(n = 1), and *Candida* spp (n = 1), and there was no difference in terms of the strains of isolated bacteria between groups I and II (data not shown).

Furthermore, the administration of additional antibiotics (18.1% vs 25.5%, P = .289) and Clavien grade ≥3a (14.9% vs 20.2%, P = .444) were similar to both groups. Postoperative hospital stays were significantly shorter in group I (median, 12 days; range, 4 to 91 days) than in group II (median, 14 days; range, 5 to 265 days) (P = .034).

Stratified analysis of infectious complications

In our previous series, we analyzed the risk factors for postoperative signs of infections and identified 3 factors (data not shown). On the basis of these results, Table 4 shows the stratified analysis of the signs of infection by the risk factors (ASA classification, intraoperative blood transfusion, and operative time). There were no significant differences between the 2 groups when stratified according to ASA classification (1 or ≥2), intraoperative blood transfusion (yes or no), and operative time (<6 or ≥6 hours).

Comments

The prevention of postoperative infections is obviously important in perioperative management, and it has been thought that antibiotic prophylaxis plays an important role. Liver resection has various disadvantages, such as glucose intolerance associated with loss of hepatic function, lowered immunity (decrease of Kuppfer cell and T-cell function), relatively long operation time, and massive bleeding.[13,14]

Only 2 RCTs on the usefulness of antibiotic prophylaxis after liver resection have been performed.[9,10] Wu et al[9] performed a comparative study of groups given and not given 7 days of cefazolin and gentamicin and found that prophylactic antibiotics did not prevent postoperative infection. In contrast, Sano et al[10] compared patients treated with cefazolin only during surgery and those treated up to POD 5 and found that antibiotic prophylaxis was useful for the prevention of infectious complications in the interim analysis. Thus, the reports are contradictory. The present RCT was performed to evaluate postoperative antibiotic prophylaxis in patients after liver resection without reconstruction of the intestine or biliary tract.

In selecting antibiotics, the target-contaminating bacteria in the field of gastroenterologic surgery are the airborne bacterium *S aureus*, the enteric bacteria *Escherichia coli* and *Klebsiella pneumoniae*, and an anaerobe, *Bacteroides fragilis*. FMOX has superior antibacterial activity against these bacteria compared with cefazolin and cefmetazole: the drug causes fewer adverse events, similar to other cephem antibiotics, and it has less effect on intestinal flora.[15,16] Togo et al[17] reported that the incidence of postoperative infection and the rate of induction of multiple-drug-resistant bacteria were low when FMOX was administered. Therefore, FMOX was considered the most appropriate antibiotic and was thus used for antibiotic prophylaxis, and no patient showed adverse side effects in this study.

From 2 hours preoperatively to directly before surgery is regarded as the most effective time of administration, as indicated in the guidelines of the Centers for Disease Control and Prevention, and administration was started 30 minutes before skin incision.[11,17] Two-hourly administration would be regarded as appropriate for maintaining the appropriate antibiotic concentration during surgery, in light of the fact that the guideline for repeat administration is twice the half-life, and the half-life of FMOX is just under 1 hour, but because this would result in excessive administration during long operations, and the infection rate does not change for up to 3 hours,[18] additional doses were given every 3 hours. One problem during the postoperative administration period is the appearance of resistant strains, and the Centers for Disease Control and Prevention guidelines recommend that antibiotic administration be stopped during the early postoperative period, while Togo et al reported that 2 days were sufficient in an RCT of 2 days and 5 days of antibiotic administration after liver resection. Three or 4 days, including the day of surgery, is normal in Japan, but regarding the question of whether 3 to 4 days of postoperative administration is actually safe, Harbarth et al[18] reported that the odds ratio for infection with a resistant strain was 1.6 times greater during ≥3 days' administration compared with ≤2 days after coronary artery bypass surgery. Terpstra et al[19] also found that *S epidermidis* on the skin developed resistance after 3-day administration, including the day of surgery, compared with same-day administration, while Takesue et al[16] reported that, for gastrotomy, with 4-day administration the intestinal bacteria *Bifidobacterium spp* decreased, whereas *P aeruginosa* and *Enterococcus faecalis* increased, meaning

Table 4 Stratified analysis of risk factors for signs of infection

Variable	Group I (n = 94)	Group II (n = 94)	P
ASA class			
1	8/45 (17.8%)	11/46 (23.9%)	.607
≥2	12/49 (24.5%)	13/48 (27.1%)	.819
Operation time (minutes)			
<360	10/68 (14.7%)	16/70 (22.9%)	.278
≥360	10/26 (38.5%)	8/24 (33.3%)	.774
Blood transfusion (mL)			
0	13/80 (16.3%)	16/79 (20.3%)	.544
>0	7/14 (50.0%)	8/15 (53.3%)	1.000

ASA = American Society of Anesthesiologists.

Postoperative antibiotic prophylaxis cannot prevent postoperative infections after liver resection, and it is thought that antibiotic prophylaxis is unnecessary and costly.

that the administration period should be kept as short as possible to prevent the appearance of resistant strains.

In addition to prevention, early detection and treatment of postoperative infection are also important in perioperative management. Systemic inflammatory response syndrome is often used as an indicator before postoperative infections become apparent, but in the present study, the focus was on the simpler measures of body temperature, white blood cell count, and further increases in C-reactive protein and white blood cell count, with the occurrence of any of these being regarded as a sign of infection. When monitoring changes over time in white blood cell count, body temperature, and C-reactive protein in patients who did not show any signs of infection, determinations were made on the basis of values after the 4th day after surgery, because these peaked on POD 4 in both groups. If any sign of infection occurred, the infection site was identified by chest x-ray, ultrasound, or computed tomography, removal of the intravenous hyperalimentation catheter and drainage were considered, and additional antibiotics were given even in the absence of a clear focus of infection. In this study, the question was investigated from a variety of angles, starting with SSI, followed by systemic inflammatory response syndrome status after POD 4, signs of infection or infectious complications, number of cases of additional antibiotic administration, and surgical intervention rate; there was no inferiority due to non–postoperative antibiotic prophylaxis but rather a tendency toward fewer signs of infection in group I, leading to a significant reduction in length of hospital stay. Furthermore, as a result of the postoperative management described above performed for this study, no patient developed liver failure caused by postoperative infection.

The prevalence of SSI or postoperative infection after liver resection is 4.6% to 25.2%,[9,20–24] with diabetes,[21,24] preoperative infection,[24] liver dysfunction,[14] body mass index,[22] age,[20,21,25] large-scale liver resection,[20] amount of hemorrhage,[21–23,25] duration of surgery,[21,23,25] blood transfusion,[21,23,26] the use of a respirator,[21] and intestinal damage[23] reported as risk factors. Before the present study, we investigated risk factors for signs of postoperative infections among patients in our institution from 2006 to 2007, and identified ASA class ≥ 2, operative time ≥ 6 hours, and intraoperative blood transfusion as significant risk factors on multivariate analysis. Thus, we stratified analysis by these 3 risk factors. In addition, we also performed subgroup analysis by stratification with age[20,21,23] >70 years,[20] body mass index >23.6 kg/m^2,[22] presence of diabetes mellitus,[21,24] and operative bleeding[21–23,25] >810 mL,[22] which have been reported as significant risk factors, but there were no significant differences between the 2 groups in the present study (data not shown).

From the findings of this study, we concluded that, in the absence of preoperative infection or severe complications, there was no increase in the risk for perioperative infection even if postoperative prophylactic antibiotic was not administered. A large-scale RCT is required in the future to provide greater reliability.

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Hepatology Research 2013; 43: 970–978 doi: 10.1111/hepr.12042

Original Article

Chymase inhibitor ameliorates hepatic steatosis and fibrosis on established non-alcoholic steatohepatitis in hamsters fed a methionine- and choline-deficient diet

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Aim: Chymase plays a role in the augmentation of angiotensin II formation, which is involved in liver fibrosis. The therapeutic effects of a chymase inhibitor, TY-51469, on established hepatic steatosis and fibrosis were investigated in a model of developed non-alcoholic steatohepatitis.

Methods: Hamsters were fed a normal diet or methionine- and choline-deficient (MCD) diet for 12 weeks. Then, treatment with TY-51469 (1 mg/kg per day) or placebo was initiated, and the treatment was continued concurrently with the MCD diet for an additional 12 weeks.

Results: At 12 weeks after initiating the MCD diet, marked hepatic steatosis and fibrosis were observed in MCD diet-fed hamsters. Malondialdehyde and gene expression levels of collagen I, collagen III, α-smooth muscle actin (α-SMA) and Rac-1 in liver extracts were also increased in the MCD-diet-fed hamsters at 12 weeks. At 24 weeks, hepatic steatosis and fibrosis were more prominent in the placebo-treated hamsters that were fed the MCD-diet for 24 weeks versus 12 weeks. Hamsters treated with TY-51469 for 12 weeks after being on a 12-week MCD diet had significant ameliorations in both hepatic steatosis and fibrosis, and there were no significant differences compared to normal diet-fed hamsters. There were significant augmentations in angiotensin II and malondialdehyde, and gene expressions of collagen I, collagen III, α-SMA and Rac-1 in the placebo-treated hamsters at 24 weeks; however, these levels were reduced to normal levels in the TY-51469-treated hamsters.

Conclusion: TY-51469 not only prevented the progression of hepatic steatosis and fibrosis, but also ameliorated hepatic steatosis and fibrosis.

Key words: angiotensin II, chymase, fibrosis, non-alcoholic steatohepatitis, steatosis

INTRODUCTION

NON-ALCOHOLIC FATTY LIVER disease (NAFLD) has been recognized as the most common liver disease observed in a simple steatosis.[1,2] NAFLD may progress into non-alcoholic steatohepatitis (NASH), which is a distinct clinical entity characterized by varying degrees of progressive steatosis, lobular inflammation and fibrosis of the liver.[3,4] The progression of NASH may induce the development of hepatocellular carcinoma.[5] However, the mechanisms underlying the transition from steatosis to steatohepatitis may involve several factors, such as inflammation and oxidative stress, and a commonly accepted therapeutic protocol has not yet been established.[6,7]

Chymase is a chymotrypsin-like serine protease located in the secretory granules of mast cells. Chymase converts angiotensin I to angiotensin II, which accelerates tissue inflammation and fibrosis.[8–10] In liver of chronic cirrhosis patients, the accumulation of chymase positive cells is observed in fibrotic regions.[11,12] Moreover, both the chymase and angiotensin II-forming activities were significantly increased in the fibrotic regions of the liver in chronic cirrhosis patients, and there were significant correlations between chymase and angiotensin II-forming activities, chymase activity

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Received 15 August 2012; revision 12 November 2012; accepted 4 December 2012.

and hepatic fibrosis, and angiotensin II-forming activity and hepatic fibrosis.[13] We also reported that a chymase inhibitor TY-51469 attenuated tetrachloride-induced liver fibrosis in hamsters.[14] These findings suggest that chymase-dependent angiotensin II formation may be involved in the development and progression of hepatic fibrosis. Conversely, angiotensin II may also play an important role in the development of hepatic steatosis.[15,16] For example, blockade of the angiotensin II receptor results in an attenuation of not only oxidative stress, but also hepatic steatosis in a rat model of NASH.[15] A significant attenuation of both oxidative stress and hepatic steatosis was also observed in angiotensin II type 1 receptor-deficient mice.[16] Recently, we demonstrated that a specific chymase inhibitor, TY-51469, significantly prevented the development of hepatic steatosis and fibrosis in hamsters fed a methionine- and choline-deficient (MCD) diet.[17] In the previous study, we initiated the chymase inhibitor treatment at the same time as the MCD diet, and the preventive effects of the chymase inhibitor were observed from normal to the development of NASH.

To clarify the ameliorative effect of chymase inhibition on fully developed NASH, a MCD diet was fed to hamsters for 12 weeks until hepatic steatosis and fibrosis were observed.[17] Then, treatment with TY-51469 was initiated concurrently with the MCD diet for an additional 12 weeks.

METHODS

Drugs

TY-51469 WAS SYNTHESIZED as a specific chymase inhibitor (Toaeiyo, Tokyo, Japan).[17,18]

Animal model

Eight-week-old male F1B hamsters ($n = 27$) were obtained from Jackson Laboratories (Bar Harbor, ME, USA) and housed in a temperature-, humidity- and light-controlled room. Normal diet- and MCD diet-fed hamsters were fed ad libitum with normal and MCD diets (Oriental Yeast, Tokyo, Japan), respectively. Twelve weeks after initiating the MCD diet, a portion of the normal- and MCD-diet hamsters ($n = 6$/group) were evaluated. The remaining normal diet-fed hamsters ($n = 5$) were further fed a normal diet for an additional 12 weeks. However, the remaining MCD diet-fed hamsters were divided into two groups, and either TY-51469 (1 mg/kg per day, $n = 5$) or saline ($n = 5$) were administrated s.c. using an Alzet osmotic minipump (model 2ML4; Durect, Cupertino, CA, USA) for an additional 12 weeks. All procedures involving animals were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals at Osaka Medical College.

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels

Measurements of AST and ALT levels in the plasma were performed by SRL (Tokyo, Japan).

Histological analysis

Liver tissue specimens were fixed with Carnoy's fixative in 10% methanol overnight. Fixed liver tissues were embedded in paraffin, and then cut at a thickness of 5 μm. The sections were mounted on silanized slides (Dako Japan, Kyoto, Japan) and deparaffinized with xylene and ethanol.

The severities of hepatic histological changes were assessed using hematoxylin–eosin (HE) staining and Sirius red staining, and were blindly measured by two observers. The lipid droplet area was measured in the HE-stained specimens. The specimens were stained with Sirius red, and the red area was defined as the fibrotic area. The area was measured with computerized morphometry using the Fuji-BSA 2000 image analyzing system (Fuji, Tokyo, Japan).[17] The degree of steatosis was quantified as the percentage of lipid droplet area from the total hepatic area. The degree of hepatic fibrosis was quantified as the percentage of fibrotic area from the total hepatic area.

Mast cells were stained with 0.05% toluidine blue (Chroma-Gesellschaft, Stuttgart, Germany) at pH 4.8.[17]

The procedures for immunohistochemical analysis of hamster chymase and angiotensin II have been previously described.[17] Sections were incubated with anti-hamster chymase antibody (raised in rabbit by immunizing animals with SPYVPWINIVIKASS, a C-terminal peptide comprising of amino acid residues 212 to 226 of hamster chymase) or rabbit polyclonal antibody against angiotensin II (IgG, Nashville, TN, USA), followed by a reaction with appropriate reagents from a streptavidin-biotin peroxidase kit (Dako LSABkit; Dako, Carpentaria, CA, USA) and 3-amino-9-ethylcarbazole, which was used for color development. The sections were lightly counterstained with hematoxylin.

Chymase activity

Tissues were minced and homogenized in 20 mM of Tris-HCl buffer at a pH 8.3, which contained 5 mM of $Mg(CH_3COO)_2$, 30 mM of KCl, 250 mM sucrose and

0.5% Nonidet P-40. The supernatant was then used for the measurement of chymase activity and levels of malondialdehyde (MDA) and angiotensin II.

Chymase activity was measured by incubating tissue extracts for 30 min at 37°C with 4 mM of angiotensin I in 150 mM borax-borate buffer (pH 8.5) containing 8 mM dipyridyl, 0.77 mM diisopropylfluorophosphate and 5 mM ethylene diamine tetraacetic acid, as previously described.[9] One unit of chymase activity was defined as the amount of enzyme that formed 1 μM of angiotensin II/min.

Protein concentrations of the extract were assayed using the bicinchoninic acid Protein Assay Reagent (Pierce, Rockford, IL, USA), with bovine serum albumin as the standard.

Levels of angiotensin II and MDA

Angiotensin II levels in the liver were measured with an enzyme immunoassay kit (Phoenix Pharmaceuticals, Burlingame, CA, USA). MDA levels, a product of lipid peroxidation, were measured by incubating liver extracts for 1 h at 100°C with 20 mM thiobarbituric acid in 300 mM phosphoric acid.[17] The reaction was terminated by cooling on ice, and the absorbance was recorded at 532 nm

Real-time polymerase chain reaction (RT–PCR)

Total RNA (1 μg) from aortas was transcribed into cDNA with Superscript III reverse transcriptase and random hexamers (Invitrogen, Carlsbad, CA, USA).[19] Levels of mRNA were measured by RT–PCR on a LightCycler with software (Roche Diagnostics, Tokyo, Japan) using TaqMan fluorogenic probes. All primers and probes for RT–PCR of collagen I, collagen III, α-smooth muscle actin (α-SMA), Rac-1, sterol regulatory element-binding protein (SREBP)-1c, fatty acid synthase (FAS) and 18S rRNA were designed by Roche Diagnostics. mRNA levels of collagen I, collagen III, α-SMA and Rac-1 were normalized to that of 18S rRNA.

Statistical analysis

Data are expressed as the mean ± standard error of the mean. Significant differences between mean values of the two groups were evaluated using Student's *t*-test for unpaired data. Significant differences among mean values for multiple groups were evaluated using one-way ANOVA followed by Fisher's test. Values of $P < 0.05$ were considered statistically significant.

RESULTS

Hepatic steatosis

REPRESENTATIVE PHOTOGRAPHS OF HE-stained liver sections obtained from normal, MCD diet-fed and placebo-treated, and MCD diet-fed and TY-51469-treated hamsters 24 weeks after initiating the diets are presented in Figure 1(a). The degree of hepatic steatosis was evaluated as a ratio of the lipid droplet area to total liver area in the livers of the normal and MCD diet-fed groups at 12 weeks (Fig. 1b). There were no lipid droplets in the normal diet-fed group, whereas lipid droplets were clearly evident in the MCD diet-fed group (Fig. 1b). At 24 weeks, the ratio of the lipid droplet area to total area in the liver was significantly higher in the placebo-treated group than in the normal group; however, the ratio was significantly lower in the TY-51469-treated group than in the placebo-treated group (Fig. 1c).

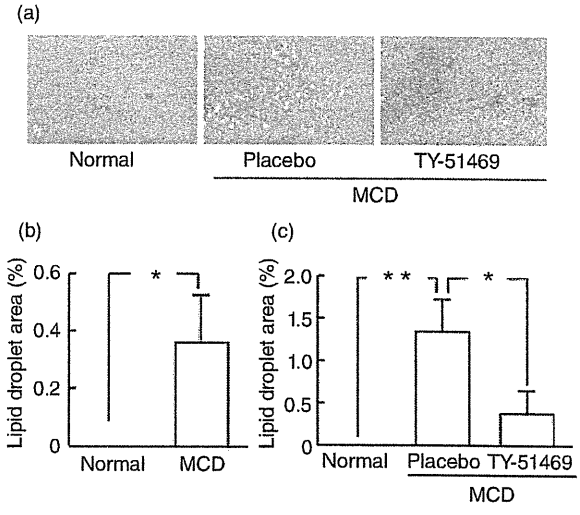

Figure 1 Representative photographs of the hematoxylin–eosin-stained liver sections from the normal diet-fed group, and methionine- and choline-deficient (MCD) diet-fed group treated with either placebo or TY-51469, 24 weeks after initiating the diet (a) (original magnification ×100). Ratio of the lipid droplet area to total liver area of the normal and MCD diet-fed groups 12 weeks after initiating the diet (b). $^{*}P < 0.05$ vs normal diet-fed group (b). Ratio of the lipid droplet area to total liver area of the normal diet-fed group, and MCD diet-fed group treated with either placebo or TY-51469, 24 weeks after initiating the diet (c). $^{*}P < 0.05$ and $^{**}P < 0.01$ vs MCD diet-fed group treated with placebo (c).

Hepatic fibrosis

Representative photographs of Sirius red-stained liver sections obtained from normal, placebo-treated and TY-51469-treated hamsters at 24 weeks (Fig. 2a). The degree of hepatic fibrosis was evaluated as a ratio of the Sirius red-stained area, which revealed the fibrotic area, to total area of the livers from the normal and MCD diet-fed groups at 12 weeks (Fig. 2b). The ratio of the fibrotic area to total hepatic area was significantly larger in the MCD diet-fed group than in the normal diet-fed group (Fig. 2b). At 24 weeks, there was also a significant augmentation of the fibrotic area to total hepatic area in the placebo-treated group compared to the normal group; however, the ratio was significantly reduced to normal levels with TY-51469 treatment (Fig. 2c).

Plasma AST and ALT levels

At 12 weeks, plasma AST levels were 46.5 ± 4.56 and 76.8 ± 12.7 U/L in the normal and MCD diet-fed groups, respectively, which were significantly different

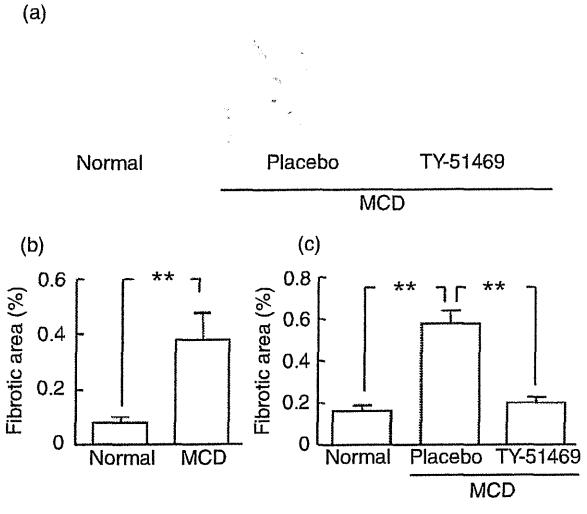

Figure 2 Representative photographs of the Sirius red-stained liver sections from the normal diet-fed group, and the methionine- and choline-deficient (MCD) diet-fed group treated with either placebo or TY-51469, 24 weeks after initiating the diet (a) (original magnification ×100). Ratio of the fibrotic area to total hepatic area of the normal and MCD diet-fed groups 12 weeks after initiating the diet (b). $^{**}P < 0.01$ vs normal diet-fed group (b). Ratio of the fibrotic area to total hepatic area of the normal diet-fed group, and the MCD diet-fed group treated with either placebo or TY-51469 24 weeks after initiating the diet (c). $^{**}P < 0.01$ vs MCD diet-fed group treated with placebo (c).

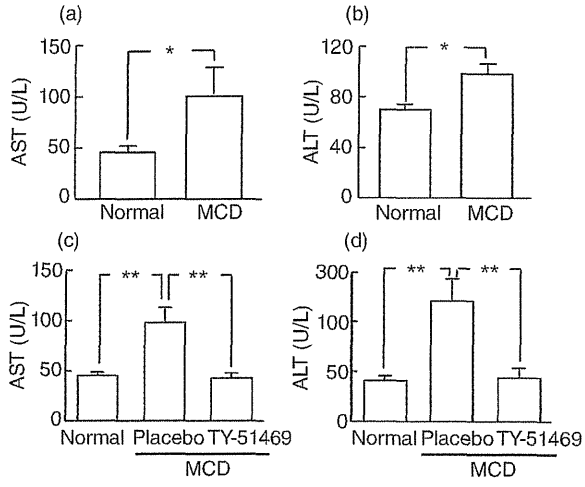

Figure 3 Plasma aspartate aminotransferase (AST) (a) and alanine aminotransferase (ALT) (b) levels of the normal and methionine- and choline-deficient (MCD) diet-fed groups 12 weeks after initiating the diet. $^{*}P < 0.05$ vs normal diet-fed group (a,b). Plasma AST (c) and ALT (d) levels of the normal diet-fed group, and the MCD diet-fed group treated with either placebo or TY-51469. $^{**}P < 0.01$ vs MCD diet-fed group treated with placebo (c,d).

(Fig. 3a). Plasma ALT levels were 69.8 ± 3.75 and 98.7 ± 8.70 U/L in the normal and MCD diet-fed groups, respectively, and these were also significantly different (Fig. 3b). At 24 weeks, plasma AST and ALT levels were 99.4 ± 14.5 and 241.2 ± 44.1 U/mL, respectively, in the placebo-treated group, and these levels were significantly higher those of the normal group (Fig. 3c). However, after TY-51469 treatment for 12 weeks, both plasma AST and ALT levels were significantly lower in the TY-51469-treated group than in the placebo-treated group (Fig. 3d).

Mast cell number, chymase activity and levels of angiotensin II in the liver

Representative photographs of toluidine blue-stained cells, which indicated mast, chymase positive and angiotensin II positive cells in liver sections obtained from the placebo-treated hamster at 12 weeks, are shown in Figure 4(a). Almost all of the mast cells co-expressed both chymase and angiotensin II, as previously observed.[17]

Mast cell number tended to be higher in the MCD diet-fed group than in the normal group at 12 weeks, although there were no significant differences (Fig. 4b). At 24 weeks, the mast cell number also tended to be

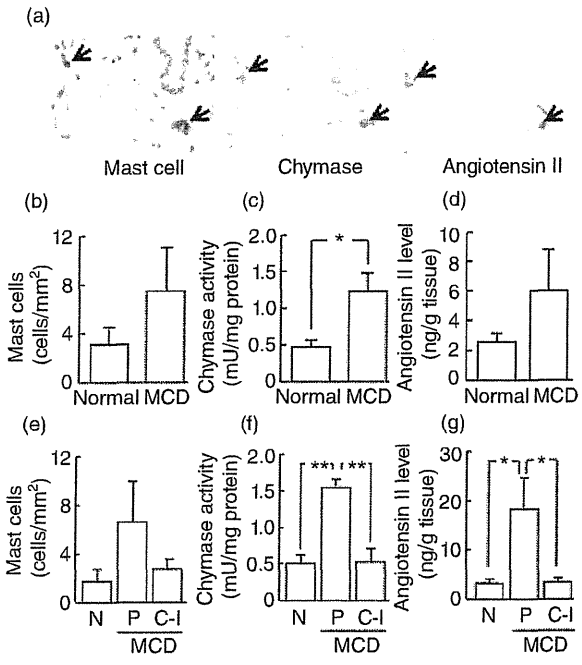

Figure 4 Representative photographs of liver sections stained with toluidine blue (mast cell), and immunostained with anti-chymase (chymase) and anti-angiotensin II antibodies (angiotensin II) of the methionine- and choline-deficient (MCD) diet-fed hamster 12 weeks after initiating the diet (a) (original magnification ×400). Mast cell number (b), chymase activity (c) and angiotensin II levels (d) in the liver extracts of the normal and MCD diet-fed groups 12 weeks after initiating the diet. $^{*}P < 0.05$ vs normal diet-fed group (c). Mast cell number (e), chymase activity (f) and angiotensin II levels (g) in the liver extracts of the normal diet-fed group (N), and the MCD diet-fed group treated with either placebo (P) or TY-51469 (C-I) 24 weeks after initiating the diet. $^{*}P < 0.05$ and $^{**}P < 0.01$ vs MCD diet-fed group treated with placebo (f,g).

higher in the placebo-treated group than in the normal group, but the number tended to be lower in the TY-512469-treated group than in the placebo-treated group (Fig. 4e). Chymase activity in liver extracts was significantly higher in the MCD diet-fed group than in the normal diet-fed group at 12 weeks (Fig. 4c). The higher chymase activity in the placebo-treated group than in the normal group continued up to 24 weeks, but chymase activity in the TY-51469-treated group was significantly reduced at 24 weeks, and there were no significant differences when compared to the normal group (Fig. 4f). Angiotensin II levels in liver extracts also tended to be higher in the MCD diet-fed group than in the normal diet-fed group; however, there were no significant differences (Fig. 4d). Angiotensin II levels at 24 weeks were significantly higher in the placebo-treated group than in the normal group, but they were significantly reduced in the TY-51469-treated group (Fig. 4g).

MDA levels in the liver

Malondialdehyde level, a marker of oxidative stress, in liver extracts was significantly higher in the MCD diet-fed group than in the normal diet-fed group at 12 weeks (Fig. 5a). The MDA level was also higher in the placebo-treated group than in the normal group at 24 weeks, but was significantly lower in the TY-51469-treated group than in the placebo-treated group (Fig. 5b).

Hepatic gene expression levels of Rac-1, SREBP-1c and FAS

Gene expression of Rac-1, which is involved in the generation of reactive oxygen species (ROS), was significantly augmented in the MCD diet-fed group at 12 weeks (Fig. 6a). Gene expression of SREBP-1c tended to be augmented in the MCD diet-fed group, and expression of FAS was significantly augmented (Fig. 6b,c). At 24 weeks, significant increases in Rac-1, FAS and SREBP-1c gene expression levels were observed in the

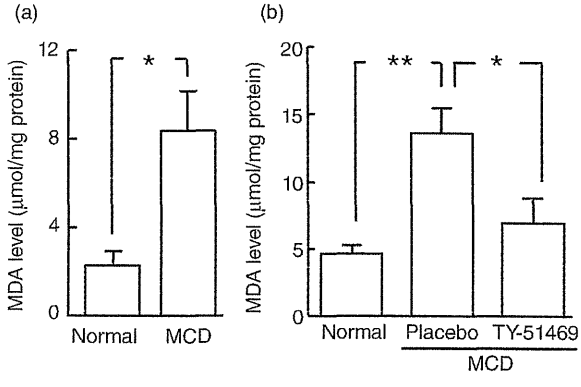

Figure 5 Malondialdehyde (MDA) level in the liver extracts of the normal and methionine- and choline-deficient (MCD) diet-fed groups 12 weeks after initiating the diet (a). $^{*}P < 0.05$ vs normal diet-fed group (a). MDA levels in the liver extracts of the normal diet-fed group, and the MCD diet-fed group treated with either placebo or TY-51469 24 weeks after initiating the diet (b). $^{*}P < 0.05$ and $^{**}P < 0.01$ vs MCD diet-fed group treated with placebo (b).

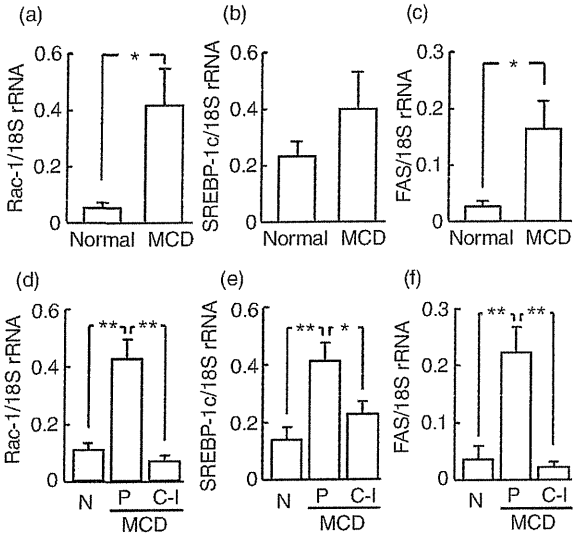

Figure 6 Gene expression levels of Rac-1 (a), sterol regulatory element-binding protein (SREBP)-1c (b) and fatty acid synthase (FAS) (c) in the liver extracts of normal and methionine- and choline-deficient (MCD) diet-fed groups 12 weeks after initiating the diet. $^{*}P < 0.05$ vs normal diet-fed group (a,c). Gene expression levels of Rac-1 (d), SREBP-1c (e) and FAS (f) in the liver extracts of the normal diet-fed group (N), and MCD diet-fed group treated with either placebo (P) or TY-51469 (c–i) 24 weeks after initiating the diet. $^{*}P < 0.05$ and $^{**}P < 0.01$ vs MCD diet-fed group treated with placebo (d–f).

placebo-treated group at 24 weeks; however, they were significantly ameliorated in the TY-51469-treated group (Fig. 6d–f).

Hepatic gene expression levels of collagen I, collagen III and α-SMA

Although there were no significant differences, collagen I gene expression tended to be higher in the MCD diet-fed group than in the normal diet-fed group at 12 weeks (Fig. 7a). There was significant augmentation of collagen III gene expression in the livers of the MCD diet-fed group compared to the normal diet-fed group (Fig. 7b). The gene expression of α-SMA, a marker of myofibroblast differentiation, was significantly augmented in the MCD diet-fed group (Fig. 7c). At 24 weeks, there were significant augmentations in collagen I, collagen III and α-SMA gene expression levels in the placebo-treated group, but they were significantly lower in the TY-51469-treated group than in the placebo-treated group (Fig. 7d–f).

DISCUSSION

TY-51469 INHIBITS CHYMASE with a half maximal inhibitory concentration of 7 nM. Chymase is a chymotrypsin-like serine protease, but TY-51469 does not inhibit other chymotrypsin-like serine proteases and an alternative angiotensin II-forming enzyme, angiotensin-converting enzyme (ACE), even at a concentration as high as 10 μM.[18] Thus, TY-51469 has a high specificity for chymase. In the present study, we used a hamster model for evaluation of TY-51469. There is a marked species difference in the angiotensin II-forming ability. For example, human chymase cleaves the Phe8-His9 bond of angiotensin I to yield angiotensin II, while rat chymase cleaves the Tyr4-Ile5 bond to form inactive fragments.[10] Monkey, dog and hamster chymases, like human chymase, cleave the Phe8-His9 bond of angiotensin I to yield angiotensin II.[10] These species differences in angiotensin II production of chymase must be considered in choosing experimental animals for evaluating the role of chymase-dependent angiotensin II function, and we chose to use a hamster model.

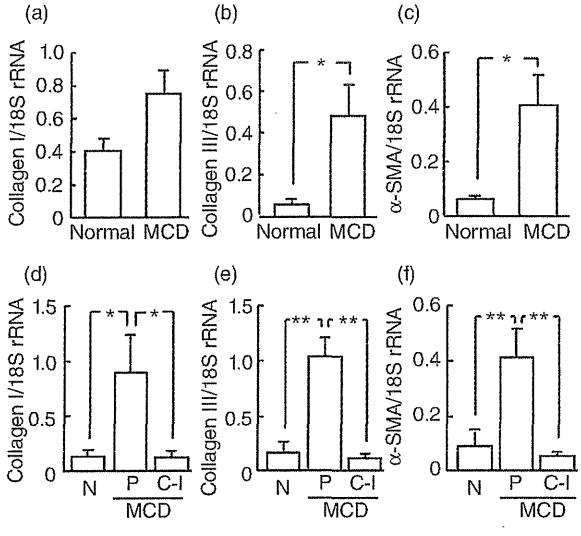

Figure 7 Gene expression levels of collagen I (a), collagen III (b) and α-smooth muscle actin (α-SMA) (c) in the liver extracts of the normal and methionine- and choline-deficient (MCD) diet-fed groups 12 weeks after initiating the diet. $^{*}P < 0.05$ vs normal diet-fed group (b,c). Gene expression levels of collagen I (d), collagen III (e) and α-SMA (f) in the liver extracts of the normal diet-fed group (N), and the MCD diet-fed group treated with either placebo (P) or TY-51469 (c–i), 24 weeks after initiating the diet. $^{*}P < 0.05$ and $^{**}P < 0.01$ vs MCD diet-fed group treated with placebo (d–f).

In the present study, we evaluated two periods in the MCD diet-fed hamster model of NASH, the early stage of NASH development and the later stage of NASH progression. Previously, we evaluated the preventive effects of TY-51469 on the development of NASH in MCD diet-fed hamsters.[17] In the previous protocol, TY-51469 (1 mg/kg per day) treatment was initiated concurrently with the MCD diet. In the present study, the administration of TY-51469 was started at 12 weeks after initiating the MCD diet, when marked hepatic steatosis and fibrosis were observed in a previous report.[17] An indicator of hepatic steatosis degree, the ratio of lipid deposit area to total area in liver, was approximately 0.36% at 12 weeks and 1.35% at 24 weeks. The degree of steatosis was increased 3.7-fold at 24 weeks. An indicator of hepatic fibrosis, the area of fibrotic area to total area in the liver, was increased roughly 1.5-fold at 24 weeks compared to 12 weeks. The degrees of both hepatic steatosis and fibrosis continually progressed on an MCD diet from week 12 to 24. At 24 weeks, there were significant attenuations of hepatic steatosis and fibrosis in the TY-51469-treated group compared to the placebo-treated group. Furthermore, after treatment with TY-51469 between week 12 and 24, the ratio of lipid deposition area to total area was 0.3%, which was lower compared to the ratio of the MCD diet-fed group at 12 weeks (i.e. just prior to TY-51469 treatment). The fibrotic area to total area in the TY-51469-treated group at 24 weeks was also decreased by approximately one half of the ratio of the MCD diet-fed group at 12 weeks. Furthermore, both plasma AST and ALT levels were significantly increased before TY-51469 treatment in the MCD diet-fed hamsters. However, these levels were significantly ameliorated back to normal levels after TY-51469 treatment. Therefore, TY-51469 appeared to have not only a preventative, but also an ameliorative effect on hepatic steatosis and fibrosis.

Chymase plays an important role in the formation of tissue angiotensin II.[8–10] However, in general, ACE is better known as an angiotensin II-forming enzyme than chymase. ACE inhibition attenuates angiotensin II functions, such as vascular constriction and sodium reabsorption, resulted in reducing blood pressure. Conversely, chymase inhibitors do not reduce blood pressure in several hypertensive models, although it reduces the formation of angiotensin II in various tissues, including liver.[20,21] Chymase is a chymotrypsin-like enzyme that is expressed in the secretory granules of mast cells, but chymase has no enzymatic activity within mast cell granules.[22,23] Chymase becomes active immediately following its release into the interstitial tissues after various stimuli in tissues, where it is associated with inflammation. Therefore, chymase inhibitors could attenuate angiotensin II formation only at the tissue level, where it occurs with inflammation, but does not interfere with blood pressure homeostasis.[20,21] We previously evaluated several parameters in an MCD-induced hamster NASH model,[17] and confirmed that ACE activity and angiotensin II levels were not significantly altered 8 weeks after initiating the MCD diet, namely, when hepatic steatosis and fibrosis were observed (Tashiro and Takai, unpubl. obs.). In that study, plasma ACE activity and angiotensin II levels were also unaffected by TY-51469 treatment, despite the fact that the preventive effects of TY-51469 against hepatic steatosis and fibrosis were observed. Therefore, chymase inhibition may only influence tissue angiotensin II levels, but not plasma angiotensin II levels. In fact, chymase inhibition has been associated with a significant reduction in tissue angiotensin II levels beyond lowering blood pressure in various experimental models.[24,25] Thus, chymase inhibitors may reduce hepatic angiotensin II levels beyond lowering blood pressure, which may result in the amelioration of hepatic steatosis and fibrosis.

In NASH, ROS derived from fatty acid oxidation plays a crucial role in hepatocytes. On the other hand, angiotensin II also induces the generation of ROS, such as superoxide and hydrogen peroxide, through the activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase in hepatic stellate cells (HSC).[26] In the present study, chymase inhibition resulted in not only a reduction in gene expression of an NADPH oxidase component, Rac-1, but also an oxidative stress marker, MDA, along with a reduction in angiotensin II levels in hepatic steatosis and fibrosis. In murine HSC, a Rac-1 inhibitor, such as an NADPH oxidase inhibitor, significantly attenuated ROS production.[27] Augmentation of ROS production induces hepatic steatosis, and an angiotensin-receptor blocker (ARB), olmesartan, showed hepatic steatosis via attenuation of ROS production.[15,28] MCD-induced steatosis was also significantly prevented in angiotensin II receptor-deleted mice.[16] SREBP-1c is an important gene in the regulation of lipogenesis via the control of lipogenic genes, such as FAS.[29] Angiotensin II upregulated the gene expression levels of SREBP-1c and FAS in the ROS augmentation *in vivo* and *in vitro* experiments.[30–32] Conversely, an ARB, irbesartan, reduced hepatic steatosis, in addition to downregulating SREBP-1c and FAS gene expression levels via ROS attenuation in a mouse NASH model.[33] In the present study, we observed a significant attenuation of SREBP-1c and FAS

gene expression with TY-51469. Therefore, amelioration of hepatic steatosis via TY-51469 may be dependent on the attenuation of ROS production through a reduction in angiotensin II formation in the liver.

In the present study, we used hamsters, which have a chymase with similar characteristics as that in humans, to evaluate the effect of chymase inhibition. Although SREBP-1c was reported to decrease in a mouse model of NASH, it increased in the hamster model.[34,35] Also in contrast to the mouse model of MCD diet-induced NASH, it has been reported that there was no weight loss in the hamster model of MCD diet-induced NASH, and that blood triglyceride levels did not decrease, but rather increase.[16,36,37] The mouse model of MCD diet-induced NASH has generally been associated with increased fatty acid uptake, decreased very low-density lipoprotein secretion and downregulation of genes involved in triglyceride synthesis.[35] While a similar mechanism may have been partially involved in the hamster model of MCD diet-induced NASH used in the present study, an increase in SREBP-1c was observed in the present study, unlike in the mouse model of MCD diet-induced NASH, suggesting that there are species differences. Further study may be required to clarify differences in pathological mechanisms among different species.

On the other hand, an ARB, losartan, reduced collagen gene expression along with Rac-1 gene expression in patients with chronic hepatitis C.[38] HSC are also recognized as the main collagen-producing cells in the liver, and an increase in the expression of α-SMA in HSC strongly augmented extracellular matrix deposition, including collagen I and collagen III.[26] Angiotensin II induces gene expressions of α-SMA in rat HSC, and blockade of angiotensin II results in the reduction of hepatic fibrosis via a reduction of α-SMA.[39] Levels of α-SMA, collagen I and collagen III in the placebo-treated group were significantly attenuated by TY-51469 in the present study. Thus, the inhibition of hepatic angiotensin II formation by TY-51469 may play an important role in ameliorating hepatic fibrosis via the reduction of angiotensin II-induced oxidative stress.

In conclusion, TY-51469 clearly ameliorated hepatic steatosis and fibrosis in a hamster model of MCD-induced NASH. Chymase inhibition may be useful for attenuating and ameliorating hepatic steatosis and fibrosis.

ACKNOWLEDGMENTS

THIS WORK WAS partially supported by Takeda Science Foundation (S. T.), and Grants-in-Aid for Scientific Research (C), 23590313 (S. T.) and 23591999 (M. H.), from the Japan Society for the Promotion of Science.

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Short-term Results of Laparoscopic versus Open Liver Resection for Liver Metastasis from Colorectal Cancer: A Comparative Study

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Laparoscopic liver resection is currently performed in an increasing number of institutions as a minimally invasive treatment. However, no randomized controlled trials have compared laparoscopic and open liver resections. Twenty-three laparoscopic and 24 open liver resections for colorectal cancer liver metastases (CRCLM) were performed, and these data for both were retrospectively compared in the short-term results. The estimated blood loss was 99 ± 207 mL in the laparoscopic group and 397 ± 381 mL in the open group ($P = 0.0018$); blood loss was significantly higher in the open group. There were no differences in the surgical procedure, blood loss, transfusion rate, pathological margins, postoperative complications, 30-day mortality, duration of intravenous drip, or hospital stay. On postoperative courses, the values of total bilirubin, white blood cell count, and C-reactive protein were significantly lower in the laparoscopic group. The data of the present series suggest the lesser invasiveness and safety of laparoscopic liver resection even for patients with CRCLM, and they showed that postoperative laboratory tests were better after laparoscopy than after the traditional open approach with better short-term results. Tumor diameter less than 5 cm appears to be the appropriate indication for laparoscopic liver resection for CRCLM.

SINCE LAPAROSCOPIC CHOLECYSTECTOMY was first performed in 1985,[1] the laparoscopic approach has spread with unequaled speed throughout the world. Today, the laparoscopic role has changed from diagnostic laparoscopy with an emphasis on diseases of the liver and the peritoneum to therapeutic laparoscopy with the development of video cameras with charge-coupled devices.[1–3] Laparoscopic liver wedge resection, first reported in 1992,[4] is currently performed in an increasing number of institutions as a minimally invasive treatment for benign and malignant liver diseases. The use of laparoscopic surgery, because of its advantages over open surgery, is expected to increase further and become standard therapy, replacing open surgery. However, to the best of our knowledge, no randomized controlled trials have compared laparoscopic and open liver resections. The benefits of a laparoscopic approach to liver resection have not been systematically defined, and only a few reports[5–9] of case–cohort-matched analyses or case series have been published to date. Given the lack of resolution of this issue, the present study retrospectively addressed the indications and evaluated the degree of invasiveness of laparoscopic liver resection for liver metastasis from colorectal cancer (CRCLM) at a single institution.

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Materials and Methods

Patient Population and Selection

In 1998, we started laparoscopic liver resection in the Department of General and Gastroenterological Surgery and at Osaka Medical College Hospital in Takatsuki City, Japan. In the initial phase, benign liver tumors were selected for the laparoscopic approach. The indication was then extended to cover malignant pathologies. From September 2008 to August 2012, a total of 214 patients underwent liver resection, and from September 2008 to September 2010, 24 open liver resections were performed, consisting of partial liver resection and left lateral segmentectomy for a maximum CRCLM size of 5 cm or less. From October 2010 to August 2012, laparoscopic liver resections for CRCLM, consisting of partial liver resection and left lateral segmentectomy for a maximum lesion size of 5 cm or less,

were performed for 23 patients who provided their informed consent. These patients underwent single liver resection without another concomitant surgical (i.e., colorectal) procedure.

The preoperative workup consisted of a specified protocol including blood examinations, abdominal ultrasound, angiocomputed tomography scan, magnetic resonance imaging, esophagogastroduodenoscopy, and spirometry. Evaluation of hepatic function was done using the Child-Pugh classification[10] of liver dysfunction. Patients with complicated cirrhosis (Child-Pugh Class B–C) or an American Society of Anesthesiology (ASA) classification greater than 4 were excluded from the study in whom liver resection would not be appropriate.

Surgical Procedure

In this series, all procedures were performed by four experienced hepatobiliary surgeons (Y.I., F.H., M.H., K.U.) during the study period. All patients received potentially curative hepatectomy with removal of gross tumor with negative macroscopic margins. With respect to hepatic hilar lymph nodes, lymph node dissection was not normally performed, because node-positive cases in this region were strongly associated with extremely poor survival in our previous experience (data not shown).

All operations were performed with the patient under general anesthesia. The detailed laparoscopic surgical technique routinely used in our department has been described in previous reports.[11] Briefly, the patients were placed in a moderate left lateral decubitus position. After the introduction of a 12-mm umbilical port using an open technique, continuous carbon dioxide pneumoperitoneum was induced at a pressure limit of 10 mmHg and flow of 6 L/min to decrease the risk of gas embolism. Five (rarely four) 5- to 12-mm trocars and a flexible laparoscope were fixed. A standard diagnostic and staging laparoscopy was performed. The liver was evaluated in all cases with the aid of intraoperative laparoscopic ultrasonography to confirm tumor extension, the number of lesions, and their positions in relation to the main intrahepatic structures. Then, mobilization of the liver was begun; the right lateral hepatic attachment and the triangular ligament were divided using the harmonic scalpel (Ultracision; Ethicon Endosurgery, Cincinnati, OH) after the round and falciform ligaments were dissected. This dissection was typically carried up to the diaphragm, allowing more effective mobilization of the liver. Next, tape was placed around the porta hepatis and passed through a 12-Fr rubber drain for use as a tourniquet to enable performance of a Pringle maneuver if necessary. Intermittent clamping was applied with 15-minute clamping and 5-minute release periods.

The parenchymal transection was performed using the ultrasonic dissector (SonoSurg System; Olympus Inc., Tokyo, Japan) and the harmonic scalpel under intraoperative ultrasonography, and small vessels were ligated or coagulated using a soft coagulation system. Intraparenchymal control of the major vessels was achieved with clips, whereas biliary and vascular radicle division was obtained with clips or stapling devices.

In cases of left lateral segmentectomy, transection of the liver parenchyma was performed together with sectioning of the vascular pedicle for segments II to III and of the left hepatic vein using consecutive linear staplers (vascular cartridge). During the resection procedure, the surgical margin was carefully confirmed using intraoperative ultrasonography to obtain a surgical margin of 5 to 10 mm when possible.

The resected, undivided specimen was placed in a plastic retrieval bag and removed through the slightly enlarged periumbilical incision. This incision was immediately closed and the abdomen reinflated. The surgical field was irrigated and checked for bleeding or bile leakage, and residual fluid was removed by suction. Monopolar electrocoagulation using a soft coagulation system was sometimes applied on the raw surface of the liver to control blood oozing from the stump while abdominal pressure (6 mmHg) was decreased. Abdominal drainage was usually omitted.

The open liver resections were performed through a right subcostal incision, extended in a few cases to the midline. Liver mobilization was performed, and intraoperative ultrasonography was performed routinely. The hepatic pedicle was always isolated to enable performance of the Pringle maneuver when needed. Parenchymal transection was achieved with the ultrasonic dissector and harmonic scalpel. Monopolar electrocoagulation was used for minor bleeding. Intraparenchymal control of the major vessels was obtained with clips or nonabsorbable sutures.

Preoperative Factors

Data examined included preoperative factors, surgical factors, and pathological factors. Preoperative factors investigated were age, sex, viral infection status, aspartate aminotransferase (AST) level, alanine aminotransferase (ALT) level, platelet count, albumin, total bilirubin, prothrombin time, Child-Pugh classification, degree of liver damage,[12] and indocyanine green retention rate at 15 minutes. Patients testing positive for hepatitis B virus surface antigen were considered positive for hepatitis B virus infection, and those testing positive for hepatitis C virus (HCV) antibody were considered positive for HCV infection.

Surgical and Pathological Factors

Surgical factors included surgical duration, intraoperative blood loss, blood transfusion requirement, and operative method. Pathological factors evaluated included size of the largest tumor, number of tumors, tumor cell differentiation (well-differentiated *vs* others), serosal invasion, vascular invasion (macroscopic and microscopic portal and/or hepatic vein invasion), surgical margin status, and background liver histology. Two specialists in pathology reviewed the specimens to confirm the pathological diagnosis. In this study, surgical margin status was defined as the distance of the lesion(s) closest to the cut surface of the liver.

Postoperative Evaluation

Morbidity was graded according to Clavien's classification.[13] Patient follow-up was based on regular outpatient clinic visits every three months and information obtained from medical records, correspondence, and telephone contact. The following parameters were evaluated: duration of operation, blood loss, transfusion rate, pathological margins, resected liver volume, postoperative complications, 30-day mortality, duration of intravenous drip, and hospital stay. Clinicopathological factors, including age, sex, ASA grade, preoperative α-fetoprotein level, hepatitis serology, esophageal varices, number of tumors (single *vs* multiple), tumor size, surgical margin, microscopic vascular invasion (defined as the presence of tumor emboli within the central veins or portal or capsular vessels), and grade of the primary tumor as defined by Edmondson and Steiner,[14] were investigated.

Statistical Analysis

Continuous variables are expressed as medians (range) or means ± standard deviation (SD). Continuous variables were compared using Student's *t* test. Categorical variables were compared by the likelihood-ratio test or Fisher's exact test, as appropriate. Factors that were found to be significant on univariate analysis were also subjected to multivariate logistic regression analysis to determine adjusted odds ratios. Univariate analyses were performed using the log-rank test. Multivariate analyses were performed by Cox proportional hazards regression. Values of $P < 0.05$ were considered significant.

Results

The demographic data of the two groups are shown in Table 1. There was no difference between the two groups in age, sex, body mass index, comorbidity, hepatitis status, cirrhosis, Child's grading, resected liver volume, or previous abdominal surgery except for the original colorectal surgery. There was also no difference in multiplicity, size, or location of the largest tumor between the two groups.

The results are reported in Table 2. In the laparoscopic group, the laparoscopic procedure was successfully completed for 23 patients. However, one patient (4.2%) was converted to open liver resection because of bleeding from a hepatic vein branch that could not be controlled laparoscopically and was excluded from the result of the laparoscopic group. The mean operative time was 204 ± 101 minutes (mean ± SD) in the laparoscopic group and 230 ± 90 minutes in

TABLE 1. *Patients' Demographic Data*

	LH (n = 23)	OH (n = 24)	*P* Value
Age (years)	66.1 ± 9.6 (41–85)	68.0 ± 9.5 (53–89)	0.5166
Sex (male/female)	11/12	13/11	0.7732
Body mass index (kg/m^2)	22.7 ± 3.1 (17.1–30.5)	23.3 ± 3.6 (18.3–31.5)	0.5440
ASA (I/II/III)	12/7/4	9/13/2	0.2375
Child's grading (A/B)	23/0	24/0	—
HBV/HCV	1/0	2/1	0.5128
Cirrhosis	0	0	—
Solitary/multiple tumor	23/0	21/3	0.2340
Size of largest tumor (cm)	2.5 ± 1.1 (1.0–5.3)	2.7 ± 0.9 (1.5–4.7)	0.5968
Tumor location			0.4082
II	2	3	
III	4	3	
IV	3	6	
V	5	3	
VI	3	3	
VII	4	3	
VIII	2	3	
Resected liver volume (g)	78 ± 75 (10–270)	99 ± 89 (15–290)	0.4374
Previous abdominal surgery	7 (30.4%)	4 (16.7%)	0.7210

LH, laparoscopic; OH, open; ASA, American Society of Anesthesiologists score; HBV, hepatitis B carrier; HCV, hepatitis C carrier.

TABLE 2. *Surgical Procedures and Results*

	LH (n = 23)	OH (n = 24)	*P* Value
Conversion	1	NA	NA
Operative time (minutes)	204 ± 101 (60–395)	230 ± 90 (100–455)	0.3659
Blood loss (mL)	99 ± 207 (0–950)	397 ± 381 (50–1500)	0.0018*
Patients with blood transfusion (%)	1 (4.3%)	4 (16.7%)	0.3475
Mortality	0	0	—
Postoperative complication	2 (8.7%)	5 (20.8%)	0.4158
Postoperative length of venous medicine (days)	4.8 ± 5.5 (1–28)	6.0 ± 5.7 (2–29)	0.4899
Type of hepatectomy (anatomic, nonanatomic)	4/19	5/19	1.0000
Partial resection	19	19	
Left lateral segmentectomy	4	5	
Surgical margin (mm)	8.9 ± 6.2 (0–30)	9.0 ± 7.4 (0–30)	0.9489
≤ 10 mm	13	9	0.3438
> 10 mm	9	12	
Tumor exposure	1	3	
Postoperative length of stay (days)	10.8 ± 11.2 (4–60)	13.9 ± 10.3 (6–56)	0.3300

LH, laparoscopic; OH, open; NA, not applicable.

the open group ($P = 0.3659$). The estimated blood loss was 99 ± 207 mL in the laparoscopic group and 397 ± 381 mL in the open group ($P = 0.0018$). Blood loss was significantly higher in the open group. However, there was no difference between the two groups in intraoperative or perioperative blood transfusions ($P = 0.3475$). Surgical margin was 8.9 ± 6.2 mm in the laparoscopic group and 9.0 ± 7.4 mm in the open group ($P = 0.9489$). In the laparoscopic group, 13 (56.5%) of the 23 patients showed a surgical margin less than 1 cm, but in these cases, the tumor was not invasive on histology. In the open group, nine (37.5%) of the 24 patients showed a surgical margin less than 1 cm ($P = 0.3438$).

The laparoscopic group had a 8.7 per cent complication rate, whereas the open group had a complication rate of 20.8 per cent, although this difference was not significant ($P = 0.4158$). The two complications that occurred in the laparoscopic group were wound infection and bile leak. In the open group, there were five complications, including two postoperative ascites, two ileuses, and one bile leak. There were no reoperations and no hospital deaths in either group.

The postoperative medical treatment was similar for the two groups, including intravenous electrolyte and balanced fluid solutions. Oral intake of fluid started on postoperative Day 2. The patients were usually given a low-sodium diet. Intravenous furosemide was given at early signs of fluid retention. The mean postoperative length of intravenous medicine was 4.8 ± 5.5 days in the laparoscopic group and 6.0 ± 5.7 days in the open group ($P = 0.4899$). In patients without postoperative complications, the mean postoperative length of intravenous medicine was 3.6 ± 1.7 days (range, 1 to 8 days) in the laparoscopic group and 5.1 ± 2.2 days (range, 2 to 10 days) in the open group ($P = 0.0243$). The overall mean postoperative length of hospital stay was 10.8 ± 11.2 days in the laparoscopic group and 13.9 ± 10.3 days in the open group ($P = 0.3300$). For patients without postoperative complications, the overall mean postoperative length of hospital stay was 8.5 ± 3.4 days (range, 4 to 17 days) in the laparoscopic group and 11.6 ± 4.1 days (range, 6 to 20 days) in the open group ($P = 0.0123$). In the points of the mean postoperative length of intravenous medicine and hospital stay, there were significant differences between the two groups without postoperative complications.

Postoperative serum AST and ALT levels peaked on Day 1 and had almost normalized on Fay 4. Postoperative serum white blood cell (WBC) counts and C-reactive protein (CRP) levels peaked on Day 2 and then gradually normalized. Although serum total bilirubin and prothrombin time peaked on Day 1, they remained within the normal ranges in both groups. Postoperatively, total bilirubin, AST, WBC counts, and CRP on postoperative course, especially on the peaked day, were significantly lower in the laparoscopic group than in the open group (Fig. 1). Mean serum ALT levels after laparoscopic surgery were lower on all postoperative courses than that of the group of patients undergoing open liver surgery, although the differences were not significant.

Discussion

Since laparoscopic liver resection was first reported in 1992,[4] laparoscopic liver resection has spread throughout the world. However, laparoscopic liver resection is a highly specialized field and is currently perceived as the most complex of all laparoscopic procedures.[15] This kind of surgery is characterized by dreadful complications such as potential massive hemorrhage or the risk of gas embolism, and the

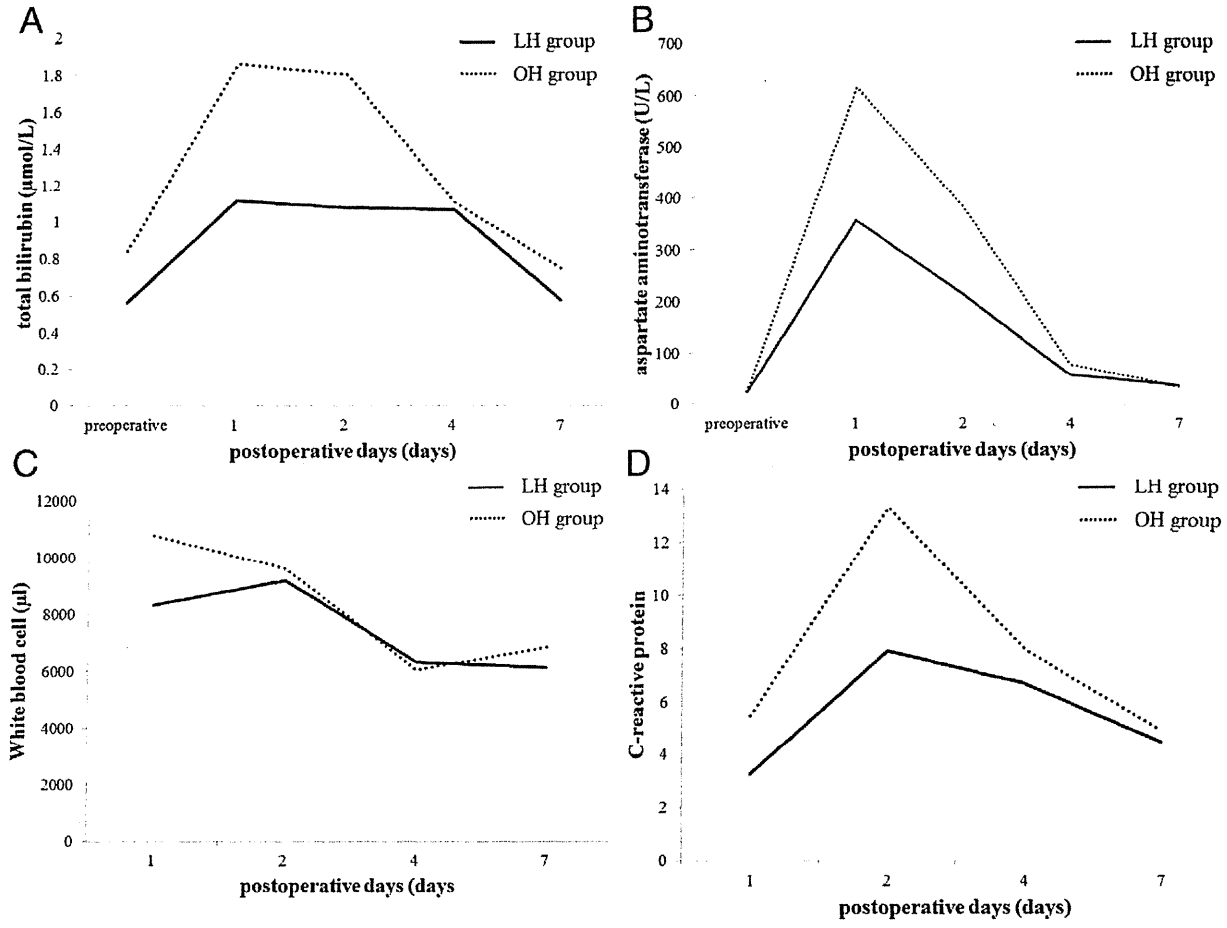

FIG. 1. Postoperative change in total bilirubin level, aspartate aminotransferase level, white blood cell, and C-reactive protein. (*A*) Total bilirubin level (μmol/L). (*B*) Aspartate aminotransferase level (U/L). (*C*) White blood cell (/μL). (*D*) C-reactive protein (mg/dL).

technical difficulty of performing various surgical maneuvers laparoscopically. However, important technological developments and improved endoscopic procedures are being established. Equipment modifications such as intraoperative ultrasonography,[16] ultrasonic dissection, and microwave coagulators have all been recognized for their efficacy in liver surgery as has the introduction of endoscopic linear staplers and laparoscopic coagulation shears. Thus, laparoscopic liver resection has been more actively performed recently.

Some recent reports[17–23] have confirmed the technical feasibility and safety of the laparoscopic technique for cirrhotic patients with liver tumors such as HCC, but an ideal prospective, randomized study comparing open and laparoscopic resections has not been performed to date.

Therefore, this report retrospectively compared the degree of invasiveness of laparoscopic and conventional open liver resections for CRCLM from the perspective of the short-term outcomes at a single institution. This comparative study, although retrospective, confirmed that laparoscopic liver resection for selected patients with CRCLM is an effective therapeutic option with less blood loss than the traditional open approach. In this study, the patient characteristics of the laparoscopic and open groups were quite similar despite our study not being a randomized controlled trial and the number of our study being small. Although the small number of patients in each arm of the study and the retrospective nature of the control group make the chance of a statistical error possible, the conclusions seem reliable. The potential historical bias is reduced by the design of the study resulting in an open group that was well matched with the laparoscopic group for all demographic data. The preoperative management of patients was also similar in both groups. However, the number of the study was small because the selection of patients was variously limited. To avoid the difference in the biological behavior of different malignant tumors, the study was limited to the selected patients with CRCLM because HCC was more likely to take bias as a result of the underlying liver disease. Moreover, to adjust for the differences in

tumor size and magnitude of operation, the short-term results were compared.

It is generally difficult to evaluate minimally invasive surgery.[24] We chose to evaluate the procedures using the clinicopathologic features, because they relate to the postoperative clinical course (Table 2; Fig. 1). The clinicopathologic features are believed to predict the postoperative morbidity and mortality by quantifying the patient's reserve. As a result, laparoscopic liver resection results in better postoperative laboratory tests such as total bilirubin, AST, prothrombin time, WBC, and CRP than the conventional open liver resection, and the postoperative length of intravenous medicine and hospital stay of the laparoscopic group for patients without postoperative complications was significantly shorter than the open group, although there was no difference in the complication rate. Laparoscopic liver resection was found to be less invasive on the postoperative clinical course.

The most important issue regarding laparoscopic liver resection for CRCLM is considered the procedure's indications. It is dangerous to broaden the indications without evidence, because such expansion could jeopardize the two goals of laparoscopic surgery, minimal invasiveness and safety. The indications for laparoscopic liver resection are essentially identical to those for open liver resection in terms of preoperative assessment of liver function. However, cirrhotic patients with relatively poor liver function can tolerate laparoscopic liver resections if the tumor is in a location affording easy access.[22, 25, 26] Therefore, in determining whether laparoscopic liver resection is indicated, the size and location of the tumor must be evaluated. In all 214 patients who underwent liver resection from 2008 to 2011, the risk factor for postoperative complications was tumor size. For tumors smaller than 5 cm, the lower invasiveness and safety of laparoscopic liver resection for patients with CRCLM were demonstrated. Therefore, laparoscopic liver resection for CRCLM is appropriate for tumors smaller than 5 cm in diameter in our institute.

In the past decade, laparoscopic devices have developed with unequaled speed. Laparoscopic techniques may become simpler with new developments. Thereby, the indications for malignant liver tumors may be broadened, although laparoscopic liver resection for HCC has been applied to treatment for limited segments.[27, 28] As for gallbladder, stomach, and colon surgery, we expect that laparoscopic liver resection will become the standard operation.

Conclusion

The data of the present series demonstrate the lower invasiveness and safety of laparoscopic liver resection even for patients with CRCLM and prove that laparoscopy offers better postoperative laboratory test results and better short-term outcomes, although there was no difference in the complication rate. Tumors with diameters smaller than 5 cm may be appropriate for laparoscopic liver resections for CRCLM.

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Hepatology Research

Hepatology Research 2013 doi: 10.1111/hepr.12196

Original Article

Outcomes and predictors of microvascular invasion of solitary hepatocellular carcinoma

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Aim: Microvascular invasion (MVI) is an important risk factor for early recurrence of hepatocellular carcinoma (HCC), but preoperative prediction of MVI is difficult.

Methods: A retrospective review was undertaken on 167 patients with primary solitary HCC who underwent initial hepatectomy. Independent predictors of MVI were identified, and factors affecting disease-free survival in patients with MVI were clarified.

Results: Of the 167 patients, 20 patients (12%) had MVI. Recurrence rates of HCC after hepatectomy in MVI patients were significantly worse than in patients without MVI ($P < 0.0361$). Univariate analysis revealed that positive L3-AFP, PIVKA-II ≥ 150 mAU/mL and tumor size ≥3 cm preoperatively were associated with positive MVI. On multivariate analysis, independent predictors of MVI were PIVKA-II ≥ 150 mAU/mL (odds ratio [OR], 5.19; 95% confidence interval [95% CI], 1.44–24.87; $P = 0.0109$) and positive L3-AFP (OR, 3.47; 95% CI, 1.19–10.75; $P = 0.0229$). Among the MVI-positive group, the 1-, 2- and 3-year disease-free survival rates were 78%, 58%, and 58% in patients with surgical margin (SM) ≥ 10 mm and 38%, 29%, and 29% in those with SM < 10 mm, respectively ($P = 0.0263$).

Conclusions: Patients with PIVKA-II ≥ 150 mAU/mL and positive L3-AFP on preoperative examination are at high risk for MVI.

Key words: AFP-L3, HCC, microvascular invasion, PIVKA-II

INTRODUCTION

HEPATOCELLULAR CARCINOMA (HCC) is associated with a high rate (70%) of recurrence[1,2] even after curative hepatectomy. The two main factors contributing to this phenomenon are multicentric recurrence and intrahepatic recurrence through the vascular route. With respect to multicentric recurrence, many studies have indicated that suppressing hepatic fibrosis with interferon or other treatments can prevent recurrence for more than 2 years postoperatively.[3] As far as intrahepatic recurrence is concerned, recurrence was seen within 2 years postoperatively in many cases.[4,5]

In order to achieve better outcomes, it is important to prevent intrahepatic recurrence after hepatectomy for HCC; however, there is currently no standard protocol to achieve this purpose. Several studies have suggested that macro- and/or microvascular invasion are uniformly associated with poor survival,[6–11] but even microvascular invasion (MVI) is associated with increased risk leading to early recurrence of HCC after curative hepatectomy.[6,12–14] Although macroscopic vascular invasion is relatively easily detected before treatment for HCC, it is often difficult to detect microvascular invasion, such as portal venous, hepatic vein and/or bile duct infiltration, prior to treatment, even using advanced imaging modalities that are applied for staging evaluation. Therefore, it is important to predict the presence of MVI as early and precisely as possible before hepatectomy.

The aim of this study was to identify preoperative predictors of MVI and to clarify the factors affecting disease-free survival of HCC patients with MVI.

METHODS

Patients

FROM APRIL 2000 to June 2010, 179 curative hepatectomies for solitary HCC were performed at Osaka Medical College Hospital. Of these 179 patients, 12

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Received 15 April 2013; revision 25 June 2013; accepted 1 July 2013.