Antinecrotic and antiapoptotic effects of hepatocyte growth factor on cholestatic hepatitis in a mouse model of bile-obstructive diseases

Zhaodong Li, Shinya Mizuno, and Toshikazu Nakamura

Division of Molecular Regenerative Medicine, Department of Biochemistry and Molecular Biology, Osaka University Graduate School of Medicine, Osaka, Japan

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Li Z, Mizuno S, Nakamura T. Antinecrotic and antiapoptotic effects of hepatocyte growth factor on cholestatic hepatitis in a mouse model of bile-obstructive diseases. Am J Physiol Gastrointest Liver Physiol 292: G639–G646, 2007. First published October 26, 2006; doi:10.1152/ajpgi.00292.2006.—Cholestasis, an impairment of bile outflux, frequently occurs in liver diseases. In this process, an overaccumulation of bile acids causes hepatocyte necrosis and apoptosis, leading to advanced hepatitis. Hepatocyte growth factor (HGF) is mitogenic toward hepatocytes, but it is still unclear whether HGF has physiological and therapeutic functions during the progression of cholestasis. Using anti-HGF IgG or recombinant HGF in mice that had undergone bile duct ligation (BDL), we investigated the involvement of HGF in cholestasis-induced hepatitis. After the BDL surgery, HGF and c-Met mRNA levels transiently increased in livers during the progression of cholestatic hepatitis. When c-Met tyrosine phosphorylation was blocked in the livers of BDL-treated mice by anti-HGF IgG, hepatic dysfunction became evident, associated with the acceleration of hepatocyte necrosis and apoptosis. Inversely, administration of recombinant HGF into the mice led to the prevention of cholestasis-induced inflammation: HGF suppressed the hepatic expression of intracellular adhesion molecule-1 and neutrophil infiltration in BDL-treated mice. As a result, parenchymal necrosis was suppressed in the HGF-injected BDL mice. In addition, HGF supplementation reduced the number of apoptotic hepatocytes in cholestatic mice, associated with the early induction of Bcl-xL. The administration of HGF enhanced hepatic repair, via accelerating G1/S progression in hepatocytes. Our study showed that 1) upregulation of HGF production is required for protective mechanisms against cholestatic hepatitis and 2) enhancement of the intrinsic defense system by adding HGF may be a reasonable strategy to attenuate hepatic inflammation, necrosis, and apoptosis under bile-congestive conditions.

cholestasis; necrosis; apoptosis; regeneration

Cholestatic liver injury occurs due to bile duct obstruction in a variety of clinical settings, such as gallstone impaction, biliary atresia, and tumor compression (8, 41). In many cases of cholestatic diseases, the initial insult is directed toward hepatocytes. Current therapeutic options for liver diseases remain inadequate, and the social economic burden of liver diseases is still high (41). To date, surgical treatment has been made on patients as the primary option to release bile duct obstruction. For example, Kasai’s operation is indicated for children with biliary atresia (22), but preexisting jaundice impairs the hepatic morphological structure, and such cholestatic conditions affect the prognosis after the release of bile obstructions. Thus further insights into the mechanisms preventing liver injury and the signals potentially linking these disease processes are needed to develop effective and rational therapies.

In the pathological process of developing hepatic failure (including cholestasis-induced hepatitis), two conceptual pathways for hepatocyte cell death under jaundice have been proposed: 1) oncotic necrosis (with cytolysis) associated with interstitial inflammatory responses, and 2) apoptosis initiated by a death receptor and/or mitochondrial stress (4, 5, 12, 23). The underlying mechanisms of liver cell injury are complex and involve the interplay of bile acids, reactive oxygen species, and leukocyte attack (6, 8, 13, 41). Recent studies have shown that necrotic cell death appears to be an important response of the liver to cholestatic injury, and necrosis is associated with inflammatory events including neutrophil extravasation (6, 7, 35). Regardless of the initial causes of cell death (necrotic vs. apoptotic), it is important to clarify the intrinsic mechanism of inhibiting the onset and progression of hepatitis under persistent cholestasis.

Hepatocyte growth factor (HGF) was originally found and cloned as a potent mitogen for mature hepatocytes in a primary culture system (28, 29). Under tissue injury or during organogenesis, HGF elicits mitogenic, motogenic, and morphogenic effects via tyrosine phosphorylation of c-Met (2, 21). In fact, HGF is a key ligand for eliciting hepatic growth and repair (9, 11). Furthermore, HGF exerts protective and anti-apoptotic functions toward hepatocytes in vitro (39) and in vivo (11, 14). In a clinical setting, blood HGF levels alter in patients with biliary atresia, and this is linked to the stage or degree of cholestasis (44). On the basis of the multifaceted functions of HGF, we hypothesized that HGF may be a physiological ligand for suppressing cholestasis-induced hepatitis, even if the biliary obstruction is persistent.

To test our hypothesis, we used a surgical technique of bile duct ligation (BDL) to induce cholestatic conditions in rodents (16). In the present study, we administrated an anti-HGF IgG or recombinant HGF into BDL-treated mice and examined the physiological and therapeutic roles of HGF under conditions of jaundice. Using this animal model of cholestasis, we provide evidence that endogenous HGF is involved in the physiological protection of hepatocyte cell death (including necrosis and apoptosis), whereas a supplement of exogenous HGF can be a medical therapy for attenuating cholestatic hepatitis.

Address for reprint requests and other correspondence: T. Nakamura, Division of Molecular Regenerative Medicine, Dept. of Biochemistry and Molecular Biology, Osaka Univ. Graduate School of Medicine, Yamadaoka 2-2-B7, Suita, Osaka 565-0871, Japan (e-mail: nakamura@onbich.med.osaka-u.ac.jp).

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**MATERIALS AND METHODS**

**BDL.** Eight-week-old female ICR mice (Slc, Hamamatsu, Japan) were anesthetized with ketamine chloride (80 mg/kg sc) and xylazine sulfate (8 mg/kg sc). Under general anesthesia, they underwent BDL, according to an established method (16). Briefly, through a laparotomy from the xiphoid to pubis, the duodenum was retracted and the common bile duct was dissected free in both BDL and sham groups. In the BDL group, the duct was ligated with non-absorbable suture and segmented between them before closure of the abdomen. For analysis of the natural course of cholestasis, 36 mice were killed at 0, 1, 2, 4, 6, and 8 days after the BDL (n = 6 mice/group). In all experiments, liver tissues were fixed in 70% ethanol or 10% formaldehyde for histological examinations, as described below.

**Neutralization or supplement of HGF.** To block the actions of endogenous HGF, we used an anti-rat HGF IgG, which has been shown to cross-react with mouse HGF and to worsen renal, pulmonary, and gastric injuries in vivo (25–27). The expression vector containing human HGF cDNA (25–27) was transfected into Chinese hamster ovary cells to generate recombinant human HGF (rh-HGF). The supernatant was used as a tissue extract. Liver tissue and plasma HGF levels were done, as reported (25–27). Briefly, tissues were homogenized in 10 volumes of a buffer composed of 20 mM Tris·HCl (pH 7.5), 2 M NaCl, 0.01% Tween 80, 1 mM phenylmethylsulfonyl fluoride, and 1 mM EDTA (Nacalai, Kyoto, Japan). The homogenate was centrifuged at 15,000 rpm for 30 min, and the supernatant was used as a tissue extract. Liver tissue and plasma HGF levels were determined using an ELISA kit for rodent HGF (Institute of Immunology, Tokyo, Japan) (25–27).

**Real-time quantitative PCR.** Total RNA was prepared from the liver, using ISOGEN (Nippon Gene, Tokyo, Japan). One microgram of total RNA was reverse transcribed into first-strand cDNA with a random hexamer by using Superscript II reverse transcriptase (Life Technologies, Rockville, MD). Quantitative PCR to detect HGF and c-Met mRNA was performed, using an ABI PRISM 7700 sequence system (Perkin-Elmer Biosystems, Foster City, CA), as reported (36).

**Immunohistochemistry.** The livers were embedded in paraffin and the tissues were cut at 4 μm and stained with hematoxylin and eosin (HE). The apoptotic nuclei were detected in 10% formaldehyde-fixed sections by a terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) kit (Takara, Tokyo, Japan). To characterize pathological events, inflammatory cells and intracellular adhesion molecule-1 (ICAM-1) were detected, by use of an anti-mouse Gr1 rat IgG (RB6–8C5) or anti-mouse ICAM-1 hamster IgG (3E2) (BD Biosciences, San Jose, CA), respectively (26). This reaction was followed by a second reaction with biotin-labeled anti-rat/hamster IgG (Vector, Burlingame, CA). An avidin-biotin coupling reaction was performed on the sections, using a kit (Elite: Vector). 

**Western blot analysis.** Liver tissues were homogenized in a lysis buffer (50 mM Tris·HCl (pH 7.5), 150 mM NaCl, 25 mM glycerophosphate, 50 mM NaF, 1 mM NaVO₃, 1% Triton X-100, 10% glycerol, 1 mM PMSF, 2 g/ml antipain, pepstatin A, and leupeptin, and 1% aprotinin, pH 7.4), incubated with anti-c-Met mouse IgG (B-2: Santa Cruz Biotechnology) at 4°C overnight, and then precipitated with protein G (Amersham-Pharmacia, Little Chalfont, UK) (27). The samples were subjected to SDS-PAGE and then transferred to polyvinylidene difluoride (PVDF) membranes. The anti-phosphorylated Met (pMet-1234/1235) rabbit IgG (Biosource, Camarillo, CA) or anti-c-Met mouse IgG (B-2) was applied to PVDF membranes, followed by a second reaction with peroxidase-labeled antibodies (Dako). The total and phosphorylated c-Met signals were visualized with an ECL kit (Amersham-Pharmacia), and then the expression levels were quantified by a densitometric method (27). Likewise, anti-mouse ICAM-1 goat IgG (R&D, Minneapolis, MN) was used to detect ICAM-1 in the livers. In addition, Bcl-XL and cyclin-D1 expression levels in the hepatic extraction were evaluated on PVDF membranes, by using the same antibodies as were used in the immunohistochemistry.

**Caspase-3 activity.** Liver lysates were obtained with a previous method (14) and incubated with DEVD-p-nitroanilide (pNA) at 37°C for 1 h, by using a kit (Takara). The assay was based on colorimetric detection of the chromophoric pNA after cleavage from the substrate DEVD-pNA. Caspase-3 activity was expressed as accumulation of free pNA, which was quantified by using a microplate reader at 405 nm.

**Statistical analysis.** Data are means ± SD. A Student’s t-test or Mann-Whitney U-test was used to compare the group means, and a value of P < 0.05 was considered to be significant.

**RESULTS**

Transient upregulation of HGF and c-Met productions during the natural course of hepatic injury in BDL-treated mice. In our murine model, plasma ALT levels increased between 1 and 6 days after the BDL surgery, within a range of 250–350 kU/dl (Fig. 1A). Consistent with the biochemical data, histopathological examinations revealed that parenchymal cellular lesions, characterized by oncocytic necrosis, were evident from 2 days after the BDL treatment (Fig. 1B). The necrotic foci were detected as round clusters at 2 days post-BDL, along with the inflammatory cells (HE staining). In addition, apoptotic changes, as evidenced by a TUNEL assay, were noted in
albumin-positive cells (i.e., hepatocytes) at 6 days after BDL. In this advanced stage, TUNEL-positive signals were also noted in cholangiocyte and sinusoidal cells but were not evident in inflammatory cells (data not shown). In this pathological process, plasma HGF levels increased, with a peak at 2 days post-BDL, but thereafter returned to near the basal levels (Fig. 1). During the development of cholestatic hepatitis, the levels of hepatic HGF and c-Met mRNA increased by three- to fivefold that of the pretreatment level, with peaks at 2 and 4 days after the BDL, respectively (Fig. 1D), and the loss of HGF production may have been due to hypoxia or jaundice, as reported (26, 47). In the early stage of cholestasis, HGF was identified in sinusoidal cells, whereas hepatocytes expressed c-Met/HGF receptor (data not shown), suggesting that such a paracrine mechanism between the interstitium and parenchyme may play a key role during the progression of cholestatic hepatitis.

Protective roles of endogenous HGF toward cholestatic hepatitis in BDL mice. To test our hypothesis, we administrated an anti-HGF rabbit IgG into BDL-treated mice (Fig. 2A). As a result, repeated injections of anti-HGF IgG led to a reduction of c-Met tyrosine phosphorylation levels, as checked at 2 days post-BDL, with no changes in c-Met expression levels (Fig. 2B). Of note, plasma ALT and AST levels increased significantly at 4 days after the BDL in the anti-HGF IgG group compared with the normal IgG group (Fig. 2C). Consistent with these changes, necrotic areas of portal hepatocytes became more extensive, associated with increases in inflammatory cells, including polymorphonuclear cells and inflammatory mononuclear cells (Fig. 2D). As a result, the necrosis score was higher in the anti-HGF IgG group than in the normal IgG group. In addition, the anti-HGF IgG treatment increased the number of TUNEL-positive and albumin-positive cells (Fig. 2E). No significant differences were seen in total bilirubin levels between normal IgG and anti-HGF IgG groups (6.14 ± 0.82 vs. 6.65 ± 0.65 mg/dl). Thus endogenous HGF is required for protection against hepatocyte necrosis and apoptosis under persistent jaundice via c-Met activation.

Suppressive effect of rh-HGF on BDL-induced hepatic injury. Inversely, we examined whether a supplement of exogenous HGF would lead to a therapeutic outcome during the progression of cholestatic hepatitis. The BDL-treated mice were injected with rh-HGF (500 μg·kg⁻¹·time⁻¹ sc) at an interval of 12 h (Fig. 3A). As a result, rh-HGF administration did not change the levels of hyperbilirubinemia, as noted in mice between 2 and 6 days after the BDL surgery (Fig. 3B). In contrast, rh-HGF supplement led to significant decreases in plasma ALT levels, as checked at 2 and 6 days after the BDL (Fig. 3C, left). ALT levels at 2 days postsurgery in the saline and rh-HGF groups were 280.1 ± 46.6 and 166.7 ± 23.9 kU/dl, respectively (P < 0.05). Likewise, injections of rh-HGF for 2 days suppressed the increase in plasma AST levels, as noted in the saline-injected BDL mice (Fig. 3C, right). These findings indicate that rh-HGF could be used as a drug to inhibit hepatic injury even if hyperbilirubinemia is not improved.

Preventions of necrosis and inflammation by rh-HGF in BDL-treated mice. In the natural course of BDL-induced hepatitis, the degree of necrosis reflected the increase in plasma ALT levels at an early stage (Fig. 1, A and B). Thus we examined the initial effects of HGF, focusing on necrotic changes. As expected, the injections of rh-HGF significantly reduced the necrotic areas in BDL mice (Fig. 4A). The hepatocyte necrotic score in the rh-HGF group was reduced to 33% of the control level at 2 days post-BDL. In the saline group, infiltration of Gr1-positive cells became evident around the small vessels, whereas the number of Gr1-positive cells was significantly decreased by rh-HGF treatments (Fig. 4B). Furthermore, rh-HGF reduced the number of mononuclear cells to a half level of the saline group (day 6: 2.38 ± 0.31 cells/×200 vs. 4.87 ± 0.51 cells/×200, P < 0.05). Given that most Gr1-positive cells (>90%) displayed the feature of polymorphonuclear cells, rh-HGF is useful for blocking infiltrations of neutrophils (and in part macrophages). It is known that induction of adhesion molecules (including ICAM-1) by the endothelium is required at an initial stage of neutrophil extravasation (6, 13, 26). In our BDL model, hepatic ICAM-1 expression...
was evident at 6 days post-BDL, whereas rh-HGF suppressed the ICMA-1 expression, as evidenced by Western blots (Fig. 4C, left). Immunohistochemical analysis revealed that ICAM-1-positive signals were detected along sinusoidal cells and, in part, on degenerative hepatocytes. The ICAM-1 expression became faint when mice were treated with rh-HGF for 6 days (Fig. 4C, right).

Antiapoptotic effect of HGF during the development of cholestasis. We next examined whether exogenous HGF alters hepatocyte apoptosis induced by BDL. In saline-treated mice, apoptotic changes, as determined by TUNEL and albumin double-staining, were noted in parenchymal hepatocytes. In contrast, such an apoptotic change was suppressed by HGF in the BDL mice (Fig. 5A). The apoptotic score at 6 days
post-BDL was reduced in HGF-treated mice to 38% of the control levels. In this process, rh-HGF treatment reduced the hepatic active caspase-3 levels, as noted in saline-treated mice at 6 days post-BDL (Fig. 5B). In saline-treated BDL mice, Bcl-xL-positive signals were noted in surviving hepatocytes near the portal areas (Fig. 5C). Serial administrations of rh-HGF into BDL mice led to an increase in Bcl-xL expression, as evidenced by Western blot analysis and immunohistochemistry, and this finding was similar to the previous studies (14, 30). The antiapoptotic effect was also noted in another exper-

Fig. 4. Improvements in necrotic and inflammatory events by adding rh-HGF in BDL-treated livers. A: inhibition of hepatocyte necrosis by rh-HGF injections. Microscopic findings at 6 days post-BDL are shown, with a quantification of histological changes (HE staining). Data are means ± SD (n = 6), *P < 0.05 and **P < 0.01 vs. identical time points of the saline group. B: attenuations of inflammatory cell infiltrations by rh-HGF, as determined by Gr1-positive cells (26). C: suppressive effect of rh-HGF injections on hepatic ICAM-1 expression in BDL-treated mice. Left: immunoblot analysis to show the decrease in ICAM-1 expression by rh-HGF at 6 days (6d) after BDL. Right: immunohistochemistry of ICAM-1 in hepatic tissues treated with saline or rh-HGF. Insert: expression of ICAM-1 by degenerating hepatocytes.

Fig. 5. Suppressive effects of exogenous HGF on progression of hepatocyte apoptosis in cholestatic mice. A: changes in apoptosis score, as evaluated on TUNEL- and albumin-stained sections. The protocol of rh-HGF therapy was same as in Fig. 3A. Data are means ± SD (n = 6), *P < 0.05 and **P < 0.01 vs. identical time points of the saline group. B: suppression of hepatic caspase-3 activity by rh-HGF. The livers were obtained at 6 days post-BDL and caspase-3 activity was expressed as the free p-nitroaniline (pNA) levels (OD405). C: rapid inductions of Bcl-xL by rh-HGF in progression of BDL-induced hepatitis. Top: immunoblot analysis to indicate an increase in Bcl-xL protein by HGF in hepatic tissues. Bottom: immunohistochemistry of Bcl-xL to show distribution and extension of Bcl-xL in the liver at 4 days (d4) post-BDL. D: antiapoptotic outcome in the delayed treatment with HGF. Left: regimen of HGF injections after the onset of BDL-induced hepatitis. The mice were treated with rh-HGF (0.5 mg/12-h ip) or saline from 2 to 6 days after BDL. Middle: microphotographs of apoptosis, as evidenced by TUNEL-positive signals (brown) in albumin-positive hepatocytes (red). Right: hepatocyte apoptosis scores in control and HGF-treated groups.
imental protocol in which HGF supplementation was performed 2–6 days after BDL. Indeed, the delayed treatment with rh-HGF significantly reduced the number of TUNEL-positive cells in the albumin-positive cells (Fig. 5D), indicating a protective effect of HGF on advancing hepatitis.

**HGF-mediated enhancement of hepatic regeneration under cholestasis in mice.** Finally, we examined the hepatotrophic roles of HGF during the progression of cholestasis, using the protocol of early treatment with rh-HGF (Fig. 3A). In saline-injected control mice, the BrdU-positive ratio ranged from 5 to 8% of total hepatocytes up to 6 days post-BDL. By contrast, the treatment with rh-HGF increased the number of BrdU-positive hepatocytes, especially near the border of the necrotic areas (Fig. 6A). Likewise, rh-HGF increased the expression levels of cyclin-D1, associated with an enhancement of c-Met tyrosine phosphorylation, as checked 2 days post-BDL by Western blot analysis (Fig. 6B). Consistently, the number of cyclin-D1-positive hepatocytes was increased at 2 days post-BDL in rh-HGF-treated mice compared with saline-treated mice (13.2 ± 2.8 vs. 8.8 ± 1.6%, P < 0.05) (Fig. 6C), thereby indicating an acceleration of G1/S progression by HGF. Together with the findings that anti-HGF IgG reduced the number of BrdU-positive hepatocytes in the BDL model (data not shown), it is likely that c-Met tyrosine phosphorylation is required for reducing hepatic injuries under cholestatic conditions.

**DISCUSSION**

Cholestasis is a common feature of bile-obstructive disorders, including biliary atresia and obstructive cholangitis (8, 41). It is known that serum HGF levels show a significant increase, especially in cases of biliary atresia with hepatic dysfunction (42, 44). Likewise, experimental studies have revealed that HGF production levels transiently increase under bile-obstructed conditions (31, 45). Of note, mice deficient for plasminogen activator inhibitor-1 had lower levels of hepatocyte cell death after the BDL than did wild-type mice, and this was linked with an increase in active HGF as well as enhancement of c-Met tyrosine phosphorylation (45). Nevertheless, there was no direct evidence to delineate a protective role of endogenous HGF during cholestatic injuries. In our BDL model, failure to sustain HGF production at a sufficient level was associated with the progression of hepatic injury, whereas an early injection of anti-HGF IgG worsened pathological conditions under jaundice. This is the first report to show that an insufficient production of HGF is, in part, involved in pathogenesis of cholestatic hepatitis.

Whether apoptosis or necrosis is responsible for the onset of hepatic failure including cholestasis has been discussed (4–7, 23). On the other hand, there is ample evidence to show that HGF administration leads to attenuation of hepatitis (11, 14, 15, 19). Thus the neutralization of or the addition of HGF may provide a clue to addressing which and how necrotic or apoptotic changes are controlled under cholestasis. In our BDL model, there was a closed relationship between plasma ALT levels and the necrotic changes. Notably, neutralization of HGF led to the accelerated necrosis of hepatocytes, and vice versa in cases of rh-HGF addition. Since hepatocyte apoptosis was not evident within 48 h post-BDL, the initial action of HGF appears to be directed to preventing necrosis. We reported that HGF inhibited the onset of septic hepatitis (15), where necrosis is involved in hepatic injury (5). Taken together, our studies may delineate the importance of “antinecrotic” effects by HGF for suppressing hepatic failure in hepatitis.

It is important to address the mechanism whereby HGF prevents necrotic cell death under cholestasis. In the process of developing necrotic injury, neutrophil- derived proteases (such as elastase) are critical for initiating tissue damage (13, 35), and neutrophil infiltration is associated with the onset of necrosis (6, 7). In mice deficient for ICAM-1, the infiltration of neutrophils is inhibited then hepatic injuries are avoided (6), indicating a key role of neutrophils in the development of hepatocyte necrosis (and in part apoptosis). In this regard, we reported that the suppression of renal ICAM-1 by HGF leads to the attenuations of neutrophil infiltration and tissue injuries in

![](https://example.com/fig6a.png)

**Fig. 6.** Enhancement of regenerative events by rh-HGF in the liver of BDL mice. A: changes in ratios of proliferating hepatocytes in saline-treated vs. rh-HGF-treated mice after BDL. Left: changes of BrdU-positive hepatocytes, as determined by counting 1,000 cells per mouse (means ± SD; n = 6). * P < 0.05 and ** P < 0.01 vs. the saline group. Right: immunohistochemical findings of BrdU intake at 2 days (2d) post-BDL. #Necrotic areas. B: acceleration of cyclin-D1 expression in livers by HGF. Immunoblot analysis was used to show the decreases in Met tyrosine phosphorylation (p-Met) and cyclin-D1 (cyc-D1). The bottom number shows the relative value of the density when control levels are as follows: 1. S, saline; H, rh-HGF; IP, immunoprecipitation; WB, Western blot. C: histochemistry of cyclin-D1 in saline-treated and rh-HGF-injected groups.
a mouse model of acute renal failure (26). This background prompted us to examine whether HGF may be involved in inflammation under cholestasis: The injections of rh-HGF led to a reduction in the numbers of inflammatory cells (i.e., neutrophils and possibly in part macrophages), and vice versa in cases of anti-HGF IgG. Concomitant with the suppression of neutrophil infiltration by rh-HGF, hepatic ICAM-1 expression was attenuated in BDL mice that had been treated with rh-HGF. Thus we speculate that the inhibition of ICAM-1 induction by HGF may underlie the molecular mechanisms of inhibiting the neutrophil extravasation, a common step in the acute inflammatory phase (6, 26).

In addition to necrotic events, hepatocyte apoptosis is notable under pathological conditions when the accumulation of bile acids is persistent (3, 6, 34): hydrophobic bile acids cause DNA fragmentation of hepatocytes in vitro (1). Furthermore, parenchymal apoptosis at 2 days post-BDL may also participate in the initial hepatic injury. Therefore, the mechanisms of bile acid-induced apoptosis are of scientific importance. In this study, we found that HGF suppressed caspase-3 activation and apoptotic cell death in hepatocytes, associated with the early induction of Bcl-xL, an essential molecule to maintain physiological functions and morphology in vivo (40). Actually, we previously reported that the upregulations of Bcl-xL by HGF lead to attenuating apoptosis of hepatocytes or myocytes in vitro (14, 30), suggesting an involvement of Bcl-xL in tissue protections. Another mechanism of HGF is toward a death receptor. A recent study indicated the importance of the Fas/Fas ligand system on the onset of hepatic failure after BDL (7), whereas the sequestration of Fas by HGF-activated c-Met leads to the arrest of death signaling in hepatocytes (46). Thus we predict that such an induction of Bcl-xL and the inhibition of the death signaling may participate in the HGF-mediated protective mechanisms. The antiapoptotic outcome was reproduced in the delayed treatment with rh-HGF, indicating the therapeutic potential of HGF for the treatment of advanced cholestatic hepatitis.

We further discuss the possible mechanisms for HGF involved in attenuating hepatocyte cell death. In BDL-treated rodents, hepatic levels of glutathione are markedly reduced, and the loss of antioxidant defense is pathogenic to accelerated hepatic injury (17, 37). On the other hand, HGF is known to increase glutathione levels in hepatocytes in vitro (43). Furthermore, administration of rh-HGF in a rat model of liver ischemia restored local glutathione levels, leading to the prevention of hepatic failure (32). Thus possible inductions of such an antioxidant capacity by HGF may also participate in local defense systems to prohibit cholestasis-related hepatic injuries. Another mechanism is that prostaglandin E1 is preventive against hepatitis (24), whereas HGF stimulates the synthesis of prostaglandins (33, 38). On the basis of all available data, we predict that such multiple functions of HGF (including anti-inflammation, antioxidant, and protective actions) will contribute to suppressing the loss of functional hepatocytes under jaundice.

During the process of BDL-induced hepatitis, hepatocyte proliferation is noted in response to necrosis and apoptosis (3, 18). In a rat BDL model, there was a correlation between hepatic HGF mRNA levels and PCNA scores: In the HGF mRNA-decreased liver, hepatic growth became mild (31). In our BDL model, rh-HGF increased the numbers of BrdU-positive hepatocytes around necrotic areas, indicating that the induction of hepatocyte replication by HGF may contribute to the reduction in necrotic areas. Similarly, it is known that the regenerative capacity of livers after 70% partial hepatectomy is suppressed in rats with hepatotoxin-induced jaundice, and this is associated with lower levels of hepatic HGF production (47). Interestingly, the administration of rh-HGF into rats with jaundice restored hepatic growth after 70% partial hepatectomy (48). Taking these facts together, it is highly possible that persistent jaundice leads to delayed and insufficient HGF production, and, as a result, hepatic growth/repair becomes faint under jaundice. Given that the hepatic regenerative response is linked with long-term survival in humans with biliary atresia (10), the induction of hepatic repair by adding HGF may lead to an improvement in pathological conditions. In other words, our findings strengthen the hypothesis that HGF is a long-sought hepatotrophic factor, as noted in acute hepatitis (9, 11) or in chronic fibrosis (20).

Finally, we conclude that HGF is a key ligand to attenuate cholestatic hepatitis, whereas the loss of local HGF levels is, in part, responsible for hepatic dysfunction. Using a BDL-treated model, in which necrotic injury is predominant for the progression of hepatitis, we revealed the cellular and molecular events, required for HGF to display an antinecrotic effect against cholestatic hepatitis. Furthermore, HGF has antiapoptotic and regenerative potential, as shown herein and elsewhere (9, 11, 14, 19, 20). Given that bile-congestive diseases can progress at an insufficient level of HGF production, restoration of lower HGF production or a supplement of HGF can be a rational option to inhibit or reverse pathological events in bile-congestive diseases, including biliary atresia before Kasai’s operation (22).

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REFERENCES


44. Wang X, DeFrances MC, Dai Y, Pediaditakis P, Johnson C, Bell A, Michalopoulos GK, Zarnegar R. A mechanism of cell survival: seques-