Table II. Characteristics of patients aged 65 years or older with HCV-RNA and without elevated baseline transaminase levels (ASAT and ALAT ≤40 IU/l) stratified by the age.

Features	65–69 years (n=79 (65.8%))	70–74 years (n=25 (20.8%))	75–80 years (n=16 (13.3%))	Differences p-value
Men	29 (36.7%)	11 (44.0%)	11 (68.8%)	0.062
Follow-up (years)	8.6 (3–31.5)	7.0 (3–12.6)	4.5 (3–17.6)	0.011
ASAT (IU/l)	27 (11–39)	35 (16–40)	28 (15–40)	0.004
ALAT (IU/l)	22 (6–40)	25 (9–40)	22 (9–37)	0.604
Albumin (g/dl)	4.1 (3.2–4.9)	4.1 (3.0–4.4)	4.0 (2.4–4.5)	0.247
Platelets ($\times 10^3/mm^3$)	193 (120–298)	177 (120–343)	182 (120–263)	0.408
HCV RNA (MEq/ml)	4.2 (<0.5–34.6)	6.5 (<0.5–120)	4.0 (<0.5–17.1)	0.181
HCV genotypes (1b:2a:2b:ND)	51:19:2:4	21:1:1:1	13:0:0:2	0.074

Abbreviations: HCV = hepatitis C virus; ASAT = aspartate aminotransferase; ALAT = alanine aminotransferase; MEq = megaequivalents; ND = not determined. Data are expressed as the number (%) or the median with the range in parentheses.

female patients aged 65 years or older. Cirrhosis tended to occur more frequently in male than in female patients. There were marked gender differences in the development of HCC. At 5 and 10 years of follow-up, HCC occurred more frequently in men than in women (18% and 25% versus 9% and 9%, respectively, p=0.033).

Complications and death in patients with the baseline transaminase levels ≦40 IU/l and ≧41IU/l

Of the 120 patients with baseline transaminase levels ≦40 IU/l, 33 (27.5%) developed complications during follow-up (hypertension in 9 (27%), diabetes in 7 (21%), both complications in 1 (3%), pulmonary disease in 4 (12%), heart disease in 4 (12%), and other illnesses in the remaining 8 (24%)). At 5, 10, and 15 years of follow-up, respectively, death occurred more frequently in the patients with complications than in those without complications (10%, 18%, and 45% versus 0%, 5%, and 5%, p=0.015) (Figure 5).

Among 9 of the 120 (7.5%) patients who died, liver disease was the cause of death in only one. Of the remaining 8 (89%) patients, 4 died of heart failure or infarction, and one each of pneumonia, cerebral hemorrhage, renal insufficiency, and decrepitude. Death was more frequent in the patients aged ≥70 years than in those aged <70 years at presentation (p=0.006) (Figure 6).

Complications and death in patients with the baseline transaminase levels ≧41 IU/l

Of the 212 patients with baseline tranasaminase levels ≧41 IU/l, 83 (39.2%) developed complications during follow-up (hypertension in 18 (22%), diabetes in 23 (28%), both complications in 10 (12%), extrahepatic malignancies in 12 (15%), and other diseases in the remaining 20 (24%)). There were no differences in the frequency of death between the patients with and those without complications, however (Figure 7).

Among 34 of the 212 (14.0%) patients who died, liver disease was the most frequent cause of death and occurred in 20 (59%); the frequency was higher than that (11% (1/9)) in the patients with transaminase levels ≦40 IU/l at baseline (p=0.021). There were no differences in the frequency of death among

Table III. Characteristics of patients with HCV-RNA aged 65 years or older and with elevated baseline transaminase levels (ASAT and/or ALAT ≥41 IU/l) stratified by the age.

Features	65–69 years (n=140 (66.0%))	70–74 years (n=48 (22.6%))	75–80 years (n=24 (11.3%))	Differences p-value
Men	63 (45.0%)	25 (52.1%)	16 (66.7%)	0.707
Follow-up (years)	9.0 (3–18.9)	8.4 (3–17.2)	7.7 (3–14.7)	0.061
ALAT (IU/l)	82 (28–496)	74 (27–440)	64 (30–269)	0.959
ASAT (IU/l)	67 (22–411)	67 (34–309)	71 (35–172)	0.201
Albumin (g/dl)	4.1 (3.2–5.3)	4.1 (3.4–4.6)	3.9 (3.4–4.7)	0.005
Platelets ($\times 10^3/cm^3$)	171 (120–313)	180 (120–289)	157 (120–263)	0.398
HCV RNA (MEq/ml)	5.9 (<0.5–44.8)	5.6 (<0.5–30.0)	3.0 (<0.5–49.0)	0.251
HCV genotypes (1b:2a:2b:ND)	121:19:8:6	37:7:4:1	18:2:0:2	0.294

Abbreviations: HCV = hepatitis C virus; ASAT = aspartate aminotransferase; ALAT = alanine aminotransferase; MEq = megaequivalents; ND = not determined.

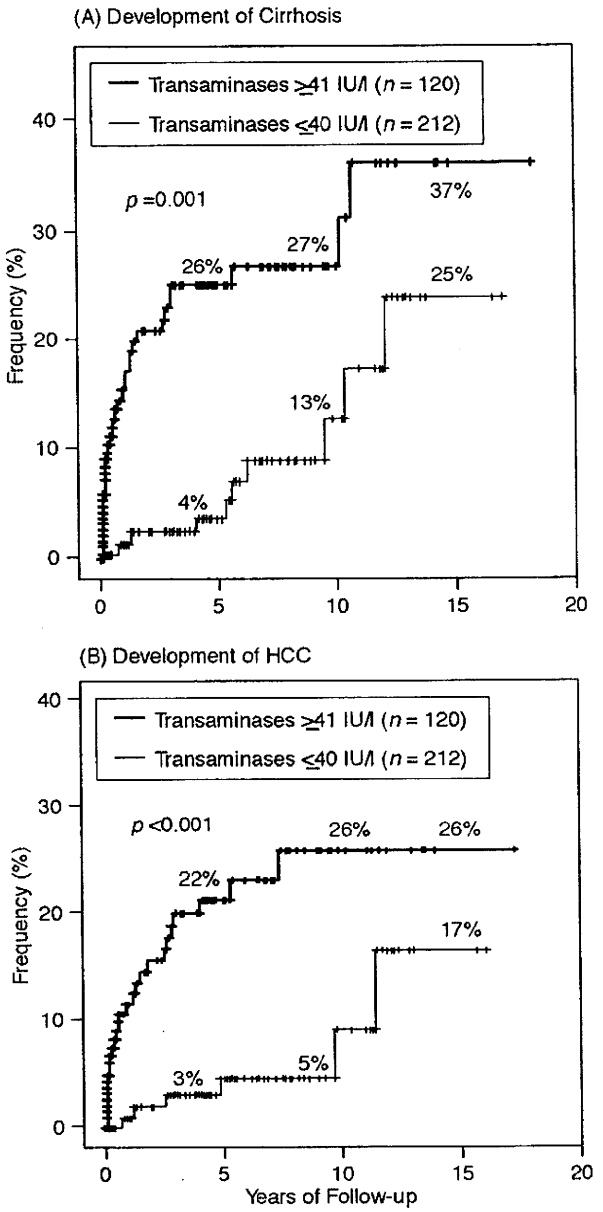

Figure 1. Development of cirrhosis (A) and HCC (hepatocelllular carcinoma) (B) in patients over 65 years of age with chronic hepatitis C who were followed-up without receiving antiviral treatment. Patients with and without elevated baseline transaminase levels are compared.

the patients in distinct age groups who had elevated baseline transaminase levels at baseline (Figure 8).

Discussion

The World Health Organization defines elderly individuals as those aged ≥65 years. In general, IFN is indicated for patients under 65 years of age, in view of frequent side effects and safety precautions. HCC develops increasingly with age and in the majority after 65 years, and in Japan approximately

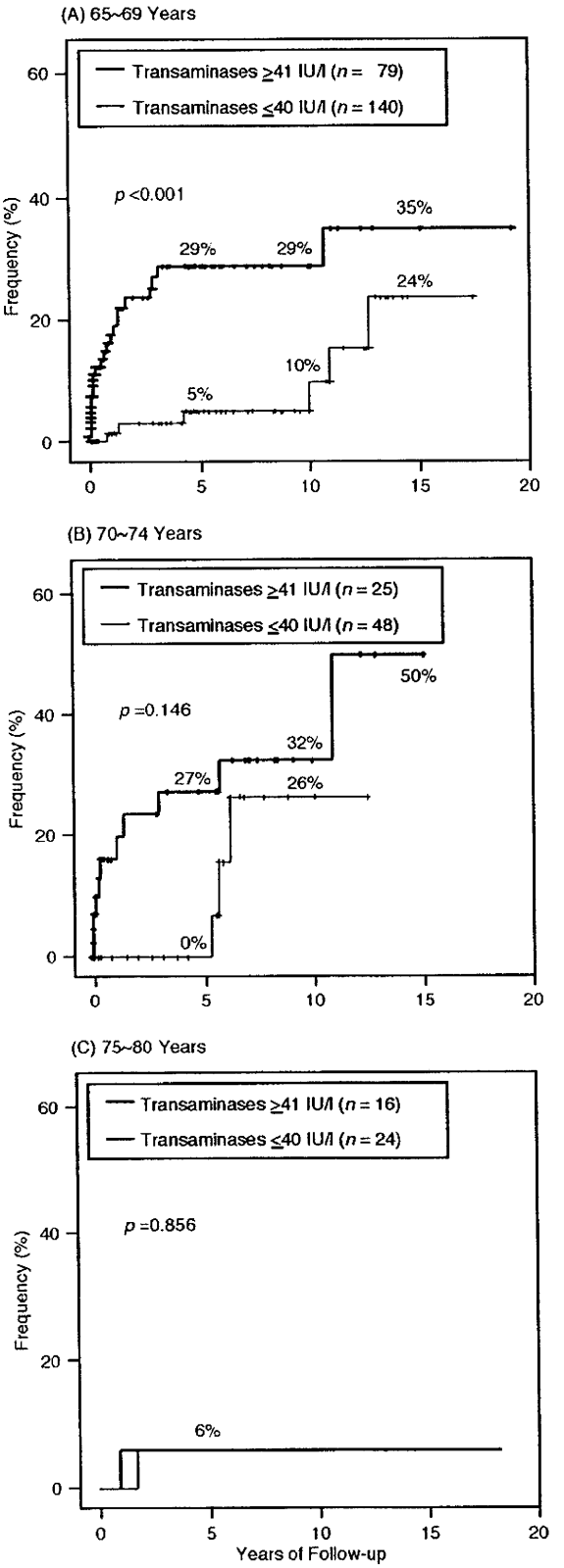

Figure 2. Development of cirrhosis in patients of more than 65 years of age with chronic hepatitis C who were followed-up without receiving antiviral treatment. Patients in different age groups are compared between those with and those without elevated transaminase levels.

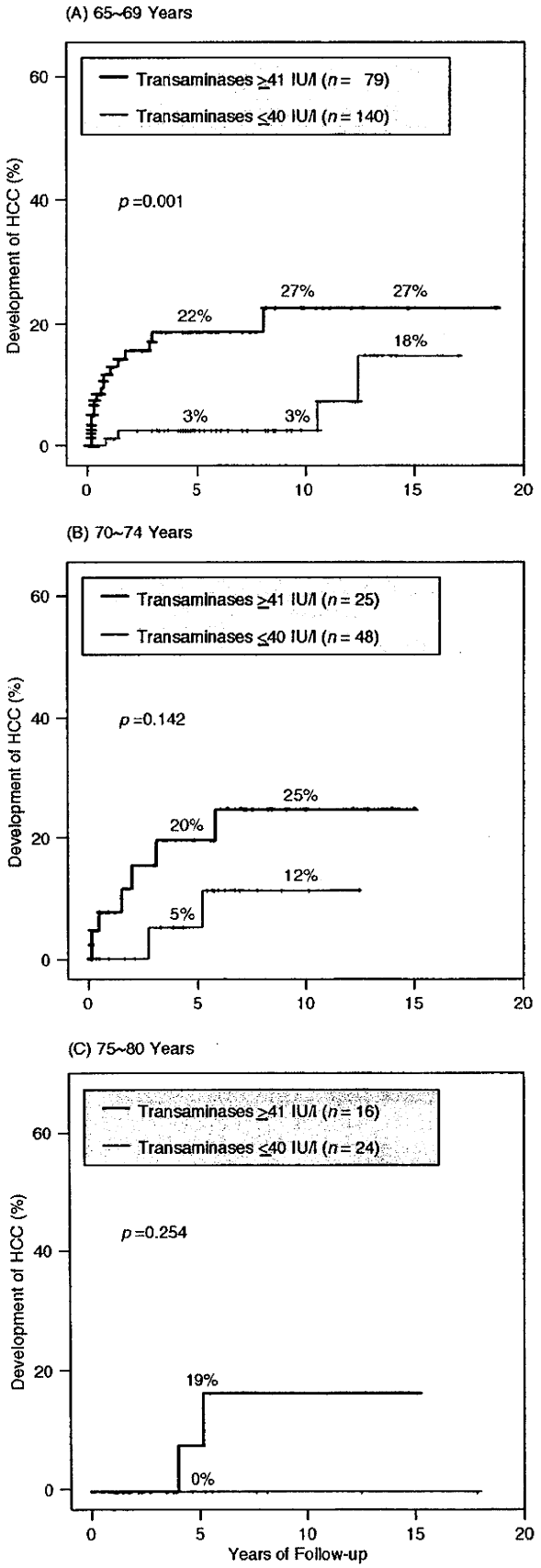

Figure 3 (*Continued*)

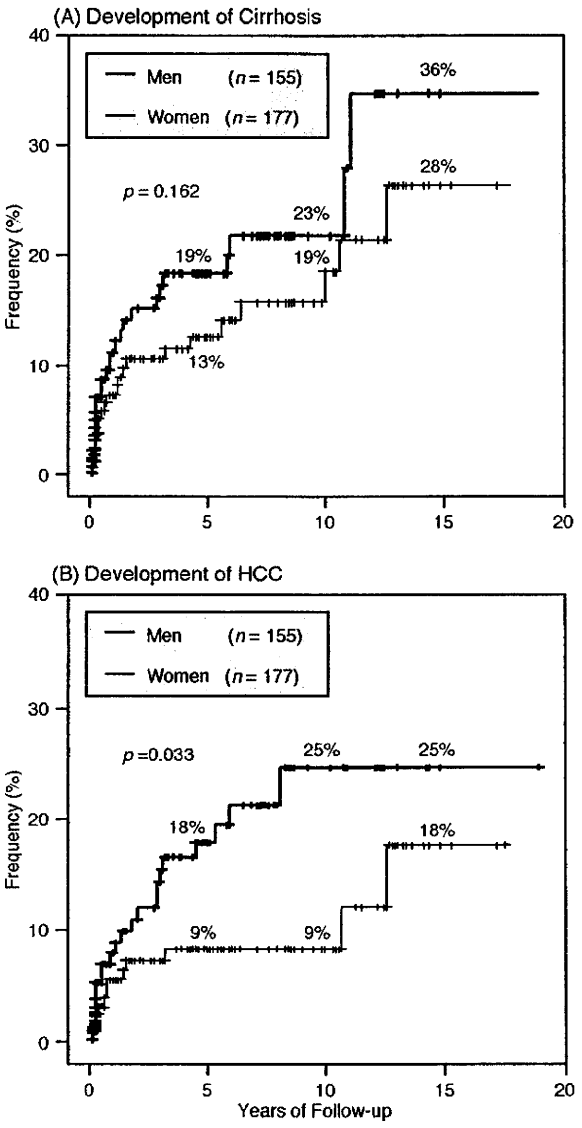

Figure 4. Development of cirrhosis (A) and HCC (hepatocelllular carcinoma) (B) in patients over 65 years of age with chronic hepatitis C who were followed-up without receiving antiviral treatment. Male and female patients are compared.

30,000 patients infected with HCV die yearly [14]. Furthermore, HCC is steadily increasing in the United States, and the incidence is expected to double or triple in the next two decades [15]. Hence, HCV carriers aged 65 years or older should be given IFN treatment, which is proven to be efficacious in preventing the development of HCC [16,17]. Previously, we have evaluated the efficacy and safety of IFN monotherapy in patients aged 65 years or older [18]. Of the 84 patients studied, the sustained virological response was reached in 30 (36%), while

Figure 3. Development of hepaptocelluar carcinoma (HCC) in patients over 65 years of age with chronic hepatitis C who were followed-up without receiving antiviral treatment. Patients in different age groups are compared between those with and those without elevated transaminase levels.

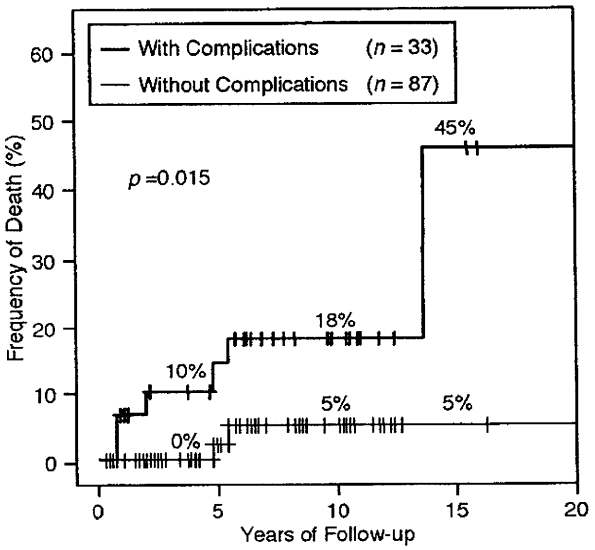

Figure 5. Deceased patients without elevated baseline transaminase levels (ASAT and ALAT <40 IU/l). Patients with and without complications other than liver disease are compared.

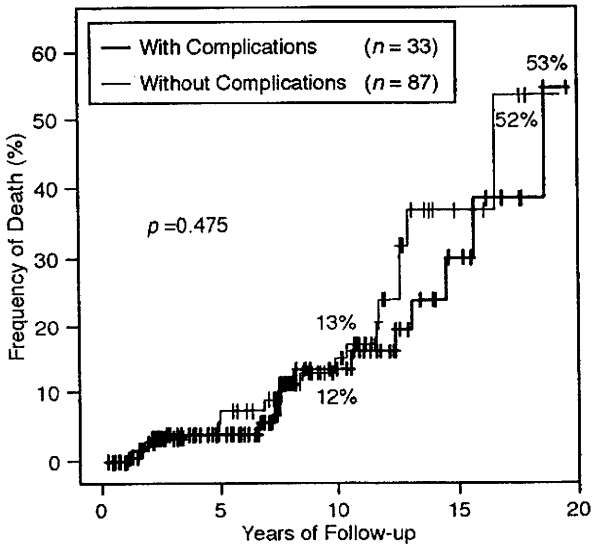

Figure 7. Deceased patients without elevated baseline transaminase levels (ASAT and ALAT <40 IU/l). Patients with and without complications other than liver disease are compared.

IFN was discontinued owing to adverse events in 11 (13%). Remarkably, the sustained virological response to combined IFN and ribavirin was comparable between the 66 patients aged ≥60 years and the 154 aged <60 years (31.8% versus 38.3%), although ribavirin had to be discontinued more frequently in the older patients (33.3% versus 20.8%, $p<0.05$) [19].

HCV spread widely in Japan around the end of World War II, at least 20 years earlier than in the other countries [4,14]. As a consequence, patients given combined IFN and ribavirin are 10–15 years older than those in Western countries [20–22]. Throughout the world, there are increasing numbers of individuals who are infected with HCV and entering the elder years. By the year 2010, the number of the elderly infected with HCV is estimated to account for 0.48 (54%) of the entire 0.89 million infected in Japan, and that in the United States for 0.78 (22%) of the 3.61 million [2–4]. These numbers will continue to increase for some time thereafter. As sequellae to this, cirrhosis and HCC will continue to increase, demanding higher medical costs. In the USA already, HCV-related

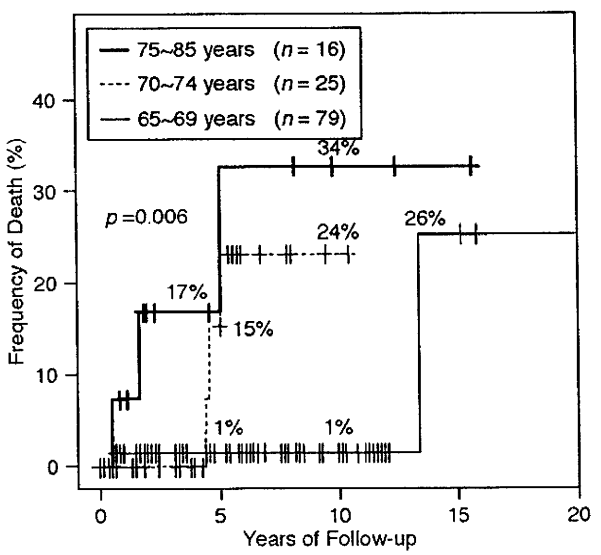

Figure 6. Deceased patients with elevated baseline transaminase levels (ASAT and/or ALAT >41 IU/l). Patients in the different age groups are compared.

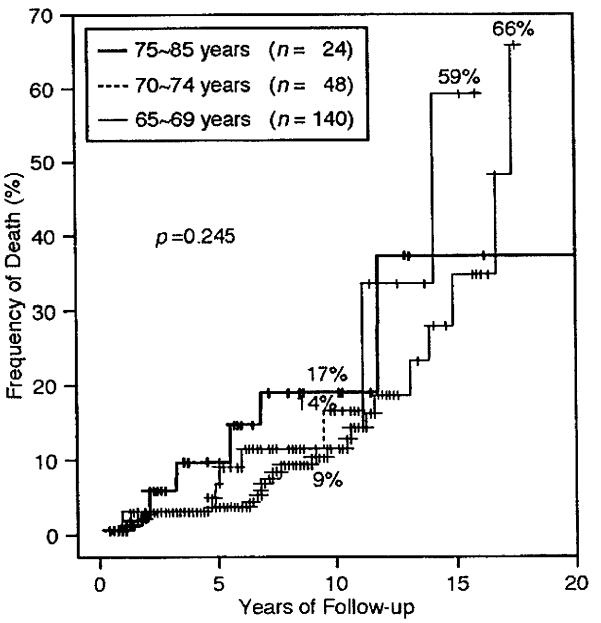

Figure 8. Deceased patients with elevated baseline transaminase levels (ASAT and/or ALAT >41 IU/l). Patients in the different age groups are compared.

end-stage liver disease is the leading cause of orthotopic liver transplantation [23]. This background demands that immediate measures should be taken to prevent fibrosis developing in the elderly with chronic hepatitis C by initiating the appropriate treatment; pegylated IFN combined with ribavirin can eliminate HCV efficiently [24,25].

Management of antiviral treatment in the elderly, however, is not without difficulties. Discontinuation of therapy or dose reduction was required frequently in the Japanese patients older than 60 years with chronic hepatitis C [21]. It is obvious that antiviral treatment needs to be administered with caution in aged patients with chronic hepatitis C, with the indication restricted to those who are likely to derive benefit from it. Early virological response at 12 weeks of treatment is predictive of sustained virological response [26]. The influence of HCV genotypes on the response to combined therapy, which increases with age [27], would have to be taken into consideration, also. In the Japanese patients infected with HCV genotype 1b, substitutions of amino acids at positions 70 and 91 are associated with a better response to combined treatment [28]. In view of the more frequent and serious side effects in elderly patients, these predictors would need to be taken into account when deciding whether to continue or discontinue combined treatment with IFN and ribavirin in elderly patients with chronic hepatitis C.

In order to plan the treatment of elderly patients, the natural history of HCV infection in these patients needs to be elucidated, which has not been done as yet. In the present study, we have followed-up treatment-naive patients aged ≥ 65 years without antiviral treatment for more than 3 years. None of them had cirrhosis at baseline. They were stratified by baseline transaminase levels $\leqq 40$ IU/l (group A ($n=120$)) and $\geqq 41$ IU/l (group B ($n=212$)) and classified further into the three age groups, 65–69, 70–74, and 75–85 years. Cirrhosis and HCC developed more frequently in the patients in group B than those in group A ($p<0.001$ for both). Of the patients aged 65–69 years at entry, in particular, cirrhosis and HCC developed more frequently in group B than in group A ($p<0.001$ and $p=0.001$, respectively). Liver-related causes of death were more common in group B than in group A (20/34 (59%) versus 1/9 (11%), $p<0.05$), and HCC developed more frequently in men than in women ($p=0.021$).

Despite the progression of fibrosis that is accelerated with age [6], liver-related deaths were infrequent in patients with normal baseline transaminase levels and much less often than in those with elevated baseline transaminase levels (1/120 (0.8%) versus 20/212 (9.4%), $p=0.002$). Development of cirrhosis or HCC was no different between patients in groups A and B who were aged 70 years or older at entry. Taken altogether, elderly patients with elevated transaminase levels who are younger than 70 years would be the best candidates for antiviral treatment. They would need to be treated, even when side effects appear, by modifying the doses of IFN and ribavirin. In contrast, antiviral treatment may not be necessary for elderly patients with normal ALAT levels, or can be discontinued in these patients when side effects emerge.

There has been some controversy over antiviral treatment for elderly patients with chronic hepatitis C, and no specific guidelines have been drawn up so far [29]. The sustained virological response to antiviral treatment in aged patients is reported to be either poorer than [30–32] or comparable with that in younger patients [19,33]. The difference is most likely ascribed to careful selection of the aged patients who would benefit from treatment [13]. Based on the natural history of elderly patients with chronic hepatitis C described herein, those with elevated transaminase levels would need treatment to prevent progression to cirrhosis and HCC, while others with normal levels may not require treatment. It is to be hoped that the results in this study might be of help in planning a reasonable treatment strategy towards the longevity, without development of cirrhosis or HCC, in elderly patients with chronic hepatitis C, whose numbers are expected to increase progressively in the foreseeable future.

Acknowledgements

This work was supported in part by grants from the Ministry of Health, Labor, and Welfare of Japan.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

[1] Cohen J. The scientific challenge of hepatitis C. Science 1999;285:26–30.

[2] Tanaka J, Kumagai J, Katayama K, Komiya Y, Mizui M, Yamanaka R, et al. Sex- and age-specific carriers of hepatitis B and C viruses in Japan estimated by the prevalence in the 3,485,648 first-time blood donors during 1995–2000. Intervirology 2004;47:32–40.

[3] Wong JB, McQuillan GM, McHutchison JG, Poynard T. Estimating future hepatitis C morbidity, mortality, and costs in the United States. Am J Public Health 2000;90:1562–9.

[4] Armstrong GL, Wasley A, Simard EP, McQuillan GM, Kuhnert WL, Alter MJ. The prevalence of hepatitis C virus infection in the United States, 1999 through 2002. Ann Intern Med 2006;144:705–14.

[5] Kenny-Walsh E. Clinical outcomes after hepatitis C infection from contaminated anti-D immune globulin. Irish Hepatology Research Group. N Engl J Med 1999;340:1228–33.

[6] Poynard T, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. Lancet 1997;349:825–32.

[7] Vogt M, Lang T, Frosner G, Klingler C, Sendl AF, Zeller A, et al. Prevalence and clinical outcome of hepatitis C infection in children who underwent cardiac surgery before the implementation of blood-donor screening. N Engl J Med 1999;341:866–70.

[8] Feray C, Gigou M, Samuel D, Paradis V, Mishiro S, Maertens G, et al. Influence of the genotypes of hepatitis C virus on the severity of recurrent liver disease after liver transplantation. Gastroenterology 1995;108:1088–96.

[9] Garcia-Samaniego J, Soriano V, Castilla J, Bravo R, Moreno A, Carbo J, et al. Influence of hepatitis C virus genotypes and HIV infection on histological severity of chronic hepatitis C. Am J Gastroenterol 1997;92:1130–4.

[10] Pageaux GP, Ducos J, Mondain AM, Costes V, Picot MC, Perrigault PF, et al. Hepatitis C virus genotypes and quantitation of serum hepatitis C virus RNA in liver transplant recipients: relationship with severity of histological recurrence and implications in the pathogenesis of HCV infection. Liver Transpl Surg 1997;3:501–5.

[11] Wali M, Harrison RF, Gow PJ, Mutimer D. Advancing donor liver age and rapid fibrosis progression following transplantation for hepatitis C. Gut 2002;51:248–52.

[12] Kumada T, Toyoda H, Honda T, Kuzuya T, Katano Y, Nakano I, et al. Treatment of chronic hepatitis C with interferon alone or combined with ribavirin in Japan. Intervirology 2006;49:112–8.

[13] Marcus EL, Tur-Kaspa R. Chronic hepatitis C virus infection in older adults. Clin Infect Dis 2005;41:1606–12.

[14] Yoshizawa H, Tanaka J, Miyakawa Y. National prevention of hepatocellular carcinoma in Japan based on epidemiology of hepatitis C virus infection in the general population. Intervirology 2006;49:7–17.

[15] El-Serag HB, Davila JA, Petersen NJ, McGlynn KA. The continuing increase in the incidence of hepatocellular carcinoma in the United States: an update. Ann Intern Med 2003;139:817–23.

[16] Arase Y, Ikeda K, Suzuki F, Suzuki Y, Kobayashi M, Akuta N, et al. Interferon-induced prolonged biochemical response reduces hepatocarcinogenesis in hepatitis C virus infection. J Med Virol 2007;79:1485–90.

[17] Ikeda K, Saitoh S, Arase Y, Chayama K, Suzuki Y, Kobayashi M, et al. Effect of interferon therapy on hepatocellular carcinogenesis in patients with chronic hepatitis type C: a long-term observation study of 1,643 patients using statistical bias correction with proportional hazard analysis. Hepatology 1999;29:1124–30.

[18] Koyama R, Arase Y, Ikeda K, Suzuki F, Suzuki Y, Saitoh S, et al. Efficacy of interferon therapy in elderly patients with chronic hepatitis C. Intervirology 2006;49:121–6.

[19] Honda T, Katano Y, Urano F, Murayama M, Hayashi K, Ishigami M, et al. Efficacy of ribavirin plus interferon-alpha in patients aged (60 years with chronic hepatitis C. J Gastroenterol Hepatol 2007;22:989–95.

[20] Davis GL, Esteban-Mur R, Rustgi V, Hoefs J, Gordon SC, Trepo C, et al. Interferon alfa-2b alone or in combination with ribavirin for the treatment of relapse of chronic hepatitis C. International Hepatitis Interventional Therapy Group. N Engl J Med 1998;339:1493–9.

[21] Iwasaki Y, Ikeda H, Araki Y, Osawa T, Kita K, Ando M et al. Limitation of combination therapy of interferon and ribavirin for older patients with chronic hepatitis C. Hepatology 2006;43:54–63.

[22] Poynard T, Marcellin P, Lee SS, Niederau C, Minuk GS, Ideo G, et al. Randomised trial of interferon alpha2b plus ribavirin for 48 weeks or for 24 weeks versus interferon alpha2b plus placebo for 48 weeks for treatment of chronic infection with hepatitis C virus. Lancet 1998;352:1426–32.

[23] Verna EC, Brown RS Jr. Hepatitis C and liver transplantation: enhancing outcomes and should patients be retransplanted? Clin Liver Dis 2008;12:637–59.

[24] Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncales FL Jr, et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 2002;347: 975–82.

[25] Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. Lancet 2001;358:958–65.

[26] Davis GL, Wong JB, McHutchison JG, Manns MP, Harvey J, Albrecht J. Early virologic response to treatment with peginterferon alfa-2b plus ribavirin in patients with chronic hepatitis C. Hepatology 2003;38:645–52.

[27] Antonucci G, Longo MA, Angeletti C, Vairo F, Oliva A, Comandini UV, et al. The effect of age on response to therapy with peginterferon alpha plus ribavirin in a cohort of patients with chronic HCV hepatitis including subjects older than 65 years. Am J Gastroenterol 2007;102:1383–91.

[28] Akuta 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;79:1686–95.

[29] Wright TL. Treatment of patients with hepatitis C and cirrhosis. Hepatology 2002;36:S185–94.

[30] Floreani A, Minola E, Carderi I, Ferrara F, Rizzotto ER, Baldo V. Are elderly patients poor candidates for pegylated interferon plus ribavirin in the treatment of chronic hepatitis C? J Am Geriatr Soc 2006;54:549–50.

[31] Hayashi J, Kishihara Y, Ueno K, Yamaji K, Kawakami Y, Furusyo N, et al. Age-related response to interferon alfa treatment in women vs men with chronic hepatitis C virus infection. Arch Intern Med 1998;158:177–81.

[32] Hiramatsu N, Oze T, Tsuda N, Kurashige N, Koga K, Toyama T, et al. Should aged patients with chronic hepatitis C be treated with interferon and ribavirin combination therapy? Hepatol Res 2006;35:185–9.

[33] Tsui JI, Currie S, Shen H, Bini EJ, Brau N, Wright TL. Treatment eligibility and outcomes in elderly patients with chronic hepatitis C: results from the VA HCV-001 Study. Dig Dis Sci 2008;53:809–14.

Epigallocatechin-3-gallate improves nonalcoholic steatohepatitis model mice expressing nuclear sterol regulatory element binding protein-1c in adipose tissue

TAKATO UENO[1], TAKUJI TORIMURA[2], TORU NAKAMURA[2], RAMADOSS SIVAKUMAR[1], HITOMI NAKAYAMA[3], SYUICHI OTABE[3], XIAOHONG YUAN[3], KENTARO YAMADA[3], OSAMU HASHIMOTO[2], KINYA INOUE[2], HIRONORI KOGA[2] and MICHIO SATA[2]

[1]Research Center for Innovative Cancer Therapy, Kurume University, Kurume, Japan; Divisions of [2]Gastroenterology; [3]Endocrinology and Metabolism, Department of Medicine, Kurume University School of Medicine, Kurume, Japan

Received February 5, 2009; Accepted April 2, 2009

DOI: 10.3892/ijmm_00000200

Abstract. We examined whether or not epigallocatechin-3-gallate (EGCG) improves liver injury of nonalcoholic steatohepatitis (NASH) model mice expressing nuclear sterol regulatory element-binding protein 1c (nSREBP-1c) in adipose tissue. nSREBP-1c transgenic C57BL6 mice aged 30 weeks were divided into group 1 (no treatment), group 2 (ascorbic acid alone), group 3 (ascorbic acid and 0.05% EGCG), and group 4 (ascorbic acid and 0.1% EGCG). At 42 weeks, we performed measurement of liver weight to body weight, biochemical assays, morphometry of liver specimens, immunohistochemistry for 8-hydro-2'-deoxyguanosine (8-OhdG), and Western blotting for insulin and TNF-α signalings. Ratio of liver weight to body weight in the high dose EGCG-treated group (group 4) was significantly lower than those of groups 1 and 2 ($p<0.05$ and <0.01, respectively). Blood ALT, glucose, total cholesterol, and triglyceride levels of group 4 were significantly low compared with those of the EGCG-non-treated group (groups 1 and 2) ($p<0.05$, respectively). The degrees of steatosis, inflammation, ballooning hepatocytes and Mallory-Denk bodies in group 4 significantly improved compared with those in other groups ($p<0.05$, respectively). The 8-OhdG immunolocalization in liver tissues of the group 4 obviously decreased compared with those of groups 2 and 3. For Western blotting, the expressions of insulin receptor substrate-1 (IRS-1) and phosphorylated IRS-1 (pIRS-1) in liver tissues of group 4 increased compared with those of groups 2 and 3. On the other hand, the expressions of pAkt, pIKKß and pNF-κB decreased compared with those of groups 2 and 3. From these results, EGCG reduces inflammation, insulin resistance and oxidative stress, and suppresses liver injury in nSREBP-1c transgenic mice.

Introduction

Although the obesity epidemic is a worldwide phenomenon, the severity of the epidemic differs greatly from region to region. The prevalence of obesity among Japanese adults is 3.4% in males and 3.8% in females, and is around one tenth of that in the USA (1). In addition, currently in Japan almost 30% of adult males and females over 50 years old are obese. This rising incidence of obesity parallels the dramatic increase in fatty liver in these age groups. Based on annual health checks, the prevalence of fatty liver diagnosed by ultrasonography increased from 10% in 1980 to 20-40% in 2000 among adults. Thus, nonalcoholic fatty liver disease (NAFLD) is now emerging as the most common liver disease in Japan (2,3). The recent worldwide rise in the number of patients with nonalcoholic steatohepatitis (NASH) tends to parallel the increase in metabolic syndrome, which includes obesity, type 2 diabetes and hyperlipidemia (4).

NAFLD mainly comprises of simple steatosis that is considered benign, however some patients have nonalcoholic steatohepatitis (NASH), which is a clinicopathological entity characterized by the development of hepatic histological changes resembling those induced by excessive alcohol intake that occur in the absence of alcohol abuse (5). Some patients with NASH progress to end-stage liver disease, such as cirrhosis and hepatocellular carcinoma in as little as a decade, and treatment of NASH is therefore very important. However, no single agent improves the histological end points and long-term outcomes (6-8).

Correspondence to: Dr Takato Ueno, Research Center for Innovative Cancer Therapy, Kurume University, 67 Asahi-machi, Kurume, 830-0011, Japan
E-mail: takato@med.kurume-u.ac.jp

Abbreviations: epigallocatechin-3-gallate, EGCG; nonalcoholic steatohepatitis, NASH; nuclear sterol regulatory element-binding protein 1c, nSREBP-1c; 8-hydro-2'-deoxyguanosine, 8-OhdG; insulin receptor substrate-1, IRS-1; phosphorylated IRS-1, pIRS-1; nonalcoholic fatty liver disease, NAFLD; nonalcoholic steatohepatitis, NASH; aspartate aminotransferase, AST; alanine aminotransferase, ALT; glycogen synthase kinase, GSK; hepatic stellate cells, HSC

Key words: nonalcoholic fatty liver disease, nonalcoholic steatohepatitis, nuclear sterol regulatory element-binding protein 1c, green tea polyphenol

Lipodystrophic mice expressing nuclear sterol regulatory element-binding protein 1c (nSREBP-1c) in the adipose tissues show severe insulin resistance, and develop NASH. These animals have marked fatty liver accompanied by hyperlipidemia, hypoleptinemia, and hypoadiponectinemia (9). Immunoreactive 8-hydroxy-2'-deoxyguanosine was observed in the livers of these model mice, suggesting that in addition to insulin resistance, oxidative stress is involved in the development of the NASH-like lesions.

On the other hand, epigallocatechin-3-gallate (EGCG), a type of green tea polyphenol, is a major component of green tea extract, and has the effects of body-fat reduction (10) and antihyperlipidemia (11,12). Therefore, EGCG is useful for NASH patients with obesity and/or hyperlipidemia, but there are no reports indicating that EGCG shows improvement of NASH. In the present study, we examined whether or not catechins improve liver injury of NASH model mice expressing nSREBP-1c in adipose tissue.

Materials and methods

Animals and treatment. The experimental protocol was approved by the Ethics Review Committee for Animal Experimentation of Kurume University School of Medicine. Transgenic C57BL6 mice (Jackson Lab, ME) expressing nSREBP-1c in adipose tissue were purchased from The Jackson Laboratory (Bar Harbor, ME) (13). We identified nSREBP-1c transgenic mice by amplifying genomic DNA isolated from tails by polymerase chain reaction using a forward primer 5'-CTACATTCGCTTTCTGCAAC-3', and used heterozygous transgenic mice in the following studies. They were bred in our laboratory, mating with wild-type C57BL6 mice (Jackson Lab), in plastic cages with wood chip bedding at a temperature of 18-22°C, moisture of 40-60%, and on a 12-h light/dark cycle. They were supplied with regular mouse chow (1450 kj/100 g, protein; 24.9 g/100 g, fat; 4.6 g/100 g, Nippon CLEA; Shizuoka, Japan) and water *ad libitum*. nSREBP-1c transgenic C57BL6 male mice aged 30 weeks, which show the typical NASH in liver histology at this age (9), were prepared and used in this study. These mice were divided into four groups [group 1, mice given distilled water alone (n=6); group 2, mice given distilled water containing 0.005% ascorbic acid, which is used to block the oxidation of EGCG (n=6); group 3, mice given distilled water containing 0.005% ascorbic acid and low dose EGCG (0.05%) (n=6); group 4, mice given distilled water containing 0.005% ascorbic acid and high dose EGCG (0.1%) (n=6)], and body weight was measured weekly for 12 weeks. After mice were anethetized with ether at week 43, body weight was measured, blood sample was collected from inferior vena cava, and these mice were examined as follows.

Measurement of body weight and liver weight at week 43. The body and liver weights of mice in each group after sacrifice were recorded and the percentage of expression of liver weight to body weight was measured.

Biochemical assays containing serum biological markers. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), glucose, total cholesterol, triglyceride, phospholipid and free fatty acid levels were measured according to the manufacturer's instructions.

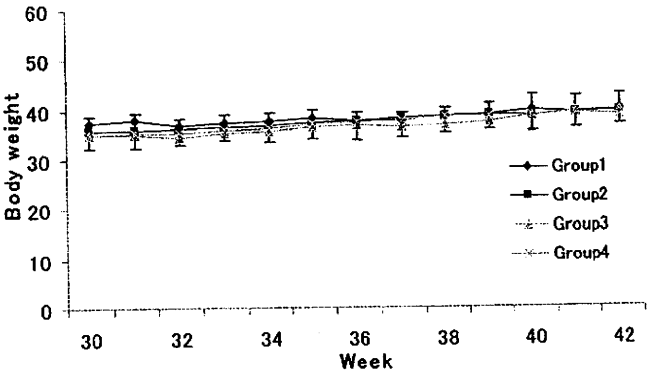

Figure 1. Changes of body weight in mice in the four groups. Body weight of mice in the four groups gradually increased throughout the course of the experiment period, but there is no significant difference among each group.

Table I. Ratio of liver weight to body weight and blood biochemical assay.

Group	1	2	3	4
Liver weight/ body weight x100 (%)	12.8±2[a]	15.2±1.8[b]	12.4±5.6	7.2±4.8
AST	200±132	183±8	248±153	187±57
ALT	321±260[a]	309±47[a]	286±257	151±67
Glucose	230±35	274±23	272±45	315±54
Triglyceride	50±23[a]	56±19[a]	35±25	30±7
Total cholesterol	156±26[a]	176±17[a]	132±54	107±42
Free fatty acid	508±171	498±68	466±116	496±126
Phospholipid	288±47[a]	309±41[a]	240±101	199±57

Group 1, mice given distilled water alone (n=6); group 2, mice given distilled water containing 0.005% ascorbic acid alone (n=6); group 3, mice given distilled water containing 0.005% ascorbic acid and low dose EGCG (0.05%) (n=6); group 4, mice given distilled water containing 0.005% ascorbic acid and high dose EGCG (0.1%) (n=6). EGCG, epigallocatechin-3-gallate; [a]$p<0.05$, [b]$p<0.01$ compared with group 4.

Histological diagnosis and morphometry of liver specimens. Paraffin-embedded sections of the liver were stained with either hematoxylin-eosin for standard microscopy and Azan-Mallory stain to observe the localization of extracellular matrix. The specimens were reviewed by two independent pathologists. Each specimen was assigned to one of the following histological subgroups for the purpose of comparative analysis, type 1, simple steatosis affecting >33% of the lobules; type 2, steatosis and lobular inflammation; type 3, steatosis and ballooning; type 4, steatosis, ballooning hepatocytes and Mallory-Denk bodies or fibrosis. We dealt with types 3 and 4 as NASH, as described previously (14). In addition, we performed morphometry of liver specimens using the histological scoring system of Kleiner *et al* (15).

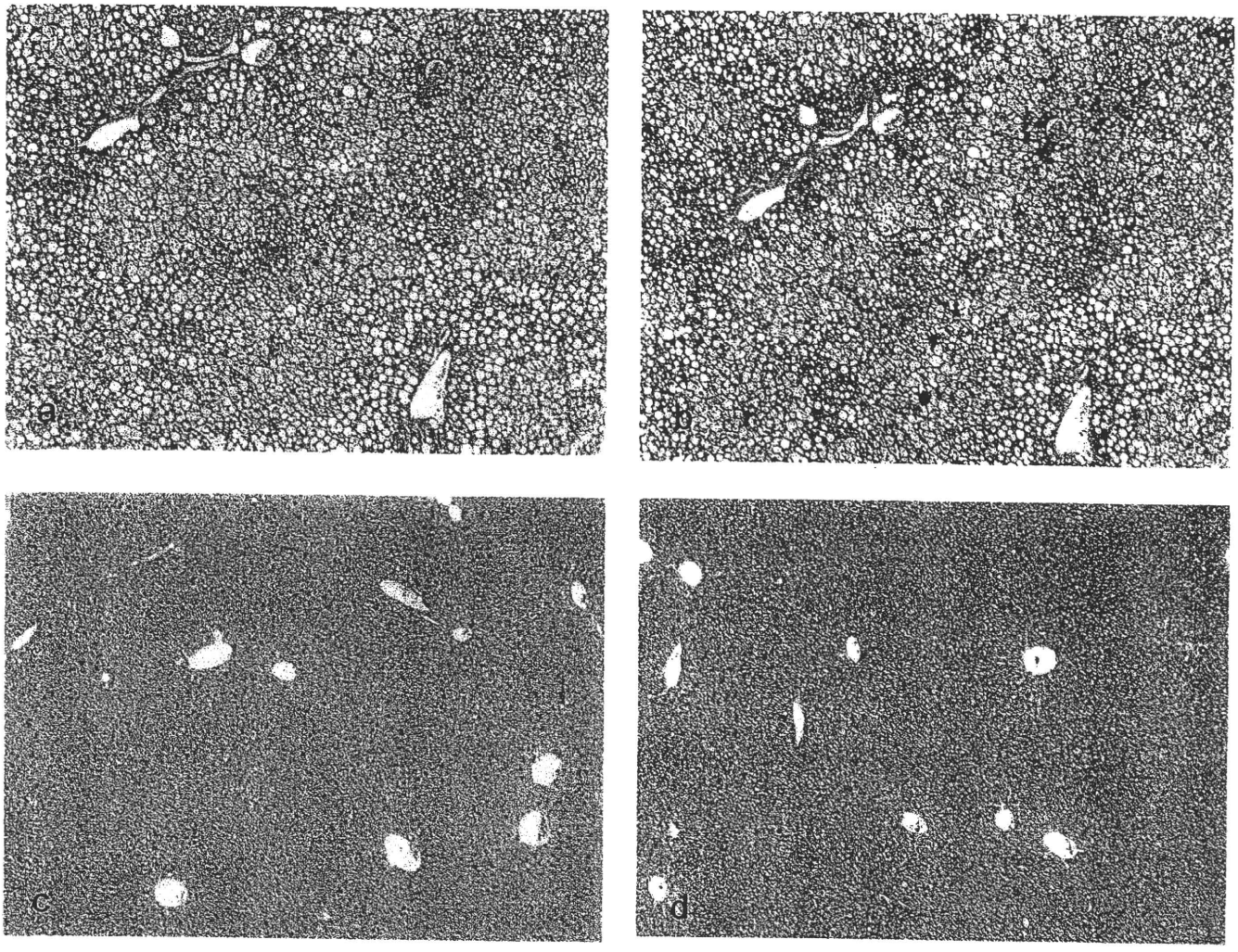

Figure 2. Histological futures of liver specimens. (a) Mouse treated with 0.005% ascorbic acid for 12 weeks. Hematoxylin-eosin stain, x100. (b) Mouse treated with 0.005% ascorbic acid for 12 weeks; Azan-Mallory stain, x100. (c) Mouse treated with 0.005% ascorbic acid and 0.1% EGCG for 12 weeks; Hematoxylin-eosin stain, x100. (d) Mouse treated with 0.005% ascorbic acid and 0.1% EGCG for 12 weeks; Azan-Mallory stain, x100. Immunoreactive products for anti-8-OhdG antibody localized in the cytoplasm of hepatocytes, especially deformed hepatocytes with fat droplets. Liver tissues of mice treated with 0.005% ascorbic acid for 12 weeks show histological features of typical NASH. However, those of mice with 0.005% ascorbic acid and 0.1% EGCG are similar to normal liver.

Immunohistochemistry. Immunoreactive products of 8-hydro-2'-deoxyguanosine (8-OhdG), a marker of oxidative DNA damage, in the liver were examined. Paraffin sections were incubated overnight with mouse monoclonal anti-8-OhgG antibody (Japan Institute for the Control of Aging, Fukuroi, Japan), followed by incubation with alkaline phosphatase-labeled horse antimouse IgG (Vector, Burlingame, CA) and visualization by diamnobenzidine. The degree of 8-OhdG immunolocalization in the liver tissues was categorized as 0, 1, 2 or 3. That is, 0, none in liver tissue; 1, <1/3 in the intrahepatic lobules; 2, 1/3-2/3 in the intrahepatic lobules; and 3, >2/3 in the intrahepatic lobules.

Western blotting. Western blotting was performed using anti-insulin receptor (IR), anti-insulin receptor substrate (IRS)-1 and anti-phosphorylated IRS-1 (pIRS-1), anti-pGSK3α/ß antibodies for insulin signaling, and using anti-Akt, -pAkt, -IκBα, -pIκBα, -NF-κB and -pNF-κB antibodies for TNF-α signaling in liver tissues. Whole extracts were prepared from liver tissues using Triton lysis buffer-containing protease and phosphatase inhibitors. Protein concentration of the extracts was determined, and 40 μg of protein was electrophorased on 10% SDS-polyacrylamide gels. The gels were then blotted onto the nitrocellulose membrane.

Statistical analysis. Numerical data were expressed as means ±SD. Student's t test was performed to assess statistical significance among each group. P-values <0.05 were considered significant.

Results

Changes of body weight of mice in the four groups. Body weight of mice in the four groups gradually increased throughout the course of the experiment period as shown in Fig. 1, but there was no significant difference among each group.

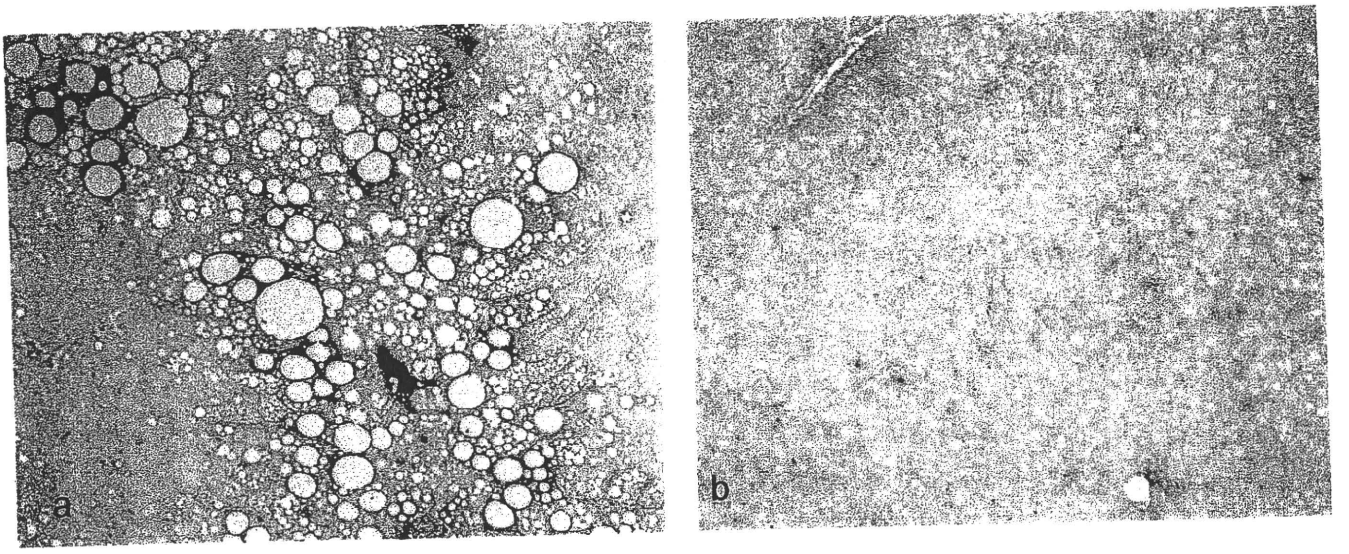

Figure 3. 8-OhdG immunolocalization in liver tissues. (a) 8-OhdG immunolocalization in the liver tissues of the 0.005% ascorbic acid-treated group is at grade 3, x100. (b) 8-OhdG immunolocalization in the liver tissues of the 0.005% ascorbic acid and 0.1% EGCG-treated group is at grade 1, x100. The degree of 8-OhdG immunolocalization in the liver tissues of the 0.005% ascorbic acid and 0.1% EGCG-treated group is low compared with that of other groups.

Ratio of liver weight to body weight at week 43. Ratio (percentage of expression) of liver weight to body weight in the high dose EGCG-treated group (group 4) was lowest compared with that of other groups (group 1, 12.8±2; group 2, 15.2±1.8; group 3, 12.4±5.6; and group 4, 7.2±4.8), and the difference between group 4 and groups 1 and 2 was significant ($p<0.05$ and <0.01, respectively) (Table I).

Biochemical assays containing serum biological markers. Blood ALT, total cholesterol, triglyceride and phospholipid levels of group 4 were significantly low compared with those of groups 1 and 2 ($p<0.05$, respectively) (Table I). The elevation of serum ALT, AST levels by EGCG treatment was not recognized. In addition, there were no significant differences between AST, free fatty acid, and glucose levels between any of the groups.

Morphometry of liver specimens. The degrees of steatosis, intralobular fibrosis, ballooning hepatocyte appearance and Mallory-Denk body appearance in group 4 significantly decreased compared with those in other groups ($p<0.05$) (Table II, Fig. 2).

Immunohistochemistry. Immunoreactive products for anti-8-OhdG antibody localized in the cytoplasm of hepatocytes, especially deformed hepatocytes with fat droplets (Fig. 3). The 8-OhdG immunolocalization in liver tissues of group 4 showed an obvious decrease compared with those of other groups, and the difference between the degree of 8-OhdG immunolocalization in group 4 and that of groups 2 and 3 was significant ($p<0.05$) (Table II, Fig. 3).

Western blotting. In Western blotting, the expressions of IR and pIRS-1 in liver tissues of group 4 increased compared with those of other groups. On the other hand, the expressions of pAkt, pIKKß and pNF-κB in liver tissues of group 4 decreased compared with those of other groups (Fig. 4).

Table II. Morphometry of liver tissues and degree of 8-OhdG immunolocalization in each group.

Group	1	2	3	4
Steatosis	2.9±0.4[a]	3.0±0[a]	2.4±0.5[a]	1.0±1.0
Ballooning hepatocyte	1.3±0.5[a]	2.0±0[a]	1.0±0[a]	0.4±0.5
Mallory-Denk body	0.9±0.4[a]	1.8±0.4[a]	0.8±0.4[a]	0.4±0.5
Fibrosis	1.3±0.8	1.0±0.7	1.2±0.4	0.8±1.1
8-OhdG localization	1.4±0.5	2.2±1.1[a]	1.8±1.3[a]	1.2±0.4

Group 1, mice given distilled water alone (n=6); group 2, mice given distilled water containing 0.005% ascorbic acid alone (n=6); group 3, mice given distilled water containing 0.005% ascorbic acid and low dose EGCG (0.05%) (n=6); group 4, mice given distilled water containing 0.005% ascorbic acid and high dose EGCG (0.1%) (n=6). EGCG, epigallocatechin-3-gallate: [a]$p<0.05$ compared with group 4.

Discussion

Nonalcoholic fatty liver disease (NAFLD) is associated with metabolic syndrome. The metabolic syndrome is characterized by insulin resistance, which is produced by a complex interaction between genetic factors, macronutrient intake and lifestyle that alters the cytokine profile, cell biology and biochemical milieu of the liver, adipose tissue and striated muscle. The resultant disequilibrium in lipid homeostasis causes triglycerides to accumulate in the liver (16). An increase in oxidative stress, due to the generation of reactive oxygen species as a result of mitochondrial abnormalities and induction of the cytochrome P-450 system is one mechanism by which the nonalcoholic fatty liver develops into NASH (4). The pathogenesis of cytologic ballooning and Mallory-Denk body formation and their role in NAFLD remain to be defined. In addition, inflammation and fibrosis are likely to be secondary to cirrhosis, hepatocellular carcinoma and death (2,3,17).

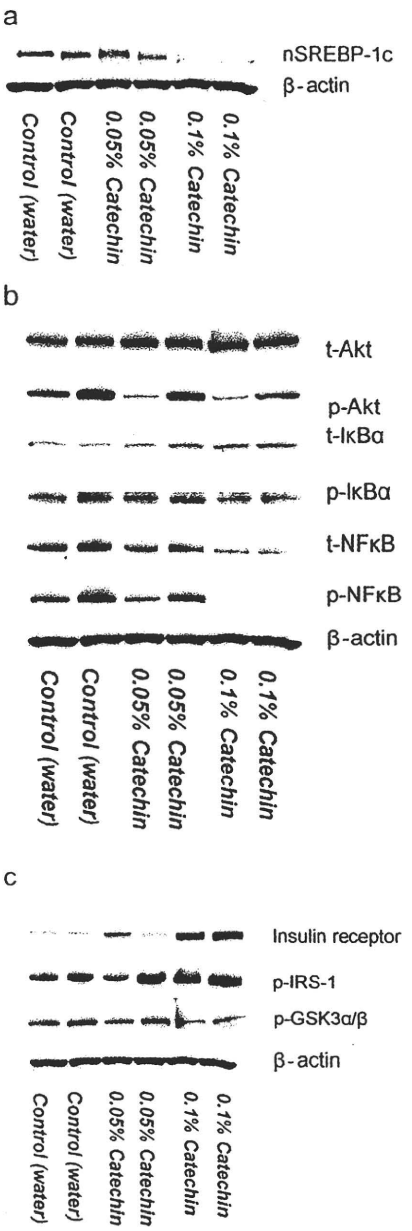

Figure 4. Western blotting. IκB, Inhibitor of κB; NF-κB: nuclear factor κB; t, total; p, phospholylated; IRS, insulin receptor substrate; GSK, glycogen synthase kinase. For Western blotting, the expressions of IR and pIRS-1 in liver tissues of group 4 increased compared with those of other groups. On the other hand, the expressions of pAkt, pIKKß and pNF-κB in group 4 decrease compared with those of other groups.

EGCG is an antioxidant and chemopreventional polyphenol that is found in green tea. It blocks activation of Ap-1 or NF-κB (18). EGCG has shown the inhibition of activation of IKKα, phosphorylation and subsequent degradation of IκBα (18). In addition, EGCG suppresses the proliferation of hepatic stellate cells and production of extracellular matrix in the hepatic fibrosis (19-21). Yumei *et al* reported that EGCG induced the *de novo* synthesis of glutathione and antifibrogenic effects in passaged rat hepatic stellate cells (22,23).

In the present study, green tea polyphenols containing EGCG showed effects of reducing inflammation, insulin resistance and oxidative stress (24-26), and improved the liver injury of transgenic mice expressing nSREBP-1c in the adipose tissue. EGCG inhibits nSREBP-1c expression in adipose tissues and Akt, IκBα and NF-κB expressions of liver tissues, and improves the insulin resistance of the liver tissues by promoting the functional recovery of the insulin receptor, insulin receptor substrate-1 (IRS-1) and glycogen synthase kinase (GSK) in the nSREBP-1c transgenic NASH model mice. The direct effect of EGCG to this model mouse is unclear. Its mechanism is likely due to the antioxidant and chemoprevention effects of EGCG (18).

Ingestion of tea rich catechins leads to a reduction in body fat and malondialdehyde-modified LDL in men (27). In obese individuals, body fat mainly accumulates in submucosal and visceral adipose tissue. Obesity alters both the cellular composition and function of adipose tissue. Adipose tissue of obese individuals contains an increased number of macrophages. Macrophages, adipocytes, and other cellular components of adipose tissue produce numerous circulating inflammatory markers including pro- and anti-inflammatory factors, chemokines, growth factors, and proteases that include a systemic inflammatory state and insulin resistance seen in individuals with increased body mass index (28). In this study, there is no significant difference among the four groups for body weight. Probably, the visceral adipose tissue of nSREBP-1c transgenic mice is less than that of wild type C57BL6 mice, and the significant difference is not recognized among each group.

The liver component of this metabolic disorder is NAFLD, which includes a spectrum of liver pathology ranging from steatosis to cirrhosis. Steatosis is often seen in obese individuals, and both presence and severity of steatosis correlate positively with adiposity. Increased hepatic free fatty acid oxidation that occurs in steatotic livers increases the generation of reactive oxygen species. Increased hepatocyte exposure to reactive oxygen species generates a state of oxidant stress and mitochondrial dysfunction, including hepatocellular injury and activation of hepatic stellate cells (HSC). In NAFLD, intestine-originated endotoxin accumulates the substance in the liver rather than to escape from liver. Increased levels of glucose and insulin up-regulate the synthesis of transforming growth factor-ß, angiotensin II, leptin, adiponectin and so on by HSC (29-31), and develop to the hepatic fibrosis.

In this study, there was no evidence of side effects by EGCG treatment. It will be important to clearly determine whether EGCG or green tea consumption can be used as a tool to prevent the development of NASH.

Acknowledgements

This research was supported by a Grant-in-Aid from the University and Society Collaboration and from COE Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

References

1. Yoshiike N and Lwin H: Epidemiological aspects of obesity and NASH/NAFLD in Japan. Hepatol Res 33: 77-82, 2005.
2. Hashimoto E, Yatsuji S, Kaneda H, Yoshioka Y, Taniai M, Tokushige K and Shiratori K: The characteristics and natural history of Japanese patients with nonalcoholic fatty liver diseases. Hepatol Res 33: 72-76, 2005.

3. Ono M and Saibara T: Clinical features of nonalcoholic steatohepatitis in Japan: evidence from the literature. J Gastroenterol 41: 725-732, 2006.
4. Harrison SA, Kadakia S, Lang KA and Schenker S: Nonalcoholic steatohepatits: what we know in the new millennium. Am J Gastroenterol 97: 2714-2724, 2002.
5. Ludwig J, Viggiano TR, McGill DB and Ott BJ: Nonalcoholic steatohepatitis Mayo Clinic experiences with a hitherto unnamed disease. Mayo Clin Proc 55: 434-438, 1980.
6. Powel EE, Cooksley WG, Hanson R, Searle J, Halliday JW and Powell JW: The natural history of steatohepatitis: a follow-up study of forty-two patients for up to 21 years. Hepatology 11: 74-80, 1990.
7. Yoshioka Y, Hashimoto E, Yatsuji S, Kaneda H, Taniai M, Tokushige K and Shiratori K: Nonalcoholic steatohepatitis: cirrhosis, hepatocellular carcinoma and burnt-out NASH. J Gastroenterol 39: 1215-1218, 2004.
8. Bugianesi E, Leone N, Vanni E, Marchesini G, Brunello F, Carucci P, Musso A, De Paolis P, Capussotti L, Salizzoni M and Rizzetto M: Expanding the natural history of NASH: from cryptogenic cirrhosis to hepatocellular carcinoma. Gastroenterology 123: 134-140, 2002.
9. Nakayama H, Otabe S, Ueno T, Hirota N, Yuan X, Fukutani T, Hashinaga T, Wada N and Yamada K: Transgenic mice expressing nuclear sterol regulatory element-binding protein 1c in adipose tissue exhibit liver histology similar to nonalcoholic steatohepatitis. Metabolism 56: 470-475, 2007.
10. Bose M, Lambert JD, Ju J, Reuhl KR and Shapses SA: The major green tea polyphenol, (-)-epigallocatechin-3-gallate, inhibits obesity, metabolic syndrome, and fatty liver disease in high-fat-fed mice. J Nutr 138: 1677-1683, 2008.
11. Lee SM, Kim CW, Kim JK, Shin HJ and Baik JH: GCG-rich tea catechins are effective in lowering cholesterol and triglyceride concentrations in hyperlipidemic rats. Lipids 43: 419-429, 2008.
12. Murase T, Nagasawa A, Suzuki J, Hase T and Tokimitsu I: Beneficial effects of tea catechins on diet-induced obesity: stimulation of lipid catabolism in the liver. Int J Obes 26: 1459-1464, 2002.
13. Shimomura I, Hammer RE, Richardson JA, Ikemoto S, Bashmakov Y, Goldstein JL and Brown MS: Insulin resistance and diabetes mellitus in transgenic mice expressing nuclear SREBP-1c in adipose tissue: model for congenital generalized lipodystrophy. Genes Dev 12: 3182-3194, 1998.
14. Matteoni CA, Younossi ZM, Gramlich T, Boparai N, Liu YC and McCullough AJ: Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity. Gastroenterology 116: 1413-1419, 1999.
15. Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, Ferrell LD, Liu YC, Torbenson MS, Unalp-Arida A, Yeh M, McCullough AJ and Sanyal AJ: Nonalcoholic Steatohepatitis Clinical Research Network. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 41: 1313-1321, 2005.
16. Korenblat KM, Fabbrini E, Mohammed BS and Klein A: Liver, muscle, and adipose tissue insulin action is directly related to intrahepatic triglyceride content in obese subjects. Gastroenterology 134: 1369-1375, 2008.
17. Sanyal AJ: Mechanisms of disease: pathogenesis of nonalcoholic fatty liver disease. Nat Clin Pra Gastroenterol Hepatol 2: 46-53, 2005.
18. Surh YJ: Cancer chemoprevention with dietary phytochemicals. Nat Rev Cancer 3: 768-780, 2003.
19. Sakata R, Ueno T, Nakamura T, Sakamoto M, Torimura T and Sata M: Green tea polyphenol epigallocatechin-3-gallate inhibits platelet-derived growth factor-induced proliferation of human hepatic stellate cell line LI90. J Hepatol 40: 52-59, 2004.
20. Chen A, Zhang L, Xu J and Tang J: The antioxidant (-)-epigallocatechin-3-gallate inhibits activated hepatic stellate cell growth and suppresses acetaldehyde-induced gene expression. Biochem J 368: 695-704, 2002.
21. Zhen M, Wang Q, Huang X, Cao L, Chen X, Sun K, Liu YJ, Li W and Zhang LJ: Green tea polyphenol epigallocatechin-3-gallate inhibits oxidative damage and preventive effects on carbon tetrachloride-induced hepatic fibrosis. J Nutr Biochem 18: 795-805, 2007.
22. Yumei F, Zhou Y, Zheng S and Chen A: The antifibrogenic effect of (-)-epigallocatechin gallate results from the induction of de novo synthesis of glutathione in passaged rat hepatic stellate cells. Lab Invest 86: 697-709, 2006.
23. Fu Y, Zheng S, Lu SC and Chen A: Epigallocatechin-3-gallate inhibits growth of activated hepatic stellate cells by enhancing the capacity of glutathione synthesis. Mol Pharmacol 73: 1465-1473, 2008.
24. Csala M, Margitti E, Senesi S, Gamberucci A, Banhegyi G, Mandl J, *et al*: Inhibition of hepatic glucose 6-phosphatase system by the green tea flavanol epigallocatechin gallate. FEBS Lett 581: 1693-1698, 2007.
25. Collins QF, Liu H, Pi J, Liu Z, Quon MJ and Cao W: Epigallocatechin-3-gallate (EGCG), a green tea polyphenol, suppresses hepatic gluconeogenesis through 5'-AMP-activated protein kinase. J Biol Chem 282: 30143-30149, 2007.
26. Igarashi K, Honma K, Yoshinari O, Nanjo F and Hara Y: Effects of dietary catechins on glucose tolerance, blood pressure and oxidative status in Goto-Kakizaki rats. J Nutr Sci Vitaminol 53: 496-500, 2007.
27. Nagao T, Komine Y, Soga S, Meguro S, Hase T, Tanaka Y and Tokimitsu I: Ingestion of a tea rich in catechins leads to a reduction in body fat and malondialdehyde-modified LDL in men. Am J Clin Nutr 81: 122-129, 2005.
28. Angulo P: NAFLD, obesity, and bariatric surgery. Gastroenterology 130: 1848-1852, 2006 .
29. Bataller R, Sancho-Bru P, Gines P and Brenner DA: Liver fibrogenesis: a new role for the rennin-angiotensin system. Antioxid Redox Signal 7: 1346-1355, 2005.
30. Ding X, Saxena NK, Lin S, Xu A, Srinivasan S and Ananina FA: The roles of leptin and adiponectin: a novel paradigm in adipocytokine regulation of liver fibrosis and stellate cell biology. Am J Pathol 166: 1655-1669, 2005.
31. Oben JA, Roskams T, Yang S, Lin H, Sinelli N, Torbenson M, Smedh U, Moran TH, Li Z, Huang J, Thomas SA and Diehl AM: Hepatic fibrogenesis requires sympathetic neurotransmitters. Gut 53: 438-445, 2004.

総説（Review Article）：膜（MEMBRANE），34（5），259-265（2009）

●特集　生体膜関連シンポジウム「脂質低下療法時代の生体膜研究」

生体膜脂質のC型肝炎ウイルス生活環における役割

相崎英樹

国立感染症研究所　ウイルス第二部　〒162-8640 東京都新宿区戸山1-23-1

Critical Role of Membrane-associated Lipid in the Life Cycle of the Hepatitis C Virus

Hideki Aizaki

Department of Virology II, National Institute of Infectious Diseases
1-23-1 Toyama, Shinjuku-ku, Tokyo 162-8640, Japan

Systems of subgenomic replicon and recombinant infectious hepatitis C virus (HCV) have been established in 1999 and 2005, and virological techniques are able to be applied to the HCV research, especially regarding molecular mechanisms on replication and virion assembly, respectively. We showed that HCV active replication complex contains membrane structures, characteristic of lipid rafts. We also recently demonstrated an important role of cholesterol and sphingolipid in HCV infection and virion maturation. Finally, inhibitors of cellular cholesterol and sphingolipid biosynthetic pathway efficiently block virion production. Association of hepatitis C virus with lipid can be also a source of new antiviral therapies.

Key words : hepatitis C virus／life cycle／subgenomic replicon／cholesterol／sphingolipid

1. はじめに

C型肝炎ウイルス（HCV）感染者は日本で約200万人，世界中で1億7000万人にのぼる[1]とされ，その多くが10～30年という長期間を経て慢性肝炎から肝硬変へと進行し，高率に肝細胞癌を発症している[2]．現在，HCV感染症に対する主要な治療法はインターフェロン（IFN）とリバビリンによる併用療法であるが，有効となるのは半数の患者にすぎない．より有効な治療法の開発が望まれているが，HCVには効率の良いウイルス培養系が存在しなかったため，HCVの基礎研究はウイルス遺伝子の発現産物の機能解析を中心に進んだ．また，実験用の感染小動物が存在しないため，HCVのウイルス学的な解析はチンパンジーを用いた感染実験に頼るしか無く，倫理的な問題やコストの面からも安易にできる実験ではなかった．このような状況がHCVの基礎研究の妨げになり，抗ウイルス薬やワクチンの開発が遅れてきた．しかし，1999年に培養細胞で自律複製する構造領域を欠くサブゲノムレプリコンが開発され[3]，これを皮切りにHCVの複製に関する研究が精力的に進められてきた．さらに，劇症肝炎患者から単離されたJFH-1株のゲノムRNAを肝癌細胞由来のHuh-7細胞に導入することにより，感染性ウイルス粒子を培養細胞で作製する技術が2005年に確立された[4]．これにより，HCVの生活環のすべてを再現可能な実験系が確立したことになり，HCV研究を急速に加速させた．

Tel : 03-5285-1111　ex. 2521
Fax : 03-5285-1161
E-mail : aizaki@nih.go.jp

2. HCVの生活環

推定されているHCVの生活環をFig. 1に示す．HCVがレセプターを介して肝細胞に感染（吸着 Attachment，侵入 Entry）し，粒子よりウイルスRNAが放出され（脱核 Cytoplasmic release），これがメッ

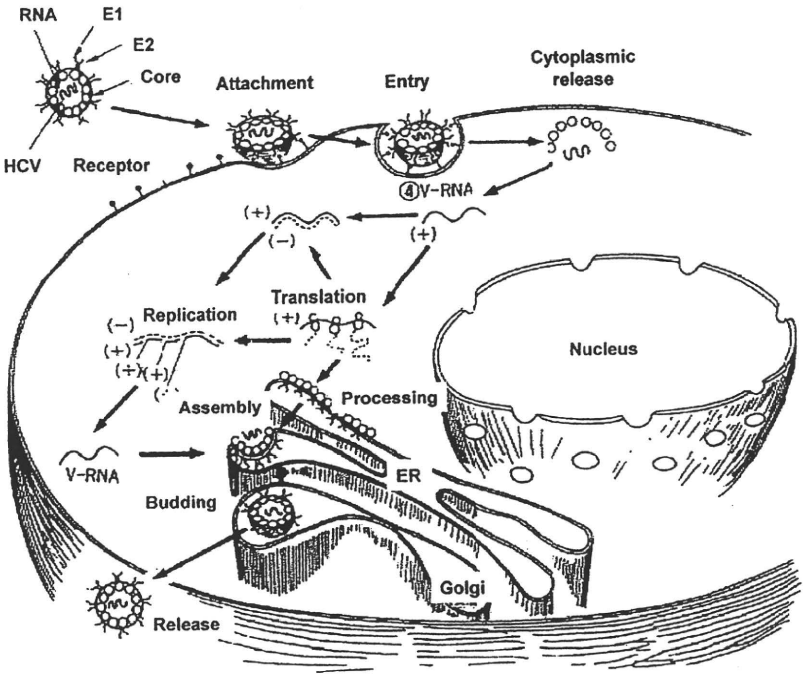

Fig. 1 HCV life cycle [18].

センジャーRNAとして働き，このRNAの5'非翻訳領域に存在するIRESから翻訳（Translation）が開始され大きな前駆体蛋白が合成される．この前駆体蛋白は，細胞のシグナラーゼによってウイルス粒子を形成する構造蛋白であるコア蛋白と2つのエンベロープ蛋白E1，E2がプロセス（Processing）される．また，ウイルス自身がコードするプロテアーゼによって，プロテアーゼ，ヘリカーゼ，RNA依存性RNAポリメラーゼ（RdRp）などウイルスの複製に必須な非構造蛋白がプロセスされる．ウイルスにコードされた酵素や宿主因子によってゲノムRNAからマイナス鎖RNAが転写され，複製複合体が形成される．これを基にしてプラス鎖RNAが合成され（複製Replication），ウイルスRNAやmRNAとして働く．ウイルスRNAがコア蛋白と結合してヌクレオカプシドを形成し，さらにエンベロープ蛋白が邂逅して小胞体（ER）でウイルス粒子が成熟し（出芽Assembly），トランスゴルジを通り細胞膜に達して細胞外へ放出（Release）されるものと考えられている．以上のようなHCV生活環のうち多くのステップでウイルスは細胞のER，ゴルジ体，形質膜といった生体膜を使っていると推定されている．

3. 生体膜脂質のウイルスゲノム複製における役割

ウイルスゲノム複製においてNS5B遺伝子のコードするRdRpが中心的な役割を担っているものと考えられている．しかしながら，強制発現させたRdRpを精製し解析したところ，その活性は鋳型特異性がなく，複製産物の長さは鋳型と異なった．一般的に，鋳型特異的なRNA合成には細胞因子や他のNS蛋白が必要と考えられている[5]．以上のことから，HCV複製の研究にはNS5Bだけでなく，他のNS蛋白や宿主因子が結合した複製複合体を維持した上での解析が重要ということが考えられる．従って，HCVレプリコンシステムはHCVゲノムの複製機構を解析する上で非常に有効と期待された．1999年，Bartenschlagerらは，本来HCVゲノムの中でウイルス粒子を形成する構造タンパク質領域を薬剤耐性遺伝子に置き換え，その下流に，より強力にHCVゲノムの内部から翻訳させる働きを有するencepharomyocarditis virus（EMCV）のIRESを挿入したRNAレプリコンを作製した[3]．このRNAをトランスフェクトした細胞を薬剤存在下で培養することで，自律複製するHCV遺伝子配列を獲得したHCVゲノムと，更にこのHCV遺伝子が複製しうる細胞を選択することを目指した．そして，このようなHCVのRNAレプリコンの複製を許容できる細胞がトランスフェクトしたヒト肝細胞癌由来Huh7細胞の一部から得られ，これによりHCVで初めてタンパク質レベルでウイルスの複製・増殖を解析できる系が確立された．

筆者らはこのレプリコン細胞内で複製しているHCV遺伝子を観察したいと考え，レプリコン細胞を

用いて，アクチノマイシンD処理して細胞内のDNA依存性RNAポリメラーゼを抑えた上で，5-bromouridine 5'-triphosphate（BrUTP）を細胞に導入し，免疫組織染色で観察した[6]．BrUTPが取り込まれた新規に合成されたHCV RNAはレプリコン細胞の核周辺の細胞質に斑点状の構造物として認められ，これらはNSタンパク質と共局在した．レプリコン細胞を電子顕微鏡で観察すると「membranous web」と呼ばれる小胞様構造物が認められることが報告されており[7]，HCVの全ての構造，非構造蛋白を強制発現させても同様の膜変化が生じることが知られている．このような変化は，HCVが感染したチンパンジーの肝細胞の電顕観察でスポンジ状の形態変化として報告されている[8]．以上のことから，HCVの複製複合体は感染細胞のmembranous webに存在しているものと思われる．

次に，生化学的手法を用いて，複製活性を維持したままのHCV複製複合体を粗精製し解析することを目指した[9]．細胞を低浸透圧液に溶解し，ホモジナイズを行った後，核画分を除き，ショ糖密度勾配法で膜とその他の細胞質成分に分画した．それぞれの画分について，多糖体でできたダイアフロー限外濾過膜を用いて限外濾過を行い，低分子量のタンパク質は除去した．HCV RNAとNSタンパク質は膜画分に検出された．lysateをNonidet P-40（NP-40）やTriton X-100（TX-100）などの非イオン性界面活性剤で処理した後，同様に分画したところ，HCV RNAとNSタンパク質の大部分は界面活性剤不溶性画分（DRM）に残った．それぞれの画分に標識化合物（CTP）を加え，この取り込みを指標にしたHCV RNA複製活性測定を行ったところ，活性はDRMにのみ検出された．以上のことから，このDRMに複製活性を保持したHCV複製複合体が存在することが判明した．界面活性剤可溶性画分（DSM）にもNSタンパク質は認められたが，限外濾過を行ったところ，検出されなくなったことから，このNSタンパク質はHCV複製複合体を形成していないと考えられた．以上のように，HCV複製複合体がDRMに検出されたことから，HCV複製複合体が脂質ラフトと結合している可能性が示唆された．そこでこのことを確かめるために，脂質ラフトの構成成分であるコレステロールを除去する働きのあるサポニン（pore-forming agent）で処理したところ，NSタンパク質は脂質ラフトのマーカーであるカベオリン2と共にDRMからDSMへと移行した．更に，HMG-CoAレダクターゼ阻害剤のロバスタチンでレプリコン細胞内のコレステロール合成を抑制するとHCV RNA複製効率も落ちることから，脂質ラフトがHCV複製複合体と結合し，

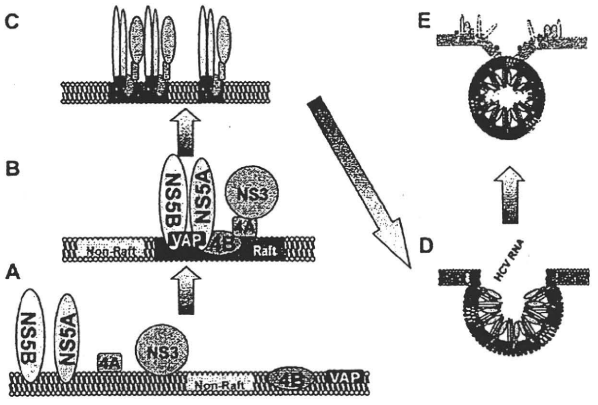

Fig. 2 Model of HCV replication [9].

HCV複製において重要な役割を果たしている可能性が示唆された[9]．また，NS蛋白が脂質ラフトに結合するのを抑制することで，各種スフィンゴ脂質合成阻害剤がウイルス複製を抑えるという報告があり，脂質ラフトの存在する膜上で複製が起こるという仮説が支持された[10]．

以上のように，HCVは細胞の生体膜上の小胞内で複製複合体を形成し，複製するものと考えられている．脂質ラフトは細胞膜上にスフィンゴ脂質とコレステロールに富んだ微小領域を示す．この脂質ラフトは，膜表面上をイカダのように漂いながら，ラフト同士が結合して島状のものになったり，小胞を形成したりと，ダイナミックに変化しながら，ラフトに結合するタンパク質の濃縮や細胞内輸送，シグナルトランスダクション，脂質代謝を担っていると考えられる[11]．また，脂質ラフトはインフルエンザウイルスの集合・出芽，ヒト免疫不全ウイルスの集合・出芽や侵入，エボラウイルスの集合，コクサッキーウイルスA9の侵入，HTLV-1の膜融合や集合，マウス白血病ウイルスの侵入，麻疹ウイルスの集合，センダイウイルスの集合，RSウイルスの集合，マーブルグウイルス，ロタウイルスの集合，ヒト単純ヘルペスウイルスの集合や侵入，エコーウイルス11の侵入，などの多くのウイルスの侵入や粒子形成に重要な役割を果たしていることが報告されている．しかしながら，ウイルスゲノム複製に影響を与えることはHCV研究で初めて示された[9]．

Fig. 2にHCV複製複合体形成モデルを示す．HCVNS蛋白はERで合成され，NS4Bは膜に，NS5Aはその5末端で，NS5Bはその3末端で膜にアンカーしている（A）．HCVNS蛋白はゴルジ体に輸送され，HCVNS蛋白同士で結合する．また，細胞内膜タンパク質の一つで，細胞内膜輸送に関わっていると考えられているthe human homologue of the 33-kDa vesi-

cle–associated membrane protein–associated protein（VAP–A）はそのN末端でNS5Bと，中央部のコイルドコイル領域でNS5Aと結合する[12]．NS5Aは脂質ラフトと弱く結合し，NS4Bは強く結合する．以上から，NS4Bが中心となって，hVAP–33やNS5Aと共に，他のNS蛋白を脂質ラフト上に誘導・固定する役割を担っているものと思われる（B）．一般的に，脂質ラフトは自由に膜上を移動し，集散を繰り返しているものと考えられている．しかしながら，NS4Bのように互いに結合する蛋白が乗っている場合，一度結合した脂質ラフト同士は安定化し，島状に次第に大きくなり，その過程で特定の蛋白を集積させる性格がある（C）．さらに，膜上の蛋白同士が結合するエネルギーにより，膜は小胞を形成するようになる（D）．既に，NS4B蛋白単独でもこの小胞構造を取ることが報告されている．ここにHCV RNAが取り込まれることにより，複製複合体を作り，複製が始まるものと考えられる（E）．以上のように，脂質ラフトはNS蛋白を集積させ，結合体を形成させるだけでなく，小胞構造をとり，膜に包まれたHCV複製の場を提供する役割があるものと想定されている．

筆者らはレプリコン細胞株からHCV複製複合体を含む画分を上記の方法で複製活性を維持したまま抽出しその構造を解析した[9]．鎖特異的PCRを用い，複製複合体中のプラス，マイナス鎖RNAのコピー数について調べたところ，マイナス鎖RNA 1に対してプラス鎖RNA10であった．分画を1% NP–40，4℃で処理後，RNA分解酵素やプロテアーゼで処理してもHCV RNAやNSタンパク質は分解されなかったが，脂質ラフトが破壊されるような強い条件（1% TX–100，37℃）で処理したところ，HCV RNAやNSタンパク質はRNA分解酵素やプロテアーゼ感受性に変化した．このことから，HCV複製複合体は脂質ラフトを含む膜小胞構造内に存在し，内部に存在するHCV RNAやNSタンパク質は外部からのRNA分解酵素やプロテアーゼに対して保護されているものと考えられた．最近の知見では，HCV複製複合体は膜小胞構造内に保護されており，外部から投与したHCV RNAは複製複合体に到達できず，既に内部に取り込まれているHCV RNAがテンプレートとなってマイナス鎖RNAが合成され，それをもとに複数のプラス鎖RNAが合成される．複製に必要なNSタンパク質は継続的に供給される必要はなく，一度HCV複製複合体を形成し，RNA複製が開始されると継続的にRNAが産生されるものと推定できる．膜小胞内にはHCVゲノムの量に対してNSタンパク質の量は1000倍以上と大量に存在しているものの，実際複製に関わっているのはほんのわずかに過ぎず，NSタンパク質の大部分は膜小胞形成の役割を果たしているものと思われる．筆者らはスタチンの一つであるロバスタチンがHCV複製を阻害することを示したが，スタチンがゲラニルゲラニル化を抑制してウイルス複製を抑える可能性も指摘されている[13]．

4. 生体膜脂質のHCV粒子形成における役割

エンベロープウイルスは小胞体，ゴルジ体，形質膜などの細胞の生体膜を被って出芽するため，細胞の膜脂質はウイルス粒子形成に重要な役割を果たしているものと考えられる．さらに，ウイルス粒子の膜脂質が宿主細胞への感染過程に関与する例も報告されている．しかし，HCV粒子に含まれる脂質成分については解析が進んでおらず，その生理学的役割も不明であった．そこで筆者らは，培養細胞で産生させたHCV JFH–1粒子を，培養上清から，限外濾過，ショ糖密度勾配超遠心，ヘパリンアフィニティクロマトグラフィを組み合わせて，濃縮，粗精製し，このHCV粒子に含まれる脂質を生化学的に解析した．その結果，コレステロール／リン脂質モル比が細胞の膜分画に比べて有意に高値を示したことから，コレステロールに富んだ生体膜からの出芽，または粒子形成，分泌過程でのコレステロールとの会合の可能性が考えられた[4]．次にこのHCV粒子上の膜脂質がどのような役割を果たしているかを調べるため，HCV粒子表面をmethyl–B–cyclodextrin（B–CD）で処理してコレステロールを除去した後感染させたところ，B–CDの用量依存的に感染性が低下し，B–CD処理した粒子にコレステロールを添加したところその感染性は回復した[4]．また，コレステロールと親和性が高いスフィンゴ脂質の主要分子スフィンゴミエリンを加水分解するsphingomyelinase（SMase）でHCV粒子を処理することにより感染性の低下を観察した．これらのことはHCV genotype 1bのエンベロープを持つシュードタイプウイルスやキメラウイルスでも確認できた．以上から，ウイルス粒子表面のコレステロールとスフィンゴ脂質はウイルスの遺伝子型によらず感染に重要な役割を果たしていることが示された．次に，HCV粒子上のコレステロールが粒子の物性に与える影響を調べた．HCV産生細胞の培養上清をショ糖密度勾配遠心分画するとCore蛋白及びHCV RNAのピークは1.17 g/ml分画，感染性のピークは1.13 g/ml分画となる．このように，感染性のピークがウイルス遺伝子のそれに比べ低密度側に存在することは培養細胞系で作製したHCVの特徴の一つであるが，濃縮したこの培養上清をB–CD処理しコレステロール除去後に同様に遠心分画を行うと，

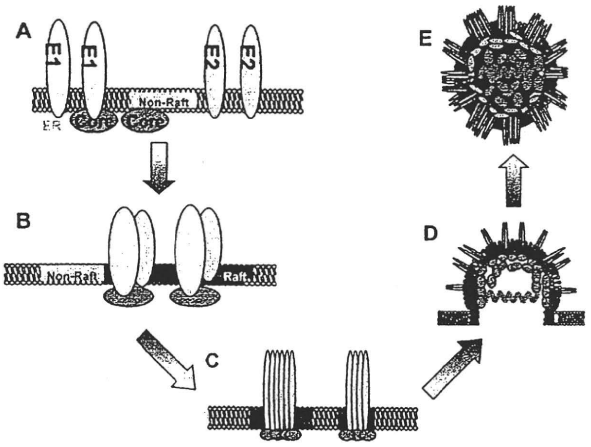

Fig. 3 Model of HCV assembly [14].

Core蛋白のピークは1.20 g/ml分画に移行し，感染性はいずれの分画も検出限界以下であった．さらに，B-CD処理後の培養上清にコレステロールを添加するとCore蛋白のピークは低密度側へシフトし感染性も回復した．このようなコレステロールの除去，および，その後の添加によるloss- and gain-of-functionは5 mg/ml B-CD処理で観察されるが，B-CD濃度を10 mg/mlへ上げた場合はコレステロール添加によって感染性の回復は見られない．これらのことから，HCV粒子表面のコレステロールは粒子構造の維持に役立っており，コレステロールを完全に除去してしまうと粒子構造は致命的なダメージを受ける，これに対し，部分的に除去した場合の構造変化は感染性を低下させるものの，その変化は再生可能なレベルである，と考えられた．次に，HCV粒子上のコレステロールまたスフィンゴ脂質が感染過程のどのステップに関与するのかを解析した．あらかじめコレステロール除去またはSMase処理を行ったHCV粒子の宿主細胞への吸着性は未処理ウイルスと同等であったのに対し，吸着後の細胞内への取り込みは，これらの前処理を施したHCVで顕著な低下が認められた．レセプター蛋白分子とともに標的細胞内へウイルスが侵入する過程に粒子コレステロール，スフィンゴ脂質が関与する可能性が示された．

前述のように，HCVゲノムは脂質ラフトの特徴である界面活性剤不溶性の膜分画で複製することが示され[6, 9]，HCV genotype 1のゲノム複製細胞またHCVが増殖するヒト肝細胞キメラマウスに脂質ラフト構成成分であるスフィンゴミエリンの合成阻害剤myriocin/ISP-1を添加，投与することによって，HCV複製効率は顕著に低下することが報告されている[10, 15]．このmyriocin/ISP-1またはセラミド輸送阻害剤HPA-12をHCV N株（genotype 1b）またJFH-1株のサブゲノムレプリコン細胞に加えることによって，N株ではHCVゲノム複製は阻害されるものの，JFH-1株では予想に反して複製の低下はほとんど認められなかった．しかしながら，興味深いことに，JFH-1のウイルス産生系では両薬剤の用量依存的にHCV産生は抑制された[14]．スフィンゴ脂質合成阻害剤の抗HCV効果の作用機序としてHCVゲノム複製阻害だけでなく粒子形成あるいは感染過程へも介入しうることが示唆された．

Fig. 3に脂質ラフトを利用したHCV粒子形成モデルを示す．HCV構造蛋白はERで合成され，E1およびE2蛋白はその3末端で膜にアンカーしている（A）．HCV構造蛋白はそれぞれ生体膜のうち脂質ラフトと結合する．さらに，コア蛋白とE1蛋白，E1とE2蛋白はそれぞれ結合する（B）．一度結合した脂質ラフト同士はコア蛋白同士の結合する力で安定化し，島状に次第に大きくなり，その過程でウイルス粒子構成の蛋白を集積させる（C）．さらに，膜上のコア蛋白同士が結合するエネルギーにより，膜は小胞を形成するようになる（D）．ここにコア蛋白と結合するHCV RNAが取り込まれることにより，HCV粒子を形成すると考えられる（E）．ウイルス粒子のコレステロールやスフィンゴ脂質を除くと感染性がなくなり，そこにコレステロールを加えると感染性が復活することから，ウイルス粒子膜上の脂質ラフトはウイルス粒子の感染性にも重要な役割を果たしているものと思われる．最近，HCV粒子形成に細胞内脂肪滴が重要な役割を果たすという発見がなされた[16]．脂肪滴に近接した生体膜でHCV粒子が形成されている可能性が考えられている．

5. おわりに

日本膜学会第31年会の生体膜関連シンポジウムのタイトルは「脂質低下療法時代の生体膜研究」ということであったので，最後に脂質低下療法によるHCV治療の可能性について考察してみたい．これまでの研究から，(i) ウイルス粒子膜は脂質に富んでおり感染に重要[14]，(ii) 細胞の形質膜の脂質も感染に重要[4]，(iii) 細胞の生体膜脂質はウイルスゲノム複製に重要[4]，(iv) 脂肪滴周辺の膜構造が粒子形成に重要[16]，などHCVはその生活環の多くのステップに脂質を必要としていることがわかってきた．そこで，筆者らはスタチン製剤でウイルス増殖を抑えることが可能かどうか調べた．ロバスタチンをHCV持続感染細胞に投与したところウイルス粒子産生量が強く抑制された．スタチン製剤は既に臨床の現場で広く使われており，安全性が確立している薬剤であり，

既にC型肝炎患者の治療も試みられている．スタチン製剤単独療法ではHCV治療に有効と無効の報告があり，意見が割れるところであるが，IFNとの併用の場合にはHCV治療に有効[17]という報告がある．

HCVはゲノム配列が多様で，大変変異しやすいウイルスである．そのエンベロープのアミノ酸配列を変えて宿主の免疫系から逃れ慢性持続感染を起こしていると考えられているだけでなく，IFNやリバビリンといった薬剤に対しても耐性を持つウイルスが出現しやすいことが知られている．新たな抗HCV薬として，ウイルスプロテアーゼやポリメラーゼといったウイルス複製に関与する酵素を標的とした薬剤の開発研究が盛んに行われているが，HIVと同様にこれらの薬剤についてもHCVは耐性変異を獲得することが報告されている．上記で報告した宿主のコレステロール産生系やスフィンゴ脂質の産生系をターゲットとし，感染した細胞側の働きを抑えてウイルス増殖を抑制する抗HCV薬の開発は耐性ウイルスが出現しにくい薬剤につながる期待がある．

謝　辞

本研究は下記の多くの研究者のご協力を得て遂行できたものであり，ここに謝意を表す：松浦善治（大阪大学微生物病研究所・分子ウイルス分野），深澤征義，花田賢太郎（国立感染症研究所・細胞化学部），西島正弘（国立医薬品食品衛生研究所），マイケル・ライ（南加大・微生物学免疫学研究室），脇田隆字，鈴木哲朗，宮村達男（国立感染症研究所・ウイルス第二部）

文　献

1) Lindenbach BD, Evans MJ, Syder AJ, Wölk B, Tellinghuisen TL, Liu CC, Maruyama T, Hynes RO, Burton DR, McKeating JA, Rice CM : Complete replication of hepatitis C virus in cell culture, *Science*, **309**, 623-626 (2005)

2) Saito I, Miyamura T, Ohbayashi A, Harada H, Katayama T, Kikuchi S, Watanabe Y, Koi S, Onji M, Ohtaet al. Y : Hepatitis C virus infection is associated with the development of hepatocellular carcinoma, *Proc. Natl. Acad. Sci. U. S. A.*, **87** (17), 6547-6549 (1990)

3) Lohmann V, Korner F, Koch J, Herian U, Theilmann L, Bartenschlager R : Replication of subgenomic hepatitis C virus RNAs in a hepatoma cell line, *Science*, **285**, 110-113 (1999)

4) Wakita T, Pietschmann T, Kato T, Date T, Miyamoto M, Zhao Z, Murthy K, Habermann A, Krausslich HG, Mizokami M, Bartenschlager R, Liang TJ : Production of infectious hepatitis C virus in tissue culture from a cloned viral genome, *Nat. Med.*, **11** (7), 791-796 (2005)

5) Lai MM : Cellular factors in the transcription and replication of viral RNA genomes: a parallel to DNA–dependent RNA transcription., *Virology*, **244** (1), 1-12 (1998)

6) Shi ST, Lee KJ, Aizaki H, Hwang SB, Lai MM : Hepatitis C virus RNA replication occurs on a detergent–resistant membrane that cofractionates with caveolin–2, *J. Virol.*, **77** (7), 4160-4168 (2003)

7) Egger D, Wolk B, Gosert R, Bianchi L, Blum HE, Moradpour D, Bienz K : Expression of hepatitis C virus proteins induces distinct membrane alterations including a candidate viral replication complex, *J. Virol.*, **76** (12), 5974-5984 (2002)

8) Pfeifer U, Thomssen R, Legler K, Bottcher U, Gerlich W, Weinmann E, Klinge O : Experimental non–A, non–B hepatitis: four types of cytoplasmic alteration in hepatocytes of infected chimpanzees, *Virchows Arch. B Cell. Pathol. Incl. Mol. Pathol.*, **33** (3), 233-243 (1980)

9) Aizaki H, Lee KJ, Sung VM, Ishiko H, Lai MM : Characterization of the hepatitis C virus RNA replication complex associated with lipid rafts, *Virology*, **324** (2), 450-461 (2004)

10) Sakamoto H, Okamoto K, Aoki M, Kato H, Katsume A, Ohta A, Tsukuda T, Shimma N, Aoki Y, Arisawa M, Kohara M, Sudoh M : Host sphingolipid biosynthesis as a target for hepatitis C virus therapy, *Nat. Chem. Biol.*, **1** (6), 333-337 (2005)

11) Simons K, Ikonen E : Functional rafts in cell membranes., *Nature*, **387** (6633), 569-572 (1997)

12) Gao L, Aizaki H, He JW, Lai MM : Interactions between viral nonstructural proteins and host protein hVAP–33 mediate the formation of hepatitis C virus RNA replication complex on lipid raft, *J. Virol.*, **78** (7), 3480-3488 (2004)

13) Kapadia SB, Chisari FV : Hepatitis C virus RNA replication is regulated by host geranylgeranylation and fatty acids, *Proc. Natl. Acad. Sci. U. S. A.*, **102** (7), 2561-2566 (2005)

14) Aizaki H, Morikawa K, Fukasawa M, Hara H, Inoue Y, Tani H, Saito K, Nishijima M, Hanada K, Matsuura Y, Lai MM, Miyamura T, Wakita T, Suzuki T : A critical role of virion–associated cholesterol and sphingolipid in hepatitis C virus infection, *J. Virol.*, **82**, 5715-5724 (2008)

15) Umehara T, Sudoh M, Yasui F, Matsuda C, Hayashi Y, Chayama K, Kohara M : Serine palmitoyltransferase inhibitor suppresses HCV replication in a mouse model, *Biochem. Biophys. Res. Commun.*, **346** (1), 67-73 (2006)

16) Miyanari Y, Atsuzawa K, Usuda N, Watashi K, Hishiki T, Zayas M, Bartenschlager R, Wakita T, Hijikata M, Shimotohno K : The lipid droplet is an important organelle for hepatitis C virus production, *Nat. Cell. Biol.*, **9** (9), 1089-1097 (2007)

17) Sezaki H, Suzuki F, Akuta N, Yatsuji H, Hosaka T, Kobayashi M, Suzuki Y, Arase Y, Ikeda K, Miyakawa Y,

Kumada H : An open pilot study exploring the efficacy of fluvastatin, pegylated interferon and ribavirin in patients with hepatitis C virus genotype 1b in high viral loads, *Intervirology*, **52**, 43-48 (2009)

18) Aizaki H, Nagomori S, Aoki Y, Ishii K, Suzuki T, Matsuura Y, Miyamura T : The utilization of human hepatocyte in the study of hepatitis C virus; establishment of the efficient replication system of hepatitis C virus, *Tiss. Cult. Res. Commun.*, **18**, 265-278 (1999)

(Received 21 July 2009 ;
Accepted 3 August 2009)

筆者略歴

相崎 英樹（あいざき ひでき）

1990年 3月 東京慈恵会医科大学医学部卒業
1992年 4月 同大研修修了，同大第一内科入局，国立感染症研究所（旧国立予防衛生研究所）ウイルス第二部 協力研究員
1992年12月 ウイルス肝炎研究財団 流動研究員
1996年 9月 科学技術振興事業団科学技術特別研究員
1999年 9月 ヒューマンサイエンス振興財団 リサーチレジデント
2000年10月 国立感染症研究所主任研究官
2002年～2004年 Howard Hughes Medical Institute, 南カルフォルニア大学微生物免疫学教室リサーチアソシエイト

Virology 386 (2009) 249–256

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

SYNCRIP (synaptotagmin-binding, cytoplasmic RNA-interacting protein) is a host factor involved in hepatitis C virus RNA replication

Helene Minyi Liu [a], Hideki Aizaki [a], Keum S. Choi [a], Keigo Machida [a], James J.-H. Ou [a], Michael M.C. Lai [a,b,c,*]

[a] Department of Molecular Microbiology and Immunology, University of Southern California, Keck School of Medicine, Los Angeles, CA 90033, USA
[b] Institute of Molecular Biology, Academia Sinica, Nankang, Taipei 115, Taiwan
[c] National Taiwan Cheng Kung University, Tainan 701, Taiwan

ARTICLE INFO

Article history:
Received 17 September 2008
Returned to author for revision
25 November 2008
Accepted 8 January 2009
Available online 20 February 2009

Keywords:
HCV RNA replication
SYNCRIP
hnRNP Q
NSAP1
HCV RNA translation
siRNA knock-down
HCV replicon

ABSTRACT

Hepatitis C virus (HCV) RNA replication requires viral nonstructural proteins as well as cellular factors. Recently, a cellular protein, synaptotagmin-binding, cytoplasmic RNA-interacting protein (SYNCRIP), also known as NSAP1, was found to bind HCV RNA and enhance HCV IRES-dependent translation. We investigate whether this protein is also involved in the HCV RNA replication. We found that SYNCRIP was associated with detergent-resistant membrane fractions and colocalized with newly-synthesized HCV RNA. Knock-down of SYNCRIP by siRNA significantly decreased the amount of HCV RNA in the cells containing a subgenomic replicon or a full-length viral RNA. Lastly, an *in vitro* replication assay after immunodepletion of SYNCRIP showed that SYNCRIP was directly involved in HCV RNA replication. These findings indicate that SYNCRIP has dual functions, participating in both RNA replication and translation in HCV life cycle.

Introduction

Hepatitis C virus (HCV) infection is a leading cause of chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma. HCV is an enveloped RNA virus with a positive-stranded RNA of 9.7 kb in length (Reed and Rice, 2000). It encodes a large polyprotein, which is then processed into structural proteins (C, E1, and E2) and nonstructural (NS) proteins, the latter of which participate in viral replication.

Besides the viral NS proteins, several host factors, including the human homologue of the 33-kDa vesicle-associated membrane protein-associated protein (hVAP-33) (Gao et al., 2004; Hamamoto et al., 2005), polypyrimidine-tract-binding protein (PTB) (Aizaki et al., 2006; Chang and Luo, 2006; Domitrovich et al., 2005), La antigen (Domitrovich et al., 2005) and host geranylgeranylated proteins and fatty acids (Kapadia and Chisari, 2005) have been shown to be involved in some steps of HCV replication cycle. Some of these host factors, such as PTB and La autoantigen, were initially found to regulate HCV protein translations (Ali and Siddiqui, 1997; Ito and Lai, 1999) by virtue of their binding to the 5′ and 3′-untranslated regions (UTR) of HCV RNA. Later studies showed that some of these host factors also directly regulate HCV RNA replication either by participating in the formation of the RNA replication complex (e.g., VAP-33) (Gao et al., 2004) or by binding to the viral RNA (e.g., La, PTB) (Ali and Siddiqui, 1995; Chang and Luo, 2006). A recent study showed that another host protein, synaptotagmin-binding, cytoplasmic RNA-interacting protein (SYNCRIP), also named NS-1-associated protein (NSAP1), binds to the N-terminal of the core protein-coding region of HCV RNA and enhances HCV Internal Ribosomal Entry Site (IRES)-dependent translation (Kim et al., 2004).

SYNCRIP is a member of cellular heterogeneous nuclear ribonucleoprotein (hnRNP) family, to which PTB also belongs. hnRNPs are well-known for their abilities to bind to cellular proteins and RNAs to facilitate many biological processes. Interestingly, SYNCRIP has previously been shown to be involved not only in cellular processes but also in mouse hepatitis virus (MHV) RNA replication (Choi et al., 2004b). Since SYNCRIP binds to HCV RNA at a site close to the 5′-end of the RNA, it is likely that SYNCRIP may also affect the RNA replication of HCV. If this is the case, SYNCRIP will have duel functions in both RNA replication and protein translation, similar to other duel-purpose hnRNPs, such as PTB. Our goal in this study is to investigate whether SYNCRIP is involved in HCV RNA replication in addition to its role in translation.

Results

SYNCRIP relocalized to detergent-resistant membrane fraction in HCV replicon cells

It has been shown that HCV RNA replication occurs in detergent-resistant membrane (DRM) fractions (Ali et al., 2002; El-Hage and Luo,

* Corresponding author. Institute of Molecular Biology, Academia Sinica, Taipei 115, Taiwan. Fax: +886 2 27826085.
E-mail address: michlai@gate.sinica.edu.tw (M.M.C. Lai).

0042-6822/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.virol.2009.01.018