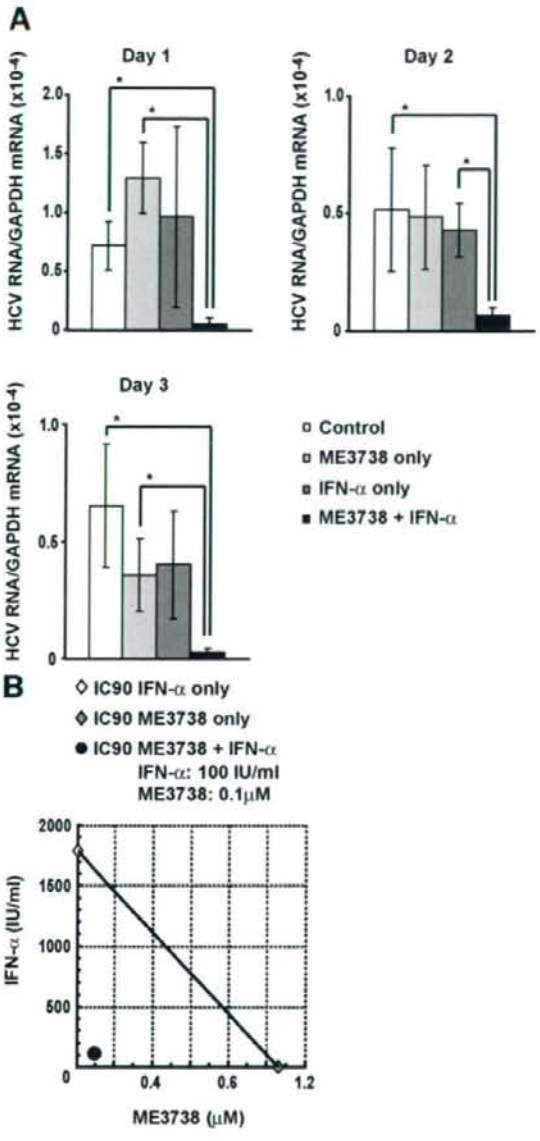

Fig. 6. Synergistic effect of ME3738 with IFN-α on HCV RNA replication. (A) HepG2 cells were infected with Ad-T7pol and then incubated with suboptimal doses of ME3738 (0.1 μM) and IFN-α (100 IU/mL). ME3738 combined with IFN-α significantly reduced HCV replication more than either alone. Data indicate means ± standard error (SE) for three replicates (*$P < 0.05$). (B) Isobole plots of 90% inhibition of HCV RNA replication on day 2 show that IC_{90} of IFN-α alone (white diamond) and of ME3738 alone (gray diamond) were 1792 IU/mL and 1.06 μM, respectively. Solid line indicates additive effect. The black circle in the isobole plot of IC_{90} of ME3738 plus IFN-α indicates synergistic, rather than additive effects of the combination.

Discussion

We showed here that ME3738, a derivative of soyasapogenol, inhibits HCV replication in hepatocyte-derived cell lines. ME3738 enhances autocrine IFN-β production, and this enhancement of IFN-β plays an important role in the anti-HCV effect of ME3738. Combining ME3738 with IFN-α synergistically reduced HCV replication, suggesting that this combination could be an effective therapeutic strategy against HCV.

Huh7 is a highly HCV replication-permissive cell line that has served as the basis of many HCV replication models *in vitro*. One reason why these cells are so permissive for HCV replication is that dsRNA-triggered IFN induction is impaired.[6,23,24] From this perspective, Huh7 might not be suitable for analyzing interactions between IFN and dsRNA, or for screening drugs associated with IFN production. We used an Adeno-T7 delivery plasmid-based HCV replication system because it works in various cell lines, including HepG2 and CV-1. We could monitor HCV replication in HepG2 cell lines using this system, and thus identify ME3738-enhanced IFN-β expression. The anti-HCV effect of ME3738 was obvious in the HepG2 cell line and the expression of both 2′-5′ OAS and MxA mRNA was also significantly and dose-dependently enhanced. Such expression was less remarkable in Huh7, and the anti-HCV effect though evident, was weaker than that in HepG2 cells. Thus, the enhanced IFN-β should be critical for the anti-HCV effect of ME3738. However, ME3738 also has other anti-HCV effects that await characterization since it is a soyasapogenol derivative. Soyasaponin extracted from soybeans (Glycine max L. Merrill) prevents liver damage and hyperlipidemia.[8] Furthermore, HCV replication is associated with cellular lipid droplets,[32] and some lipid metabolic factors are associated with the effect of anti-HCV therapy with IFN.[33] Thus, an indirect effect through lipid metabolism could contribute to the anti-HCV effect of ME3738. Furthermore, soyasaponin has antiviral effects against herpes simplex virus, human cytomegalovirus, influenza virus and human immunodeficiency virus by inhibiting gene and viral protein synthesis.[9,34] The anti-viral effects of ME3738 against HCV could be similar. These issues require further study.

Genotype 1 strains are principally associated with liver diseases worldwide. The most effective antiviral therapy against HCV is presently pegylated IFN combined with ribavirin. However, this strategy eliminates genotype 1 HCV in only about 50% of patients.[2] The remaining 50% requires a more effective therapy with but less side effects to eliminate HCV. ME3738 has anti-HCV effects for genotype 1 HCV.

The present study evaluated the anti-HCV effects of ME3738 alone, as well as in combination with IFN-α. Suboptimal doses of these agents together, remarkably increased anti-HCV activity sooner than either alone.

Since IFN-β induction represents the immediate response of cells to viral infection and precedes the transcription of most IFN-α species, IFN-β enhancement appears to be important for the anti-HCV effect of ME3738. Autocrine IFN-β is recognized as important in viral elimination. Infection with HCV can induce the IFN-β signaling pathway,[35] but HCV has some mechanisms that disrupt this pathway.[27,28] If ME3738 can overcome such disruption, it could increase the antiviral effectiveness against HCV. Moreover, not only hepatocytes, but also immune cells would be affected by autocrine IFN-β,[36] and those influences would help to eliminate HCV. As already demonstrated clinically, the combination of IFN-α and IFN-β enhances antiviral activity against HCV.[37] Therefore, the upregulation of IFN-β by ME3738 could also contribute to reducing HCV replication by combination with IFN-α. Our results demonstrated that ME3738 and IFN combination therapy has synergistic anti-HCV effects, and suggest an additional potential approach toward HCV elimination. Moreover, ME3738 should ameliorate liver damage induced by HCV.[10] Altogether, ME3738 could be a useful anti-HCV strategy, as well as for ameliorating liver damage. These findings support the further evaluation of ME3738 as a promising drug for treating chronic infection with hepatitis C.

Acknowledgment: We thank Ms. Satomi Yamanaka and Ms. Chie Matsugi for valuable contributions and cooperation. We also thank Dr. Robert Lanford for providing the Huh7 cells.

References

1. Alter MJ, Kruszon-Moran D, Nainan OV, McQuillan GM, Gao F, Moyer LA, et al. The prevalence of hepatitis C virus infection in the United States, 1988 through 1994. N Engl J Med 1999;341:556-562.
2. Feld JJ, Hoofnagle JH. Mechanism of action of interferon and ribavirin in treatment of hepatitis C. Nature 2005;436:967-972.
3. Wakita T, Pietschmann T, Kato T, Date T, Miyamoto M, Zhao Z, et al. Production of infectious hepatitis C virus in tissue culture from a cloned viral genome. Nat Med 2005;11:791-796.
4. Zhong J, Gastaminza P, Cheng G, Kapadia S, Kato T, Burton DR, et al. Robust hepatitis C virus infection in vitro. Proc Natl Acad Sci U S A 2005;102:9294-9299.
5. Yi MK, Villanueva RA, Thomas DL, Wakita T, Lemon SM. Production of infectious genotype 1a hepatitis C virus (Hutchinson strain) in cultured human hepatoma cells. Proc Natl Acad Sci U S A 2006;103:2310-2315.
6. Li K, Chen Z, Kato N, Gale M Jr, Lemon SM. Distinct Poly(I-C) and Virus-activated Signaling Pathways Leading to Interferon-β Production in Hepatocytes. J Biol Chem 2005;280:16739-16747.
7. Hiasa Y, Blackard JT, Lin W, Kamegaya Y, Horiike N, Onji M, et al. Cell-based models of sustained, interferon-sensitive hepatitis C virus genotype 1 replication. J Virol Methods 2006;132:195-203.
8. Ohminami H, Kimura Y, Okuda H, Arichi S, Yoshikawa M, Kitagawa I. Effects of soyasaponins on liver injury induced by highly peroxidized fat in rats. Planta Med 1984;50:440-441.
9. Hayashi K, Hayashi H, Hiraoka N, Ikeshiro Y. Inhibitory activity of soyasaponin II on virus replication in vitro. Planta Med 1997;63:102-105.
10. Kuzuhara H, Nishiyama S, Minowa N, Sasaki K, Omoto S. Protective effects of soyasapogenol A on liver injury mediated by immune response in a concanavalin A-induced hepatitis model. Eur J Pharmacol 2000;391: 175-181.
11. Sasaki K, Minowa N, Kuzuhara H, Nishiyama S. Preventive effects of soyasapogenol B derivatives on liver injury in a concanavalin A-induced hepatitis model. Bioorg Med Chem 2005;13:4900-4911.
12. Chung RT, He W, Saquib A, Contreras AM, Xavier RJ, Chawla A, et al. Hepatitis C virus replication is directly inhibited by INF-a in a full-length binary expression system. Proc Natl Acad Sci U S A 2001;98:9847-9852.
13. Contreras AM, Hiasa Y, He W, Terella A, Schmidt EV, Chung RT. Viral RNA mutations are region specific and increased by ribavirin in a full-length hepatitis C virus replication system. J Virol 2002;76:8505-8517.
14. Yanagi M, Purcell RH, Emerson SU, Bukh J. Transcripts from a single full-length cDNA clone of hepatitis C virus are infectious when directly transfected into the liver of a chimpanzee. Proc Natl Acad Sci U S A 1997;94:8738-8743.
15. Chung RT, Kaplan LM. Heterogenous nuclear ribonucleoprotein I (hnRNP-1/PTB) selectively binds the conserved 3' terminus of hepatitis C viral RNA. Biochem Biophys Res Commun 1999;254:351-362.
16. Blackard JT, Smeaton L, Hiasa Y, Horiike N, Onji M, Jamieson DJ, et al. Detection of hepatitis C virus (HCV) in serum and peripheral blood mononuclear cells of HCV-monoinfected and HIV/HCV-coinfected persons. J Infect Dis 2005;192:258-265.
17. Castet V, Fournier C, Soulier A, Brillet R, Coste J, Larrey D, et al. Alpha interferon inhibits hepatitis C virus replication in primary human hepatocytes infected in vitro. J Virol 2002;76:8189-8199.
18. Lanford RE, Chavez D, Chisari FV, Sureau C. Lack of detection of negative-strand hepatitis C virus RNA in peripheral blood mononuclear cells and other extrahepatic tissues by the highly strand-specific rTth reverse transcriptase PCR. J Virol 1995;69:8079-8083.
19. Laskus T, Radkowski M, Wang LF, Cianciara J, Vargas H, Rakela J. Hepatitis C virus negative strand RNA is not detected in peripheral blood mononuclear cells and viral sequences are identical to those in serum: a case against extrahepatic replication. J Gen Virol 1997;78:2747-2750.
20. Hiasa Y, Kamegaya Y, Nuriya H, Onji M, Kohara M, Schmidt EV, et al. PKR is increased and is functional in hepatitis C virus-related hepatocellular carcinoma. Am J Gastroenterol 2003;98:2528-2534.
21. Dobson AT, Margolis TP, Sedarati F, Stevens JG, Feldman LT. A latent, nonpathogenic HSV-1-derived vector stably expresses b-galactosidase in mouse neurons. Neuron 1990;5:353-360.
22. Bouvier-Alias M, Patel K, Dahari H, Beaucourt S, Larderie P, Blatt L, et al. Clinical utility of total HCV core antigen quantification: a new indirect marker of HCV replication. HEPATOLOGY 2002;36:211-218.
23. Lanford RE, Guerra B, Lee H, Averett DR, Pfeiffer B, Chavez D, et al. Antiviral effect and virus-host interactions in response to alpha interferon, gamma interferon, poly(I)-poly(C), tumor necrosis factor alpha, and ribavirin in hepatitis C virus sugenomic replicons. J Virol 2003;77:1092-1104.
24. McCormick CJ, Challinor L, Macdonald A, Rowlands DJ, Harris M. Introduction of replication-competent hepatitis C virus transcripts using a tetracycline-regulable baculovirus delivery system. J Gen Virol 2004;85: 429-439.
25. Levy DE, Marie I, Prakash A. Ringing the interferon alarm: differential regulation of gene expression at the interface between innate and adaptive immunity. Curr Opin Immunol 2003;15:52-58.
26. Yoneyama M, Kikuchi M, Natsukawa T, Shinobu N, Imaizumi T, Miyagishi M, et al. The RNA helicase RIG-I has an essential function in double-stranded RNA-induced innate antiviral responses. Nat Immunol 2004;5:730-737.
27. Foy E, Li K, Sumpter R Jr, Loo YM, Johnson CL, Wang C, et al. Control of antiviral defenses through hepatitis C virus disruption of retinoic acid-inducible gene-I signaling. Proc Natl Acad Sci U S A 2005;102:2986-2991.
28. Li K, Foy E, Ferreon JC, Nakamura M, Ferreon AC, Ikeda M, et al. Immune evasion by hepatitis C virus NS3/4A protease-mediated cleavage of the Toll-like receptor 3 adaptor protein TRIF. Proc Natl Acad Sci U S A 2005;102:2992-2997.

29. Kumar H, Kawai T, Kato H, Sato S, Takahashi K, Coban C, et al. Essential role of IPS-1 in innate immune responses against RNA viruses. J Exp Med 2006;203:1795-1803.
30. Ikeda M, Abe K, Yamada M, Dansako H, Naka K, Kato N. Different anti-HCV profiles of statins and their potential for combination therapy with interferon. HEPATOLOGY 2006;44:117-125.
31. Tallarida RJ. Drug synergism: its detection and applications. J Pharmacol Exp Ther 2001;298:865-872.
32. Miyanari Y, Atsuzawa K, Usuda N, Watashi K, Hishiki T, Zayas M, et al. The lipid droplet is an important organelle for hepatitis C virus production. Nat Cell Biol 2007;9:1089-1097.
33. Acuta N, Suzuki F, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, et al. Predictors of viral kinetics to peginterferon plus ribavirin combination therapy in Japanese patients infected with hepatitis C virus genotype 1b. J Med Virol 2007;79:1686-1695.
34. Simoes CM, Amoros M, Girre L. Mechanism of antiviral activity of triterpenoid saponins. Phytother Res 1999;13:323-328.
35. Kanda T, Steele R, Ray R, Ray RB. Hepatitis C virus infection induces interferon-β signaling pathway in immortalized human hepatocytes. J Virol 2007;81:12375-12381.
36. Qian C, An H, Yu Y, Liu S, Cao X. TLR agonists induce regulatory dendritic cells to recruit Th1 cells via preferential IP-10 secretion and inhibit Th1 proliferation. Blood 2007;109:3308-3315.
37. Okuse C, Rinaudo JA, Farrar K, Wells F, Korba BE. Enhancement of antiviral activity against hepatitis C virus in vitro by interferon combination therapy. Antiviral Res 2005;65:23-34.

Hepatology Research 2008; **38**: 1172–1177 doi: 10.1111/j.1872-034X.2008.00389.x

Original Article

Efficacy of splenectomy for hypersplenic patients with advanced hepatocellular carcinoma

Masashi Hirooka,[1] Kiyotaka Ishida,[1] Yoshiyasu Kisaka,[1] Takahide Uehara,[1] Yuji Watanabe,[2] Yoichi Hiasa,[1] Kojiro Michitaka,[1] and Morikazu Onji[1]

[1]*Department of Gastroenterology and Metabology, and* [2]*The Department of Organ Regenerative Surgery, Graduate School of Medicine, Ehime University, Onsen, Japan*

Aim: Chemotherapy for advanced hepatocellular carcinoma (HCC) patients with hypersplenism is generally unsatisfactory, as a lower-dose therapy is usually administered. Splenectomy may represent a better approach to overcoming the complication due to hypersplenism in patients with advanced HCC. This retrospective study was conducted to evaluate whether HCC patients who undergo splenectomy show improved prognosis.

Methods: We examined 34 HCC patients. Twenty-two had thrombocytopenia and/or leucopenia and underwent laparoscopic splenectomy. The completion rate of full-dose drug regimens, the response rate, the toxicity of chemotherapy and the cumulative survival rate were compared between the splenectomy and non-splenectomy groups.

Results: The response rate and the cumulative survival rate in the splenectomy group were significantly better than that in the non-splenectomy group.

Conclusions: Splenectomy is an efficient method for advanced HCC patients with hypersplenism treated by chemotherapy.

Key words: Hepatocellular carcinoma, chemotherapy, splenectomy

INTRODUCTION

HEPATOCELLULAR CARCINOMA (HCC) is one of most common malignant tumors. The development of imaging modalities including ultrasonography, computed tomography (CT), and magnetic resonance imaging (MRI) has enabled the early diagnosis of HCC. Thus, curative treatments, such as liver transplantation, hepatic resection, percutaneous ethanol injection, and radiofrequency ablation therapy, are effective in patients with early stage HCC. However, advanced HCC has poor prognosis and a standard therapy has not been established. Advanced HCC is usually associated with liver cirrhosis[1–4] and patients often have hypersplenism due to portal hypertension resulting in anemia, leucopenia, or thrombocytopenia.[5–10] Advanced HCC is widely treated by chemotherapy,[11–13] however as chemotherapy restricts bone marrow function, advanced HCC patients with hypersplenism do not receive a sufficient dose of chemotherapy drugs to be effective. Thus, these patients have been considered to have an even poorer prognosis than advanced HCC patients with splenomegaly who received a more sufficient dose of chemotherapy. Splenectomy may be a better method to overcome the problems of leucopenia and thrombocytopenia in patients with advanced HCC.

We are unable to find in the literature an analysis of the efficacy of splenectomy as an alternative for chemotherapy in cirrhotic patients. This retrospective study aims to evaluate whether HCC patients who undergo splenectomy show improved prognosis.

Correspondence: Dr Morikazu Onji, Department of Gastroenterology and Metabology, Graduate School of Medicine, Ehime University, Touon-Shi, Ehime-ken 791-0295, Japan. Email: onjimori@m.ehime-u.ac.jp

Received 26 November 2007; revision 23 April 2008; accepted 23 April 2008.

PATIENTS AND METHODS

Patients

WE EXAMINED 34 patients (29 males, 5 females; 35–76 years, mean age 59.9 ± 8.7 years) who had been admitted to the Third Department of Internal

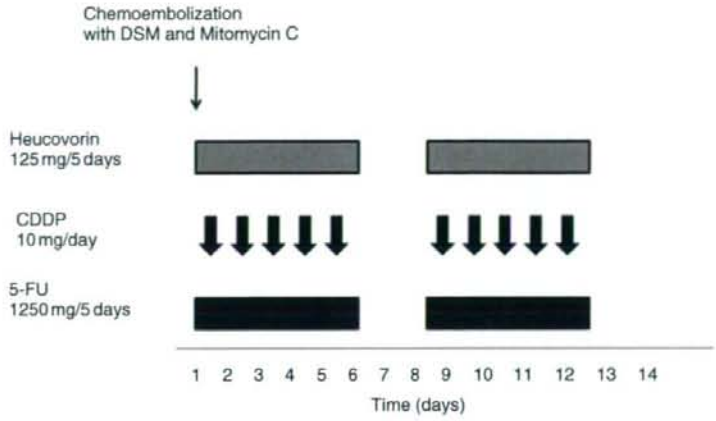

Figure 1 The chemotherapeutic regimen of this study. A schematic presentation of the chemotherapeutic regimen is shown. CDDP, cisplatin; DSM, degradable starch microspheres; 5-FU, 5-fluorouracil.

Medicine, Ehime University Hospital, Japan, between January 2002 and December 2006. The criteria for this study included: (i) a performance status of 0–2; (ii) successful implantation of intra-arterial catheter and drug delivery system; (iii) existence of tumor thrombosis of the portal vein or giant nodule greater than 5 cm in size; and (iv) absence of extra-hepatic metastasis. A diagnosis of HCC was made using imaging analysis, including helical dynamic CT and MRI. The patients with HCC were confirmed to have elevated levels of alpha-fetoprotein (AFP; mean 12241.7 ± 31529.0 ng/mL) and/or des-γ-carboxy-prothrombin (DCP; mean 15428.0 ± 43324.7 mAU/mL). Splenectomy was performed according to the following criteria: (i) thrombocytopenia (platelet count $< 8 \times 10^4$ /mm^3); (ii) leucopenia (white blood cell count $< 2 \times 10^3$/mm^3); or (iii) in the case of consenting splenectomy. Of the 34 patients, 22 with thrombocytopenia and/or leucopenia underwent laparoscopic splenectomy after informed, written consent was obtained (splenectomy group). The splenectomy was performed about 4 weeks before the start of chemotherapy. The remaining 12 patients with advanced HCC who did not consent to a splenectomy were the historical control group (non-splenectomy group). These 12 patients also has thrombocytopenia.

Chemotherapy

A five French heparin-coated catheter was introduced intraluminally to the right femoral artery with a subcutaneously implanted reservoir and was positioned in the proper or common hepatic artery. The gastroduodenal artery and right gastric artery were occluded with steel coils to prevent gastroduodenal injury from the anticancer agents. Patients received regional chemotherapy via the hepatic artery through a subcutaneously implanted port. As shown in Figure 1, one course of chemotherapy consisted of daily administration of cisplatin (CDDP; 10 mg on days 1–5, 8–12) and leucovorin (LV; 25 mg on days 1–5, 8–12) followed by 5-fluorouracil (5-FU; 250 mg on days 1–5, 8–12). In addition, Mitomycin C (8 mg) was emulsified into degradable starch microspheres (DSM), and injected on day 1. This drug regime was repeated weekly for a fortnight and the course of treatment was repeated several times unless the tumor progressed during the therapy.

METHODS

THE CLINICAL PARAMETERS of the patients in each group are shown in Table 1. Laboratory tests were performed before splenectomy in the splenectomy group. All patients had liver cirrhosis. The pathogenesis of liver cirrhosis was hepatitis B virus (HBV) in six patients, hepatitis C virus (HCV) in 27, and unknown etiology in one. Using the Child-Pugh scale, 22 patients were classified as class A cirrohsis and 12 class B. The mean maximum diameter of the HCC nodules was 54.4mm ± 26.0 (20.0–140.0mm). According to the tumor stage defined by the Liver Cancer Study Group of Japan,[14] five patients were in stage 3 and 29 in stage 4.

The efficacy of chemotherapy was assessed by CT during angiography. The response criteria used were those outlined in the Response Evaluation Criteria in Solid Tumors (RECIST)[15–17] guidelines, which propose using only the change in maximal diameter. This led to

Table 1 Characteristics of the patients in the splenectomy and non-splenectomy groups

	Splenectomy group ($n = 22$)	Non-splenectomy group ($n = 12$)	P value
Age (years)	55.8 ± 10.3	62.1 ± 7.0	NS
Gender (M:F)	17:5	12:0	NS
Etiology (HBV:HCV:other)	3:18:1	3:9:0	NS
Child-Pugh grade (A:B)	14:8	8:4	NS
Clinical stage (3:4)	4:18	1:11	NS
Tumor thrombus in main portal branch	8	5	NS
Spleen volume pre-splenectomy (cm^3)	402.8 ± 135.3	361.7 ± 94.4	NS
Platelet count (mm^3)	6.1 ± 1.4	7.3 ± 0.4	0.04
Median white blood cells (mm^3)	3200 (1420–8500)	3900 (2530–5750)	NS
Median tumor diameter (mm)	50.0 (20.5–104.0)	62.0(25.0–78.5)	NS

HBV, hepatitis B virus; HCV hepatitis C virus; NS, not significant.

four categories of response in the target lesion, evaluated as follows: (i) complete response (CR); (ii) total disappearance (TD); (iii) partial response (PR; at least a 30% decrease in the sum of the longest diameter of the target lesion); (iv) progressive disease (PD; at least a 20% increase in the sum of the longest diameter of the target lesion); and (v) stable disease (SD; neither a sufficient shrinkage to qualify for partial response, nor a sufficient increase to qualify for progressive disease). To be assigned a status of CR or PR, changes in tumor measurements needed to be confirmed by assessment performed 4 weeks after the criteria for response were first met. In non-target lesions, response was evaluated as follows: (i) CR (the disappearance of all non-target lesions and normalization of tumor marker level); (ii) incomplete response (SD; the persistence of one or more non-target lesions and/or the maintenance of tumor marker levels above normal limits); (iii) PD (the appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions).

The toxicity of chemotherapy was assessed and graded according to the Common Terminology Criteria for Adverse Events (CTCAE) version 3.0.[18] The completion rate by full dose drugs, the response rate, the toxicity of chemotherapy, and the cumulative survival rate were compared between the splenectomy and non-splenectomy groups. The follow up of albumin, bilirubin, and prothrombin time was performed after splenectomy.

Statistical analysis

Continuous parameters were expressed as medians and ranges (10th and 90th percentiles) or means and standard deviations. Statistical analysis was performed using Student's t-test for paired and unpaired data, Mann–Whitney U-test, χ^2 test, Fisher's exact tests, or Wilcoxon signed-rank test as applicable. The survival curves were plotted using the Kaplan–Meier methodology and were compared using the log-rank test. Multivariate analysis was performed using the Cox proportional hazards regression model. $P < 0.05$ was considered to represent statistical significance.

RESULTS

APART FROM PLATELET count, no significant differences in age, gender, etiology, Child–Pugh grade, clinical stage, tumor diameter, spleen volume before splenectomy or chemotherapy, and white blood cell count (WBC) count were observed between the two groups. The mean platelet counts significantly increased one month after splenectomy ($6.1 \pm 1.4 \times 10^4/mm^3$ vs $18.0 \pm 9.1 \times 10^4/mm^3$, $P < 0.001$). Median WBC counts also increased (3200 [1420–8500] vs. 4600 [2560–7820]). Of the 22 patients in splenectomy group, 20 (90.9%) were able to be treated with the full-dose chemotherapy regimen. Most of those in the non-splenectomy group (8/12, 66.6%) were unable to receive a full therapeutic chemotherapy dose due to thrombocytopenia. In the splenectomy group, two patients were not able to be treated with the full-dose chemotherapy regimen due to infection at the site of the subcutaneously implanted reservoir port.

Table 2 shows the tumor responses. In the splenectomy group, CR was achieved in three patients, PR in 12 patients, yielding a response rate of 68.1%. In the non-splenectomy group, PR was achieved in 2 patients, SD and PD in 10 patients, yielding a response rate of 16.6%. Thus, the response rate of the splenectomy group was significantly better than that of the non-splenectomy group ($P < 0.001$).

Table 2 Comparison of therapeutic response between the splenectomy and non-splenectomy groups

	Splenectomy group ($n = 22$)	Non-splenectomy group ($n = 12$)	*P* value
Treatment cycle (weeks)	2.9 ± 0.9	2.3 ± 1.0	NS
Full regimen completion (%)	90.9	33.3	<0.001
CR	3	0	
PR	12	2	
SD, PD	7	10	
Response rate (%) 68.1	68.1	16.6	<0.001

CR, complete response; NS, not significant; PD, progressive disease; PR, partial response; SD, stable disease

The cumulative survival rate is shown in Figure 2. The 1-year survival rates for the splenectomy and non-splenectomy groups were 86.3% and 41.6%, respectively, while the 2-year survival rates were 42.5% and 0%, respectively. The cumulative survival rate was significantly better in the splenectomy group than the non-splenectomy group ($P < 0.001$). In all patients, the cause of death was related to the exacerbation of HCC. The most important factors influencing overall survival were splenectomy and the existence of portal thrombus (Table 3). After splenectomy the hazard ratio was 8.89 (CI 2.21–18.82, $P < 0.001$). Post-operative complications consisted of portal thrombosis in only two patients. However the liver function of these patients remained unchanged. Prothrombin time was the only parameter to improve after splenectomy (Table 3).

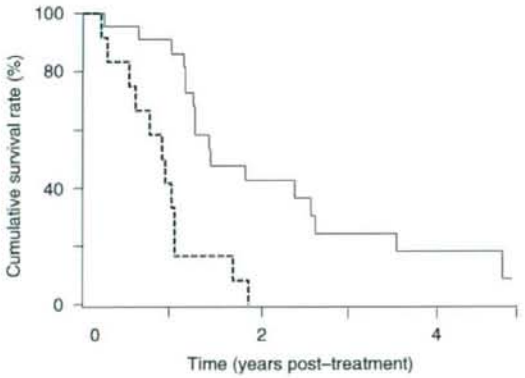

Figure 2 Comparison of cumulative survival rates of the splenectomy (—) and non-splenectomy (- - -) groups. The survival rate of the splenectomy group was significantly higher than that of the non-splenectomy group.

DISCUSSION

THE PROGNOSIS OF advanced HCC has been improved by chemotherapy treatment, especially via hepatic arterial infusion. There are several reports indicating that chemotherapy patients survive more than 3-years post-treatment.[11–13] Hypersplenism, due to portal hypertension, can result in decreased WBC and platelet counts. This is considered a contraindication to chemotherapy, and hypersplenism precludes aggressive chemotherapy. In an attempt to obtain more satisfactory results in the treatment of HCC with hypersplenism, we performed splenectomy prior to chemotherapy. To address the problems caused by hypersplenism, open

Table 3 Multivariable analysis for overall survival

	Hazard ratio (95%CI)	*P* value
Splenectomy	8.89 (2.21–18.82)	<0.001
Age		
<50	1	
50–59	1.07 (0.21–5.11)	NS
60–69	0.85 (0.15–4.14)	NS
70–	0.47 (0.06–3.52)	NS
Etiology		
HBV	1	
HCV	1.54 (0.47–5.97)	NS
NBNC	1.90 (0.30–4.72)	NS
Child-Pugh		
A	3.44 (0.93–13.05)	NS
B	1	
Tumor diameter		
<50 mm	1	
50 mm–	0.93 (0.30–2.58)	NS
Portal thrombus		
(−)	1	
(+)	2.21 (1.09–4.80)	0.002

HBV, hepatitis B virus; HCV hepatitis C virus; NS, not significant.

Table 4 the comparison of liver function tests pre- and post-splenectomy

	Pre-splenectomy	24 weeks post-splenectomy	*P*
Prothrombin time (%)	87.9	94.7	0.03
Albumin (g/dL)	2.9 ± 0.4	3.1 ± 0.4	NS
Median total bilirubin (mg/dL)	1.05 (0.50–2.07)	0.90 (0.46–2.24)	NS

NS, not significance.

splenectomy was performed. However, open surgery is a highly invasive method, and thus partial splenic arterial embolization (PSE) has been performed in previous patients with HCC to reduce spleen volume and control hypersplenism.[19,20] PSE, via interventional radiology, is less invasive than open splenectomy but platelet counts post-PSE are less than that after splenectomy. As it is necessary that platelet counts be more than about $10 \times 10^4/mm^3$ for treatment with a full-dose chemotherapy regimen, some PSE patients will not meet this criteria. In such a case, a control trial is necessary.

The WBC and platelet counts of all patients in the splenectomy group increased following surgery, and thus (excepting two cases) were able to be treated with the full-dose chemotherapy regimen. Most patients in the non-splenectomy group (8/12, 66.6%) were unable to receive a full therapeutic dose of chemotherapy. Consequently, the response rate in the splenectomy group was significantly better than that the non-splenectomy group. The response rate in the splenectomy group was very high (68.1%). In this study, patients without portal thrombus were included, achieving a response rate of 61.1%.

The 2-year survival rate was also significantly better in the splenectomy group, suggesting that splenectomy should be performed in order to improve the prognosis of HCC patients with leucopenia and thrombocytopenia. A prospective study will be necessary in the future.

Splenectomy has been reported to be associated with a high risk of overwhelming post-splenectomy syndrome, bleeding, and portal thrombosis. In the present study, the only complications were two cases of portal thrombosis (9.0%), indicating that splenectomy is a safe and feasible approach in this clinical situation. Recently, patients with hypersplenism, due to liver cirrhosis, have undergone interferon therapy for the eradication of the HCV and reduction of occurrence of HCC. Splenectomy may also be useful for these patients.

Splenectomy might be not only be useful for increasing platelet counts, but also in improving liver function. There are several reports indicating that splenectomy could improve liver function for patients with liver cirrhosis. In our patients, only the prothrombin time was significantly increased after splenectomy. The progression of cancer and degree of nutrition might have precluded improvements in other liver function parameters (Table 4).

In conclusion, splenectomy allowed an increased proportion of the patients studied to complete full-dose chemotherapy, increased the proportion of patients whose tumor(s) responded to chemotherapy and prolonged survival.

REFERENCES

1 Ikeda K, Saitoh S, Koida I *et al.* A multivariate analysis of risk factors for hepatocellular carcinogenesis: a prospective observation of 795 patients with viral and alcoholic cirrhosis. *Hepatology* 1993; **18**: 47–53.
2 Yoshida H, Shiratori Y, Moriyama M *et al.* Interferon therapy reduces the risk for hepatocellular carcinoma: national surveillance program of cirrhotic and noncirrhotic patients with chronic hepatitis C in Japan. IHIT Study Group. Inhibition of Hepatocarcinogenesis by Interferon Therapy. *Ann Int Med* 1999; **131**: 174–81.
3 Nishiguchi S, Kuroki T, Nakatani S *et al.* Randomised trial of effects of interferon-alpha on incidence of hepatocellular carcinoma in chronic active hepatitis C with cirrhosis. *Lancet* 1995; **346**: 1051–5.
4 Torzilli G, Makuuchi M, Inoue K *et al.* No-mortality liver resection for hepatocellular carcinoma in cirrhotic and noncirrhotic patients: is there a way? A prospective analysis of our approach. *Arch Surg* 1999; **134**: 984–92.
5 Sugawara Y, Yamamoto J, Shimada K *et al.* Splenectomy in patients with hepatocellular carcinoma and hypersplenism. *J Am Coll Surg* 2000; **190**: 446–50.
6 Bolognesi M, Merkel C, Sacerdoti D, Nava V, Gatta A. Role of spleen enlargement in cirrhosis with portal hypertension. *Dig Liver Dis* 2002; **34**: 144–50.
7 Piscaglia F, Zironi G, Gaiani S *et al.* Systemic and splanchnic hemodynamic changes after liver transplantation for cirrhosis: a long-term prospective study. *Hepatology* 1999; **30**: 58–64.
8 Tomikawa M, Hashizume M, Akahoshi T *et al.* Effect of splenectomy on liver volume and prognosis of cirrhosis in patients with esophageal varices. *J Gastroenterol Hepatol* 2002; **17**: 77–80.

9 Cao ZX, Chen XP, Wu ZD. Effects of splenectomy in patients with cirrhosis undergoing hepatic resection for hepatocellular carcinoma. *World J Gastroenterol* 2003; **9**: 2460–3.
10 Chen XP, Wu ZD, Huang ZY, Qiu FZ. Use of hepatectomy and splenectomy to treat hepatocellular carcinoma with cirrhotic hypersplenism. *Br J Surg* 2005; **92**: 334–9.
11 Ando E, Yamashita F, Tanaka M, Tanikawa K. A novel chemotherapy for advanced hepatocellular carcinoma with tumor thrombosis of the main trunk of the portal vein. *Cancer* 1997; **79**: 1890–6.
12 Yamasaki T, Kurokawa F, Shirahashi H *et al.* Novel arterial infusion chemotherapy using cisplatin, 5 – fluorouracil, and leucovorin for patients for patients with advanced hepatocellular carcinoma. *Hepatol Res* 2002; **23**: 7–17.
13 Sakon M, Nagano H, Dono K *et al.* Combined intraarterial 5-fluorouracil and subcutaneous interferon-alpha therapy for advanced hepatocellular carcinoma with tumor thrombi in the major portal branches. *Cancer* 2002; **94**: 435–42.
14 Liver Cancer Study Group of Japan. *Classification of Primary Liver Cancer*, 1st edn. Tokyo: Kanehara, 1997.
15 Therasse P, Arbuck SG, Eisenhauer EA *et al.* New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst* 2000; **92**: 205–16.
16 Mazumdar M, Smith A, Schwartz LH. A statistical simulation study finds discordance between WHO criteria and RECIST guideline. *J Clin Epidemiol* 2004; **57**: 358–65.
17 Park JO, Lee SI, Song SY *et al.* Measuring response in solid tumors: comparison of RECIST and WHO response criteria. *Jpn J Clin Oncol* 2003; **33**: 533–7.
18 Trotti A, Colevas AD, Setser A *et al.* CTCAE v3.0: development of a comprehensive grading system for the adverse effects of cancer treatment. *Semin Radiat Oncol* 2003; **3**: 176–81.
19 Hirai K, Kawazoe Y, Yamashita K *et al.* Transcatheter partial splenic arterial embolization in patients with hypersplenism: a clinical evaluation as supporting therapy for hepatocellular carcinoma and liver cirrhosis. *Hepatogastroenterology* 1986; **33**: 105–8.
20 Sangro B, Bilbao I, Herrero I *et al.* Partial splenic embolization for the treatment of hypersplenism in cirrhosis. *Hepatology* 1993; **18**: 309–14.

肝動注癌化学療法における脾摘術とPSEの功罪

広岡昌史* 上原貴秀* 恩地森一*

索引用語：脾臓摘出術，PSE，肝動注癌化学療法，肝細胞癌

1 はじめに

肝細胞癌は慢性肝障害を背景に発症する．慢性肝障害の多くは門脈圧亢進症を合併しており，その結果種々の臨床症状を現す．具体的な症状として食道・胃静脈瘤，門脈圧亢進症性胃腸症，腹水，肝性脳症，脾機能亢進症などがある．

近年，肝動脈塞栓療法により治療効果が得られない症例や脈管浸潤をきたした進行肝癌症例に対し肝動注化学療法が普及しその有用性が報告されている(表1)．肝細胞癌患者の多くに血球減少があり，抗癌剤を連日使用することやインターフェロン(IFN)の使用によりさらに血球減少をきたし，しばしば治療を中断せざるをえない．門脈圧亢進症において，血球減少などの合併症を改善する目的に脾臓摘出術，部分的脾動脈塞栓術(partial splenic embolization; PSE)が用いられ始めている．

本稿では肝動注化学療法患者における脾臓摘出術とPSEの適応，治療効果，また両治療法の功罪について述べる．

2 肝動注化学療法施行患者における血球減少

進行肝癌に対する肝動注化学療法は2つに大別される．1つはリザーバーカテーテルと皮下留置型ポートを使用し持続的に抗癌剤を動注するもの[1~6]，もう1つはシスプラチンなどを使用し単回の抗癌剤動注を繰り返す方法である[7]．特にリザーバーシステムによる肝持続動注療法では複数の抗癌剤の連日使用を行ったり，IFNを使用したりすることにより血球減少をきたしやすい．当科におけるリザーバーシステムによる肝持続動注療法を施行した際，治療開始前より血球減少を呈していることも重なり，特に血小板において半数を超える症例に高度の減少がみられた．術前の血小板が10万/μlの症例であってもgrade 3以上の血小板低下がみられた．これらの患者は治療の中断を余儀なくされる．実際に抗癌剤による十分な効果もみられなかった．以上より抗癌剤投与中の血球減少，特に

Masashi HIROOKA et al: Splenectomy and PSE for advanced hepatocellular carcinoma
*愛媛大学大学院先端病態制御内科学［〒791-0295 愛媛県東温市志津川454］

表1　進行肝細胞癌に対する肝動注化学療法

投与薬剤	報告者(年)	奏功率(%)	1年生存率/2年生存率(%)
IFN α, 5FU	Ota[1] (2002)	13/29 (45)	49 / 29
IFN α, 5FU	Obi[2] (2006)	61/116 (53)	34 / 18
CDDP, 5FU	Ando[3] (2002)	23/48 (48)	45 / 31
CDDP, 5FU Leucovorin	Yamasaki[4] (2005)	14/29 (48)	48 / 24
CDDP, エトポシド	Sangro[5] (2002)	10/26 (38)	33 / 24
MTX, CDDP 5FU, IFN α	Kaneko[6] (2002)	13/29 (45)	− / 15
CDDP	吉川[7] (2007)	27/80 (33.8)	67.5 / 50.8

表2　脾臓摘出術，PSEの適応

汎血球減少
腹水
門脈圧亢進性胃腸症
食道胃静脈瘤
肝性脳症

血小板減少は抗癌剤の不十分な投与につながり，結果として奏功が得られない原因の1つとなっていると考えている．

3 肝細胞癌の肝動注化学療法施行患者における脾臓摘出術とPSEの適応

門脈圧亢進症患者に対する脾臓摘出術またはPSEの適応を示す(表2)．これらの中で化学療法を施行する際に問題となるのは血球減少である．当科では化学療法施行前の白血球2,000/μl未満または血小板が8万/μl以下の症例は，同意が得られた場合抗癌剤投与前に積極的に脾臓摘出術またはPSEを行うようにしている．肝動注療法施行中または休薬中にNCI-CTCAE分類にてgrade 3以上の血球減少をきたし治療の中断を余儀なくされる

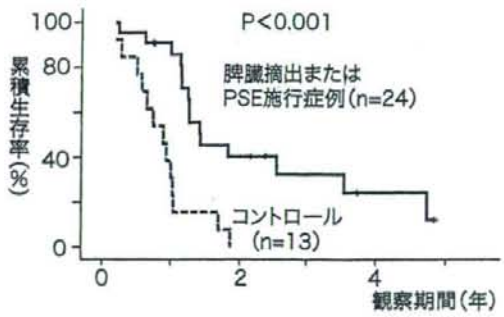

図1

症例についても脾臓摘出術またはPSEの適応としている．PSEの施行時期は可能であればカテーテル留置時に行っている．血管造影に要する回数が1回で終了することと化学療法を早く開始できるためである．PSE施行後血小板上昇に2〜3週を要するため化学療法の開始日はそれ以降になるためである．PSEは田尻らの方法に準じて塞栓物質のみで行っている[11]．塞栓物質によるPSEは簡便であり30分程度の時間で施行可能であるためカテーテル留置に手間がかからなければ同時に行ってもよいものと思われる．血行改変に時間を要する症例では患者の負担を考え，後日

表3　肝硬変症例に対する脾臓摘出術とPSE

治療	報告者（年）	術前血小板/白血球（/μL）	術後血小板/白血球（/μL）	主な合併症
脾臓摘出	緒方[8]（2005）	4.2±1.5万/2,712±1,354	15.3±4.8万/5,131±1,187	発熱22%，門脈血栓17%，腹水7%
脾臓摘出	Sugawara[9]（2000）	4.7±0.3万/記載なし	23.1±2.6万/記載なし	門脈血栓23%
脾臓摘出	Watanabe[10]（2007）	3.3±1.2万/2,500±1,100	22.0±15万/5,600±1,800	門脈血栓18.8%，腹水8%，胸水4%
脾臓摘出	自験例	5.1±3.8万/	22.1±6.5万/μl/	門脈血栓13.6%，腹水9.1%，胸水4.5%
PSE	Tajiri[11]（2007）	8.2±3.9万/記載なし	16.7±6.9万/記載なし	記載なし
PSE	渡辺[12]（2007）	3.8万/記載なし	8.6万/記載なし	脾膿瘍2例
PSE	Hayashi[13]（2007）	4.5±1.2万/記載なし	11.6±5.1万/記載なし	発熱・左側腹部痛（程度不明）100% 胸水7.1%，腹水2.4%，胃潰瘍2.4%
PSE	Sangro B[14]（1993）	5.5±2.0万/3,016±1,317	18.04±7.7万/9,901±3,007	15日以上の発熱7.5%，胸水10% 腹水30%
PSE	自験例	4.5±2.3万/2,510±950	14.2±5.5万/4,300±1,100	15日以上の発熱5.0%，腹水15%，胸水5%

PSEを施行するようにしている．

4 脾臓摘出術，PSE施行患者における肝動注化学療法の治療成績

2002年1月から2007年6月までに，肝動注化学療法患者に対し22例脾臓摘出術，2例PSEを施行した．同時期に脾臓摘出術またはPSEを施行せずに肝動注化学療法を施行した患者をコントロールとして完遂率，奏功率，生存率を比較した．脾臓摘出術またはPSEを施行した症例では，カテーテルトラブルによる2例を除く全症例で完遂が可能であった．十分な抗癌剤投与を行うことにより，奏功率は向上し，生存率は脾臓摘出術やPSEを行った症例が有意に良好であった（図1）．

5 肝動注化学療法における脾臓摘出術，PSEの功罪

表3に門脈圧亢進症患者における脾臓摘出術とPSEによる血球改善の報告を示す．両治療法ともに有意な血球改善がみられている．当科においてもIFN投与や肝癌局所療法施行前などにも脾臓摘出術やPSEを施行している．これらの患者を含めた当科で施行した脾臓摘出，PSEの血小板回復は両治療ともに血小板数は10万/μl以上に改善したが，脾臓摘出症例はPSEに比べ高い上昇をきたした（脾臓摘出22.1±6.5万/μl，14.2±5.5万/μl）．さらに術前血小板数により血小板の改善の程度を比較した場合，術前血小板数3.5万/μl未満の症例では脾臓摘出術では十分な血小板上昇が得られたのに対し（18.9±

表4　肝癌化学療法患者における脾臓摘出術とPSEの長所と短所

	長所	短所
脾臓摘出術	・血球数改善が確実 ・肝予備能の改善	・術後重篤な感染症，門脈血栓症発生の危険性がある ・PSEに比べ侵襲あり
PSE	・手技が簡便 ・術後感染症，門脈血栓は稀 ・肝予備能の改善	・血球改善が不十分な症例あり

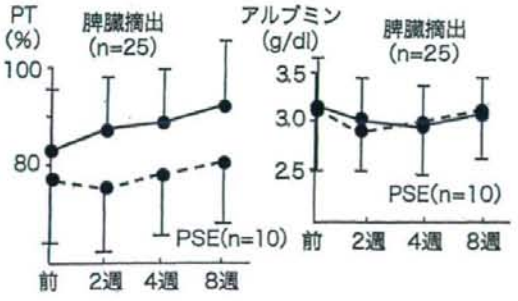

図2

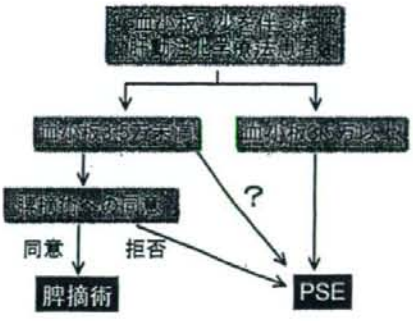

図3

5.0万/μl)，PSE後では10万前後への上昇しか得られなかった(10.1±3.4万/μl)．以前の報告も同様でありWatanabeらの報告[10]では，脾臓摘出により術前平均3.3万/μlの血小板が平均22万/μlに改善したのに対し，渡辺らの報告ではPSEは平均3.8万/μlの血小板は8.6万/μl程度にしか改善できていなかった[12]．術前血小板脾臓摘出血小板10万の症例までgrade 3以上の血小板減少がみられていることから肝持続動注を主眼とした場合，使用薬剤や投与方法によっては術前3.5万/μl未満の症例においてPSEでは血小板の上昇程度は不十分であることが示唆される．したがって術前血小板数が3.5万/μlを下回るような極めて血小板数が低い症例は脾臓摘出術が行われるべきである．一方，脾臓摘出術は肺炎球菌ワクチン投与や術後管理の工夫によりその頻度は減少しているものの，門脈血栓症や術後重症感染症の危険性は存在する．表3に示す通り脾臓摘出術では高率に門脈血栓を合併している．逆にPSEでは門脈血栓症は稀である．現在ステロイドやNSAIDs投与の工夫により以前に比べPSEは負担の少ない治療になっている．術前血小板数が3.5万/μl以上の症例では簡便で低侵襲なPSEの良い適応と考えられる．脾臓摘出術による術後門脈血栓症や重篤な感染症のリスクを考えた場合，術前血小板数が3.5万/μlを超えるようなある程度血小板数が保たれている症例ではPSEが選択されるべきである(図2)．3.5万/μlという数字が脾臓摘出術とPSEの選択基準として妥当か否かは，今後症例を集積しさらに検討が必要である．これらの処置は肝予備能を改善することが報告されている[8~14]．両治療法の間にPT，アルブミンで両治療法ともに改善はみられたが，その改善の程度に差はみられなかった(図3)．

6 おわりに

血球数減少を合併した肝動注化学療法患者では，脾臓摘出術やPSEを行うことにより予後の改善が期待できると考えられる．

血球消費を抑制する観点から脾臓に対する有用性を述べたが，その一方で血球産生を向上する治療も重要である．白血球についてはG-CSFが広く使用され，今後はPEG製剤が普及してくるものと思われる．血小板産生については，現在トロンボポエチンレセプター活性化作用を持つ第2世代の血小板増加薬が開発され臨床試験が順調に進んでいる．特に経口投与可能なEltrombopag (SB-497115) [15,16]は臨床試験が進行中である．これらの薬剤[17]が日本でも使用されるようになれば，今後脾臓摘出術やPSEを施行する意義が問われる可能性はある．数年後には脾臓に対する治療自体が再考されるものと考えられる．

文　献

1) Ota H, Nagano H, Sakon M et al : Treatment of hepatocellular carcinoma with major portal vein thrombosis by combined therapy with subcutaceous interferon – alpha and intra – arterial 5-fluorouracil; role of type 1 interferon receptor expression. Br J cancer 93 : 557–564, 2005
2) Obi S, Yoshida H, Toune R et al : Combination therapy of intraarterial 5 –fluorouracil and systemic interferon – alpha for advanced hepatocellular carcinoma with portal venous invasion. Cancer 106 : 1990–1997, 2006
3) Ando E, Tanaka M, Yamashita F et al : Hepatic infusion chemotherapy for advanced hepatocellular carcinoma with portal vein thrombosis: analysis of 48 cases. Cancer 95 : 588–595, 2002
4) Yamasaki T, Kurokawa F, Shirahashi H et al : Novel arterial infusion chemotherapy using cisplatin, 5 – fluorouracil, and leucovorin for patients for patients with advanced hepatocellular carcinoma. Hepatology Research 23 : 7–17, 2002
5) Sangro B, Rios R, Bilbao I et al : Efficacy and toxicity of intra arterial cisplatin and etoposide for advanced hepatocellular carcinoma. Oncology 62 : 293–298, 2002
6) Kaneko S, Urabe T, Kobayashi K : Combination chemotherapy for advanced hepatocellular carcinoma complicated by major portal vein thrombosis. Oncology 62 (Suppl 1) : 69–73, 2002
7) 吉川正治，須永雅彦，岡部真一郎，他：アイエーコール（CDDP），肝胆膵 55：813–821, 2007
8) 緒方俊郎，奥田康司，守永暁生，他：巨大脾腫を伴う肝硬変における門脈圧亢進症の治療―脾摘の効果―．日門亢会誌 11：249–255, 2005
9) Sugawara Y, Yamamoto J, Shimada K et al : Splenectomy in patients with hepatocellular carcinoma and hypersplenism. J Am Coll Surg 190 : 446–450, 2000
10) Watanabe Y, Horiuchi A , Yoshida M et al : Significance of laparoscopic splenectomy in patients with hypersplenism. World J Surg 31 : 549–555, 2007
11) Tajiri T, Onda M, Yoshida H et al : Long – term hepatological and biochemical effects of partial splenic embolization in hepatic cirrhosis. Hepatogastroenterology 49 : 1445–1448, 2002
12) 渡辺勲史，白石光一，松崎松平：部分的脾動脈塞栓術（PSE）を中心とした肝硬変の治療戦略．日門亢会誌 10：165–169, 2004
13) Hayashi H, Beppu T, Masuda T et al ; Predictive factors for platelet increase after partial splenic embolization in liver cirrhosis patients. J Gastroenterology and hepatology 22 : 1638–1642, 2007
14) Sangro B, Bilbao I, Herrero I et al : Partial splenic embolization for the treatment of hypersplenism in cirrhosis. Hepatology 18 : 309–314, 1993
15) Bussel JB, Cheng G, Saleh MN et al : Eltrombopag for the treatment of chronic idiopathic thrombocytopenic purpura. N Engl J Med 357 : 2237–2247, 2007
16) Jenkins JM, Williams D, Deng Y et al : Phase 1 clinical study of eltrombopag, an oral, nonpeptide thrombopoietin receptor agonist. Blood 109 : 4739–4741, 2007
17) McHutchison JG, Dusheiko G, Shiffman ML et al : Eltrombopag for thrombocytopenia in patients with cirrhosis associated with hepatitis C. N Engl J Med 357 : 2227–2236, 2007

Hepatology Research 2008; **38** (Suppl. 1): S102–S106 doi: 10.1111/j.1872-034X.2008.00434.x

Long-term outcome of branched-chain amino acid treatment in patients with liver cirrhosis

Hisataka Moriwaki, Makoto Shiraki, Hideki Fukushima, Masahito Shimizu, Junpei Iwasa, Takafumi Naiki and Masahito Nagaki

Department of Internal Medicine, Gifu University School of Medicine

Clinical impact of protein-energy malnutrition (PEM) on the outcome of liver cirrhosis is well documented. As a candidate interventional modality to improve PEM in cirrhosis, effects of branched-chain amino acid (BCAA) supplementation on event-free survival and quality of life (QOL) was first reported by Yoshida *et al.* in 1989. Although critical arguments still continue regarding the effects of BCAA, several randomized trials in the last 5 years have brought positive results, and seem to have settled the discussion in a favorable direction for the efficacy of BCAA in liver cirrhosis. Actually, The European Society for Clinical Nutrition and Metabolism (ESPEN) upgraded the recommendation of BCAA supplementation in decompensated liver cirrhosis in the latest revision of its guidelines in 2006, by referring to the literatures from Italy and Japan. Particularly in these two long-term randomized studies with 1–2 years-supplementation, event-free survival was estimated by employing composite endpoints such as aggravation of hepatic failure (ascites, peripheral edema, hepatic encephalopathy, and jaundice), rupture of esophageal or gastric varices, development of liver cancer, and death from any cause. Both trials agreed on the effect of BCAA to reduce the incidence of hepatic failure, thus contributing to the rise in the event-free survival. Quality of life is another essential marker of outcome survey. Marchesini, Muto, and Nakaya reported the improved QOL in cirrhotics with BCAA supplementation. In particular, quantitative analysis of QOL measured by Short Form 36 (SF-36) questionnaire demonstrated a significant recovery of general heath perception score in BCAA supplemented patients in a randomized trial. In this article, the long-term outcome of BCAA treatment in liver cirrhosis will be reviewed with its action mechanisms. In addition, the effects of BCAA treatment on the incidence of liver cancer in obese patients with type C liver cirrhosis, significance of obesity as a risk factor for type C liver cancer, and a possible role of Body Mass Index to estimate the histological grade of fat deposition in the liver will be briefly discussed.

Key words: branched-chain amino acids (BCAA), liver cancer, liver cirrhosis, liver failure, obesity, survival

INTRODUCTION

IT IS WELL known that the liver plays a central role in the nutrient metabolism. After intestinal absorption, the majority of both macronutrients, consisting of carbohydrate, lipid and protein, and micronutrients, such as vitamins and trace minerals, are taken up by the liver, stored there, and transported to peripheral tissues depending on its demand. In addition, carrier proteins of these nutrients are also produced by the liver. Hence, nutrient metabolism is often impaired in liver diseases, leading to protein and energy malnutrition (PEM). The incidence of PEM varies among liver diseases, but reaches up to 85% particularly in advanced cirrhosis.[1,2]

Correspondence: Professor Hisataka Moriwaki, Department of Internal Medicine, Gifu University School of Medicine, 1-1 Yanagido, Gifu 501-1194, Japan. Email: hmori@gifu-u.ac.jp

Conflict of Interest: No conflict of interest has been declared by the author(s).

CLINICAL SIGNIFICANCE OF PROTEIN-ENERGY MALNUTRITION (PEM) IN THE OUTCOME OF LIVER CIRRHOSIS

PROTEIN NUTRITIONAL STATE is commonly estimated anthropometrically by arm muscle circumference (AMC)[1] or blood biochemically by serum albumin level.[2] Similarly, energy nutritional state is measured by triceps skinfold thickness (TSF)[1] or by indirect calorimetry.[2] The grade of PEM progresses in parallel with the increasing severity of liver cirrhosis,[2] and is regarded as a most significant factor that determines the survival rate of the patients with liver cirrhosis.[1,2] As possible interventions for PEM of cirrhotics, current guidelines recommend branched-chain amino acid

(BCAA) supplementation for protein malnutrition[3] and divided meals including a late evening snack (LES) for energy malnutrition.[3,4] However, effects of such nutritional support on the survival rate itself have long been controversial until recently.

EFFECTS OF BRANCHED-CHAIN AMINO ACID TREATMENT ON EVENT-FREE SURVIVAL AND QUALITY OF LIFE IN LIVER CIRRHOSIS

BENEFICIAL EFFECTS OF BCAA supplementation on the survival rate of cirrhotic patients was first reported by Yoshida *et al.* in 1989.[5] Although critical arguments still continue regarding such effects of BCAA,[6] several randomized trials in the last 5 years have brought positive results,[7–10] and seem to have settled the discussion in a favorable direction toward the efficacy of BCAA in liver cirrhosis. Actually, the European Society for Clinical Nutrition and Metabolism (ESPEN) upgraded the recommendation of BCAA supplementation in decompensated liver cirrhosis in the latest revision of its guidelines in 2006,[1] by referring to the literature, references 7 and 9 in this paper. Particularly in these two long-term randomized studies with 1–2 years-supplementation,[7,9] event-free survival was estimated by employing composite endpoints including aggravation of hepatic failure (ascites, peripheral edema, hepatic encephalopathy, and jaundice), rupture of esophageal or gastric varices, development of liver cancer, and death from any cause. Both trials agreed on the effect of BCAA in reducing the incidence of hepatic failure, thus contributing to the rise in the event-free survival.[7,9]

Quality of life (QOL) is another essential marker of outcome survey.[11] Marchesini,[7] Muto,[9] and Nakaya[10] reported the improved QOL in cirrhotics with BCAA supplementation. In particular, quantitative analysis of QOL measured by the Short Form 36 (SF-36) questionnaire demonstrated a significant recovery of general health perception scores in BCAA-supplemented patients in a randomized trial.[9]

EFFECTS OF BRANCHED CHAIN AMINO ACID TREATMENT ON INCIDENCE OF LIVER CANCER IN TYPE C LIVER CIRRHOSIS

AMONG COMPOSITE ENDPOINTS as described before, development of liver cancer was significantly inhibited by BCAA supplementation in type C cirrhotic patients with body mass index above 25.[12] In contrast, BCAA showed no inhibitory effect on the incidence of liver cancer in lean cirrhotics with a BMI below 25.[12]

RISK FACTORS FOR TYPE C LIVER CANCER: SIGNIFICANCE OF OBESITY

CONCURRENT PRESENCE OF diabetes mellitus,[13–19] hyperinsulinemia, or obesity[1,7,13,20–24] is regarded as a significant risk factor for the development of liver cancer. This concern has not been appreciated sufficiently in Japan, since the proportion of obese patients among all cirrhotics was low until the year 1995[2] (see Fig. 1). However, the cirrhotic cohort registered in 2000 showed the proportion as high as that in the age- and sex-matched general population[12] (Fig. 1). These data suggest that obesity prevailed in such a short period in patients with liver cirrhosis.

In such a patient cohort, obesity was a significant risk factor for liver carcinogenesis in addition to other risk factors including sex (male), lower serum albumin concentration, higher serum alpha-fetoprotein concentration and the presence of diabetes mellitus.[17]

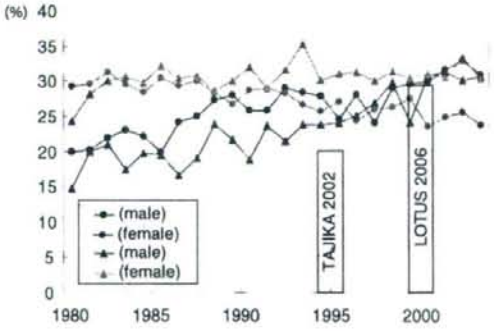

Figure 1 Proportion of overweight/obese subjects in patients with liver cirrhosis and in the general population in Japan. Overweight and obese were defined by body mass index above 25 and 30, respectively. Lines indicate the proportion of overweight/obese subjects in the general population according to National Health Survey conducted by the Ministry of Health, Labor, and Welfare of Japan. Bars indicate the proportion of overweight/obese patients in cirrhotics in the year 1995 (Tajika M, *et al.*[2]) and 2000 (LOTUS trial,[11]), respectively. (–●–) 50–59 years (male); (–●–) 50–59 years (female); (–▲–) 60–69 years (male); (–▲–) 60–69 years (female)

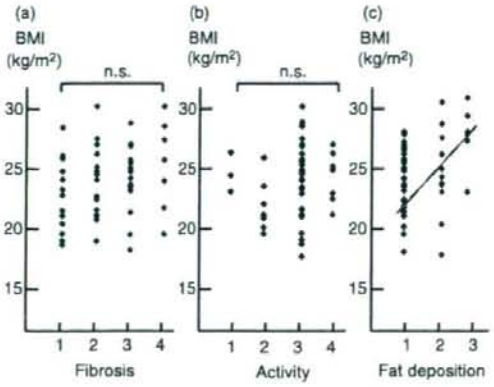

Figure 2 Correlations between body mass index (BMI) and the histological grades of fibrosis (a), inflammatory activity (b) and fat deposition (c) in the liver of type C chronic hepatitis and cirrhosis. The grade of hepatic fat deposition was defined as 1 with <10% of the total parenchymal area, two with 10–30%, and three with >30%. $P < 0.01$ for panel C by Spearman's rank correlation test. n.s., not significant.

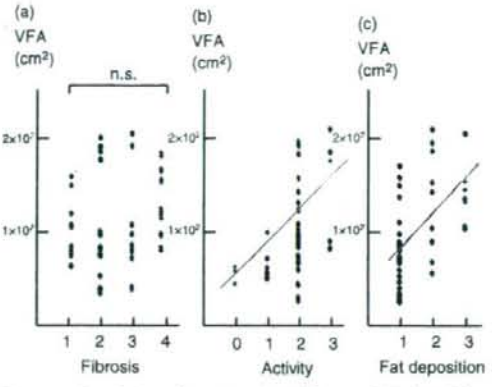

Figure 3 Correlations between visceral fat area (VFA) and the histological grades of fibrosis (a), inflammatory activity (b) and fat deposition (c) in the liver of type C chronic hepatitis and cirrhosis. $P < 0.01$ for panels B and C by Spearman's rank correlation test. n.s., not significant.

BODY MASS INDEX ESTIMATES THE HISTOLOGICAL GRADE OF FAT DEPOSITION IN THE LIVER

OBESITY IS USUALLY defined by body mass index above 25 in Japan and above 30 in western countries. The promotional role of obesity in liver carcinogensis can be hypothesized in two ways; (i) obesity induces fat deposition in the liver, leading to generation of lipid peroxiside and reactive oxygen species (ROS) and (ii) obesity induces general insulin resistance. Reactive oxygen species damage DNA and may act at the very early stage of carcinogenesis, while insulin resistance and resulting hyperinsulinemia promote the growth of cancer cells.

Figure 2 indicates correlations between body mass index (BMI) and the histological grades of fibrosis (Fig. 2a), inflammatory activity (Fig. 2b) and fat deposition (Fig. 2c) in the liver of type C chronic hepatitis and cirrhosis. These data suggest the direct relation between obesity and fat deposition in the liver as described above.

Figure 3 indicates correlations between visceral fat area (VFA) and the histological grades of fibrosis (A), inflammatory activity (B) and fat deposition (C) in the liver of type C chronic hepatitis and cirrhosis. Visceral fat area is generally accepted as the most important factor that affects the development of metabolic syndrome. However, it is interesting that a more simple and easy-to-measure parameter, BMI, showed a higher correlation coefficient with hepatic fat deposition than the visceral fat area (Table 1).

Figure 4 indicates that hepatic fat deposition affects both the histological grades of liver fibrosis and inflammatory activity in type C chronic hepatitis and cirrhosis. Although these findings do not directly support the relation between obesity and liver carcinogenesis, hepatic fat deposition seems to determine the progression of the activity of liver disease.

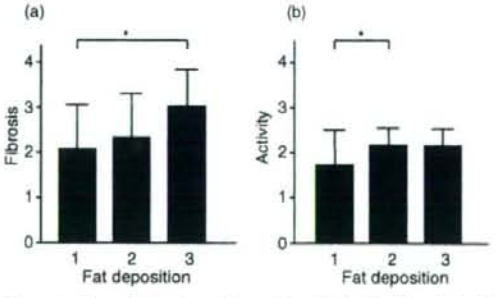

Figure 4 Hepatic fat deposition affects both the histological grades of liver fibrosis and inflammatory activity in type C chronic hepatitis and cirrhosis. Values are expressed as mean and standard deviation. *$P < 0.05$ by Kruskal-Wallis test.

Table 1 Correlations between blood biochemical parameters and the histological grade of fat deposition in the liver

	Spearman correlation coefficient		Multiple regression coefficient	
	r	*P* value	*r*	*P* value
Triglyceride	0.386	<0.01		
FBS	0.348	<0.01	0.008	<0.0001
T-bil	0.103	n.s.		
AST	0.160	n.s.		
ALT	0.092	n.s.		
T-chol	0.047	n.s.		
HbA1c	0.217	n.s.		
Hyaluronic acid	0.284	n.s.		
P3NP	0.341	n.s.		
Type 4 collagen	0.081	n.s.		
HCV-RNA	−0.009	n.s.		
Serum-Fe	0.236	n.s.		
Ferritin	0.277	n.s.		
Platelet count	0.007	n.s.		
BMI	0.579	<0.01	0.100	<0.0001
Visceral fat	0.555	<0.01	0.084	<0.0001

FBS, fasting blood sugar; T-bil, total bilirubin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; T-chol, total cholesterol; HbA1c, hemoglobin A1c; P3NP, amino terminal propeptide of type 3 procollagen; HCV-RNA, hepatitis C virus-ribonucleic acid; BMI, body mass index.

CONCLUSION

LONG-TERM TREATMENT with BCAA improves the clinical outcome of cirrhotic patients by reducing the event of liver failure and, in obese patients, by inhibiting liver carcinogenesis. The mechanism of such action by BCAA and, furthermore, the role of obesity in liver carcinogenesis will be a very important and interesting target of future basic and clinical studies.

ACKNOWLEDGMENTS

THIS WORK WAS supported in part by Grants-in-aid from the Ministry of Health, Labor, and Welfare of Japan.

REFERENCES

1 Alberino F, Gatta A, Amodio P *et al.* Nutrition and survival in patients with liver cirrhosis. *Nutrition* 2001; **17**: 445–50.
2 Tajika M, Kato M, Mohri H *et al.* Prognostic value of energy metabolism in patients with liver cirrhosis. *Nutrition* 2002; **18**: 229–34.
3 Plauth M, Cabre E, Riggio O *et al.* ESPEN guidelines on enteral nutrition: liver disease. *Clin Nutr* 2006; **25**: 285–94.
4 ASPEN Board of Directors and the Clinical Guidelines Task Force. Guidelines for the use of parenteral and enteral nutrition in adult and pediatric patients. *JPEN* 2002; **26** (Suppl 1): 73–6.
5 Yoshida T, Muto Y, Moriwaki H, Yamato M. Effect of long-term oral supplementation with branched-chain amino acid granules on the prognosis of liver cirrhosis. *J Gastroenterol* 1989; **24**: 692–8.
6 Charlton M. Branched-chain amino acid-enriched supplements as therapy for liver disease: Rasputin lives. *Gastroenterology* 2003; **124**: 1980–2.
7 Marchesini G, Bianci G, Merli M *et al.* Nutritional supplementation with branched-chain amino acids in advanced cirrhosis: a double-blind, randomized trial. *Gastroenterology* 2003; **124**: 1792–801.
8 Poon RTP, Yu WC, Fan ST *et al.* Long-term oral branched chain amino acids in patients undergoing chemoembolization for hepatocellular carcinoma: a randomized trial. *Aliment Pharmacol Ther* 2004; **19**: 779–88.
9 Muto Y, Sato S, Watanabe A *et al.* Effects of oral branched-chain amino acid granules on event-free survival in patients with liver cirrhosis. *Clin Gastroenterol Hepatol* 2005; **3**: 705–13.
10 Nakaya Y, Okita K, Suzuki K *et al.* BCAA-enriched snack improves nutritional state of cirrhosis. *Nutrition* 2007; **23**: 113–20.
11 Sanyal AG, Koff RS. Methodologies in outcomes research. *Hepatology* 1999; **29**: 1S–2S.
12 Muto Y, Sato S, Watanabe A *et al.* Overweight and obesity increase the risk for liver cancer in patients with liver cirrhosis and long-term oral supplementation with branched-chain amino acid granules inhibits liver carcinogenesis in heavier patients with liver cirrhosis. *Hepatol Res* 2006; **35**: 204–14.
13 El-Serag HB. Hepatocellular carcinoma: trends in the United States. *Gastroenterology* 2004; **127** (Suppl 1): S27–34.
14 Kiyosawa K, Umemura T, Ichijo T *et al.* Hepatocellular carcinoma: recent trends in Japan. *Gastroenterology* 2004; **127** (Suppl 1): S17–26.
15 Fattovich G, Stroffelini T, Zagni I, Donato F. Hepatocellular carcinoma in cirrhosis: incidence and risk factors. *Gastroenterology* 2004; **127** (Suppl 1): S35–50.
16 Lagiou P, Kuper H, Stuver SO, Tzonou A, Trichopoulas D, Adami HO. Role of diabetes mellitus in the etiology of hepatocellular carcinoma. *J Natl Cancer Inst* 2000; **92**: 1096–9.
17 Hassan MM, Hwang LY, Hatten CJ *et al.* Risk factors for hepatocellular carcinoma: synergism of alcohol with viral

hepatitis and diabetes mellitus. *Hepatology* 2002; **36**: 1206–13.
18 El-Serag HB, Tran T, Everhart JE. Diabetes increase the risk of chronic liver disease and hepatocellular carcinoma. *Gastroenterology* 2004; **126**: 460–8.
19 Davila JA, Morgan RO, Shaib Y, McGlynn KA, El-Serag HB. Diabetes increases the risk of hepatocellular carcinoma in the United States: a population based case control study. *Gut* 2005; **54**: 533–9.
20 Calle EE, Rodriguez C, Walker-Thurmond K, Thun MJ. Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. *N Engl J Med* 2003; **348**: 1625–38.
21 Caldwell SH, Crespo DM, Kang HS, Al-Osaimi AM. Obesity and hepatocellular carcinoma. *Gastroenterology* 2004; **127** (Suppl 1): S97–103.
22 Oh SW, Yoon YS, Shin SA. Effects of excess weight on cancer incidences depending on cancer sites and histologic findings among men: Korea National Health Insurance Corporation Study. *J Clin Oncol* 2005; **23**: 4742–54.
23 Moore MA, Park CB, Tsuda H. Implications of the hyperinsulinemia-diabetes-cancer link for preventive efforts. *Eur J Cancer Prev* 1998; **7**: 89–107.
24 Balkau B, Kahn HS, Courbon D, Eschwege E, Ducimetiere P. Hyperinsulinemia predicts fatal liver cancer but is inversely associated with fatal cancer at some other sites: the Paris Prospective Study. *Diabetes Care* 2001; **24**: 843–9.

International Immunology Advance Access published May 16, 2008

International Immunology 2008; 1 of 11
doi:10.1093/intimm/dxn046

Role of Vα14$^+$ NKT cells in the development of Hepatitis B virus-specific CTL: activation of Vα14$^+$ NKT cells promotes the breakage of CTL tolerance

Hiroyasu Ito[1,2,*], Kazuki Ando[1,3,*], Tetsuya Ishikawa[4], Toshinori Nakayama[5], Masaru Taniguchi[6], Kuniaki Saito[1,7], Michio Imawari[8], Hisataka Moriwaki[9], Takashi Yokochi[2], Shinichi Kakumu[10] and **Mitsuru Seishima[1]**

[1]Department of Informative Clinical Medicine, Gifu University Graduate School of Medicine, 1-1 Yanagido, Gifu 501-1194, Japan
[2]Department of Microbiology and Immunology, Aichi Medical University, Nagakute, Aichi 480-1195, Japan
[3]Goto Clinic, Gifu 503-0899, Japan
[4]Cancer Immunotherapy Center, Nagoya Kyoritsu Hospital, 1-172 Hokke, Nakagawa, Nagoya 454-0933, Japan
[5]Department of Immunology, Graduate School of Medicine, Chiba University, 1-8-1 Inohana, Chuo-ku, Chiba 260-8670, Japan
[6]RIKEN Research Center for Allergy and Immunology, RIKEN Yokohama Institute, 1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama City, Kanagawa 230-0045, Japan
[7]Human Health Sciences, Graduate School of Medicine and Faculty of Medicine, Kyoto University, 53 Kawahara-cho, Shogoin, Sakyo, Kyoto 606-8507, Japan
[8]Second Department of Internal Medicine, Showa University School of Medicine, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142-8666, Japan
[9]First Department of Internal Medicine, Gifu University Graduate School of Medicine, 1-1 Yanagido, Gifu 501-1194, Japan
[10]Division of Gastroenterology, Department of Internal Medicine, Aichi Medical University, Nagakute, Aichi 480-1195, Japan

Keywords: CTL, hepatitis B virus, NKT cell

Abstract

CTLs are thought to be major effectors for clearing viruses in acute infections including hepatitis B virus (HBV). Persistent HBV infection is characterized by a lack of or a weak CTL response to HBV, which is thought to reflect tolerance to HBV antigens. In the present study, we found that alpha-galactosylceramide (α-GalCer), a ligand for Vα14-positive NKT cells, strongly enhanced the induction and proliferation of HBV-specific CTLs by HBsAg. In HBsAg transgenic mice, which are thought to be tolerant to HBV-encoded antigens, administration of HBsAg or α-GalCer alone failed to induce HBsAg-specific CTLs, but they were induced by co-administration of both compounds. Furthermore, by limiting dilution analysis, we confirmed the existence of HBsAg-specific CTL precursors in the HBsAg transgenic mice immunized with HBsAg and α-GalCer. A blocking experiment using antibodies to cytokines and CD40 ligand showed that IL-2 and CD40–CD40L interaction mediate the enhancement of CTL induction caused by α-GalCer through NKT cell activation. Our results may open up a new method for clearing the virus from patients with persistent HBV infection.

Introduction

Most perinatal hepatitis B virus (HBV) infections become persistent due to the failure to mount an effective immune response. Individuals with such persistent infection usually become asymptomatic carriers (ASCs) who are thought to be immunologically tolerant to HBV-encoded antigens. However, this tolerance is eventually broken and the development of chronic hepatitis (CH) is observed. Once CH has been developed, a weak CTL response to HBV becomes detectable and is thought to form an important part of the pathogenesis of CH, liver injury and down-regulation of the vial replication (1).

In many types of viral infections, CTLs have been shown to play critical roles on the clearance of the viruses (2–4). The same scenario is applicable in HBV infection. However, the clearance or continuous suppression of HBV is not observed in all the cases with CH possibly because of the

*These authors contributed equally to this study.

Correspondence to: H. Ito; E-mail: hito@gifu-u.ac.jp

Transmitting editor: H. Karasuyama

Received 10 August 2007, *accepted* 16 April 2008