ELSEVIER

Hepatology Research 24 (2002) 174–183

Hepatology Research

www.elsevier.com/locate/ihepcom

Anti-transforming growth factor-β1 antibody improves survival rate following partial hepatectomy in cirrhotic rats

Kazunori Ohara *, Mitsuo Kusano

The Second Department of Surgery, School of Medicine, Showa University, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142-8666, Japan

Received 21 December 2001; received in revised form 15 February 2002; accepted 20 February 2002

Abstract

In a cirrhotic liver, regenerative ability is so impaired that massive resection easily complicates postoperative liver dysfunction, which frequently leads to life-threatening multiple-organ failure. Transforming growth factor-β1 (TGF-β1) is considered to be a key cytokine regulating both hepatocyte proliferation and matrix expression in fibrosis. Therefore, we investigated the effect of TGF-β1 inhibitors by using a neutralizing antibody on 70% partial hepatectomy (PHx) in cirrhotic rats. Cirrhosis was induced by intraperitoneal injections of dimethylnitrosamine (DMN) three times per week for 3 weeks. Since the cirrhotic rats died within 48 h after PHx, anti-TGF-β1 antibody or phosphate buffered saline (PBS) was administered to the rats from subcutaneously implanted osmotic pump preoperatively. Twenty-four hours after PHx, the rats were sacrificed. The anti-TGF-β1 antibodies suppressed the elevation of TGF-β1 mRNA, and increased both relative liver weight ratio and the hepatocellular DNA synthesis. The blood chemical analysis indicated that the anti-TGF-β1 antibodies significantly suppressed postoperative hyperbilirubinemia. As a result, it improved the survival rate of the rats after PHx. In the present study, we firstly demonstrated that preoperative continuous administration of anti-TGF-β1 antibodies significantly accelerates liver regeneration after PHx in DMN-treated cirrhotic rats.

Keywords: Transforming growth factor-β1 (TGF-β1); Dimethylnitrosamine (DMN); Liver regeneration; Hyperbilirubinemia; MIB-5; TGF-β1 mRNA

1. Introduction

Most of the primary hepatocellular carcinomas (HCC) reported in Japan are associated with chronic hepatitis or liver cirrhosis (LC). Since functionally reserved capacity of the liver in LC patients is reduced, a major hepatectomy is not feasible for most LC patients due to postoperative hepatic failure associated with insufficient remnant liver regeneration. The factors related to regeneration in LC following a hepatectomy are still not well understood.

Hepatocyte growth factor (HGF) was originally identified as the most potent stimulator of DNA synthesis in primary hepatocytes [1,2]. HGF stimulates liver regeneration [3–5], improves hepatic

* Corresponding author. Tel.: +81-3-3784-8541; fax: +81-3-3784-5835

E-mail address: kazu-o@fk9.so-net.ne.jp (K. Ohara).

1386-6346/02/$ - see front matter
PII: S1386-6346(02)00031-1

function on partially hepatectomized rats [6] and protects hepatocytes [7–9]. Moreover, HGF prevents or improves rat experimental hepatic fibrosis/cirrhosis [10,11].

Transforming growth factor-β1 (TGF-β1) was firstly identified in the culture medium conditioned by transformed cells and was named for its ability to induce a transformed phenotype in rodent fibroblasts [12,13]. It is a potent inhibitor of growth of cultured epithelial cells, including hepatocytes [14–19]. Nakamura et al. [17] reported that epidermal growth factor (EGF)-or HGF-stimulated DNA synthesis was inhibited even at a level of 0.1 ng/ml of TGF-β1 in hepatocytes in primary culture. In a rat model, Russell et al. [19] found that liver regeneration after partial hepatectomy (PHx) was suppressed by the administration of TGF-β1. The expression of TGF-β1 mRNA in the liver increases during liver regeneration in rats [20]. Therefore, TGF-β1 seems to be a key regulator of liver regeneration. In addition to being an inhibitor of DNA synthesis by hepatocytes, TGF-β1 is associated with hepatic fibrosis [21]. TGF-β1 increases the production of extracellular matrix (ECM) proteins and their receptors and inhibits the synthesis of matrix-degrading proteolytic enzymes. In the liver, TGF-β1 induces collagen synthesis in lipocytes. These cells, located in subendotherial spaces, are believed to be activated in hepatic fibrosis. Kaido et al. reported that a continuous administration of HGF with portal branch ligation improves the survival rate after extensive hepatectomy in cirrhotic rats [22]. On the other hand, Armendariz-Borunda et al. reported that the administration of TGF-β1 antibodies extends the proliferative response of the regenerating liver [23]. Thus, we examined the effect of a neutralizing antibody against TGF-β1 on cirrhotic liver induced by dimethylnitrosamine (DMN) with 70% PHx in rats.

2. Materials and methods

2.1. Animals

Male F344 rats aged 6 weeks (Saitama Experimental Animals Supply Co., Saitama, Japan) were fed a nutritionally balanced rodent diet and water ad libitum. During all experimental procedures, the animals were treated in accordance with the guidelines outlined in *Guide for the Care and Use of Laboratory Animals*.

2.2. Experimental design

As an experimental model of cirrhosis, we used rats treated with DMN (Sigma Chemical Co., St Louis, MO), a reproducible animal model of hepatic cirrhosis [24,25]. One percent DMN dissolved in saline was given intraperitoneally at 1 ml/kg body weight for 3 consecutive days per week for 3 weeks. Anti-TGF-β1 antibody (Genzyme/Techne., Mineapolis, MN) was prepared as described previously [23]. Anti-TGF-β1 antibody was dissolved in 10 mmol/l phosphate buffered saline (PBS) (pH 7.4), containing 0.7 mol/l NaCl, with the final concentration adjusted to 1 mg/ml. We found that the cirrhotic rats died within 48 h after PHx, when performed within 5 days after the last DMN injection in our preliminary study. Five days after the last injection, PHx was performed under ether anesthesia according to the method described by Higgins and Anderson [26]. Twenty-four hours before PHx, anti-TGF-β1 antibodies were continuously administered to the rats in the anti-TGF-β1 antibody treated group from an osmotic pump implanted subcutaneously (Alzet model 1003D; capacity: 100 μl; pump rate: 1 μl/h; Alza, Palo Alto, CA). In control cirrhotic rats, the same volume of PBS was administered similarly. Five rats in each group were sacrificed and blood was collected at 0 and 24 h postoperatively. Their livers excised and weighed. Since control cirrhotic rats died within 48 h after PHx, the survival rate in each group was observed during 48 h after PHx for all hepatectomized cirrhotic rats. The above schedule of the surgical procedure and anti-TGF-β1 antibody treatment is summarized in Fig. 1.

2.3. Assessment of postoperative acute liver dysfunction

The amounts of serum glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), total serum bilirubin, and serum

albumin were quantitated for evaluation of postoperative hepatocellular damage. Serum hyaluronic acid (HA) level was quantitated for evaluation of sinusoidal endothelial cellular dysfunction. All parameters were measured using standard laboratory methods.

2.4. Histological examination

For histological analysis, sections from every rat were stained with hematoxyline-eosin and immunohistostaining using antibodies against collagen type IV (PROGEN BIOTECHNIK GMBH, Heiderberg, Germany).

2.5. Measurement of labeling index

The MIB-5 labeling index, using a novel antibody reactive with the equivalent Ki-67 protein, to detect all active phase of the cell cycle (G1, S, G2 and M), was determined immunohistochemically, using the indirect enzyme-linked antibody method. The labeling indices of the hepatocytes were determined by the random evaluation of more than 2000 hepatocytes, and were expressed as the percentage of immunochemically labeled cells.

2.6. Total RNA isolation

Total RNA was isolated from frozen liver tissues using the ISOGEN reagent according to manufacture's description (NIPPON GENE, Toyama, Japan). Average RNA yield was approximately 1 μg RNA/mg rat liver tissue.

2.7. DNA probes and polymerase chain reaction primers

Hybridization probes for real-time PCR analysis were created using the following gene specific primers and reverse transcription polymerase chain reaction (RT-PCR) amplification: rat TGF-β1: 213 bp-fragment (rat TGF-β1 forward 5′-ATG ACA TGA ACC GAC CCT-3′; rat TGF-β1 reverse: 5′-AGA AGT TGG CAT GGT AGC-3′; hybridization probes: 5′-GAG AGC CCT GGA TAC CAA CTA CTG C-3′ -Fluorescein; 5′-LCRed640-TCA GCT CCA CAG AGA AGA ACT GCT G-3′-Phosphorylated).

2.8. RT-PCR and real time quantitative PCR analysis

One microgram of RNA was reverse transcribed for 30 min at 42 °C (cDNA-synthesis), and 5 min at 95 °C (enzyme denaturation) using 1 ml (0.125 μM) of oligo-dT-primer (TaKaRa RNA PCR Kit (AMV) ver. 2.1, Kyoto, Japan). The reaction was immediately put on ice and was stored at −20 °C. PCR analysis was performed using standard protocols. Real time quantitative RT-PCR was undertaken using the LightCycler from Roche Diagnostics GmbH. With this setting, it is possible to detect the amount of template online after each cycle of PCR amplification. Therefore, in addition to the two amplification primers two further template-specific ohgonucleotides, which are fluorescently labeled according to the FRET-principal (for details see manufacturer's instructions), are included in the PCR reaction. As a standard reaction, cDNA

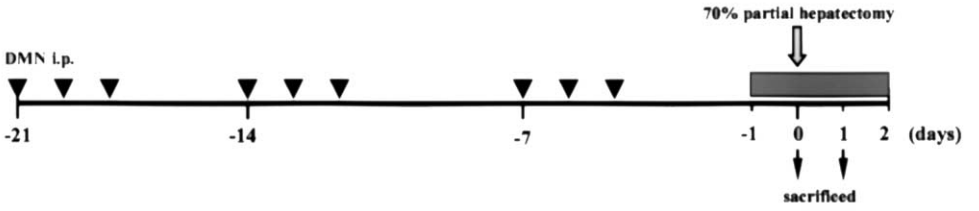

Fig. 1. Experimental schedule. DMN was given intraperitoneally for 3 consequtive days per week for 3 weeks (black arrowheads). Four days after the last DMN injection, anti-TGF-β1 antibody or PBS was continuously administered subcutaneously from an osmotic pump (■) in each groups. The rats were sacrificed at 0 and 24 h after PHx (white arrowheads).

corresponding to 10^4, 10^3, 10^2, or 10 copy total RNA of one sample was examined. Subsequently, cDNAs of different samples were analyzed, each corresponding to 50 ng reverse transcribed total RNA. To determine the absolute copy number of the target transcript, a cloned plasmid DNA for TGF-β1 was used to generate a standard curve. The plasmid DNA was purified according to the manufacture's directons. According to the standard curve, the copy numbers for all unknown samples were obtained automatically. The cycling steps were as follows: one cycle of 95 °C, 10 min; and 45 cycles of 95 °C, 15 s; 60 °C, 15 s; and 72 °C, 9 s.

2.9. Statistical examination

All values are expressed as means ± S.D. Student's *t*-test and Mann–Whitney *U*-test were used to analyze for significant differences. The survival rates were analyzed statistically using the log-rank test. All analyses were performed using the computer-assisted STAT VIEW program (SAS Institute Inc., Gary, NC, USA) and $P < 0.05$ was considered to be significant.

3. Result

3.1. Anti-TGF-β1 antibodies suppressed hyperbilirubinemia after PHx

The total serum bilirubin level were no significant difference between anti-TGF-β1-administred animals (0.30 ± 0.07) and control cirrhotic animals (0.32 ± 0.22) at the time of PHx (Fig. 2). However, in control cirrhotic animals, it increased at the level of 5.28 ± 1.67 mg/dl, anti-TGF-β1 antibodies dramatically suppressed serum total bilirubin elevation at the level of 0.90 ± 0.89 mg/dl following PHx ($P < 0.001$) (Fig. 2).

3.2. Continuous preoperative anti-TGF-β1 antibodies administration histologically prevented postoperative liver damage

Twenty-four hours after PHx, centrilobular damage consisting of hemorrhagic necrosis, congestion, and inflammatory small-round cell infiltrations were observed in the control cirrhotic livers (Fig. 3A), while those findings were decreased in anti-TGF-β1-antibodies administered livers (Fig. 3D). The accumulation of fibrous components in Glisson's shealth and around hepatocytes might be more potently decreased in anti-TGF-β1-administered livers than in control cirrhotic livers (Fig. 3B and E).

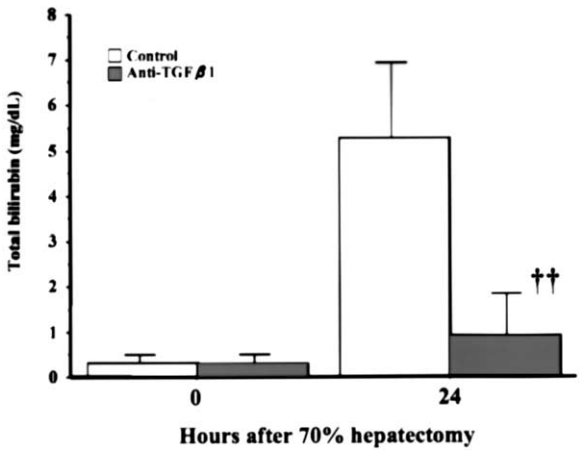

Fig. 2. Serum total bilirubin levels at 0 and 24 h after PHx in the control (white bars) and anti-TGF-β1-treated (shaded bars) groups. Each point represents the mean ± S.D. for five animals, ††, $P < 0.001$ vs. control.

3.3. Anti-TGF-βl antibodies accelerated liver regeneration

DNA synthesis of hepatocytes in regenerated liver was stimulated in the anti-TGF-β1-treated animal group at 24 h after PHx (Fig. 3C and F). The labeling index of hepatocytes in the anti-TGF-β1-treated animal group at 24 h following PHx was 3.2 times higher than that of control cirrhotic animal group ($P < 0.05$) (Fig. 4). Fig. 5 show the increased pattern of regenerated liver weights ratio in both groups. Relative liver weights (remnant liver weights/body weights in %) were no different between anti-TGF-β1-treated animals (0.94 ± 0.09) and control cirrhotic animals (0.96 ± 0.04) at the time of PHx. However, after PHx, relative liver weights of anti-TGF-β1-administered animals increased more promptly

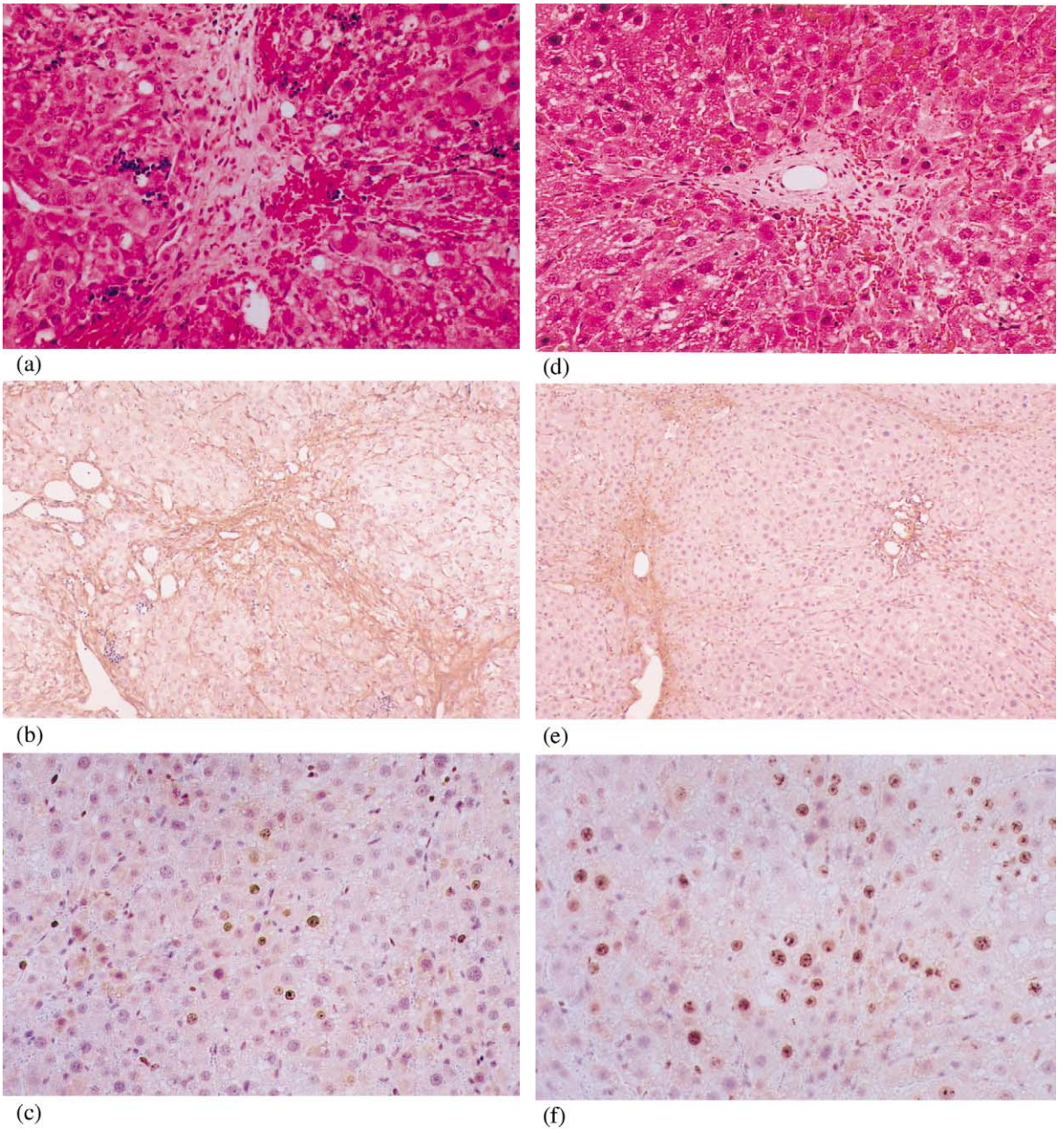

Fig. 3. Histological findings of the liver at 24 h after PHx in the control (A, B, C) or in the anti-TGF-β1-treated (D, E, F) groups. Centrilobular damage consisting of hemorrhagic necrosis, congestion, and inflammatory small-round cell infiltrations shown in panel A was less severe in panel D. Fibrous connective tissue bridges shown in panel B might be decreased in panel E. MIB-5 labeled hepatocytes shown in panel C was increasing in panel F. (Hematoxylin and eosin, original magnification; [A, D] × 200. Collagen type IV, original magnification; [B, E] × 40. MIB-5, original magnification; [C, F] × 100.)

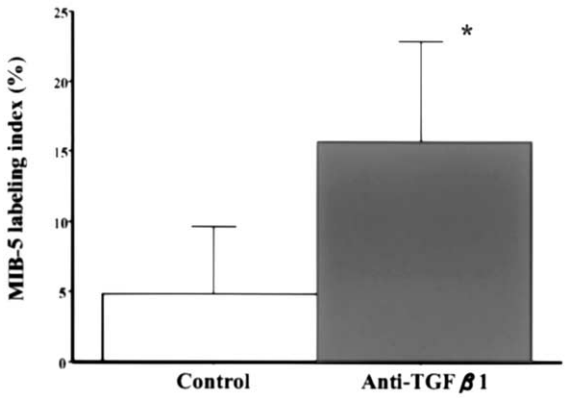

Fig. 4. MIB-5 labeling indices of hepatocytes at 24 h after PHx in the control (white bars) and anti-TGF-β1-treated (shaded bars) groups. The value represents the mean ± S.D. for five animals. *, $P < 0.05$ vs. control.

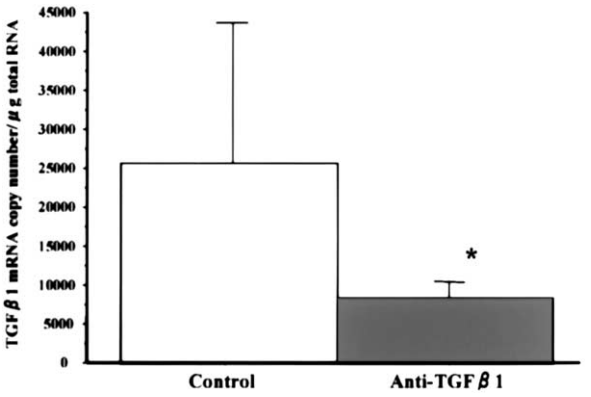

Fig. 6. mRNA expressions analysis of TGF-β1. Light Cycler real time RT-PCR quantification is shwn. Reaction conditions, primers, and hybridization oligonucleotides are described in Section 2. The absolute copy numbers of TGF-β1 were determined. Quantitative data ($n = 5$) for TGF-β1 mRNA expression after PHx was displayed graphically. *, $P < 0.05$ vs. control.

than those of control cirrhotic animals. Those of control animals, 24 h following PHx were 1.02 ± 0.15, whereas those of anti-TGF-β1-administrered animals were 1.30 ± 0.17 ($P < 0.05$).

3.4. Various liver functions after PHx

Liver-specific cytosolic enzyme activities (GOT, GPT) and albumin concentrations were no significant differences between the two groups at each time point (data not shown). Since HA is cleared out from the blood mostly by sinusoidal endothelial cells (SECs), it is often used as a marker of the SECs-specific function. Although there were no significant differences between the two groups at each time point, the elevation of HA level was suppressed in anti-TGF-β1-administered animals (data not shown).

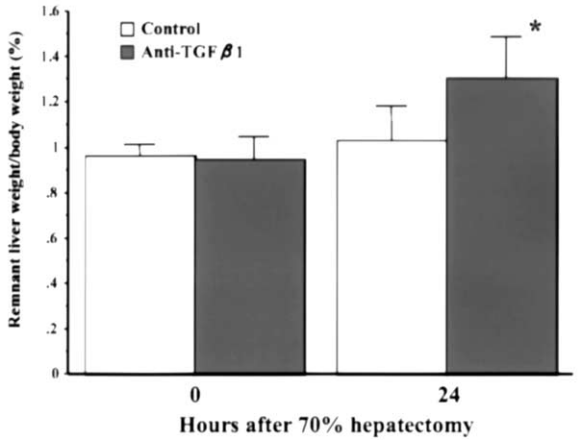

Fig. 5. Relative liver weights at 0 and 24 h after PHx in the control (white bars) and anti-TGF-β1-treated (shaded bars) groups. Relative liver weight was calculated as remnant liver weight/body weight in %. Each point represents the mean ± S.D. for five animals. *, $P < 0.05$ vs. control.

3.5. Analysis of TGF-β1 mRNA expression in remnant liver at 24 h after PHx

To investigate the expression of TGF-β1 in remnant liver, we analyzed mRNA using five animals in each groups at 24 h after PHx. Quantification was performed by real time PCR product analysis using two labeled hybridization probes and Light Cycler technology as described in the Section 2. The absolute copy number of TGF-β1 mRNA in the anti-TGF-β1-administered group was 8373 ± 2150 ($P < 0.05$) while that of control cirrhotic groups was 25 581 ± 18 072 per μg tissue 24 h following PHx (Fig. 6).

3.6. Anti-TGF-β1 antibodies improved survival rate after PHx

To assess possible beneficial effect of anti-TGF-β1 antibodies on the survival rate of cirrhotic rats after PHx, survival studies were performed using ten rats per group. All ten control hepatectomized cirrhotic rats, without anti-TGF-β1 antibodies, died within 48 h (Fig. 7). In contrast, eight of ten hepatectomized cirrhotic rats (80%) with anti-TGF-β1 antibodies survived until experiments were terminated (Fig. 7). These data suggest that anti-TGF-β1 antibodies extremely improves the survival rate in hepatectomized cirrhotic rats.

4. Discussion

In the present study, we showed that anti-TGF-β1 antibodies suppressed hyperbilirubinemia and resultant improved survival rate following PHx in cirrhotic rats. Four possible mechanisms are implicated in the favorable results.

Firstly, anti-TGF-β1 antibodies improved hepatic inflammation. Histologically, inflammatory cell infiltrates were improved by the anti-TGF-β1 administration at 24 h after PHx. TGF-β1, which plays a role in inflammation and wound repair, has been shown to inhibit the proliferation of rat hepatocytes in vitro [17] and in vivo [19]. It has been reported that TGF-β1 is strongly chemotactic for neutrophils, T cells, monocytes, and fibroblasts [27–29]. Moving to the site of the injury, these cells become activated as they encounter higher concentrations of TGF-β1. Monocytes begin secreting fibroblasts growth factor, tumor necrosis factor, and interleukin-1, and fibroblasts increase their synthesis of ECM proteins [27]. TGF-β1 also induces both infiltrating cells and resident cells to produce more of itself. This autoinduction amplifies the biologic effects of TGF-β1 at the injury site and may have a central role in chronic fibrosis [30].

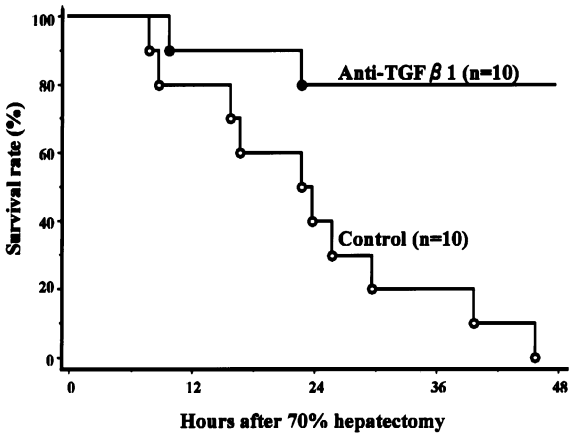

Fig. 7. Effect of preoperative continuous administration of anti-TGF-β1 antibodies on survival rate after PHx for DMN-treated cirrhotic rats ($n = 10$ in each group). Anti-TGF-β1 antibodies significantly improved the survival rate of the rats ($P < 0.001$).

Secondly, preoperative administration of anti-TGF-β1 antibodies accelerated the regeneration of cirrhotic liver after PHx. The regenerative activity has been shown clinically and experimentally to be fairly impaired in cirrhotic liver compared with normal liver. Concerning the time-course of DNA synthesis, Mitsue et al. reported that BrdU-labeled hepatocytes were increased in number within 24 h after PHx in CCl_4 induced cirrhotic rats [31]. Although we examined the MIB-5 labeling indices only at 24 h after PHx, the rate of DNA synthesis was account for the increase in the remnant liver weight. It has been reported that TGF-β is an inhibitor of the proliferation of hepatocytes in vivo [32,33], and that at higher concentrations, TGF-β induces oxidative stress leading to hepatocytes apoptosis [33]. Thus, an attenuation of the action of TGF-β might well accelerate the process of hepatic regeneration and/or prevent hepatocyte damage (such as apoptosis) from occurring. We did not examine TGF-β1 activity; however, TGF-β1 mRNA expression after the hepatectomy correlated well with the suppression of liver cell proliferation [31]. TGF-β1 mRNA expression is believed to reflect TGF-β1 activity. In the present study, whether anti-TGF-β1 antibodies do or do not directly inhibit TGF-β1 mRNA expression should be addressed; however, consequent down-regulation of TGF-β1 mRNA expression may be one of the important mechanisms for the preventive effect of anti-TGF-β1 antibodies on the onset of liver apoptosis.

Thirdly, exogenous anti-TGF-β1 antibodies might protect SECs from injuries caused by PHx. In the present study, the elevation of HA level was suppressed in anti-TGF-β1-administered animals. Regarding SECs, the finding that administration of anti-TGF-β1 antibodies improved the clearance of serum HA suggests that it could prevent the impairment of SECs. The destruction of SECs causes fibrin deposition in hepatic sinusoids [34]. Since fibrosis in the Disse's spaces may block the exchange of molecules between the sinusoidal spaces and the hepatocytes [35], a cessation (or even a partial reduction) of the accumulation of ECM proteins in these spaces may be critical to maintain or improve liver function. Nakamura et al. reported that anti-TGF-β molecular intervention decreased deposition of ECM proteins in the Disse's spaces of the livers during the course of the DMN administration [36]. Recently, the use of anti-TGF-β antibody against the fibrosis has started to draw attention, however, experimental data are only beginning to accumulate [37]. In a preliminary study, the administration of a neutralizing TGF-β antibody decreased rat liver fibrosis produced by bile duct ligation [38], However, the effect was only observed chronic experiments with a more than 3-week administration of anti-TGF-β. Histologically, improvement of collagen IV expression was not evident in the anti-TGF-β1-treated group at 24 h after PHx. Thus, the antifibrogenic effect of anti-TGF-β1 on cirrhotic livers would not play such an important role in the present study.

Finally, it is possible that the anti-TGF-β1 antibodies promoted early restoration of the normal bile excretory system. Whereas the anti-TGF-β1 antibodies could not improve the level of blood chemical data such as albumin and transaminase, it dramatically suppressed hyperbilirubinemia in the present study. The reason for this difference might be that cholestasis caused by this model of hepatectomized cirrhotic rats was dependent upon not only hepatocellular dysfunction but also damage of the bile excretory system. These findings suggest that biliary epithelial cells as well as hepatocytes possess TGF-β1 receptors. Therefore, it would be reasonable to suppose that neutralizing antibody against TGF-β1 promote the repair of cellular damage caused by hepatotoxins and major hepatectomy, leading to early restoration of the normal bile excretory system.

To determine the effect of anti-TGF-β1 antibodies on cirrhotic liver after PHx, we selected a DMN-induced cirrhotic model from among many models of cirrhotic liver. The reason we preferred this cirrhotic model to other models (e.g. CCl_4) is that the administration of DMN causes very early deposition of ECM proteins in the liver, particularly collagen and also severe hepatocellular necrosis that might be useful as a rapid tool for screening antifibrotic agents [39]. The reason we could not observe survival rate for only 48 h after PHx, was its high mortality in this model.

In conclusion, our result demonstrated that the administration of excess amount of anti-TGF-β1 antibodies reduces the serum bilirubin level after PHx, to increase remnant liver weight accompanied with stimulating hepatocyte DNA synthesis. Therefore, a preoperative administration of anti-TGF-β1 antibodies may be useful for clinical application in the case of a hepatectomy with damaged liver.

Acknowledgements

We thank Nobukazu Nishino and Yoko Hoashi of our department for their helpful discussion and expert technical assistance.

References

[1] Matsumoto K, Nakamura T. Hepatocyte growth factor: molecular structure, roles in liver regeneration and other biological functions. Crit Rev Oncog 1992;3:27–54.
[2] Rubin JS, Bottaro DP, Aaronson SA. Hepatocyte growth factor/scatter factor and its receptor, the c-met protooncogene product. Biochim Biophys Acta 1993;1155:357–71.
[3] Fujiwara K, Nagoshi S, Ohno A, et al. Stimulation of liver growth by exogenous human hepatocyte growth factor in normal and partially hepatectomized rats. Hepatology 1993;18:1443–9.
[4] Shiota G, Wang TC, Nakamura T, et al. Hepatocyte growth factor in transgenic mice: effects on hepatocyte growth, liver regeneration and gene expression. Hepatology 1994;19:962–72.

[5] Sakata H, Takayama H, Sharp R, et al. Hepatocyte growth factor/scatter factor overexpression induces growth, abnormal development, and tumor formation in transgenic mouse livers. Cell Growth Differ 1996;7:1513–23.
[6] Ishii T, Sato M, Sudo K, et al. Hepatocyte growth factor stimulates liver regeneration and elevates blood protein level in normal and partially hepatectomized rats. J Biochem Tokyo 1995;117:1105–12.
[7] Ishiki Y, Ohnishi H, Muto Y, et al. Direct evidence that hepatocyte growth factor is a hepatotrophic factor for liver regeneration and has a potent anti-hepatitis effect in vivo. Hepatology 1992;16:1227–53.
[8] Kaido T, Yamaoka S, Tanaka J, et al. Continuous HGF supply from HGF-expressing fibroblasts transplanted into spleen prevents CCU_4-induced acute liver injury in rats. Biochem Biophys Res Commun 1996;218:1–5.
[9] Kaido T, Yamaoka S, Seto S, et al. Continuous hepatocyte growth factor supply prevents lipopolysaccharide-induced liver injury in rats. FEBS Lett 1997;411:378–82.
[10] Matsuda Y, Matsumoto K, Ichida T, et al. Hepatocyte growth factor suppresses the onset of liver cirrhosis and abrogates lethal hepatic dysfunction in rats. J Biochem Tokyo 1995;118:643–9.
[11] Yasuda H, Imai E, Shiota A, et al. Antifibrotic effect of a deletion variant of hepatocyte growth factor on liver fibrosis in rats. Hepatology 1996;24:636–42.
[12] Moses HL, Braun EL, Proper JA, et al. Transforming growth factor production by chemically transformed cells. Cancer Res 1981;41:2842–8.
[13] Roberts AB, Anzano MA, Lamb LC, et al. New class of transforming growth factors potentiated by epidermal growth factor: isolation from non-neoplastic tissues. Proc Natl Acad Sci USA 1981;78:5339–43.
[14] Tucker RF, Shipley GD, Moses HL, et al. Growth inhibitor form BSC-1 cells closely related to platelet type β transforming growth factor. Science 1984;226:705–7.
[15] Moses HL, Tucker RF, Leof EG, et al. Type-β transforming growth factor is a growth stimulator and a growth inhibitor. Cancer Cells 1985;3:65–71.
[16] Carr BI, Hayashi I, Braun EL, et al. Inhibition of DNA synthesis in rat hepatocytes by platelet-derived type β transforming growth factor. Cancer Res 1986;46:2330–4.
[17] Nakamura T, Tomita Y, Hirai R, et al. Inhibitory effect of transforming growth factor-β on DNA synthesis of adult rat hepatocytes in primary culture. Biochem Biophys Res Commun 1985;133:1042–50.
[18] McMahon JB, Richards WL, del Campo AA, et al. Differential effects of transforming growth factor-β on proliferation of normal and malignant rat liver epithelial cells in culture. Cancer Res 1986;46:4665–71.
[19] Russell WE, Coffey RJ Jr, Quellette AJ, et al. Type β transforming growth factor reversibly inhibits the early proliferative response to partial hepatectomy in the rat. Proc Natl Acad Sci USA 1988;85:5126–30.
[20] Braun L, Mead JE, Panzica M, et al. Transforming growth factor β mRNA increases during liver regeneration: a possible paracrine mechanism of growth reguration. Proc Natl Acad Sci USA 1988;85:1539–43.
[21] Czaja MJ, Weiner FR, Flanders KC, et al. In vitro and in vivo association of transforming growth factor-β1 with hepatic fibrosis. J Cell Biol 1989;108:2477–82.
[22] Kaido T, Yoshikawa A, Seto S, et al. Portal branch ligation with a continuous hepatocyte growth factor supply makes extensive hepatectomy possible in cirrhotic rats. Hepatology 1998;28:756–60.
[23] Armendariz-Borunda J, Katai H, Jones CM, et al. Transforming growth factor β gene expression is transiently enhanced at a critical stage during liver regeneration after CCl_4 treatment. Lab Invest 1993;69:283–94.
[24] Jenkins SA, Grandison A, Baxter JN, et al. A dimethylnitrosamine-induced model of cirrhosis and portal hypertension in the rat. J Hepatol 1985;1:489–99.
[25] Jezequel AM, Mancini R, Rinaldesi ML, et al. A morphological study of the early stages of hepatic fibrosis induced by low doses of dimethylnitrosamine in the rat. J Hepatol 1987;5:174–81.
[26] Higgins GM, Anderson RM. Experimental pathology of liver: restoration of liver of the white rat following partial surgical removal. Arch Pathol 1931;12:186–202.
[27] Roberts AB, Joyce ME, Bolander ME, et al. Transforming growth factor-β (TGF-β): a multifunctional effector of both soft and hard tissue regeneration. In: Westermark B, Betsholtz C, Hökfelt B, et al., editors. Growth factors in health and disease: basic and clinical aspects. Amsterdam: Excepta Medica, 1990:89–101.
[28] Wahl SM, Hunt DA, Wakefield LM, et al. Transforming growth factor type β induces monocyte chemotaxis and growth factor production. Proc Natl Acad Sci USA 1987;84:5788–92.
[29] Postlethwaite AE, Keski-Oja J, Moses HL, et al. Stimulation of the chemotactic migration of human fibroblasts by transforming growth factor β. J Exp Med 1987;165:251–6.
[30] Kim SJ, Angel P, Lafyatis R, et al. Autoinduction of transforming growth factor β1 is mediated by the AP-1 complex. Mol Cell Biol 1990;10:1492–7.
[31] Mitsue S, Hamanoue M, Tnanabe G, et al. Expression of HGF and TGF-β1 mRNA after partial hepatectomy in rats with liver cirrhosis. Surg Today 1995;25:237–43.
[32] Carr BI, Hayashi I, Branum EL, et al. Inhibition of DNA synthesis in rat hepatocytes by platelet-derived type β transforming growth factor. Cancer Res 1986;46:2330–4.
[33] Sanchez A, Alverz AM, Benito M, et al. Apoptosis induced by transforming growth factor-β in fetal hepatocyte primary cultures. J Biol Chem 1996;271:7416–22.
[34] Hirata K, Ogata I, Ohta Y, et al. Hepatic sinusoidal cell destruction in the development of intravascular coagulation in acute liver failure of rats. J Pathol 1989;158:157–65.
[35] Friedman SL. The cellular basis of hepatic fibrosis. New Engl J Med 1993;328:1828–35.
[36] Nakamura T, Sakata R, Ueno T, et al. Inhibition of

transforming growth factor β revents progression of liver fibrosis and enhances hepatocyte regeneration in dimethylnitrosamine-treated rats. Hepatology 2000;32:247–55.
[37] Mavier P, Mallet A. Perspectives in the treatment of liver fibrosis. J Hepatol 1995;22(Suppl. 2):111–5.
[38] Strobel D, Wittekind C, Ruoslahti E, et al. In vivo application of a neutralizing TGF-β antibody in experimental liver fibrosis. J Hepatol 1993;18(Suppl. 1):S63.
[39] George J, Ramesh Rao K, Stern R, et al. Dimethylnitrosamine-induced liver injury in rats: the early deposition of collagen. Toxicology 2001;156:129–38.