion of HGF increases with hepatic damage (*14,15*) and that HGF promotes DNA synthesis or proliferation of hepatocytes (*16*). Consequently, HGF is considered an important factor in liver regeneration. Recombinant HGF stimulated DNA synthesis of both human and rat hepatocytes in primary culture as well as native HGF (*20*). Few studies have reported the effects of in vivo administration of rhHGF. For example, rhHGF increased the labeling index of hepatocytes in normal rats (*29*), suppressed the onset of liver cirrhosis and abrogated lethal hepatic dysfunction in DMN-treated rats (*30*). In contrast, little is known about the effect of rhHGF on the GSA uptake of individual hepatocyte.

To clarify the role of HGF in GSA uptake activity, we administered rhHGF to normal rats and assessed the GSA uptake per hepatocyte. GSA clearance was increased after 4 days of administration of rhHGF, although this effect did not

TABLE 2
Effects of 4-Day Infusion of Recombinant Human Hepatocyte Growth Factor (rhHGF) on Liver and Galactosyl Human Serum Albumin (GSA)

Group	Liver weight (g)	Total number of hepatocytes ($\times 10^9$)	Area of hepatic nucleus (μm^2)	Initial removal rate of GSA per hepatocyte ($\times 10^8$ μg/min)
Untreated	18.0 ± 1.1	2.88 ± 0.20	51.0 ± 3.4	5.54 ± 0.52
rhHGF-treated	17.1 ± 1.5	2.59 ± 0.56	44.9 ± 7.2	8.81 ± 1.84*

*$p < 0.05$, compared with the untreated group.
Values are means ± s.d.; n = 4 per group.

persist after the rhHGF was stopped. There was also no appreciable difference in the number of hepatocytes between the rhHGF-administered rats and the control group. These results demonstrated that the increase in GSA clearance with rhHGF administration was caused by an increase in the ASGPR uptake activity per hepatocyte rather than hepatocyte proliferation. Ishii et al. (*21*) reported that protein synthesis of hepatocytes was stimulated by rhHGF addition before the increase in the number of cells. These findings are consistent with the results of our studies with rhHGF administration and support the conclusion that HGF increases individual hepatocyte functions such as uptake of GSA. Further studies are required to clarify the mechanism of HGF activation of the ASGPR.

CONCLUSION

This study was designed to measure GSA uptake per hepatocyte in the convalescent stage after hepatic injury and during rhHGF stimulation. In the convalescent stage, the decrease in total liver GSA clearance is mainly due to hepatocyte loss. However, there was an associated increase in GSA uptake per hepatocyte. Stimulation by rhHGF affected not only the proliferation of hepatocytes but also enhanced the GSA uptake per hepatocyte. These results suggest that, during the convalescent stage after hepatic injury, some hepatotrophic factors such as HGF may enhance the function of individual hepatocytes to compensate for the decreased cell number.

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REFERENCES

1. Morell AG, Irvine RA, Sternlieb I, Scheinberg IH. Physical and chemical studies on ceruloplasmin. V. Metabolic studies on sialic acid-free ceruloplasmin in vivo. *J Biol Chem* 1968;243:155–159.
2. Ha-Kawa SK, Tanaka Y. A quantitative model of technetium-99m-DTPA-galactosyl-HSA for the assessment of hepatic blood flow and hepatic binding receptor. *J Nucl Med* 1991;32:2233–2240.
3. Galli G, Maini CL, Orlando P, Deleide G, Valle G. A radiopharmaceutical for the study of the liver: ^{99m}Tc-DTPA-asialo-orosomucoid. I. Radiochemical and animal distribution studies. *J Nucl Med Allied Sci* 1988;32:110–116.
4. Galli G, Maini CL, Orlando P, et al. A radiopharmaceutical for the study of the liver: ^{99m}Tc-DTPA-asialo-orosomucoid. II. Human dynamic and imaging studies. *J Nucl Med Allied Sci* 1988;32:117–126.
5. Ashwell G, Steer CJ. Hepatic recognition and catabolism of serum glycoproteins. *JAMA* 1981;246:2358–2364.
6. Stockert RJ, Morell AG. Hepatic binding protein: the galactose-specific receptor of mammalian hepatocytes. *Hepatology* 1983;3:750–757.
7. Sawamura T, Kawasato S, Siozaki Y, Sameshima Y, Nakada H, Tashiro Y. Decrease of a hepatic binding protein specific for asialoglycoproteins with accumulation of serum asialoglycoproteins in galactosamine-treated rats. *Gastroenterology* 1981;81: 527–533.
8. Sawamura T, Nakada H, Hazama H, Shiozaki Y, Sameshima Y, Tashiro Y. Hyperasialoglycoproteinemia in patients with chronic liver diseases and/or liver cell carcinoma: asialoglycoprotein receptor in cirrhosis and liver cell carcinoma. *Gastroenterology* 1984;87:1217–1221.
9. Appel M, Potrat P, Feger J, Mas-Chamberlin C, Durand G. In vivo quantification of removal of asialo-orosomucoid from the circulation in anaesthetized streptozotocin-diabetic rats. *Diabetologia* 1986;29:383–387.
10. Dodeur M, Durand D, Dumont J, Durand G, Feger J, Agneray J. Effects of streptozotocin-induced diabetes mellitus on the binding and uptake of asialoorosomucoid by isolated hepatocytes from rats. *Eur J Biochem* 1982;123:383–387.
11. Howard DJ, Stockert RJ, Morell AG. Asialoglycoprotein receptors in hepatic regeneration. *J Biol Chem* 1982;257:2856–2858.
12. Tworek BL, Tuma DJ, Casey CA. Decreased binding of asialoglycoproteins to hepatocytes from ethanol-fed rats. *J Biol Chem* 1996;271:2531–2538.
13. Wanson JC, Bernaert D, May C, Deschuyteneer M, Prieels JP. Isolation and culture of adult rat hepatocytes and preneoplastic nodules from diethylnitrosamine treated livers: glucose-6-phosphatase distribution, albumin synthesis and hepatic binding protein activity. *Ann N Y Acad Sci* 1980;349:413–415.
14. Kinoshita T, Tashiro K, Nakamura T. Marked increase of HGF mRNA in non-parenchymal liver cells of rats treated with hepatotoxins. *Biochem Biophys Res Commun* 1989;165:1229–1234.
15. Hamanoue M, Kawaida K, Takao S, et al. Rapid and marked induction of hepatocyte growth factor during liver regeneration after ischemic or crush injury. *Hepatology* 1992;16:1485–1492.
16. Gohda E, Tsubouchi H, Nakayama H, et al. Purification and partial characterization of hepatocyte growth factor from plasma of a patient with fulminant hepatic failure. *J Clin Invest* 1988;81:414–419.
17. Miyazawa K, Tsubouchi H, Naka D, et al. Molecular cloning and sequence analysis of cDNA for human hepatocyte growth factor. *Biochem Biophys Res Commun* 1989;163: 967–973.
18. Nakamura T, Nishizawa T, Hagiya M, et al. Molecular cloning and expression of human hepatocyte growth factor. *Nature* 1989;342:440–443.
19. Yoshiyama Y, Arakaki N, Naka D, et al. Identification of the N-terminal residue of the heavy chain of both native and recombinant human hepatocyte growth factor. *Biochem Biophys Res Commun* 1991;175:660–667.
20. Strain AJ, Ismail T, Tsubouchi H, et al. Native and recombinant human hepatocyte growth factors are highly potent promoters of DNA synthesis in both human and rat hepatocytes. *J Clin Invest* 1991;87:1853–1857.
21. Ishii T, Sato M, Sudo K, et al. Hepatocyte growth factor stimulates liver regeneration and elevates blood protein level in normal and partially hepatectomized rats. *J Biochem* 1995;117:1105–1112.
22. Jézéquel AM, Mancini R, Rinaldesi ML, Macarri G, Venturini C, Orlandi F. A morphological study of the early stages of hepatic fibrosis induced by low doses of dimethylnitrosamine in the rat. *J Hepatol* 1987;5:174–181.
23. Okamoto K, Sugie S, Ohnisi M, et al. Chemopreventive effects of taurine on diethylnitrosamine and phenobarbital-induced hepatocarcinogenesis in male F344 rats. *Jpn J Cancer Res* 1996;87:30–36.
24. Gates GA, Henley KS, Pollard HM, Schmidt E, Schmidt FW. The cell population of human liver. *J Lab Clin Med* 1961;57:182–184.
25. Bucher NLR. Experimental aspects of hepatic regeneration. *N Engl J Med* 1967;277: 686–696.
26. Bucher NLR. Experimental aspects of hepatic regeneration (concluded). *N Engl J Med* 1967;277:738–746.
27. Ryoo JW, Buschmann RJ. A morphometric analysis of the hypertrophy of experimental liver cirrhosis. *Virchows Arch* [A] 1983;400:173–186.
28. Hildebrand R. Nuclear volume and cellular metabolism. *Adv Anat Embryol Cell Biol* 1980;60:1–54.
29. Fujiwara K, Nagoshi S, Ohno A, et al. Stimulation of liver growth by exogeneous human hepatocyte growth factor in normal and partially hepatectomized rats. *Hepatology* 1993;18:1443–1449.
30. Matsuda Y, Matsumoto K, Ichida T, et al. Hepatocyte growth factor suppresses the onset of liver cirrhosis and abrogates lethal hepatic dysfunction in rats. *J Biochem* 1995;118:643–649.