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Effect of Hepatocyte Growth Factor on the Proliferation of Intrasplenically Transplanted Hepatocytes in Rats

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The effect of human hepatocyte growth factor on the proliferation of intrasplenically transplanted hepatocytes was investigated. A human hepatocyte growth factor (360 μg/kg BW) was administered intravenously every 24 hr for 5 days following hepatocyte transplantation in rats. A significant increase in hepatocyte proliferation was observed morphologically and immunohistochemically ($p < 0.05$). The bromodexyuridine labeling index was significantly greater in human hepatocyte growth factor treated rats than in the control rats ($p < 0.05$). Hepatocyte growth factor may promote the proliferation of intrasplenically transplanted hepatocytes.

Hepatocyte growth factor (HGF) is a multifunctional cytokine with mitogenic, monogenic and morphogenic actions, that represents the most potent stimulation of hepatocyte growth and DNA synthesis identified. HGF is becoming recognized as one of the most important factors in the regulation of liver regeneration after surgical resection or clinical damage. Experimental hepatocyte transplantation (HTx) is receiving increasing attention as a possible treatment for acute hepatic failure or enzyme-deficient diseases (1,2). This therapeutic approach is based on the assumption that transplanted hepatocytes provide metabolic support for deficient hepatic functions (3). Previously we have reported that the ODS-od/od rat, a rat strain which can not synthesize ascorbic acid (ASA) due to a lack of L-gulonolactone oxidase in the liver microsomes, can be treated successfully with intrasplenic hepatocyte transplantation (4).

Recent reports of successful intrasplenic transplantation have suggested that many of the hepatocytes transplanted into the spleen actually migrate to the liver (5,6). Furthermore, it has been noted that severe liver damage and extensive necrosis leading to early mortality can take place following intraportal cell transplantation (7). Therefore, intrasplenic hepatocyte transplantation is limited by the relatively small number of cells (3,4), hence rapid growth of hepatocytes transplanted into the spleen is required. In this study, we investigated the proliferative effect of recombinant human HGF (hHGF) on intrasplenically transplanted hepatocytes in a rat model.

MATERIALS AND METHODS

Animals

Adult inbred Wistar rats, weighing approximately 200 g, were used as hepatocyte transplant donors and recipients. These rats were obtained from the Agriculture Cooperative Association for Laboratory Animals (Shizuoka, Japan). The rats received humane care according to our institution's guidelines.

Isolation of Hepatocytes

Preparation of isolated hepatocytes involved a modification of the two-step perfusion technique of Seglen (8). After cannulation of the portal vein, the liver was perfused with approximately 250 mL Ca^{++}-free Hank's balanced salt solution (HBSS) at a flow rate of 20–25 mL/min. Subsequently, perfusion was started with a 0.05% collagenase (type I, Sigma Chemicals, St. Louis, MO, USA) and 5 mM Ca^{++}-ion, containing HBSS. After 10 min of collagenase digestion, the capsule was removed, freeing isolated liver cells. The cells were then counted and resuspended in HBSS containing 10 mg/mL fatty-acid-free bovine serum albumin (Sigma Chemicals) to a concentration of 10^7 viable cells/mL. Cell viability was assessed by the trypan blue exclusion test, and ranged from 85% to 95%. Within 1 hr to 3 hr after hepatocyte isolation, 1

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mL of the cell suspension was injected into the spleen of the recipient rat under ether anesthesia. Hilar vessels of the spleen were clamped during the intrasplenic injection. The rats that underwent transplants were maintained for 5 days in a sterile environment with free access to food and water. No immunosuppressant was given to recipient rats prior or following hepatocyte transplantation.

Morphologic Evaluation of Intrasplenically Transplanted Hepatocytes

After the animals were killed on day 6 following hepatocyte transplantation, the spleen was sectioned, embedded in paraffin, and excised and the tissue was stained with hematoxylin and eosin for light microscopic examination.

DNA Synthesis by Hepatocytes in the Spleen

Bromodeoxyuridine (BrdU; Becton Dickinson, San Jose, CA, USA) was administered to determine the rates of DNA synthesis by intrasplenically transplanted hepatocytes and the incorporation of BrdU into DNA was detected immunohistochemically. Six days after transplantation, BrdU was diluted with 0.9% NaCl (20 mg/mL), and 0.5 mL (50 mg/kg) of this solution was administered intravenously to rats 1 hr before death. Harvested spleens were sectioned, fixed in 70% ethanol, embedded in paraffin, and stained via an immunohistochemical technique (9,10) using anti-BrdU monoclonal antibodies, avidin-biotinylated anti-mouse IgG and a VECTASTAIN ABC-Kit (Vector Lab., Burlingame, CA, USA). The number of labeled nuclei of hepatocytes was counted under light microscopy, and the labeling index (L.I.) was expressed as the number of labeled cells per 1000 hepatocytes. Recombinant human hepatocyte growth factor (hHGF), a generous gift from the Research and Development Division of the Mitsubishi Corporation, was diluted in phosphate buffer (10 mM/L, pH 7.5) and administered at a daily dose of 360 μg/kg intravenously. Dextran sulfate, another gift from the Mitsubishi Corporation, was diluted in 0.15 M NaCl and administered intravenously at in a daily dose of 1 mg/kg mixed with hHGF (360 μg/kg) (11).

Experimental Design

Control group. Control rats underwent intrasplenic hepatocyte transplantation consisting of a single dose of 5×10^7 cells (HTx).

HGF group. These rats underwent HTx in the same manner as controls. In addition, they received 360 μg/kg of hHGF intravenously every 24 hr for 5 days after HTx.

HGF+DS group. After HTx, these rats received of 360 μg/kg BW of hHGF mixed with dextran sulfate (1 mg/kg) intravenously every 24 hr for 5 days.

Statistical Analysis

Each assay was performed in seven rats. Data are expressed as the mean ± SD. Within each group, differences between the control and experimental animals were statistically analyzed using Student's t test.

RESULTS

Morphologic and Immunohistochemical Evaluation of Intrasplenically Transplanted Hepatocytes

Figure 1 shows the appearance of intrasplenically transplanted hepatocytes in the control (A) and HGF (B) groups 6 days following transplantation. Large masses of hepatocytes were present in the red pulp. A larger quantity of hepatocytes was observed in the spleens of rats in the HGF group than in controls. In most instances, large confluent masses of hepatocytes were observed around the splenic white pulp. BrdU-labeled hepatocytes were observed in the spleens of rats in the control (A) and HGF (B) groups (Fig. 2).

Evaluation of Number of Transplanted Cells

Transplanted hepatocytes could be easily identified via light microscopy, even at a low magnification. Quantitation of the number of hepatocytes revealed a mean of 14.9 ± 7.7 cells/mm^2 in the control group versus 45.5 ± 5.0 cells/mm^2 in the HGF group ($p < 0.05$) and 58.6 ± 3.8 cells/mm^2 in the HGF+DS group ($p < 0.01$). There was a significant difference between counts in the HGF and HGF+DS groups ($p < 0.05$) (Fig. 3A).

Evaluation of the BrdU Labeling Index

The BrdU labeling index of hepatocytes in the spleen was of 1.8 ± 0.7 in the control group versus 5.9 ± 1.0 ($p < 0.05$) in the HGF group and 6.3 ± 0.9 in the HGF + DS group ($p < 0.01$) (Fig. 3B).

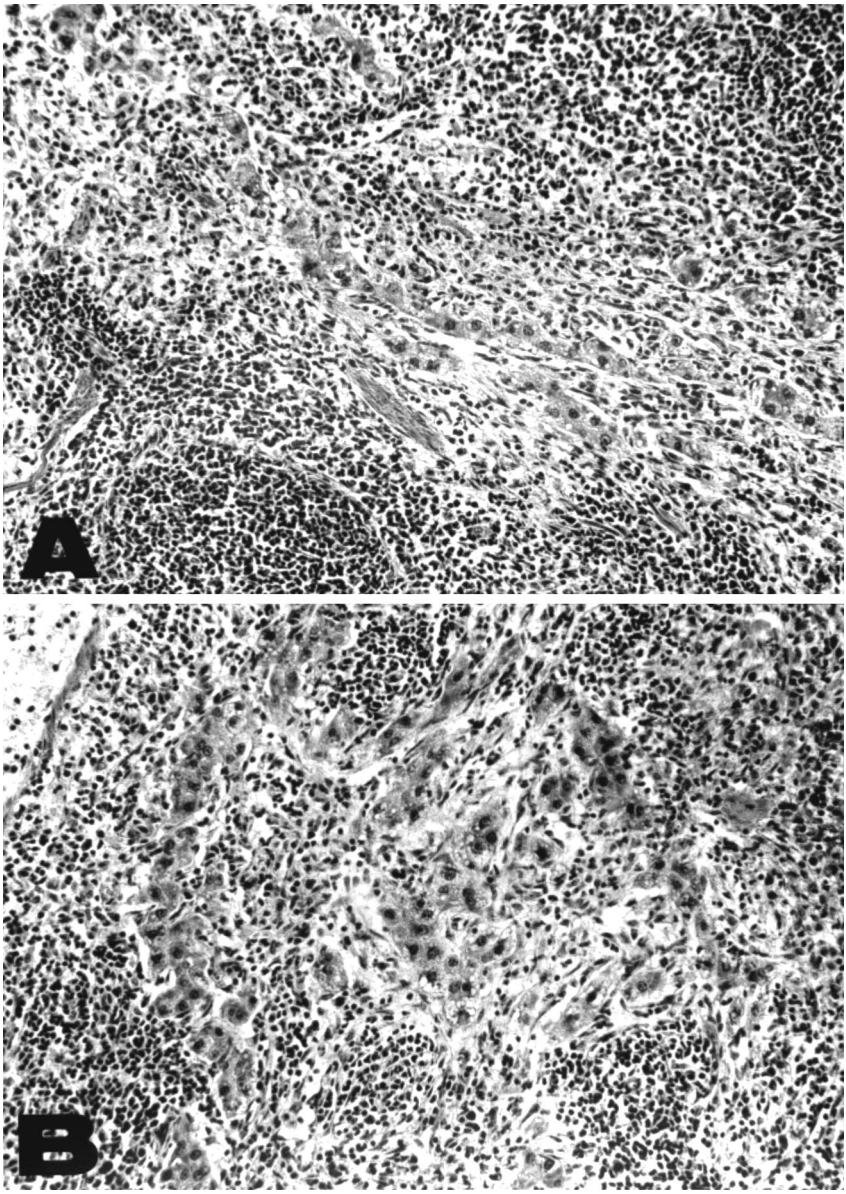

FIG. 1. Photomicrographs showing the morphologic appearance of intrasplenically transplanted fetal hepatocytes. Large masses of hepatocytes are visible in the red pulp with cord-like structures and marked proliferation of the bile ducts. Oval-shaped hepatocytes surrounded by lymphocytes and red blood cells are visible on close examination (× 100). **A:** Control group, **B:** Hepatocyte growth factor (HGF) group.

DISCUSSION

The potential therapeutic implications of hepatocyte transplantation into the spleen for the treatment of enzyme deficiency diseases or acute liver failure has received attention in several laboratories (12). However, Mito et al. found that it took almost the entire life span of the recipient rats for intrasplenically transplanted hepatocytes to occupy approximately 42% of the cut surface of the spleen (13). Therefore, the methods used in some recent studies have aimed at promoting the rapid proliferation of transplanted hepatocytes (14,15,16). Liver regeneration after partial hepatec-

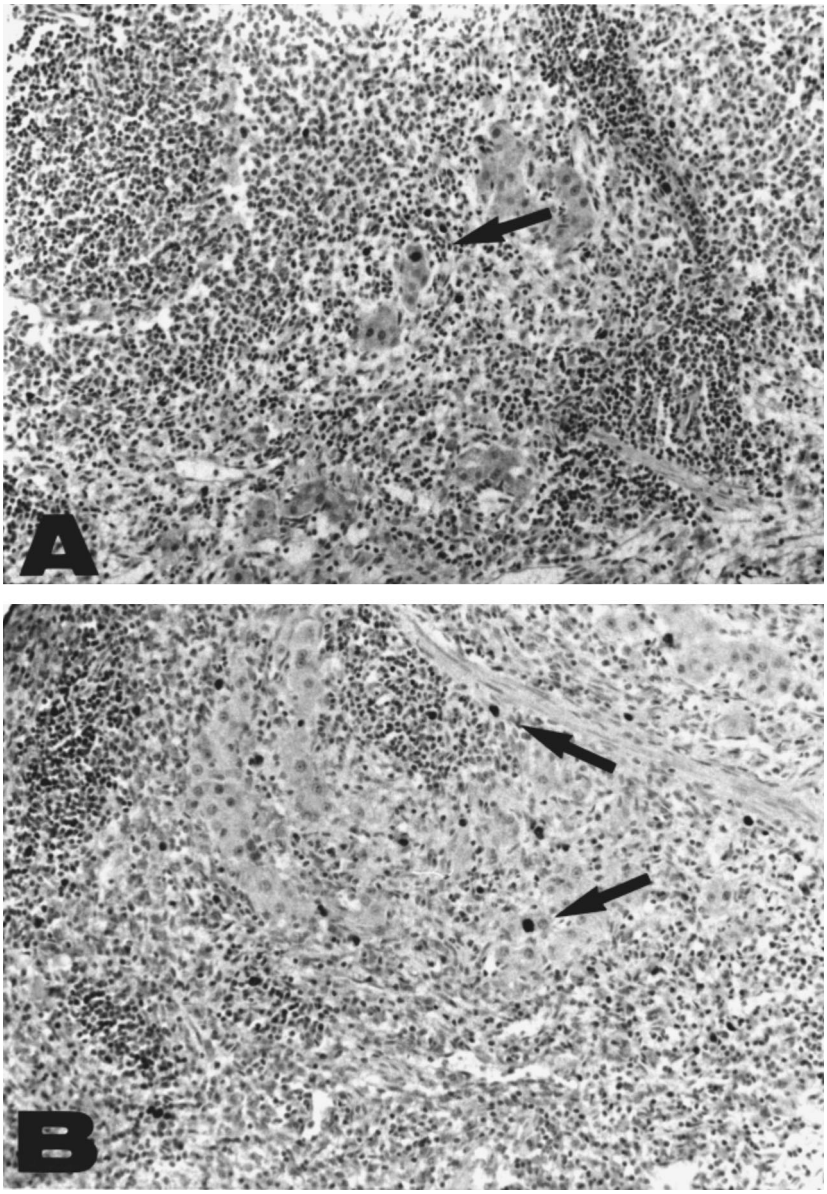

FIG. 2. Sections of spleen stained via an immunohistochemical technique employing anti-bromodeoxridine monoclonal antibodies, demonstrating proliferating hepatocytes (× 100). **A:** Control group, **B:** Hepatocyte growth factor (HGF) group.

tomy is the best example of induced regenerative growth (17). Partial hepatectomy is the strongest stimulus for during hepatocyte proliferation (18), and partial host-liver hepatectomy may stimulate a regenerative response in the graft. However, this effect is transient and no additional increase in the number of intrasplenically transplanted hepatocytes has been observed (16). Previously, we have reported that hepatic stimulatory substance (HSS), from regenerating procaine liver enhanced intrasplenically transplanted hepatocyte proliferation in rats. The clusters of intrasplenic hepatocytes contained more than 100 cells, and formed cord structures at 2 wk after transplantation, and the hepatocytes still survived at 6 wk in the HSS-treated rats (19). However the present study is the first report that hepatocyte proliferation can be induced more rapidly in the spleen by administering

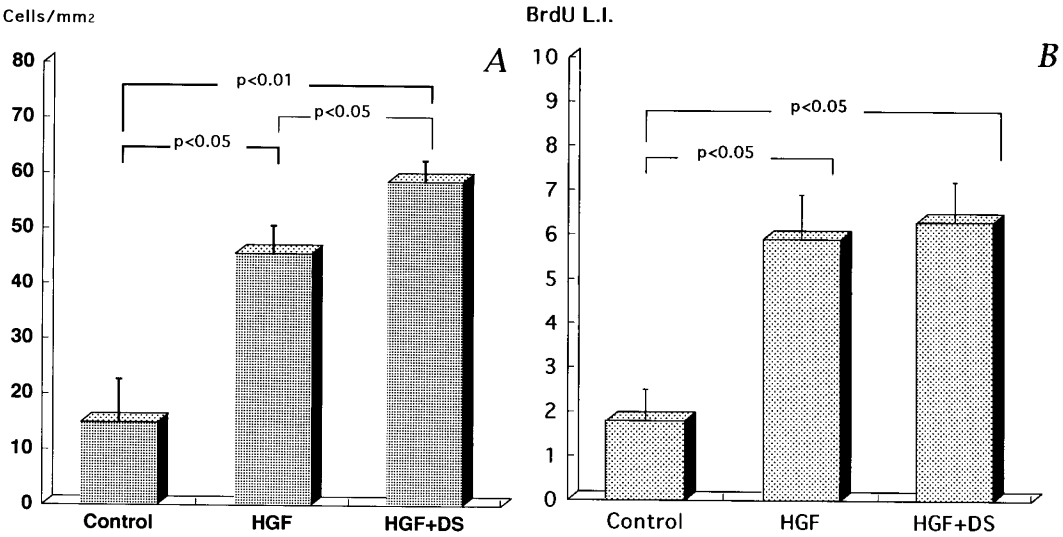

FIG. 3. Cell number (A) and BrdU labeling index (B) of intrasplenically transplanted hepatocytes. HGF group: The rats underwent HTx and then received hHGF intravenously. HGF+DS group: After HTx, rats received hHGF mixed with dextran sulfate.

recombinant hHGF. Furthermore, this enhancing effect of hepatocyte proliferation was stronger in the HGF + DS group. Roos (11) has reported that combining dextran sulfate with HGF markedly increased the plasma concentration of HGF. Ito (20) has reported that heparin-binding EGF-like growth factor stimulated DNA synthesis in rat hepatocytes in primary culture. The local concentrations of heparin-like growth factors were likely regulated, in part, by binding to heparin sulfate proteoglycan. In our study, the serum HGF level might have been maintained at a higher level in HGF + DS group in a similar manner. This continuously higher level of serum HGF enhanced proliferation in intrasplenically transplanted hepatocytes without any pretreatment in the host animal.

We concluded that recombinant hHGF may promote the rapid proliferation of hepatocytes transplanted to the spleen.

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