World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v18.i19.2320

World J Gastroenterol 2012 May 21; 18(19): 2320-2333
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Matrix metalloproteinase-9 contributes to parenchymal hemorrhage and necrosis in the remnant liver after extended hepatectomy in mice

Norifumi Ohashi, Tomohide Hori, Florence Chen, Sura Jermanus, Christopher B Eckman, Akimasa Nakao, Shinji Uemoto, Justin H Nguyen

Norifumi Ohashi, Akimasa Nakao, Gastroenterological Surgery, Nagoya University Graduate School of Medicine, Nagoya, Aichi 466-8550, Japan
Tomohide Hori, Shinji Uemoto, Divisions of Hepato-Pancreato-Biliary, Transplant and Pediatric Surgery, Department of Surgery, Kyoto University Graduate School of Medicine, Kyoto 606-8507, Japan
Florence Chen, Sura Jermanus, Christopher B Eckman, Department of Neuroscience, Mayo Clinic, Jacksonville, FL 32224, United States
Justin H Nguyen, Division of Transplant Surgery, Department of Transplantation, Mayo Clinic, Jacksonville, FL 32224, United States
Author contributions: Nguyen JH and Eckman CB designed the study; Ohashi N performed the surgery, the assays and the statistical analysis, and wrote the initial draft; Hori T performed the additional surgery for the second assays to confirm the initial results; Chen F and Jermanus S helped with the assays; Nguyen JH and Hori T contributed to further drafts; Nakao A and Uemoto S provided important advice for the research; Nguyen JH supervised the research.
Supported by Grants to Nguyen JH from the Deason Foundation, Sandra and Eugene Davenport, Mayo Clinic CD CRT-II, and from the National Institutes of Health, No. R01NS051646-01A2; a grant to Hori T from the Uehara Memorial Foundation, No. 200940051, Tokyo 171-0033, Japan
Correspondence to: Tomohide Hori, PhD, MD, Divisions of Hepato-Pancreato-Biliary, Transplant and Pediatric Surgery, Department of Surgery, Kyoto University Graduate School of Medicine, 54 Shogoinkawara-cho, Sakyo-ku, Kyoto 606-8507, Japan. horit@kuhp.kyoto-u.ac.jp
Telephone: +81-75-7513651 Fax: +81-75-7513106
Received: August 26, 2011 Revised: October 27, 2011
Accepted: February 27, 2012
Published online: May 21, 2012

Abstract

AIM: To investigate the effect of matrix metalloproteinase-9 (MMP-9) on the remnant liver after massive hepatectomy in the mouse.

METHODS: Age-matched, C57BL/6 wild-type (WT), MMP-9(-/-), and tissue inhibitors of metalloproteinases (TIMP)-1(-/-) mice were used. The mice received 80%-partial hepatectomy (PH). Samples were obtained at 6 h after 80%-PH, and we used histology, immunohistochemical staining, western blotting analysis and zymography to investigate the effect of PH on MMP-9. The role of MMP-9 after PH was investigated using a monoclonal antibody and MMP inhibitor.

RESULTS: We examined the remnant liver 6 h after 80%-PH and found that MMP-9 deficiency attenuated the formation of hemorrhage and necrosis. There were significantly fewer and smaller hemorrhagic and necrotic lesions in MMP-9(-/-) remnant livers compared with WT and TIMP-1(-/-) livers ($P < 0.01$), with no difference between WT and TIMP-1(-/-) mice. Serum alanine aminotransaminase levels were significantly lower in MMP-9(-/-) mice compared with those in TIMP-1(-/-) mice (WT: 476 ± 83 IU/L, MMP-9(-/-): 392 ± 30 IU/L, TIMP-1(-/-): 673 ± 73 IU/L, $P < 0.01$). Western blotting and gelatin zymography demonstrated a lack of MMP-9 expression and activity in MMP-9(-/-) mice, which was in contrast to WT and TIMP-1(-/-) mice. No change in MMP-2 expression was observed in any of the study groups. Similar to MMP-9(-/-) mice, when WT mice were treated with MMP-9 monoclonal antibody or the synthetic inhibitor GM6001, hemorrhagic and necrotic lesions were significantly smaller and fewer than in control mice ($P < 0.05$). These results suggest that MMP-9 plays an important role in the development of parenchymal hemorrhage and necrosis in the small remnant liver.

CONCLUSION: Successful MMP-9 inhibition attenuates the formation of hemorrhage and necrosis and might

be a potential therapy to ameliorate liver injury after massive hepatectomy.

Key words: Matrix metalloproteinase; Liver remnant; Hepatectomy; Liver failure; Necrosis

Peer reviewers: Dr. Assy Nimer, MD, Assistant Professor, Liver Unit, Ziv Medical Centre, PO Box 1008, Safed 13100, Israel; Ta-Sen Yeh, MD, PhD, Department of Surgery, Chang Gung Memorial Hospital, Taipei, 5 Fu-Hsing Street, Kwei-Shan, Taoyuan 886, Taiwan, China

Ohashi N, Hori T, Chen F, Jermanus S, Eckman CB, Nakao A, Uemoto S, Nguyen JH. Matrix metalloproteinase-9 contributes to parenchymal hemorrhage and necrosis in the remnant liver after extended hepatectomy in mice. *World J Gastroenterol* 2012; 18(19): 2320-2333 Available from: URL: http://www.wjgnet.com/1007-9327/full/v18/i19/2320.htm DOI: http://dx.doi.org/10.3748/wjg.v18.i19.2320

INTRODUCTION

In the field of hepatobiliary surgery, liver resection is considered the standard treatment for primary liver tumor and colorectal liver metastases[1-3]. Major liver resections have been associated with an increased morbidity and mortality compared with more limited resections. In particular, the outcomes are worsened when a chronic underlying liver disease is present[4]. Recent studies have shown that the volume of the remnant liver is correlated with perioperative morbidity and mortality[5-7], and an insufficient hepatic remnant (IHR) after extended hepatectomy is a critical issue.

On the other hand, liver transplantation (LT) is the treatment of choice for end-stage liver diseases. However, in the LT field, donor shortage and small-for-size grafts (SFSGs) are critical issues. Although split-liver transplantation (SOLT) is promising, failure of SFSGs in adult LT recipients[8,9] and after extensive hepatectomy for metastatic cancer hinders its application[10]. SFSG failure results in postoperative coagulopathy, cholestasis, ascites, encephalopathy, and death[11,12]. The mechanisms of SFSG failure remain incompletely understood.

One mechanism that has been implicated in the early development of SFSG failure is the formation of parenchymal hemorrhage and necrosis[13,14]. Previous investigators used electron microscopic studies to demonstrate sinusoidal breakdown leading to hemorrhage and necrosis after chemically or endotoxin-induced liver failure[15-17], hepatic ischemia-reperfusion injury[18-19], extensive hepatectomy[18,20,21], and LT with SFSG or SOLT[22-25]. These findings suggested that although the inciting factors might have different causes, the pathogenesis of sinusoidal breakdown leading to hemorrhage and necrosis might be similar.

Matrix metalloproteinases (MMPs) comprise a family of zinc-dependent neutral proteases that can degrade the extracellular matrix and basement membrane. MMPs play significant roles in cellular regulation, cell-cell communication, and tumor progression[26,27]. MMP-2 and MMP-9 have been implicated in liver injury and remodeling. Knocking out MMP-9 and other MMPs attenuate liver injury associated with interferon treatment[28]. MMP-9 contributes to the pathogenesis of experimental acute liver failure[29]. Specifically, MMP-9 has been implicated in sinusoidal breakdown leading to extravasation of circulating cells and hemorrhage[14,30]. MMP-9 plays a pivotal role in ischemia-reperfusion injury[31-33]. When MMP-9 is blocked with a synthetic inhibitor or by MMP-9 gene deletion, it successfully reverses ischemia-reperfusion injury[34,35] and cold preservation-warm reperfusion injury after LT[35]. Collectively, these data show that MMP-9 is involved in sinusoidal injury in liver failure. However, the role of MMP-9 in the pathogenesis of failure in IHR or SFSGs is not well known.

We hypothesized that MMP-9 plays an important role in the development of liver parenchymal hemorrhage and necrosis in IHR/SFSG failure. Tissue inhibitors of metalloproteinases (TIMP)-1 are physiological tissue inhibitors of MMP-9, which increase MMP-9 activity when deleted[36,37]. We hypothesized that a deletion or decrease in MMP-9 has a beneficial effect after hepatectomy. In the current study, we examined this hypothesis in IHR after 80%-partial hepatectomy (PH) in wild-type (WT), MMP-9(-/-), and TIMP-1(-/-) mice. We also examined the efficacy of MMP-9 blockade with a monoclonal antibody and synthetic MMP-9 inhibitor.

MATERIALS AND METHODS

Animals

Male C57BL/6 mice (10-14 wk old) were obtained from Jackson Laboratory (Bar Harbor, ME), housed with a 12-h light/dark cycle, and given food and water *ad libitum*. MMP-9(-/-) mice, obtained from Dr. Robert Senior (Washington University, St. Louis, MO), and TIMP-1(-/-) mice, purchased from Jackson Laboratory, both with a C57BL/6 background, were bred in our facility. The study was institutionally approved in accordance with the National Institutes of Health "Guide for the Care and Use of Laboratory Animals".

PH in mice

Our institutional procedures for PH and postoperative care have been described elsewhere in detail[38]. Left and right posterior and left and right anterior segments were resected for an 80%-PH, similar to that described in a previous report[39]. In brief, under general anesthesia, liver lobes were mobilized. A hemostatic clip (Teleflex Medical, Triangle Park, NC) was applied across the pedicle, and the liver lobes were removed, without any obstruction of portal flow and hepatic venous drainage[38]. Laparotomy alone was performed for sham operations. Before closure, 2 mL of warm saline was administered

intraperitoneally. Cephalexin (30 mg/kg) and buprenorphine (0.1 mg/kg) were given subcutaneously. Postoperatively, the mice were kept in a temperature-controlled environment with free access to food and water. All mice were sacrificed 6 h after 80%-PH. The remnant livers were harvested, and blood samples were collected. We chose the extended hepatectomy model in mice because we hypothesized that MMP-9 plays an important role in the development of liver parenchymal hemorrhage and necrosis in IHR/SFSG failure.

MMP-9 inhibition by anti-MMP-9 monoclonal antibody and the MMP inhibitor GM6001

WT mice received 3 mg/kg of anti-MMP-9 neutralizing monoclonal antibody intravenously (clone 6-6B; EMD Chemicals, Gibbstown, NJ) 1 h before 80%-PH (MMP-9 mAb, $n = 6$). Control mice received normal IgG (EMD, Gibbstown, NJ) (control IgG, $n = 6$). The broad-spectrum MMP-inhibitor GM6001 (Millipore, Billerica, MA) at a concentration of 100 mg/kg in 10% dimethyl sulfoxide (DMSO) was administrated intraperitoneally 2 h before 80%-PH ($n = 10$), and controls received DMSO only ($n = 10$). An inhibitor of MMP-9 itself may affect liver regeneration after PH. Therefore, for the inhibition of MMP-9, we employed two inhibitory methods (i.e., a monoclonal antibody and inhibitor) and used MMP-9(-/-) mice in this study.

Biochemical analysis

Serum levels of aspartate aminotransferase (AST) and alanine aminotransaminase (ALT) were determined by using a kinetic detection kit (Pointe Scientific, Inc, Canton, MI), and total bilirubin was determined by using the QuantiChrom™ Bilirubin Assay Kit (BioAssay Systems, Hayward, CA).

Western blotting analysis

Liver samples were homogenized in a buffer containing 10 mmol/L Tris-HCl (pH 7.4), 150 mmol/L NaCl, 1% Triton-X, 0.1% sodium dodecyl sulfate (SDS), 1 mmol/L ethylene diamine tetra-acetic acid (EDTA), 1 mmol/L ethylene glycol tetra-acetic acid, 1 mmol/L phenylmethyl-sulfonyl fluoride, and protease and phosphatase inhibitors. Homogenates were centrifuged at 105 000 × *g* for 1 h at 4 ℃. Supernatants were collected, and protein concentration was determined by bicinchoninic acid assay (Pierce, Rockford, IL). Forty micrograms of protein was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a polyvinylidene fluoride membrane (Millipore, Bedford, MA). Membranes were blocked with 5% nonfat milk in Tris-buffered saline with Tween 20 [20 mmol/L Tris-buffered saline (pH 7.4), 500 mmol/L NaCl, and 0.05% Tween 20] and probed using the antibody for MMP-9 (R and D Systems, Minneapolis, MN), and were then incubated with peroxidase-conjugated secondary antibodies (Santa Cruz Biotechnology, Santa Cruz, CA) followed by enhanced chemi-luminescence (ECL) or ECL Plus reagent (Amersham Biosciences, Piscataway, NJ). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served as control (Imgenex Corporation, San Diego, CA). Signals were quantified by using ImageQuant software (Molecular Dynamics, Sunnyvale, CA).

Gelatin zymography

Liver homogenates were analyzed by gelatin zymography with affinity chromatography[40]. In brief, 400 μg of liver extract samples were incubated with 100 μL of gelatin-Sepharose 4B (GE Healthcare, Piscataway, NJ) and equilibrated buffer containing 50 mmol/L Tris-HCL pH 7.5, 150 mmol/L NaCl, 5 mmol/L $CaCl_2$, 0.02% Tween 20, and 10 mmol/L EDTA for 2 h at 4 ℃. After being washed three times, gelatin-Sepharose beads were resuspended in the same volume of 2 × zymography sample buffer (Bio-Rad Laboratories, Inc., Hercules, CA) and loaded onto a 10% SDS-PAGE gel containing 1 mg/mL of gelatin (Bio-Rad Laboratories, Inc.). After electrophoresis, the gel was washed twice for 30 min with 2.5% Triton X-100 for renaturing and then incubated in development buffer (Bio-Rad Laboratories, Inc.) for 20 h at 37 ℃. The gel was then fixed and stained with 0.5% Coomassie Blue R-250 (Bio-Rad Laboratories, Inc.) for 1 h and destained with 10% acetic acid in 40% methanol solution. Gelatinase zymography standards (Millipore, Billerica, MA) were used as a positive control.

Histology and immunohistochemical staining

Formalin-fixed, paraffin-embedded liver specimens in 5-μm sections were stained with hematoxylin and eosin. Immunohistochemical staining for CD11b (MAC-1) and CD68 was performed on frozen sections, and staining for desmin, myeloperoxidase, and MMP-9 was performed on paraffin-embedded sections after heat-induced antigen retrieval. We used 0.3% H_2O_2 to quench the endogenous peroxidase activity. An ABC kit (Vector Laboratories, Inc, Burlingame, CA) was used according to the manufacturer's instructions for HRP-based staining with 3,3' diaminobenzidine (DAB) tetrahydrochloride (Dako, Carpinteria, CA). Antibodies for MMP-9 [antigen affinity-purified, lyophilized from a 0.2 μm filtrated solution in phosphate buffered saline (PBS) with trehalose; AF909; R and D Systems, Minneapolis, MN], CD11b (clone M1/70, R and D Systems), CD68 (clone FA-11, Abcam Inc., Cambridge, MA), desmin (Abcam Inc.), and myeloperoxidase (Ab-1) (Thermo Scientific, Fremont, CA) were incubated for the primary reaction. Alexa Fluor 568 donkey anti-goat IgG (H + L), Alexa Fluor 488 donkey anti-rabbit IgG, and Alexa Fluor 488 donkey anti-rat antibody (Invitrogen, Carlsbad, CA) were used for secondary reactions.

Histological analysis

The HE-stained sections were scanned with a Scanscope XT system and analyzed with Aperio Imagescope software (Aperio Technologies, Inc., Vista, CA). Histologically positive areas were counted and measured by investigators blinded to the study group in three randomly

selected 3-mm^2 fields. DAB-positive staining of MMP-9 was calculated using the positive-pixel-count function of Imagescope software in 10 randomly selected fields. The value was quantified as the percentage of positive pixels per total pixels. The number of infiltrated neutrophils in the remnant liver was evaluated in five randomly captured 40 × high-power fields (hpf) with an Olympus BX50 fluorescence microscope (Olympus Optical, Tokyo, Japan).

In situ zymography

In situ gelatinolytic activity was determined using 20-μm cryostat sections for the EnzChek Gelatinase assay kit (Molecular Probes, Eugene, OR)[40]. Sections were incubated with 20 μg/mL of fluorescence-conjugated gelatin in reaction buffer (50 mmol/L Tris-HCl, 150 mmol/L NaCl, 5 mmol/L $CaCl_2$, and 0.2 mmol/L sodium azide) for 2 h at 37 ℃. After three washes with PBS, they were fixed in 4% paraformaldehyde. The sections were mounted with Vectashield (Vector Laboratories Inc., Burlingame, CA). Gelatinase activity was visualized using fluorescence microscopy (Olympus BX50; Olympus Optical, Tokyo, Japan).

Preliminary study

In our institution, 90%-PH (10% of the liver remnant with an omental segment), 80%-PH (20% of the liver remnant with right middle and omental segments) and 75%-PH (25% of the liver remnant with right middle and posterior segments) are available[38]. Initially, we performed 90%-PH in WT, MMP-9(-/-) and TIMP-1(-/-) mice, as a preliminary study. A survival study after 90%-PH was performed in each murine strain. Moreover, liver damage scores in HE staining[41], serum AST and ALT levels, and the ratios of terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling-positive nuclei/all nuclei per mm^2 and caspase 3-positive nuclei/all nuclei per mm^2 in immunohistological staining were assessed in serum or liver samples 6 h after 90%-PH in each murine strain. The results for each factor did not reach significant differences between WT and MMP-9(-/-) mice and between WT and TIMP-1(-/-) mice. Even in WT mice (control group), all mice received 90%-PH without any surgical issues but showed considerable damage and subsequently died in the early postoperative period. We therefore considered that 90%-PH is not suitable to investigate the effect of MMP-9 and TIMP-1 on the hepatic remnant after hepatectomy. To obtain relevant data according to clinical background[1-10], we finally employed 80%-PH in each strain for further experiments in this study. We decided to evaluate 80%-PH because mice could survive for a long time after surgery.

Statistical analysis

Data are presented as the mean ± SE. Statistical comparisons were performed using analysis of variance followed by a two-sample *t*-test with Bonferroni adjustment. A *P* value less than 0.05 was considered statistically significant.

RESULTS

Parenchymal hepatic hemorrhage and necrosis occurs soon after 80%-PH

First, 80%-PH was performed in WT mice. Six hours after 80%-PH, the animals were killed and divided into two groups according to the status of parenchymal hemorrhage and necrosis in the remnant livers. Murine behavior in hepatic failure has been well described[42,43]. The animals with hemorrhage and necrosis were observed to be sick and inactive, whereas the mice without such injury were asymptomatic and active. As shown in Figure 1A and B, the groups consisted of mice with minimal or no hemorrhage and necrosis (group Ⅰ, $n = 6$) or those with multifocal hemorrhage and necrosis (group Ⅱ, $n = 5$). Group Ⅰ had a significantly greater area of necrosis (2.97% ± 0.92% *vs* 0.11% ± 0.08%, $P < 0.05$) (Figure 1C) and number of necrotic foci compared with group Ⅱ (3.60 ± 0.87 *vs* 0.26 ± 0.17, $P < 0.01$) (Figure 1D). Group Ⅱ had significantly higher AST (893 ± 72 IU/L *vs* 28 ± 12 IU/L, $P < 0.05$) (Figure 1E), ALT (744 ± 35 IU/L *vs* 32 ± 77 IU/L, $P < 0.05$) (Figure 1F) and total bilirubin levels than those in group Ⅰ (4.45 ± 0.63 mg/dL *vs* 1.41 ± 0.19 mg/dL, $P < 0.01$) (Figure 1G). These results suggested that group Ⅱ mice were in the process of liver failure, and these results are consistent with previous reports[13,44-46].

Focal and/or patchy necrosis is an important finding after hepatectomy[13,44]. Progressive necrosis is found from the early postoperative period[13,44-46]. We observed that parenchymal hemorrhage and necrosis occurred as early as 1 h after 80%-PH, and increased in number and size at 12 h and 24 h (data not shown), consistent with recent reports[13,44-46].

MMP-9 is upregulated in the remnant liver with hemorrhage and necrosis

MMP-9 has been implicated in acute liver failure[14,29]. We investigated MMP-9 expression in the remnant livers of both groups Ⅰ and Ⅱ 6 h after 80%-PH. Western blotting analysis demonstrated that MMP-9 was upregulated in group Ⅱ compared with group Ⅰ ($P < 0.01$) (Figure 2A and B). When we examined immunohistochemical staining for MMP-9, hepatocytes showed no expression of MMP-9. However, MMP-9 was mainly observed in round-shaped cells and in stellate-shaped cells (Figure 3A and B). To evaluate the association between necrosis and MMP-9, we compared the expression of MMP-9 in 10 randomly selected fields of 3-mm^2 in area with and without hemorrhage and necrosis. MMP-9 expression in the foci of hemorrhage and necrosis was higher than that in normal liver parenchyma ($P < 0.05$) (Figure 3C). These results suggest that MMP-9 expression is associated with the development of hemorrhage and necrosis in the remnant liver after 80%-PH, which is consistent with previous reports[14,29].

CD11b-positive neutrophils are the main source of MMP-9 in the remnant liver

To determine the source of upregulated MMP-9 in the

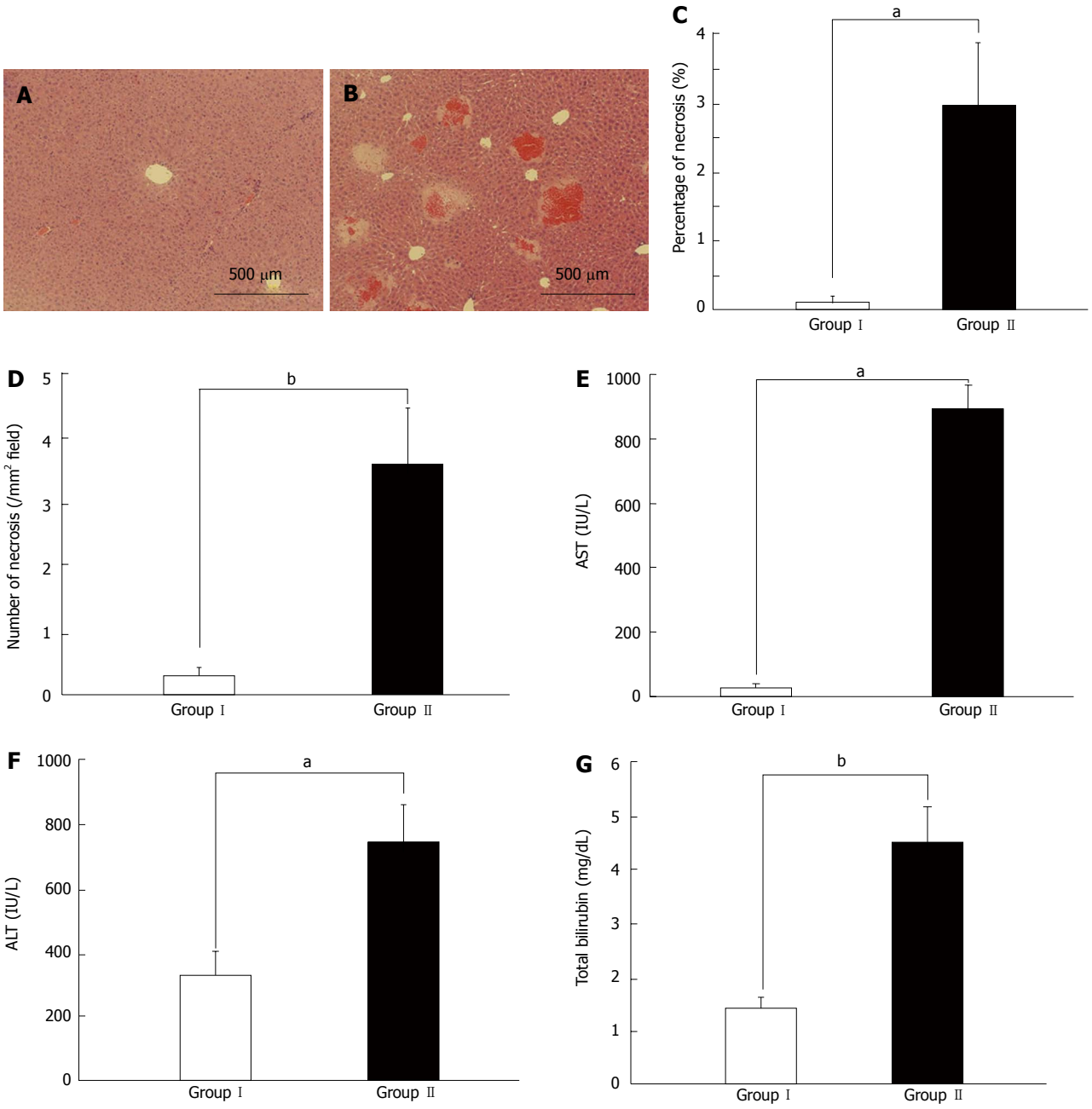

Figure 1 Histological and hematological differences between groups Ⅰ and Ⅱ at 6 h after 80%-partial hepatectomy. A: Histological findings in group Ⅰ mice; B: Representative images of remnant liver histology demonstrating significant multiple foci of hemorrhage and necrosis in group Ⅱ mice; C: Quantitation of the size (area percentage) of hemorrhage and necrosis: Significantly larger areas and more hemorrhagic and necrotic foci were observed in group Ⅱ mice than in group Ⅰ mice ($^aP < 0.05$); D: Quantitation of the number of foci of hemorrhage and necrosis per mm^2: Significantly larger areas and more hemorrhagic and necrotic foci were observed in group Ⅱ mice than in group Ⅰ mice ($^bP < 0.01$); E: Aspartate aminotransferase (AST) levels were significantly higher in group Ⅱ mice than those in group Ⅰ mice ($^aP < 0.05$); F: Alanine aminotransaminase (ALT) levels were significantly higher in group Ⅱ mice than those in group Ⅰ mice ($^aP < 0.05$); G: Total bilirubin levels were significantly higher in group Ⅱ mice than those in group Ⅰ mice ($^bP < 0.01$).

remnant liver, we performed double immunofluorescent analysis with MMP-9 and cell marker antibodies. CD68 and desmin were used for Kupffer cells and hepatic stellate cells, respectively. MMP-9 was not observed in CD68-positive Kupffer cells (Figure 4A-C). Weak immunoreactivity to MMP-9 was observed in desmin-positive hepatic stellate cells (Figure 4D-F). In contrast, we consistently found MMP-9 expression in CD11b-positive cells (Figure 4G-I). These results suggest that infiltrating neutrophils are the source of MMP-9 found in the foci of hemorrhage and necrosis in the remnant liver after 80%-PH, which is consistent with previous reports[28,35].

MMP-9 deletion reduces parenchymal hemorrhage and necrosis

To further confirm the role of MMP-9 in the development of liver hemorrhage and necrosis after 80%-PH, we performed liver resections in WT, MMP-9(-/-), and

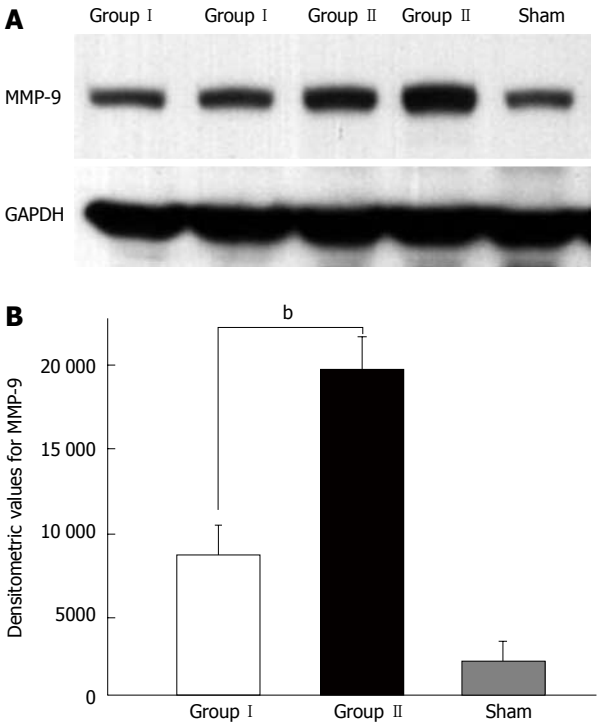

Figure 2 Western blotting analysis for matrix metalloproteinase-9 in remnant livers with (group Ⅱ) and without (group Ⅰ) foci of hemorrhage and necrosis after 80%-partial hepatectomy. Representative immunoblot (A) and histogram (B) show enhanced expression of matrix metalloproteinase (MMP)-9 protein in group Ⅱ mice compared with that in group Ⅰ mice and sham controls ($^{b}P < 0.01$).

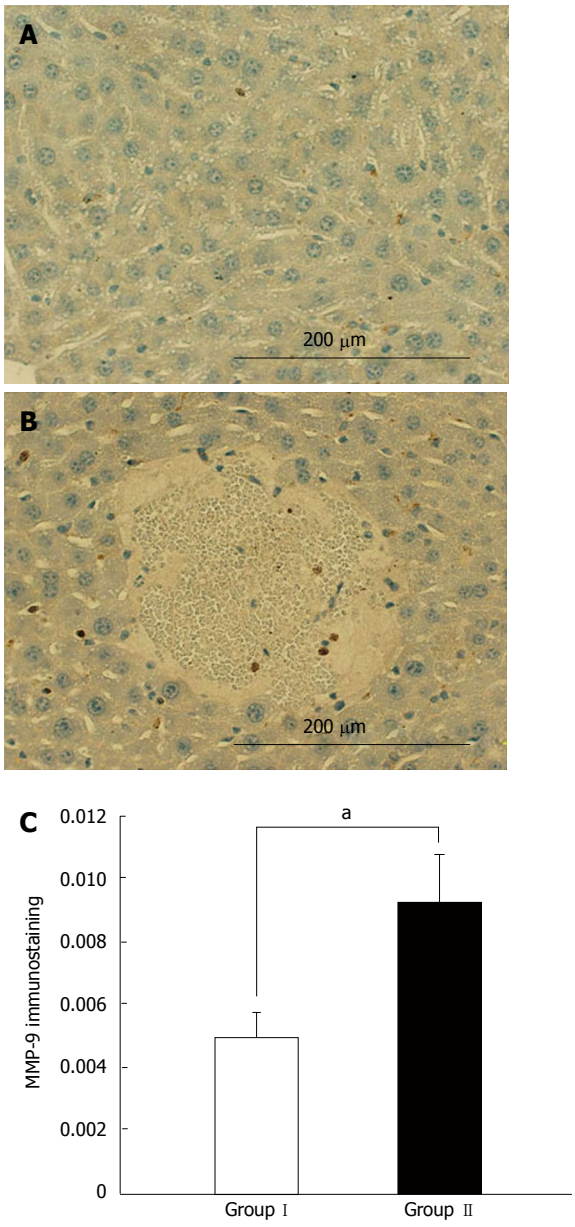

Figure 3 Immunohistochemical staining for matrix metalloproteinase-9 in the remnant liver. A: Representative images of matrix metalloproteinase (MMP)-9 staining in a non-necrotic area in group Ⅱ; B: Representative images of MMP-9 staining in a necrotic area in group Ⅱ; C: Histogram of MMP-9 expression as described in the material and methods section: Enhanced MMP-9 expression was observed in areas close to the focus of necrosis ($^{a}P < 0.05$).

TIMP-1(-/-) mice. TIMP-1 is a physiological tissue inhibitor of MMP-9, which increases MMP-9 activity when deleted[36,37]. We compared the size (area percentage) and the number of hemorrhagic and necrotic foci in the remnant livers. There were significantly fewer and smaller hemorrhagic and necrotic lesions in MMP-9(-/-) mice compared with WT and TIMP-1(-/-) mice (Figure 5A-C), (necrotic area: 0.49% ± 0.14% *vs* 1.74% ± 0.27% *vs* 1.82% ± 0.40%, $P < 0.01$, Figure 5D) (number of necrotic foci: 0.64 ± 0.18 *vs* 1.50 ± 0.21 *vs* 1.76 ± 0.39, $P < 0.05$, Figure 5E). There were no differences in the size and number of necrotic foci in WT and TIMP-1(-/-) animals.

Western blotting and gelatin zymography showed that MMP-9 expression and activity were present in WT and TIMP-1(-/-) mice but were absent in MMP-9(-/-) mice (Figure 6A and B). There was no difference in MMP-2 expression, a gelatinase, which is closely related to MMP-9, in the three groups of mice. In situ gelatin zymography showed significantly less MMP-9 activity in the remnant liver of MMP-9(-/-) mice compared with that in WT and TIMP-1(-/-) mice (Figure 6C-E). These results support our other findings that MMP-9 contributes to the development of parenchymal hemorrhage and necrosis in the remnant liver after 80%-PH.

MMP-9 deletion inhibits hepatic infiltration of neutrophils

To evaluate the effect of MMP-9 deficiency on hepatic neutrophil infiltration, we performed double immunofluorescent analysis of MMP-9 and myeloperoxidase, as previously described[47]. As shown in Figure 7A-C, we identified myeloperoxidase-positive neutrophils by their characteristic staining pattern in cytoplasmic azurophilic granules[48]. There were significantly fewer infiltrated neutrophils in MMP-9(-/-) mice than in WT and TIMP-1(-/-) mice (3.3 ± 0.4 *vs* 7.0 ± 1.1 *vs* 6.0 ± 0.8 per hpf, $P < 0.05$) (Figure 7D). These results suggest that MMP-9 deletion inhibits neutrophil infiltration of the remnant liver after massive hepatectomy.

Inhibition of MMP-9 with MMP-9 mAb or GM6001 ameliorates liver hemorrhage and necrosis

We performed an MMP-9 inhibition experiment with a

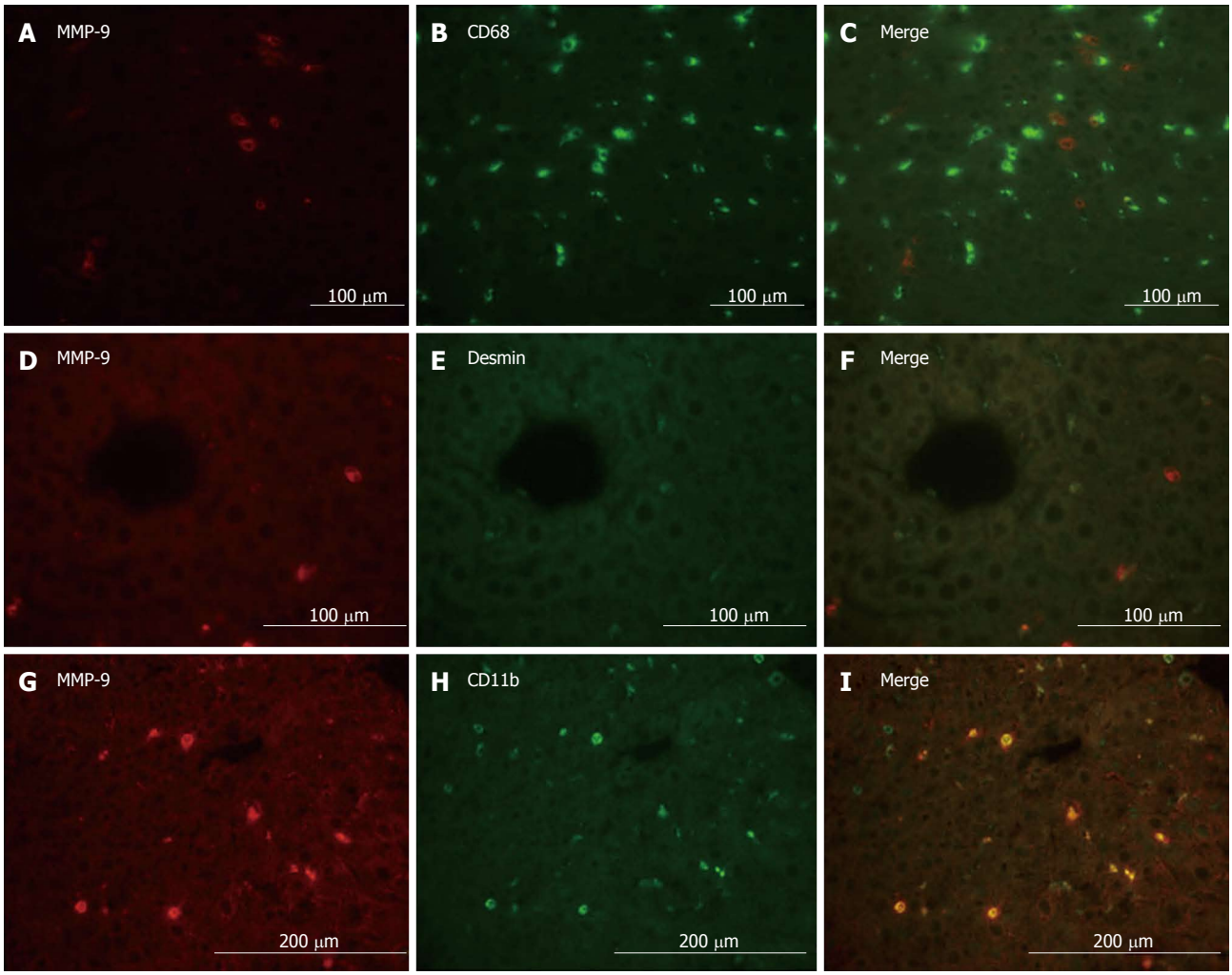

Figure 4 Co-localization analysis by dual immunofluorescence in the remnant liver after 80%-partial hepatectomy. Co-localization analysis by dual immunofluorescence in the remnant liver for (A) matrix metalloproteinase (MMP)-9 labeled in red (Alexa Fluor 568), (B) CD68 labeled in green (Alexa Fluor 488), and (C) both MMP-9 and CD68; for (D) MMP-9 labeled in red (Alexa Fluor 568), (E) desmin labeled in green (Alexa Fluor 488), and (F) both MMP-9 and desmin; and for (G) MMP-9 labeled in red (Alexa Fluor 568), (H) CD11b labeled in green (Alexa Fluor 488), and (I) both MMP-9 and CD11b. These co-localization results demonstrate that MMP-9 protein expression is mainly localized in CD11b-positive cells, and to a lesser extent in desmin-positive cells.

specific MMP-9 monoclonal antibody (MMP-9 mAb) and the broad-spectrum MMP inhibitor GM6001, as previously described[40]. There were significantly smaller and fewer necrotic areas in the remnant liver in mice treated with MMP-9 mAb than in control IgG mice (necrotic area: 0.17% ± 0.15% *vs* 1.81% ± 0.66%, $P < 0.05$; number of necrotic foci: 0.23 ± 0.16 *vs* 1.23 ± 0.44, $P < 0.05$) (Figure 8A and B). We showed inhibition of gelatinolytic activity with *in situ* gelatin zymography[40], which demonstrated significantly reduced MMP-9 activity in the MMP-9 mAb-treated group compared with that in the IgG-treated group (Figure 8C and D). Similarly, the number of infiltrated neutrophils was significantly lower in the group treated with MMP-9 mAb than that in the group treated with IgG (4.3 ± 1.1 *vs* 9.1 ± 1.9 per hpf, $P < 0.05$) (Figure 8E-G).

We observed similar results with GM6001. Treatment with GM6001 showed significant suppression of liver necrosis compared with the DMSO vehicle (necrotic area: 0.19% ± 0.13% *vs* 1.04% ± 0.36%, $P < 0.05$; number of necrotic foci: 0.35 ± 0.19 *vs* 1.12 ± 0.31, $P < 0.05$). Neutrophil infiltration was significantly suppressed in the GM6001 group compared with that in the DMSO group (5.5 ± 1.0 *vs* 9.2 ± 1.3 per hpf, $P < 0.05$). In situ zymographic analysis showed a significant reduction in gelatinolytic activity in the GM6001 group compared with that in the DMSO group (data not shown). Collectively, these results demonstrate that MMP-9 inhibition plays an important role in protecting mice from developing parenchymal hemorrhage and necrosis after 80%-PH.

DISCUSSION

IHR after extended liver resection or a SFSG after SOLT is prone to develop parenchymal hemorrhage and necrosis. Uncontrolled progression of the foci of hemorrhage and necrosis leads to liver failure. The role of MMP-9 in liver injury in the hepatic remnant has not been investigated. In this study, we investigated the role of MMP-9 in the development of hemorrhage and necrosis in IHR

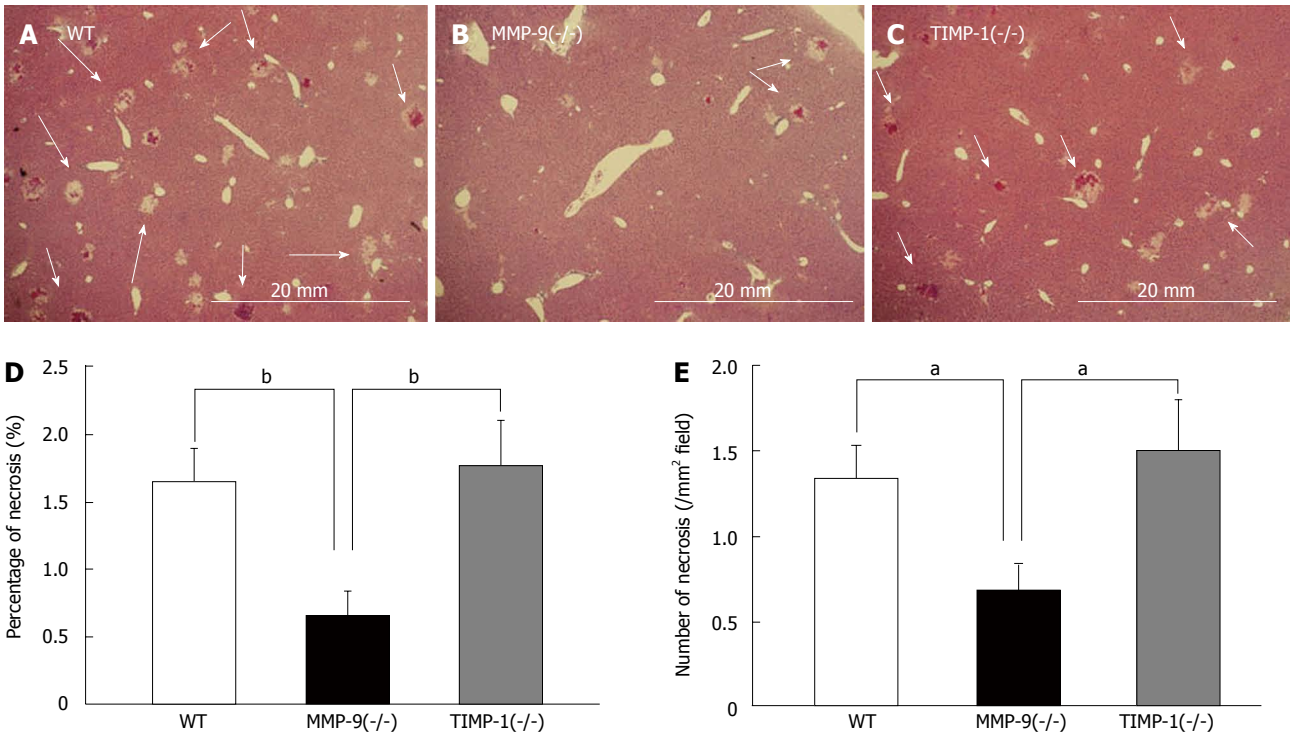

Figure 5 Histological analysis in each strain. Histological analysis between wild-type (WT, *n* = 15) (A), matrix metalloproteinase (MMP)-9(-/-) (*n* = 15) (B), and tissue inhibitors of metalloproteinases (TIMP)-1 (-/-) mice (*n* = 14) (C) at 6 h after 80%-partial hepatectomy (PH). Representative images of remnant liver histology at 6 h after 80%-PH in WT, MMP-9(-/-), and TIMP-1(-/-) mice. Foci of hemorrhage and necrosis are denoted by white arrows. The percentage of the necrotic area (D) and the number of necrotic foci per mm^2 (E) in remnant livers of study mice are shown. Significantly smaller and fewer necrotic foci in the remnant liver were observed in MMP-9(-/-) mice compared with WT and TIMP-1(-/-) mice ($^aP < 0.05$, $^bP < 0.01$).

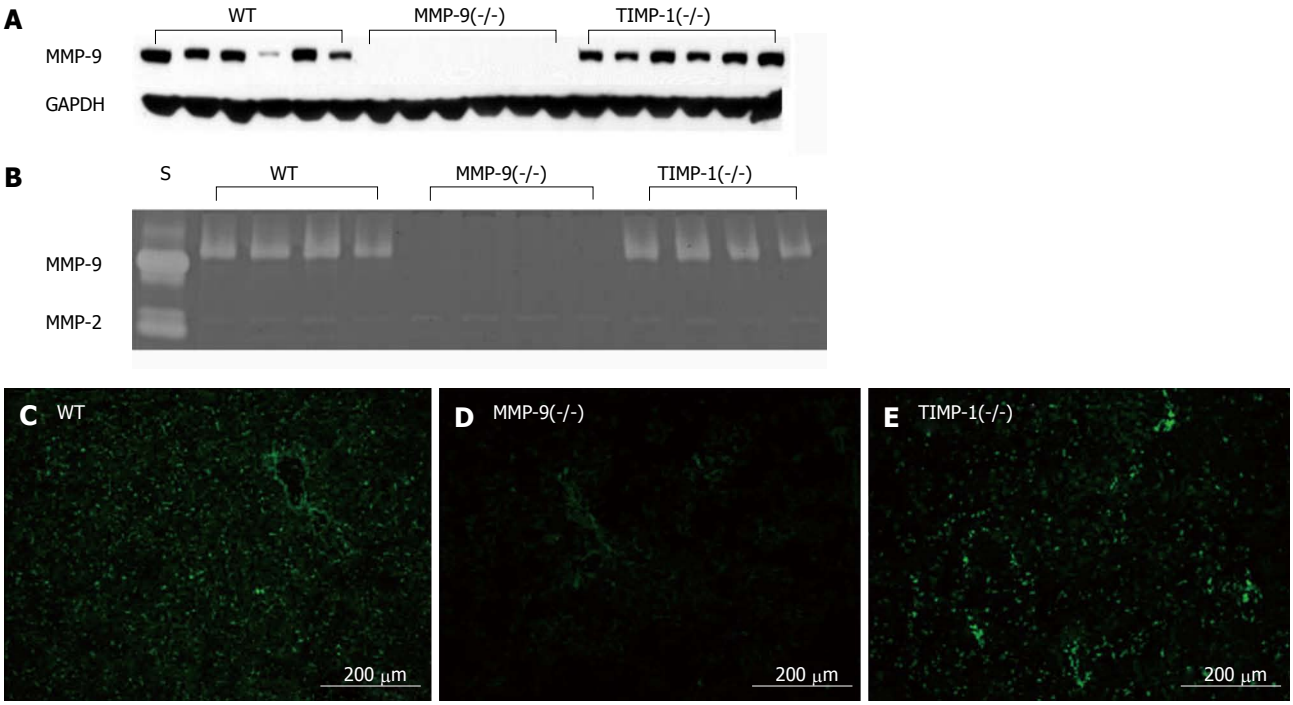

Figure 6 Expression and activity of matrix metalloproteinase-9. Expression and activity of matrix metalloproteinase (MMP)-9 were determined using (A) western blotting analysis for MMP-9 with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as a control, (B) polyacrylamide gel electrophoresis gelatin zymography with human MMP-9 and -2 standards (S), and *in situ* fluorescence gelatin zymography in the remnant livers of wild-type (WT), MMP-9(-/-), and tissue inhibitors of metalloproteinases (TIMP)-1(-/-) mice at 6 h after 80%-partial hepatectomy. We observed MMP-9 protein expression and activity in WT mice (C), but they were absent in MMP-9(-/-) mice (D). Enhancement of MMP-9 protein expression and activity in TIMP-1(-/-) mice was observed (E).

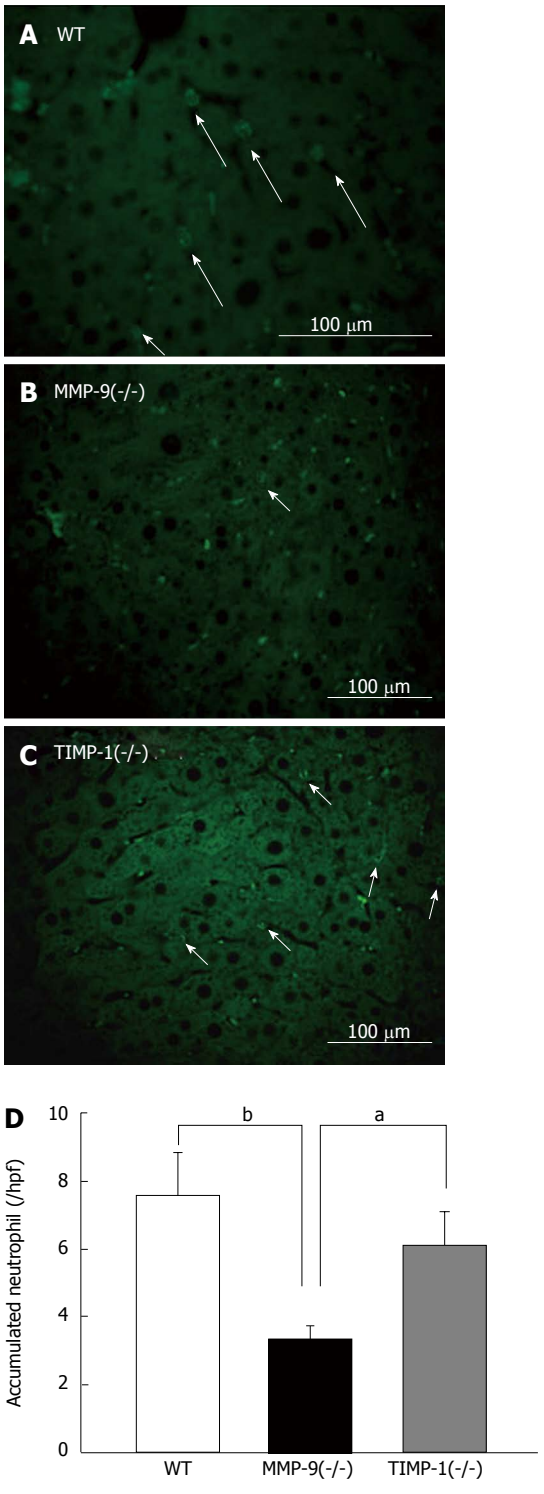

Figure 7 Immunohistochemical analysis of accumulated neutrophils with myeloperoxidase staining in remnant livers of wild-type, matrix metalloproteinase-9(-/-), and tissue inhibitors of metalloproteinases-1(-/-) mice at 6 h after 80%-partial hepatectomy. Representative images of myeloperoxidase staining, labeled in green (Alexa Fluor 488), of cytoplasmic azurophilic granules in neutrophils (arrows) in wild-type (WT) mice in remnant liver sections in (A) WT, (B) matrix metalloproteinase (MMP)-9(-/-), and (C) tissue inhibitors of metalloproteinases (TIMP)-1(-/-) mice. A histogram shows the number of accumulated neutrophils in the remnant liver (D). Significantly fewer neutrophils were observed in the remnant liver in MMP-9(-/-) mice than in WT and TIMP-1(-/-) mice ($^{a}P < 0.05$, $^{b}P < 0.01$).

after 80%-PH in mice. We demonstrated that in IHR 6 h postoperatively, MMP-9 was upregulated in infiltrating neutrophils associated with the foci of hemorrhage and necrosis. Blocking MMP-9 expression by using a synthetic inhibitor, a specific monoclonal antibody, or gene deletion significantly ameliorated the formation of parenchymal hemorrhage and necrosis.

In the current study, we found that the number and percentage of necrotic foci were decreased in MMP-9(-/-) mice compared with WT mice. While TIMP-1(-/-) mice appeared to show slightly increased necrosis, there were no statistical differences between WT and TIMP-1(-/-) mice. TIMP-1 is a physiological tissue inhibitor of MMP-9[36,37], and therefore, MMP-9 activity is increased under the absence of TIMP-1[36,37]. Our results demonstrated that a decrease or absence of MMP-9 prevented liver injury, and that a physiological inhibitor of MMP-9, i.e., TIMP-1, caused similar liver damage in WT mice with IHR after 80%-PH. One possible explanation for the similar damage between WT and TIMP-1(-/-) mice is that liver injury after 80%-PH is very severe in these mice.

In our study of IHR after 80%-PH in mice, we found that infiltrating neutrophils carry MMP-9, which appears to be responsible for the development of hemorrhage and necrosis. This is consistent with previous reports[14,28,35]. Wielockx and colleagues showed that MMPs, including MMP-9, play a central role in the destruction of sinusoidal integrity, resulting in hemorrhage and necrosis[28]. They speculated that MMPs could be derived from infiltrating neutrophils, resident hepatic stellate cells, and Kupffer cells. Recently, hepatic stellate cells have been shown to play a critical role in acute liver failure induced by lipopolysaccharide and beta-galactosamine or carbon tetrachloride by releasing MMP-9, which disrupts sinusoid integrity leading to sinusoidal collapse and liver failure[29]. Similarly, in hepatic warm ischemia-reperfusion injury, hepatic MMP-9 and MMP-2 induce sinusoidal injury in a manner that is independent of neutrophil cytotoxicity[35]. Together, these data suggest that decreasing MMP-9 expression is useful to reduce early sinusoidal injury in IHR and SFSGs.

Inhibition of MMP-9 appears to be a potential therapeutic option in ischemic liver injury. In a previous study in rats, 70% of the liver was rendered ischemic for 90 minutes followed by reperfusion; a phosphinic MMP inhibitor attenuated the liver injury and necrosis[49]. Gene deletion of MMP-9 mitigates liver injury after 90 minutes of warm ischemia and reperfusion in mice[34,35]. Inhibition of MMP-9 has been shown to protect rat livers from cold preservation-warm reperfusion injury[34,35]. Interestingly, after cold ischemic preservation and warm reperfusion, it was found that MMP-9 and MMP-2 were not of hepatocellular origin but from an extrahepatic source[34,35]. Therefore, collectively, these data support MMP-9 reduction as a potential pathway to improve parenchymal integrity and function in IHR after extended liver resection or a SFSG after SOLT.

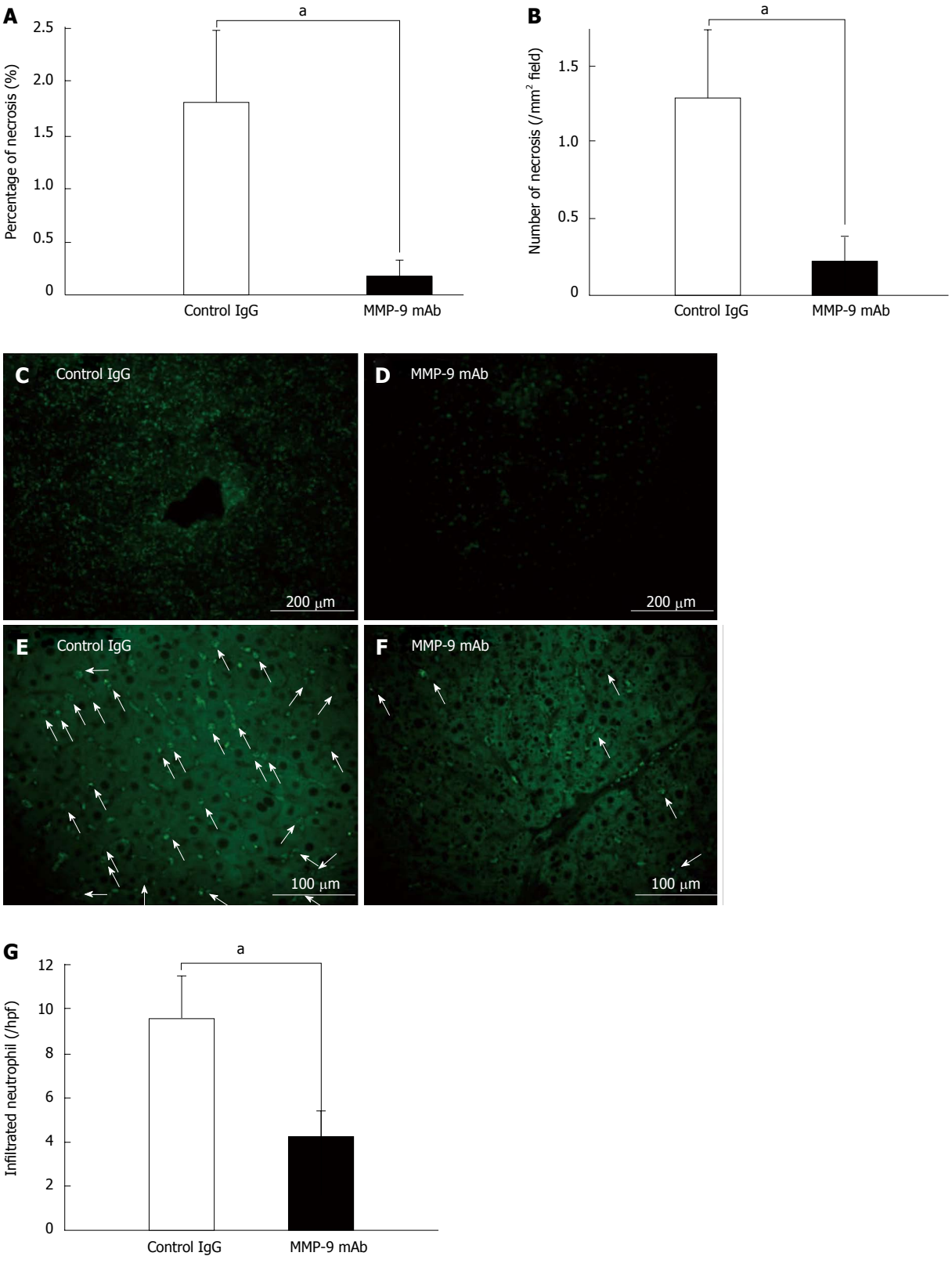

Figure 8 Effect of matrix metalloproteinase-9 monoclonal antibody on 80%-partial hepatectomy. A, B: Treatment with matrix metalloproteinase-9 monoclonal antibody (MMP-9 mAb) in mice with 80%-partial hepatectomy (PH) resulted in a significant reduction in the size (percent area) and number of hemorrhagic and necrotic foci 6 h after 80%-PH compared with treatment using control IgG ($^aP < 0.05$); C, D: Suppression of MMP-9 activity as determined by *in situ* fluorescence gelatin zymography compared with control IgG-treated mice; E, F: There was significantly less myeloperoxidase staining (arrows) in remnant liver sections in mice treated with MMP-9 mAb compared with that in control IgG-treated mice; G: A histogram shows the number of accumulated neutrophils in the remnant liver 6 h after 80%-PH. Significantly fewer neutrophils were observed in MMP-9 mAb-treated mice than in control IgG-treated animals ($^aP < 0.05$).

Although MMP-9 can cause liver injury in IHR after extended liver resection or SFSGs after SOLT, MMP-9 is also essential for liver regeneration[50]. It has been shown that MMP-9 releases hepatic growth factor from the hepatic extracellular matrix during the priming phase of liver regeneration[51,52]. Together with its physiological tissue inhibitor, TIMP-1, MMP-9 participates in regulating the hepatocellular cell cycle and proliferation[51]. MMP-9 is temporally correlated to angiogenesis and regeneration in the liver. Therefore, prolonged inhibition of MMP-9 might be clinically undesirable. Recent studies have suggested that inhibition of MMP-9 to ameliorate the early formation of parenchymal hemorrhage and necrosis should probably be started within and be limited to the first 2 d after extended liver resection or SOLT with SFSGs[50,52]. However, the use of MMP-9 inhibition in the clinical management of IHR or SFSGs requires further investigation and understanding to optimize its therapeutic effectiveness.

After extended liver resection or SOLT with SFSGs, portal hyperperfusion is likely to occur. Although increased portal hypertension is important in liver regeneration, uncontrolled acute portal hypertension with portal hyperperfusion can be detrimental[21,25,53]. In SOLT with SFSGs, portal hypertension and hyperperfusion are known to contribute to SFSL failure[25,54,55]. In experimental models[25,53,56] and in clinical LT with SFSGs[8,9,25,54,55], decompression of portal hyperperfusion has been shown to significantly attenuate hepatic hemorrhage and necrosis[57,58]. Evidence suggests that surgical and anatomical reduction of portal hyperperfusion is important in the management of IHR and SFSGs.

The molecular mechanisms by which portal hyperperfusion affects the outcome of IHR after extended hepatectomy and SFSGs after SOLT are poorly understood. Shear stress has been shown to induce nitric oxide production in microvascular endothelial cells[59], which might upregulate MMP-9 synthesis and production[60]. Reduction of portal hyperperfusion could thus indirectly mitigate the effect of MMP-9 production. However, it is not known how portal hyperperfusion relates to MMP-9 with respect to sinusoidal injury leading to parenchymal hemorrhage and necrosis. Other potential important mediators in the injury of IHR or SFSGs include oxidative stress[61] and tumor necrosis factor[62]. Understanding the role of MMP-9 and other signaling pathways will increase our ability to develop therapeutic options to heal and prevent liver injury associated with IHR and SFSGs.

Neutrophils have been consistently implicated in ischemia-reperfusion injury[19,35]. Neutrophils might produce and release MMPs that contribute to initiating sinusoidal injury[14]. Conversely, hepatic MMP-9 might be derived from hepatic stellate cells or sinusoidal cells, including endothelial cells and Kupffer cells[29]. Hepatic-derived MMP-9 might then attract neutrophils[30] into the liver after an injury. Neutrophils could then initiate and/or propagate sinusoidal hemorrhage and progressive necrosis[35]. Therefore, targeting neutrophils for treatment could be important in some diseases and conditions.

The limitations of this study include the following. Although MMP-9 is implicated in the formation of parenchymal hemorrhage and necrosis in IHR after 80%-PH, our study did not address how and where MMP-9 acts on the sinusoidal elements. The cascade of nitric oxide synthetase, which is important in upregulating MMP-9, was not examined[59,60]. Even though we did not observe constitutive MMP-9 in association with parenchymal hemorrhage and necrosis, we could not rule out the possibility that hepatic cells, including sinusoidal endothelial cells, Kupffer cells, and hepatic stellate cells[30], produced MMP-9 at a minute level that was adequate to initiate liver injury with subsequent recruitment of circulating neutrophils. The potential role of T cells was not evaluated[63]. The possibility of myeloperoxidase-associated oxidative inactivation of TIMP-1, thus activating MMP-9, was not considered in this study[64]. We consider that the effects of upregulation of MMP-9 on sinusoidal and endothelial cells should be investigated in detail.

Initially, we hypothesized that MMP-9 plays an important role in parenchymal hemorrhage and necrosis in the small remnant liver. We found significantly less hemorrhagic and necrotic lesions in MMP-9(-/-) remnant livers than in WT and TIMP-1(-/-) remnant livers. Similar results were observed with MMP-9 monoclonal antibody or the synthetic inhibitor GM6001. In conclusion, we demonstrated that MMP-9 plays an important role in the development of hepatic hemorrhage and necrosis in the small liver remnant 6 h after extensive partial hepatectomy. Moreover, we showed that infiltrating neutrophils are critical to the process of liver injury. Targeting MMP-9 could provide a clinically useful tool to optimize the function of a small liver volume, i.e., IHR after extended hepatectomy or SFSGs after SOLT.

ACKNOWLEDGMENTS

We are very grateful to Dickson W Dennis, Monica Castanedes-Casey, Virginia R Phillips, Linda G Rousseau and Melissa E Murray (Department of Neuroscience, Mayo Clinic Florida, Jacksonville, FL, United States) for their diagnostic and technical support for histopathological and immunohistological assessment, and to Kathleen Norton and Lisa Maroski for their editorial assistance.

COMMENTS

Background

In the field of hepatobiliary surgery, liver resection is considered the standard treatment for primary liver tumors and colorectal liver metastases. Major liver resections have been associated with an increased morbidity and mortality compared with more limited resections. In particular, the outcomes are worsened when a chronic underlying liver disease is present. Recent studies have shown that the volume of the remnant liver is correlated with perioperative morbidity and mortality, and an insufficient hepatic remnant (IHR) after extended hepatectomy is a critical issue.

Research frontiers

Matrix metalloproteinases (MMPs) comprise a family of zinc-dependent neutral proteases that can degrade the extracellular matrix and basement membrane.

MMPs play significant roles in cellular regulation, cell-cell communication, and tumor progression. MMP-2 and MMP-9 have been implicated in liver injury and remodeling. Knocking out MMP-9 and other MMPs attenuates liver injury associated with interferon treatments. MMP-9 contributes to the pathogenesis of experimental acute liver failure. Specifically, MMP-9 has been implicated in sinusoidal breakdown leading to extravasation of circulating cells and hemorrhage. MMP-9 plays a pivotal role in ischemia-reperfusion injury. When MMP-9 is blocked with a synthetic inhibitor or by MMP-9 gene deletion, it successfully reverses ischemia-reperfusion injury. Collectively, these data show that MMP-9 is involved in sinusoidal injury in liver failure. However, the role of MMP-9 in the pathogenesis of failure in IHR is not well understood.

Innovations and breakthroughs

Initially, the authors hypothesized that MMP-9 plays an important role in parenchymal hemorrhage and necrosis in the small remnant liver. In this study, the authors found significantly less hemorrhagic and necrotic lesions in MMP-9(-/-) remnant livers than in wild-type and tissue inhibitors of metalloproteinases (TIMP)-1(-/-) remnant livers. Similar results were observed with a MMP-9 monoclonal antibody or synthetic inhibitor GM6001.

Applications

The authors demonstrated that MMP-9 plays an important role in the development of hepatic hemorrhage and necrosis in the small remnant liver 6 h after extensive partial hepatectomy. Moreover, the authors showed that infiltrating neutrophils are critical to the process of liver injury.

Terminology

Targeting MMP-9 could provide a clinically useful tool to optimize the function of a small liver volume, i.e., IHR after extended hepatectomy.

Peer review

Using MMP-9(-/-), wild type, and TIMP-1(-/-) mice, the authors demonstrated that in IHR at 6 h postoperatively MMP-9 is upregulated in the infiltrating neutrophils associated with the foci of hemorrhage and necrosis. Blocking MMP-9 expression by using a synthetic inhibitor, specific monoclonal antibodies, or gene deletion significantly ameliorated the formation of parenchymal hemorrhage and necrosis. The authors finally proposed that MMP is tightly associated with liver parenchymal hemorrhage and necrosis for insufficient liver remnant, which always leads a lethal outcome clinically.

REFERENCES

1 **Simmonds PC**, Primrose JN, Colquitt JL, Garden OJ, Poston GJ, Rees M. Surgical resection of hepatic metastases from colorectal cancer: a systematic review of published studies. *Br J Cancer* 2006; **94**: 982-999

2 **Jaeck D**, Bachellier P, Oussoultzoglou E, Weber JC, Wolf P. Surgical resection of hepatocellular carcinoma. Post-operative outcome and long-term results in Europe: an overview. *Liver Transpl* 2004; **10**: S58-S63

3 **Nagino M**, Kamiya J, Nishio H, Ebata T, Arai T, Nimura Y. Two hundred forty consecutive portal vein embolizations before extended hepatectomy for biliary cancer: surgical outcome and long-term follow-up. *Ann Surg* 2006; **243**: 364-372

4 **Belghiti J**, Hiramatsu K, Benoist S, Massault P, Sauvanet A, Farges O. Seven hundred forty-seven hepatectomies in the 1990s: an update to evaluate the actual risk of liver resection. *J Am Coll Surg* 2000; **191**: 38-46

5 **Shoup M**, Gonen M, D'Angelica M, Jarnagin WR, DeMatteo RP, Schwartz LH, Tuorto S, Blumgart LH, Fong Y. Volumetric analysis predicts hepatic dysfunction in patients undergoing major liver resection. *J Gastrointest Surg* 2003; **7**: 325-330

6 **Ferrero A**, Viganò L, Polastri R, Muratore A, Eminefendic H, Regge D, Capussotti L. Postoperative liver dysfunction and future remnant liver: where is the limit? Results of a prospective study. *World J Surg* 2007; **31**: 1643-1651

7 **Seyama Y**, Makuuchi M. Current surgical treatment for bile duct cancer. *World J Gastroenterol* 2007; **13**: 1505-1515

8 **Hori T**, Uemoto S, Gardner LB, Sibulesky L, Ogura Y, Nguyen JH. Left-sided grafts for living-donor liver transplantation and split grafts for deceased-donor liver transplantation: their impact on long-term survival. *Clin Res Hepatol Gastroenterol* 2012; **36**: 47-52

9 **Ogura Y**, Hori T, El Moghazy WM, Yoshizawa A, Oike F, Mori A, Kaido T, Takada Y, Uemoto S. Portal pressure < 15 mm Hg is a key for successful adult living donor liver transplantation utilizing smaller grafts than before. *Liver Transpl* 2010; **16**: 718-728

10 **van den Broek MA**, Olde Damink SW, Dejong CH, Lang H, Malagó M, Jalan R, Saner FH. Liver failure after partial hepatic resection: definition, pathophysiology, risk factors and treatment. *Liver Int* 2008; **28**: 767-780

11 **Emond JC**, Renz JF, Ferrell LD, Rosenthal P, Lim RC, Roberts JP, Lake JR, Ascher NL. Functional analysis of grafts from living donors. Implications for the treatment of older recipients. *Ann Surg* 1996; **224**: 544-552; discussion 552-554

12 **Dahm F**, Georgiev P, Clavien PA. Small-for-size syndrome after partial liver transplantation: definition, mechanisms of disease and clinical implications. *Am J Transplant* 2005; **5**: 2605-2610

13 **Rudich N**, Zamir G, Pappo O, Shlomai Z, Faroja M, Weiss ID, Wald H, Galun E, Peled A, Wald O. Focal liver necrosis appears early after partial hepatectomy and is dependent on T cells and antigen delivery from the gut. *Liver Int* 2009; **29**: 1273-1284

14 **Ito Y**, Abril ER, Bethea NW, McCuskey MK, Cover C, Jaeschke H, McCuskey RS. Mechanisms and pathophysiological implications of sinusoidal endothelial cell gap formation following treatment with galactosamine/endotoxin in mice. *Am J Physiol Gastrointest Liver Physiol* 2006; **291**: G211-G218

15 **Toth B**, Wilson RB. Early vascular injury induced by 1,2-dimethylhydrazine dihydrochloride. II. Gross and electron microscopic findings. *Arch Pathol* 1972; **93**: 427-434

16 **Chosay JG**, Essani NA, Dunn CJ, Jaeschke H. Neutrophil margination and extravasation in sinusoids and venules of liver during endotoxin-induced injury. *Am J Physiol* 1997; **272**: G1195-G1200

17 **Takenaka K**, Sakaida I, Yasunaga M, Okita K. Ultrastructural study of development of hepatic necrosis induced by TNF-alpha and D-galactosamine. *Dig Dis Sci* 1998; **43**: 887-892

18 **Wack KE**, Ross MA, Zegarra V, Sysko LR, Watkins SC, Stolz DB. Sinusoidal ultrastructure evaluated during the revascularization of regenerating rat liver. *Hepatology* 2001; **33**: 363-378

19 **Tsuchihashi S**, Ke B, Kaldas F, Flynn E, Busuttil RW, Briscoe DM, Kupiec-Weglinski JW. Vascular endothelial growth factor antagonist modulates leukocyte trafficking and protects mouse livers against ischemia/reperfusion injury. *Am J Pathol* 2006; **168**: 695-705

20 **Yachida S**, Kokudo Y, Wakabayashi H, Maeba T, Kaneda K, Maeta H. Morphological and functional alterations to sinusoidal endothelial cells in the early phase of endotoxin-induced liver failure after partial hepatectomy in rats. *Virchows Arch* 1998; **433**: 173-181

21 **Kuroda H**, Kawarada Y, Das BC, Iwata M, Isaji S. Ultrastructural study of the remnant liver after extensive hepatectomy in dogs; especially morphological alterations of sinusoidal endothelial cells. *Hepatogastroenterology* 2000; **47**: 450-454

22 **Man K**, Lo CM, Ng IO, Wong YC, Qin LF, Fan ST, Wong J. Liver transplantation in rats using small-for-size grafts: a study of hemodynamic and morphological changes. *Arch Surg* 2001; **136**: 280-285

23 **Tian Y**, Rüdiger HA, Jochum W, Clavien PA. Comparison of arterialized and nonarterialized orthotopic liver transplantation in mice: prowess or relevant model? *Transplantation* 2002; **74**: 1242-1246

24 **Yao A**, Li X, Pu L, Zhong J, Liu X, Yu Y, Zhang F, Kong L, Sun B, Wang X. Impaired hepatic regeneration by ischemic preconditioning in a rat model of small-for-size liver transplantation. *Transpl Immunol* 2007; **18**: 37-43

25 **Wang H**, Ohkohchi N, Enomoto Y, Usuda M, Miyagi S, Masuoka H, Sekiguchi S, Kawagishi N, Fujimori K, Sato A, Satomi S. Effect of portocaval shunt on residual extreme small

liver after extended hepatectomy in porcine. *World J Surg* 2006; **30**: 2014-2022; discussion 2023-2024
26 **Sternlicht MD**, Werb Z. How matrix metalloproteinases regulate cell behavior. *Annu Rev Cell Dev Biol* 2001; **17**: 463-516
27 **Cauwe B**, Van den Steen PE, Opdenakker G. The biochemical, biological, and pathological kaleidoscope of cell surface substrates processed by matrix metalloproteinases. *Crit Rev Biochem Mol Biol* 2007; **42**: 113-185
28 **Wielockx B**, Lannoy K, Shapiro SD, Itoh T, Itohara S, Vandekerckhove J, Libert C. Inhibition of matrix metalloproteinases blocks lethal hepatitis and apoptosis induced by tumor necrosis factor and allows safe antitumor therapy. *Nat Med* 2001; **7**: 1202-1208
29 **Yan C**, Zhou L, Han YP. Contribution of hepatic stellate cells and matrix metalloproteinase 9 in acute liver failure. *Liver Int* 2008; **28**: 959-971
30 **Hanumegowda UM**, Copple BL, Shibuya M, Malle E, Ganey PE, Roth RA. Basement membrane and matrix metalloproteinases in monocrotaline-induced liver injury. *Toxicol Sci* 2003; **76**: 237-246
31 **Padrissa-Altés S**, Zaouali MA, Franco-Gou R, Bartrons R, Boillot O, Rimola A, Arroyo V, Rodés J, Peralta C, Roselló-Catafau J. Matrix metalloproteinase 2 in reduced-size liver transplantation: beyond the matrix. *Am J Transplant* 2010; **10**: 1167-1177
32 **Ma ZY**, Qian JM, Rui XH, Wang FR, Wang QW, Cui YY, Peng ZH. Inhibition of matrix metalloproteinase-9 attenuates acute small-for-size liver graft injury in rats. *Am J Transplant* 2010; **10**: 784-795
33 **Coito AJ.** Leukocyte transmigration across endothelial and extracellular matrix protein barriers in liver ischemia/reperfusion injury. *Curr Opin Organ Transplant* 2010; Epub ahead of print
34 **Tarrats N**, Moles A, Morales A, García-Ruiz C, Fernández-Checa JC, Marí M. Critical role of tumor necrosis factor receptor 1, but not 2, in hepatic stellate cell proliferation, extracellular matrix remodeling, and liver fibrogenesis. *Hepatology* 2011; **54**: 319-327
35 **Defamie V**, Laurens M, Patrono D, Devel L, Brault A, Saint-Paul MC, Yiotakis A, Barbry P, Gugenheim J, Crenesse D, Dive V, Huet PM, Mari B. Matrix metalloproteinase inhibition protects rat livers from prolonged cold ischemia-warm reperfusion injury. *Hepatology* 2008; **47**: 177-185
36 **Fujimoto M**, Takagi Y, Aoki T, Hayase M, Marumo T, Gomi M, Nishimura M, Kataoka H, Hashimoto N, Nozaki K. Tissue inhibitor of metalloproteinases protect blood-brain barrier disruption in focal cerebral ischemia. *J Cereb Blood Flow Metab* 2008; **28**: 1674-1685
37 **Tejima E**, Guo S, Murata Y, Arai K, Lok J, van Leyen K, Rosell A, Wang X, Lo EH. Neuroprotective effects of overexpressing tissue inhibitor of metalloproteinase TIMP-1. *J Neurotrauma* 2009; **26**: 1935-1941
38 **Hori T,** Ohashi N, Chen F, Baine AMT, Gardner LB, Hata T, Uemoto S, Nguyen JH. Simple and reproducible hepatectomy in the mouse using the clip technique. *World J Gastroenterol* 2012; In press
39 **Makino H**, Togo S, Kubota T, Morioka D, Morita T, Kobayashi T, Tanaka K, Shimizu T, Matsuo K, Nagashima Y, Shimada H. A good model of hepatic failure after excessive hepatectomy in mice. *J Surg Res* 2005; **127**: 171-176
40 **Nguyen JH**, Yamamoto S, Steers J, Sevlever D, Lin W, Shimojima N, Castanedes-Casey M, Genco P, Golde T, Richelson E, Dickson D, McKinney M, Eckman CB. Matrix metalloproteinase-9 contributes to brain extravasation and edema in fulminant hepatic failure mice. *J Hepatol* 2006; **44**: 1105-1114
41 **Hori T,** Uemoto S, Zhao X, Chen F, Baine AMT, Gardner LB, Ohashi N, Conkle F, Castanedes-Casey M, Phillips VR, Rousseau LG, Murray M, Kamo N, Nguyen JH. Surgical guide including innovative techniques for orthotopic liver transplantation in the rat: Key techniques and pitfalls in whole and split liver grafts. *Annals Gastroenterol* 2010; **23**: 270-295
42 **Matkowskyj KA**, Marrero JA, Carroll RE, Danilkovich AV, Green RM, Benya RV. Azoxymethane-induced fulminant hepatic failure in C57BL/6J mice: characterization of a new animal model. *Am J Physiol* 1999; **277**: G455-G462
43 **Bélanger M**, Côté J, Butterworth RF. Neurobiological characterization of an azoxymethane mouse model of acute liver failure. *Neurochem Int* 2006; **48**: 434-440
44 **Panis Y**, McMullan DM, Emond JC. Progressive necrosis after hepatectomy and the pathophysiology of liver failure after massive resection. *Surgery* 1997; **121**: 142-149
45 **Mitchell C**, Willenbring H. A reproducible and well-tolerated method for 2/3 partial hepatectomy in mice. *Nat Protoc* 2008; **3**: 1167-1170
46 **Jin X**, Zhang Z, Beer-Stolz D, Zimmers TA, Koniaris LG. Interleukin-6 inhibits oxidative injury and necrosis after extreme liver resection. *Hepatology* 2007; **46**: 802-812
47 **Shen ZY**, Zhu ZJ, Deng YL, Zheng H, Pan C, Zhang YM, Shi R, Jiang WT, Zhang JJ. Liver retransplantation: report of 80 cases and review of literature. *Hepatobiliary Pancreat Dis Int* 2006; **5**: 180-184
48 **Shen XD**, Ke B, Zhai Y, Gao F, Anselmo D, Lassman CR, Busuttil RW, Kupiec-Weglinski JW. Stat4 and Stat6 signaling in hepatic ischemia/reperfusion injury in mice: HO-1 dependence of Stat4 disruption-mediated cytoprotection. *Hepatology* 2003; **37**: 296-303
49 **Cursio R**, Mari B, Louis K, Rostagno P, Saint-Paul MC, Giudicelli J, Bottero V, Anglard P, Yiotakis A, Dive V, Gugenheim J, Auberger P. Rat liver injury after normothermic ischemia is prevented by a phosphinic matrix metalloproteinase inhibitor. *FASEB J* 2002; **16**: 93-95
50 **Alwayn IP**, Verbesey JE, Kim S, Roy R, Arsenault DA, Greene AK, Novak K, Laforme A, Lee S, Moses MA, Puder M. A critical role for matrix metalloproteinases in liver regeneration. *J Surg Res* 2008; **145**: 192-198
51 **Mohammed FF**, Pennington CJ, Kassiri Z, Rubin JS, Soloway PD, Ruther U, Edwards DR, Khokha R. Metalloproteinase inhibitor TIMP-1 affects hepatocyte cell cycle via HGF activation in murine liver regeneration. *Hepatology* 2005; **41**: 857-867
52 **Olle EW**, Ren X, McClintock SD, Warner RL, Deogracias MP, Johnson KJ, Colletti LM. Matrix metalloproteinase-9 is an important factor in hepatic regeneration after partial hepatectomy in mice. *Hepatology* 2006; **44**: 540-549
53 **Ueno S**, Kobayashi Y, Kurita K, Tanabe G, Aikou T. Effect of prior portosystemic shunt on early hepatic hemodynamics and sinusoids following 84% hepatectomy in dogs. *Res Exp Med* (*Berl*) 1995; **195**: 1-8
54 **Kiuchi T**, Kasahara M, Uryuhara K, Inomata Y, Uemoto S, Asonuma K, Egawa H, Fujita S, Hayashi M, Tanaka K. Impact of graft size mismatching on graft prognosis in liver transplantation from living donors. *Transplantation* 1999; **67**: 321-327
55 **Demetris AJ**, Kelly DM, Eghtesad B, Fontes P, Wallis Marsh J, Tom K, Tan HP, Shaw-Stiffel T, Boig L, Novelli P, Planinsic R, Fung JJ, Marcos A. Pathophysiologic observations and histopathologic recognition of the portal hyperperfusion or small-for-size syndrome. *Am J Surg Pathol* 2006; **30**: 986-993
56 **Ku Y**, Fukumoto T, Nishida T, Tominaga M, Maeda I, Kitagawa T, Takao S, Shiotani M, Tseng A, Kuroda Y. Evidence that portal vein decompression improves survival of canine quarter orthotopic liver transplantation. *Transplantation* 1995; **59**: 1388-1392
57 **Troisi R**, Ricciardi S, Smeets P, Petrovic M, Van Maele G, Colle I, Van Vlierberghe H, de Hemptinne B. Effects of hemiportocaval shunts for inflow modulation on the outcome of small-for-size grafts in living donor liver transplantation. *Am J Transplant* 2005; **5**: 1397-1404
58 **Umeda Y**, Yagi T, Sadamori H, Matsukawa H, Matsuda H, Shinoura S, Mizuno K, Yoshida R, Iwamoto T, Satoh D,

Tanaka N. Effects of prophylactic splenic artery modulation on portal overperfusion and liver regeneration in small-for-size graft. *Transplantation* 2008; **86**: 673-680

59 **Dumont O**, Loufrani L, Henrion D. Key role of the NO-pathway and matrix metalloprotease-9 in high blood flow-induced remodeling of rat resistance arteries. *Arterioscler Thromb Vasc Biol* 2007; **27**: 317-324

60 **Misra S**, Fu AA, Anderson JL, Sethi S, Glockner JF, McKusick MA, Bjarnason H, Woodrum DA, Mukhopadhyay D. The rat femoral arteriovenous fistula model: increased expression of matrix metalloproteinase-2 and -9 at the venous stenosis. *J Vasc Interv Radiol* 2008; **19**: 587-594

61 **Moon KH**, Hood BL, Mukhopadhyay P, Rajesh M, Abdelmegeed MA, Kwon YI, Conrads TP, Veenstra TD, Song BJ, Pacher P. Oxidative inactivation of key mitochondrial proteins leads to dysfunction and injury in hepatic ischemia reperfusion. *Gastroenterology* 2008; **135**: 1344-1357

62 **Tian Y**, Jochum W, Georgiev P, Moritz W, Graf R, Clavien PA. Kupffer cell-dependent TNF-alpha signaling mediates injury in the arterialized small-for-size liver transplantation in the mouse. *Proc Natl Acad Sci USA* 2006; **103**: 4598-4603

63 **Khandoga A**, Hanschen M, Kessler JS, Krombach F. CD4+ T cells contribute to postischemic liver injury in mice by interacting with sinusoidal endothelium and platelets. *Hepatology* 2006; **43**: 306-315

64 **Wang Y**, Rosen H, Madtes DK, Shao B, Martin TR, Heinecke JW, Fu X. Myeloperoxidase inactivates TIMP-1 by oxidizing its N-terminal cysteine residue: an oxidative mechanism for regulating proteolysis during inflammation. *J Biol Chem* 2007; **282**: 31826-31834

S- Editor Cheng JX **L- Editor** A **E- Editor** Zhang DN