

Hepatocyte Growth Factor Facilitates Colonic Mucosal Repair in Experimental Ulcerative Colitis in Rats

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ABSTRACT

Hepatocyte growth factor (HGF) modulates intestinal epithelial cell proliferation and migration, serving as a critical regulator of intestinal wound healing. In this study, we examined the effect of administration of recombinant human HGF on colonic mucosal damage *in vivo*. Acute colitis was induced in rats by feeding with 5% dextran sulfate sodium (DSS) for 7 days, and colitis was subsequently maintained by feeding with 1% DSS. On the 5th day of DSS administration, osmotic pumps releasing recombinant human HGF (200 μ g/day) were implanted into the peritoneum of the rats. Continuous intraperitoneal delivery of HGF led to both increased serum human HGF levels and c-Met tyrosine phosphorylation within the colonic mucosa. Compared

with mock-treated rats, those administered human HGF showed a reduction in colitis-associated weight loss, large intestinal shortening, and improved colonic erosions. Enhanced epithelial regeneration and cellular proliferation were observed in rats treated with recombinant human HGF. The weights of the liver, kidneys, and spleen were not affected by HGF administration. These results indicate that HGF administration accelerates colonic mucosal repair in rats with DSS-induced colitis and suggest that recombinant human HGF may be a useful therapeutic tool to facilitate intestinal wound healing in patients with ulcerative colitis.

Ulcerative colitis, particularly the chronic persistent form, is an intractable disease. The persistence of active inflammation within the colonic mucosa and disturbances in mucosal healing both contribute to the intractability of ulcerative colitis (Podolsky, 1991). A variety of therapeutic agents, including salazosulfapyridine, mesalazine, and corticosteroids, have been developed to suppress the chronic inflammation present in ulcerative colitis. Recent studies have sought to clarify the mechanisms involved in promoting intestinal mucosal wound repair; roles in intestinal mucosal remodeling have been described for growth factors such as epidermal growth factor, transforming growth factor- α , and trefoil factors (Murphy, 1998; Beck and Podolsky, 1999; Dignass and Sturm, 2001). Despite our increased understanding of mucosal homeostasis, therapeutic modalities facilitating colonic mucosal repair have not yet been established.

Dextran sulfate sodium (DSS)-induced colitis is a well established experimental model that mimics many of the signs

and symptoms of human ulcerative colitis, including diarrhea, bloody feces, weight loss, mucosal ulceration, and shortening of the large intestine (Okayasu et al., 1990; Elson et al., 1995). DSS-induced colitis is very reproducible, thus it is a useful tool that facilitates the study of the pathogenesis of ulcerative colitis. Furthermore, these properties will allow the study of potential therapies *in vivo*.

Hepatocyte growth factor (HGF), a multifunctional polypeptide secreted by mesenchymal cells, functions as a mitogen, morphogen, and/or motogen for multiple subsets of epithelial cells, including gastrointestinal epithelial cells (Gohda et al., 1988; Igawa et al., 1991; Joplin et al., 1992; Takahashi et al., 1993, 1995a). HGF activator (HGFA) and HGF activator inhibitor type-1, HGF-associated molecules involved in the activation of HGF in injured tissues, are associated with colonic mucosal repair (Itoh et al., 2000; Kataoka et al., 2000b). Additionally, HGF expression was reported to be up-regulated in inflamed colonic mucosal tissue in patients with ulcerative colitis (Kitamura et al., 2000), and plasma HGF levels are increased in animal models of acute colitis (Dignass et al., 1994; Takahashi et al., 1995b; Matsuo et al., 1997; Nishimura et al., 1998; Ortega-Cava et al., 2002). Recombinant human HGF will soon be available for administration to patients with severe liver disease. Both the clinical availability of HGF and its documented role in

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ABBREVIATIONS: DSS, dextran sulfate sodium; HGF, hepatocyte growth factor; ELISA, enzyme-linked immunosorbent assay; PBS, phosphate-buffered saline; PCNA, proliferative cell nuclear antigen.

mucosal repair suggest that HGF has the potential to be an important new treatment modality that promotes intestinal mucosal repair in patients with inflammatory bowel diseases. To identify the role that HGF plays in colonic mucosal repair *in vivo*, we examined changes in mucosal tissue following the administration of recombinant human HGF to rats with DSS-induced colitis.

Materials and Methods

Animals. Male Wistar rats, 6 weeks of age and weighing between 200 and 210 g, were obtained from Kyudo Co., Ltd. (Kumamoto, Japan). Rats were maintained under constant room temperature (25°C) and provided with free access to a standard diet and tap water in accordance with institutional guidelines; rats were acclimatized to the conditions for 7 days before any involvement in experimental studies. The Ethics Committee of Miyazaki Medical College (Miyazaki, Japan) approved all aspects of this study. Acute colitis was induced in rats by feeding with 5% DSS (50,000 mol. wt.) (Bio Research Corp. of Yokohama, Kanagawa, Japan) dissolved in drinking water for 7 days (days 0–7). Colitis was maintained by feeding with 1% DSS (Fig. 1A). The number of rats used in each experiment is summarized in Table 1. To evaluate the severity of colitis, the rats' body weights were examined either daily or every other day. Rats were sacrificed on days 5 and 11. Following sacrifice, the length of the large intestines from the colocolic junction to the anal verge and the weights of the liver, kidneys, and spleen were measured.

Administration of HGF and Measurement of Serum HGF Concentration. After 5 days of 5% DSS administration, either recombinant human HGF (200 µg/day) in phosphate-buffered saline (PBS) or PBS alone was delivered intraperitoneally for 6 days (days 5 to 11) by implanted osmotic pumps (Fig. 1A). Serum human HGF levels were measured using a commercially available enzyme-linked

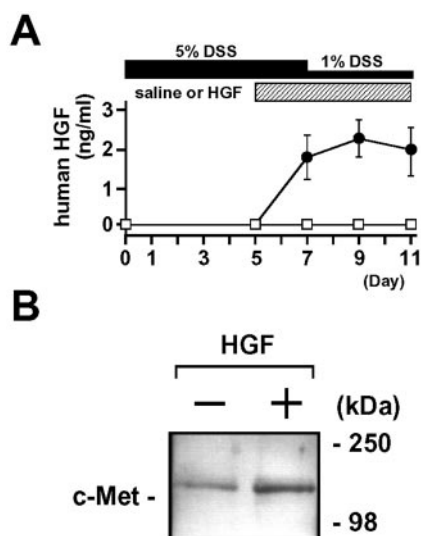


Fig. 1. Intrapерitoneal delivery of HGF increased serum HGF levels and stimulated tyrosine phosphorylation of c-Met in colonic mucosa. A, acute colitis was induced by the administration of 5% DSS for 7 days and maintained by feeding with 1% DSS. Serum HGF levels were examined by ELISA capable of detecting only exogenously administered human HGF as described under *Materials and Methods* (Tsubouchi et al., 1991). Rats treated with human HGF (closed circle; $n = 3$) or PBS alone (open square; $n = 3$) are shown. Human HGF was detected in the serum of treated rats but not in mock-treated animals. B, tyrosine phosphorylation of c-Met in colonic mucosal tissues. Colonic mucosal tissue of colitis-induced rats in the presence or absence of HGF was solubilized in lysate buffer containing protease inhibitors. Cell lysates were immunoprecipitated with an anti-c-Met antibody and blotted with an anti-phosphotyrosine antibody. Intrapерitoneal administration of human HGF stimulated c-Met tyrosine phosphorylation in the colonic mucosal tissue.

TABLE 1

The number of rats used in each experiment

	PBS-Treated	HGF-Treated
Measurement of serum HGF	3	3
Measurement of rats' body weights (surviving animals)	35 (28)	30 (23)
Areas of colonic mucosal erosions		
Day 5		3
Day 11	15	16
Length of large intestines		
Day 5		3
Day 11	17	13
Histological scoring	4	5
Weight of the liver, kidneys, and spleen	5	4

immunosorbent assay (ELISA) kit, in which only human HGF, but not rat HGF, was detected (Tsubouchi et al., 1991).

Tyrosine Phosphorylation of c-Met. Tyrosine phosphorylation of c-Met was evaluated by Western blotting. Large intestinal mucosal tissues were solubilized in lysis buffer containing 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1% NP40, 0.5% sodium deoxycholate, 1 mM Na_3VO_4 , 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, and 10 µg/ml leupeptin, aprotinin, and pepstatin A. Post-nuclear supernatants were precleared with protein A-agarose and immunoprecipitated with anti-c-Met antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) and protein A-agarose. Immunoprecipitated materials were washed five times with 0.1% NP40 and 0.05% sodium deoxycholate and eluted by boiling in Laemmli sample buffer (Bio-Rad, Hercules, CA). Samples were separated by 8% SDS-polyacrylamide gel electrophoresis and blotted onto a nitrocellulose filter. After blocking membranes with 1% bovine serum albumin, filters were incubated with horseradish peroxidase-conjugated anti-phosphotyrosine antibody and subjected to ECL Western blotting detection (Amersham Biosciences, Buckinghamshire, UK).

Measurement of the Areas of Colonic Erosions. The large intestines of treated rats were obtained on days 5 and 11 and opened longitudinally. Following measurement of colon lengths from the colocolic junction to the anal verge, large intestines were fixed with 10% formalin for 7 days and stained for 20 min with 1% Alcian Blue 8GX (Nacalai Tesque, Kyoto, Japan) diluted in 3% acetic acid. After washing with 3% acetic acid, the areas of erosions were measured using an Olympus video micrometer, VM-30 (Olympus, Tokyo, Japan).

Histological Examination and Immunohistochemistry. The entire colon was excised postmortem and fixed in 10% buffered formalin for histological analysis. Three colon rings were obtained at 1.0, 1.5, and 2.0 cm from the anal rings, and paraffin sections were stained with hematoxylin and eosin. Histological scoring was assessed independently by two investigators blinded to the protocol as a combined score of inflammatory cell infiltration (0–3) and tissue damage (0–3) (Siegmund et al., 2001). For inflammatory cell infiltration, the presence of rare inflammatory cells in the lamina propria was scored as 0, increased numbers of inflammatory cells in the lamina propria as 1, confluence of inflammatory cells extending into the submucosa as 2, and transmural extension of the inflammatory cell infiltrate as 3. For epithelial damage, absence of mucosal damage was scored as 0, discrete focal lymphoepithelial lesions were counted as 1, mucosal erosion or ulceration as 2, and extensive mucosal damage and extension through deeper structures of the bowel wall as 3. The two subscores were added, and the combined histological score ranged from 0 (no change) to 6 (extensive cell infiltration and tissue damage).

To evaluate proliferation of the colonic epithelium, cells undergoing DNA synthesis were identified by immunohistochemistry for proliferative cell nuclear antigen (PCNA) using an anti-PCNA monoclonal antibody (DAKO Japan, Kyoto, Japan).

Statistical Analysis. Unless specified, data are expressed as mean \pm S.D. Statistical parameters were ascertained with Statview

J-4.5 software (Abacus Concepts, Inc., Berkeley, CA). Differences between means were assessed by the unpaired Student's *t* test. Significance level was set at $p < 0.05$.

Results

Intraperitoneal Delivery of Human HGF Led to Increased Serum HGF Concentrations and Stimulation of c-Met Tyrosine Phosphorylation in the Colonic Mucosa. Acute colitis was induced by feeding with 5% DSS for 7 days, and colitis was maintained by feeding with 1% DSS (Fig. 1A). To confirm the ability of intraperitoneally implanted osmotic pumps to deliver recombinant growth factor, serum levels of human HGF were measured by ELISA (Fig. 1A). The ELISA used could not detect endogenous rat HGF. Human HGF was not detected in the serum of rats treated with PBS alone at any time point. In contrast, serum levels of human HGF significantly increased after implantation of osmotic pumps releasing recombinant human HGF and continued to increase (1–3 ng/ml) throughout the experimental period.

c-Met, a specific receptor for HGF, is expressed on epithelial cells within the gastrointestinal tract and phosphorylated in response to ligation by HGF (Di Renzo et al., 1991). Expression of c-Met in colonic mucosa was observed immunohistochemically in colonic mucosal epithelial cells despite colonic mucosa injury with 5% DSS (data not shown). Therefore, c-Met tyrosine phosphorylation in the colonic mucosal epithelium was examined by Western blotting (Fig. 1B). As DSS-induced colonic mucosal injury increases endogenous rat HGF (Ortega-Cava et al., 2002), some phosphorylated c-Met was detected within the injured colonic mucosa of rats treated with PBS alone. After the administration of human HGF to rats with DSS-induced colitis, c-Met tyrosine phosphorylation in colonic mucosal tissues was enhanced.

These results indicate that recombinant human HGF was released effectively from osmotic pumps implanted within the peritoneal cavity, leading to the presence of human HGF in rat serum, and also suggest that the administered human HGF was capable of ligating and inducing tyrosine phosphorylation of rat c-Met in colonic epithelial cells.

HGF Administration Abrogated Colitis-Associated Weight Loss in Rats with DSS-Induced Colitis. To examine the effect of intraperitoneally administered HGF on the severity of DSS-induced colitis, the body weights of HGF or mock-treated rats were measured (Fig. 2). Initially, 65 rats

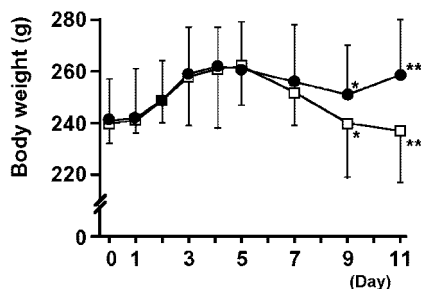


Fig. 2. Administration of HGF reduced colitis-associated weight loss in rats with DSS-induced colitis. The body weights of rats with DSS-induced colitis with (closed circle; $n = 23$) or without (open square; $n = 28$) intraperitoneal HGF administration were periodically measured throughout the experimental period (up to 11 days). Administration of recombinant human HGF abrogated the colitis-associated weight loss seen in mock treated animals (*, $p = 0.033$; **, $p = 0.012$).

were treated with 5% DSS, and were divided into PBS- or HGF-treated groups, consisting of 35 or 30 animals, respectively (Table 1). Seven rats from each group died within 2 days after osmotic pump implantation (on days 6 and 7), but all rats survived the subsequent 1% DSS administration (from days 8 to 11). The body weights of rats with DSS-induced colitis gradually increased during the 5 days of 5% DSS administration, and liquid stool and gross bleeding from the rectum were induced by this treatment. Intraperitoneal administration of PBS or recombinant human HGF was started on day 5. Although gross bleeding disappeared regardless of HGF treatment during 4 days of 1% DSS administration, persistent liquid stool and wasting were observed in rats treated with PBS, resulting in severe weight loss. Rats treated with recombinant human HGF, however, suffered less severe diarrhea and weight loss. The body weights of HGF-treated rats on days 9 and 11 (248 ± 17.9 and 259.0 ± 26.0 g, respectively) were significantly higher than those of PBS-treated rats (238.1 ± 31.0 and 235.7 ± 24.4 g, respectively) ($p = 0.033$ and $p = 0.012$, respectively). These results indicate that the administration of recombinant human HGF significantly abrogated colitis-associated weight loss in rats with DSS-induced colitis.

Administration of Recombinant Human HGF Improved Colonic Mucosal Injury and Reduced Colitis-Induced Large Intestinal Shortening. The area of mucosal erosions and lengths of the large intestines were evaluated in rats before and after 6 days of HGF treatment (on days 5 and 11, respectively) (Fig. 3). Without HGF treatment, orally administered 1% DSS induced persistent colitis. In these rats no difference was detected in the area of mucosal erosions between day 5 (186.7 ± 95.0 mm²) and day 11 (191.4 ± 85.9 mm²) (Fig. 3, A and B), but the large intestine was shortened significantly over this period of time (Fig. 3C). In rats with DSS-induced colitis treated with recombinant human HGF for 6 days and examined on day 11, the eroded areas within the large intestine were significantly reduced in size (52.5 ± 72.7 mm²) compared with those without HGF treatment on days 5 and 11 ($p = 0.012$ and 0.0001 , respectively) (Fig. 3, A and B), and the colon was significantly longer (20.0 ± 1.92 cm) than that seen in untreated rats (17.4 ± 2.3 cm) ($p = 0.0001$) (Fig. 3C).

HGF Administration Enhances the Regeneration of the Colonic Epithelium and Decreases the Inflammatory Infiltrate in Rats with DSS-Induced Colitis. We evaluated the effect of HGF on DSS-induced colonic mucosal injury in rats by histological analysis. Following the administration of 5% DSS, extensive mucosal damage and inflammatory cell infiltrates developed by day 5. Although DSS treatment was reduced to 1% on day 7, similar histological findings were also observed on day 11 (Fig. 4A). This indicates that colitis was maintained effectively by 1% DSS treatment throughout the experimental period. When treated with recombinant human HGF for 6 days, rats with DSS-induced colitis exhibited increased development of a regenerative epithelium. Moreover, HGF administration decreased the histological score of colitis severity from 6.0 in rats with colitis in the absence of HGF to 2.75 ± 0.96 in HGF-treated rats ($p = 0.007$; Fig. 4B).

To evaluate the proliferation of colonic epithelium, we performed immunohistochemistry for PCNA. An increase in PCNA-positive cells within the colonic mucosal epithelium

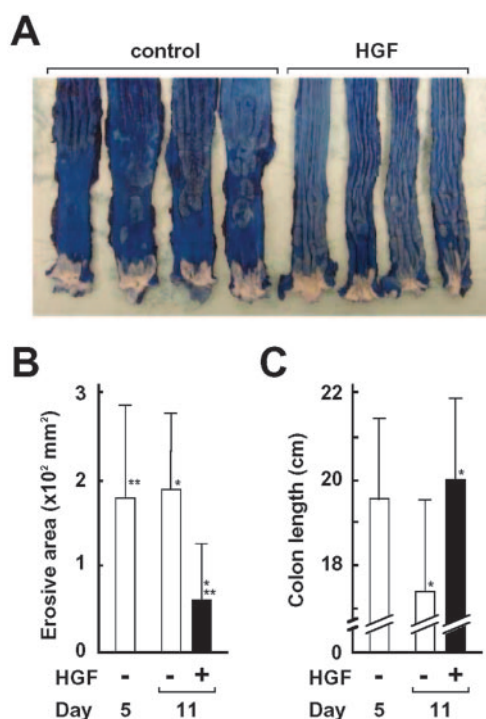


Fig. 3. Treatment with HGF reduced colonic mucosal erosions and prevented colon shortening in rats with DSS-induced colitis. A, macroscopic views of the large intestine of rats with DSS-induced colitis with or without HGF treatment on day 11. The large intestines were opened longitudinally and stained with 1% Alcian Blue 8GX as described under *Materials and Methods*. B, the areas of the erosive sites were measured on day 5 (before HGF administration; $n = 3$) and day 11 (with or without 6-day HGF administration; $n = 16$ or 15, respectively) using a video micrometer as described under *Materials and Methods*. Erosive areas were significantly reduced by intraperitoneal administration of HGF (*, $p = 0.012$; **, $p = 0.0001$). C, colon lengths were measured from the colocolic junction to the anal verge at day 5 (before HGF administration; $n = 3$) and day 11 (with or without 6-day HGF administration; $n = 13$ or 17, respectively) as described under *Materials and Methods*. HGF administration reversed the shortening of the large intestine in rats with DSS-induced colitis (*, $p = 0.0001$).

following HGF administration was observed compared to rats with colitis treated with PBS (Fig. 4C).

Intraperitoneally Administered HGF Did Not Affect the Weights of the Liver, Kidneys, and Spleen. HGF stimulates liver regeneration in normal and partially hepatectomized rats (Fujiwara et al., 1993); this growth factor also exerts a mitogenic activity on renal tubular epithelial cells. When recombinant human HGF was injected intravenously, human HGF was distributed primarily in the liver and partly in other organs such as the kidneys and spleen (data not shown). Therefore, we examined the weights of the liver, kidneys, and spleen in rats with or without HGF administration (Fig. 5). Although continuous delivery of recombinant human HGF from intraperitoneal pumps led to a sustained increase in serum HGF levels (Fig. 1A), the weights of the liver, kidneys, and spleen were not affected by this treatment.

Discussion

Mucosal repair involves both the rapid migration of cryptal enterocytes into the injured area of the mucosa and the replacement of the mucosa by cell replication (Silen and Ito, 1985). Various peptide growth factors regulate epithelial cell

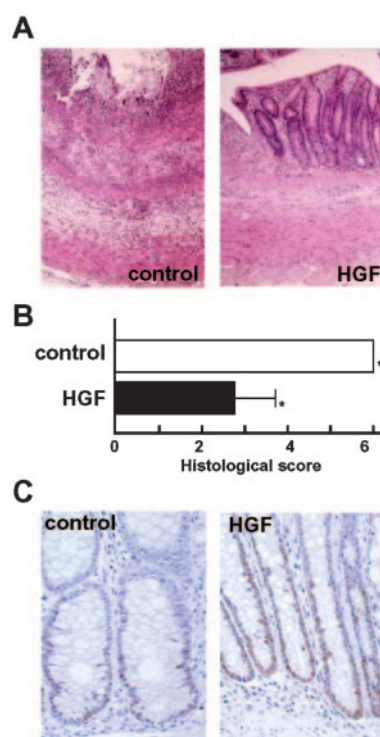


Fig. 4. HGF administration enhances the regeneration of the colonic epithelium and decreases the inflammatory infiltrate in rats with DSS-induced colitis. Distal colon, 1.0, 1.5, and 2.0 cm from the anal rings were used for histological and immunohistochemical evaluation. A, colonic tissues on day 11 (with or without 6-day HGF administration) were stained with hematoxylin and eosin. Following DSS administration, extensive mucosal damage and inflammatory cell infiltration were observed without HGF administration. Treatment with HGF led to enhanced development of regenerative epithelium and reduced inflammatory cell infiltrates. (Original magnification 100 \times .) B, histological scoring was performed in a blind manner as a combined score of inflammatory cell infiltration (0–3) and tissue damage (0–3) (Siegmund et al., 2001). All rats treated with PBS alone were assigned the maximum score of 6 ($n = 4$), although HGF administration decreased the histological score to 2.75 ± 0.96 ($n = 5$) (*, $p = 0.007$). C, the proliferation of colonic epithelium in rats with colitis were examined on day 11 with or without 6-day HGF administration by immunohistochemistry using anti-PCNA monoclonal antibody as described under *Materials and Methods*. An increase in PCNA-positive cells was observed within mucosal epithelium in colitis-induced rats administered HGF. (Original magnification 400 \times .)

function within the mucosal epithelium of the gastrointestinal tract. Recently, Dignass et al. (1994) demonstrated that HGF modulates intestinal epithelial cell proliferation and migration, thus enhancing epithelial cell restitution, the initial step of gastrointestinal wound healing, in *in vitro* models. We demonstrate that administration of recombinant human HGF lessens colitis-associated weight loss in rats without increasing the weights of liver, kidneys, and spleen, as well as effectively improving signs of colitis *in vivo*. We continuously administered a low concentration (1%) of DSS to rats to maintain colitis after a 7-day treatment with a high concentration (5%) to induce colitis. In this rat model, the mucosal erosion observed on day 5 was maintained through day 11, whereas the colon lengths were reduced on day 11 compared with day 5. Overall, the macroscopic and histologic changes seen in rats treated with this protocol resemble the changes observed in chronic persistent colitis in humans. Importantly, the intraperitoneal administration of recombinant human HGF enhanced epithelial regeneration, leading to a reduction in the size of colonic mucosal erosions, even

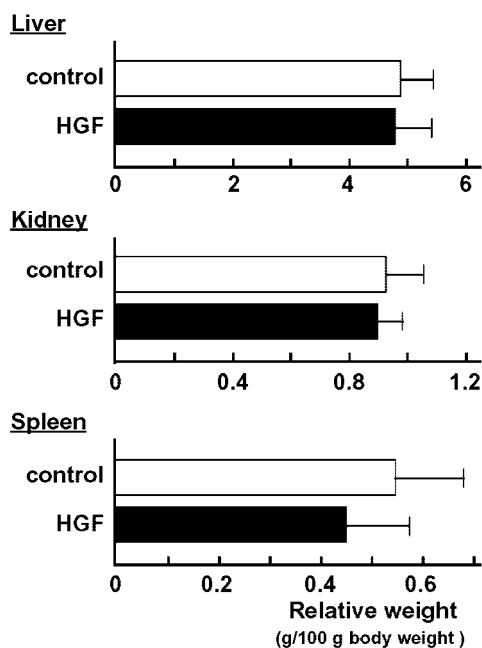


Fig. 5. Administration of HGF did not affect the weight of the liver, kidneys, or spleen in colitis-induced rats. We examined the relative weights of the liver, kidneys, and spleen (g/100 g body weight) of colitis-induced rats with (closed columns; $n = 4$) or without (open columns; $n = 5$) HGF treatment on day 11. The weight of the liver, kidneys, or spleen was not affected by treatment with recombinant human HGF.

though HGF and DSS were applied concomitantly. Additionally, in colitis-induced rats treated with recombinant human HGF, the crypts of the colonic mucosa were enlarged, and enhanced proliferation of colonic epithelial cells was observed by PCNA immunostaining. Conversely, HGF has been shown to rescue animals from lethal hepatic failure through its antiapoptotic activity (Kosai et al., 1998; Mori et al., 1999). Recent studies demonstrated that HGF reduces mucosal damage in the gastrointestinal tract through antiapoptotic effects on intestinal epithelial cells in animal models of ischemia-reperfusion injury and acute graft-versus-host disease (Kuenzler et al., 2002; Kuroiwa et al., 2001). In the present study, it seems likely that the antiapoptotic effects of HGF may have partly contributed to the observed enhanced colonic mucosal repair.

Colon length is a morphometric measure for the degree of colonic inflammation and correlates with the pathologic changes signifying the severity of colitis. Continuous administration of low-dose DSS maintained persistent colitis with both colonic mucosal erosions and shortening of the large intestine at the end of the experiments (day 11). HGF administration, however, reduced inflammatory cell infiltrate and reversed the shortening of the large intestines. The observed reduction in inflammation in HGF-treated rats is in agreement with previous reports that HGF protects the intestinal epithelium from graft-versus-host disease injury by inhibiting the inflammatory cytokine cascade mediated by interleukin-12, interferon- γ , and tumor necrosis factor- α (Kuroiwa et al., 2001). Additionally, enhanced mucosal repair allows for the more rapid recovery of epithelial barrier function leading to reduced exposure to various luminal agents that contribute to persistent colitis. Accordingly, HGF treatment should reduce the inflammatory response to these luminal stimuli.

Several studies have described increased serum HGF lev-

els in animals with experimental colitis (Dignass et al., 1994; Takahashi et al., 1995b; Matsuo et al., 1997; Nishimura et al., 1998; Ortega-Cava et al., 2002). In the present study, c-Met tyrosine phosphorylation was also observed in colonic tissue of rats with DSS-induced colitis in the absence of HGF treatment, suggesting that endogenous rat HGF was also induced. Continuous intraperitoneal delivery of recombinant human HGF allowed for the detection of human HGF within rat serum and was successful in further stimulating tyrosine phosphorylation of c-Met within the colonic mucosal tissue. These observations indicate that c-Met expressed by colonic epithelial cells was exposed to recombinant human HGF circulating in the blood. Recently, Ortega-Cava et al. (2002) demonstrated that, although both an increase in serum HGF and enhanced expression of HGF mRNA in colonic tissue were observed in DSS-induced colitis rats, HGF protein levels in colonic tissue were reduced. HGF also interacts with low-affinity receptors, heparin-like molecules such as heparin sulfate proteoglycan, which are commonly found at the cell surface and within extracellular matrix that serve to retain HGF in tissues (Zarnegar et al., 1990; Naldini et al., 1991; Arakaki et al., 1992; Komada et al., 1992). Since DSS administration causes the damage and loss of not only the colonic epithelial cells but also extracellular matrix, local HGF concentration in injured colonic tissue should be reduced in rats with DSS-induced colitis. Therefore, although endogenous rat HGF levels increased, the additional systemic administration of recombinant human HGF was effective in reducing colonic damage induced by DSS.

In colorectal mucosa affected by ulcerative colitis, active inflammation is persistent. The mucosal injury and repair cycle is therefore continually repeated, leading to an increase in the risk of colorectal cancer (Collins et al., 1987; Ekobom et al., 1990). Recently, Kitamura et al. (2000) demonstrated that HGF and c-Met expression are increased in inflamed colonic mucosa in patients with ulcerative colitis and that c-Met is overexpressed in ulcerative colitis-associated colorectal cancer. In sporadic colorectal carcinomas, activation of HGF was enhanced in colorectal carcinoma tissues compared with that in corresponding normal mucosa (Kataoka et al., 2000b). Although it will be necessary to determine the details of the functional interaction between HGF and c-Met activation in ulcerative colitis-associated colorectal cancer, these observations suggest that the HGF/c-Met system may function in both repair of the inflamed mucosa and development of colorectal cancer in patients with ulcerative colitis. Therefore, the administration of recombinant human HGF may increase the risk of colorectal carcinomas in patients with inflammatory bowel disease. Conversely, HGF facilitates colonic mucosal remodeling and ameliorates colitis, thus breaking the cycle of repeated mucosal injury and repair. Because these cycles of repeated injury and repair increase the risk of colonic carcinogenesis, HGF treatment may instead prevent ulcerative colitis-associated colorectal cancer. Although it is still not clear whether HGF administration increases the risk of carcinogenesis in patients with ulcerative colitis, further investigation of the influence of recombinant human HGF on colitis-associated colorectal cancer is in progress. Because of the short half-life of recombinant human HGF, treatment with it is considered safer than HGF gene transduction, in which HGF is constitutively expressed within a limited area.

HGF is also known to reduce fibrosis in animal models of

cirrhosis, as well as in those of renal and pulmonary fibrosis (Yasuda et al., 1996; Yaekashiwa et al., 1997; Mizuno et al., 1998). Fibrosis is a major complication of inflammatory bowel disease and is mediated by intestinal fibroblasts. An activated subpopulation of these fibroblasts in both ulcerative colitis and Crohn's disease has recently been reported (Lawrance et al., 2001). Therefore, HGF-induced antifibrotic effects may contribute to the reduction of fibrotic tissue deposition leading to amelioration of intestinal stricture formation, especially in patients with Crohn's disease.

In this study, we continuously administered a low concentration of DSS to rats to maintain colitis, following its induction with a high concentration of DSS. Persistent colitis was maintained without a reduction of erosive areas, and shortening of large intestines was detectable at the end of the experiments. Both of these conditions closely resemble chronic persistent colitis in humans. Using this model, we demonstrated that intraperitoneally administered human HGF reduced the severity of colitis and accelerated colonic mucosal repair in the DSS-induced rat model of colitis. Recombinant human HGF will be available for patients with fatal liver disease in the near future in Japan; the results presented here indicate that HGF may be a potent candidate for a new therapeutic modality accelerating intestinal mucosal repair in patients with inflammatory bowel disease.

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