biphasic signaling through the DNA damage response and p38MAPK pathways.

Experimental procedures

Cell culture, preparation of MEFs and reagents

TIG-3 cells were purchased from Health Science Research Resources Bank (Tokyo, Japan), and REF52 cells were provided by Dr M. Nakamura (Tokyo Medical and Dental University).

Generation and characterization of $Nox1^{KO}$ ($Nox1^{-/Y}$) mice followed previously described methods (Matsuno *et al.* 2005). MEF cells were prepared from day 13 embryos derived from crosses between $Nox1^{KO}$ mice as described previously (Serrano *et al.* 1997) and were used between P2 and P6. Four independent wild-type and $Nox1^{KO}$ mouse embryos were used. DPI was obtained from Calbiochem and apocynin from Sigma-Aldrich.

Retroviral gene transfer

pBabe H-RasV12 was a gift from Dr. S. W. Lowe (Cold Spring Harbor Laboratory). pBabe (mock) or pBabe H-RasV12 was stably transfected into PT67 packaging cells (Clone Tech), and the stable clones were isolated. For infection, the first culture supernatants from the virus producing PT67 cells were inoculated into target fibroblasts for 12 h and the infection process was repeated with the second supernatant according to the manufacturer's protocol. The infected cells were then selected with puromycin (0.5 μg/mL for REF52; 1 μg/mL for TIG-3) for 2–3 days and reseeded for various assays. pBabe vectors carrying human Nox1 and Nox4 were constructed and transfected into HEK293 or 293T cells together with VSVenv and Gag/Pol using the calcium phosphate precipitation method. The culture supernatants were harvested 48 h later and inoculated into target cells as described above. For disruption of Nox1 and Nox4, target fibroblasts were first transfected with pSilencer vectors carrying Nox1 siRNA, Nox4 siRNA or scrambled siRNA as described before (Mitsushita *et al.* 2004; Yamaura *et al.* 2009) and 24 h later infected with pBabe H-RasV12 retroviral vectors.

Senescence-associated β-galactosidase

SA-β-gal staining was performed as described previously (Serrano *et al.* 1997).

Growth curve

Cells were plated into 24-well plates and transfected with pSilencer vectors carrying Nox1 siRNA, Nox4 siRNA or scrambled siRNA 24 h before pBabe H-RasV12 or control virus infection. After selection with puromycin for 2 days, cells were reseeded (day 0) and counted at the indicated time points.

RT-PCR

Total RNAs were extracted from cells, and PCR was performed by using specific primers for Noxs as described previously (Mitsushita *et al.* 2004).

Immunoblotting

Cells were lysed in RIPA buffer and subjected to immunoblotting as described (Shinohara *et al.* 2010). The following antibodies were used: rabbit anti-p53 and rabbit anti-Nox4 from Santa Cruz Biotechnology, rabbit anti-phospho-p38 (Thr180/Tyr182), mouse anti-p21 and rabbit anti-Nox1 from Sigma, rabbit anti-p16, rabbit anti-H2A.X, rabbit anti-γ-H2A.X and rabbit anti-Ras from Cell Signaling, and chicken anti-$p19^{Arf}$ from Gene Tex. Rabbit anti-Nox1 antibodies were also generated (Komatsu *et al.* 2008).

Measurement of ROS production

Cell suspensions were incubated with 200 μM luminol and 1 unit horseradish peroxidase for 20 min at room temperature as described (Komatsu *et al.* 2008). Luminescence was quantified by a Luminometer Lumat LB9507 (Berthold). Alternatively, MEF cells were loaded with 10 μM 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA; Molecular Probes) for 30 min at 37 °C. Images were obtained, and fluorescence intensity of approximately 100 random cells was quantified as described (Yamaura *et al.* 2009).

Statistics

Differences or correlations between two groups were assessed by Student's *t*-test with a P value < 0.05 being considered significant.

Acknowledgements

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Supporting Information/Supplementary Material

Additional Supporting Information may be found in the online version of this article at the publisher's web site:

Figure S1 Expression of cell cycle regulatory proteins in H-RasV12-expressing REF52 and TIG-3 cells.

Figure S2 Effects of rotenone on Ras-induced senescence.

Figure S3 Nox4 mediates Ras-induced senescence of human embryonic lung IMR90 cells.

Figure S4 Treatment of TIG-3 cells with H_2O_2 induces senescence.

Figure S5 Overexpression of Nox1 and Nox4 induces ROS production.

Figure S6 Disruption of Nox1 expression in MEF cells.

Figure S7 The effect of knockdown of both Nox1 and Nox4 on Ras-induced growth arrest in REF52 cells.

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Original Article

Characteristics and prediction of hepatitis B e-antigen negative hepatitis following seroconversion in patients with chronic hepatitis B

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Aim: We analyzed the characteristics of alanine aminotransferase (ALT) abnormality after achieving hepatitis B e-antigen (HBeAg) seroconversion (SC) and other factors associated with the occurrence of HBeAg negative hepatitis.

Methods: We followed 36 patients with chronic hepatitis B from 3 years prior to at least 3 years after SC (mean, 11.6 years) and examined ALT, hepatitis B virus (HBV) DNA, HB surface antigen, HB core-related antigen (HBcrAg) levels and mutations related to HBeAg SC.

Results: ALT normalization (<31 IU/L for at least 1 year) was primarily observed until 2 years following SC, after which it became more infrequent. We next divided patients into abnormal (≥31 IU/L, $n = 20$) and normal (<31 IU/L, $n = 16$) groups based on integrated ALT level after the time point of 2 years from SC, and considered the former group as having HBeAg negative hepatitis in the present study. Although changes in median levels of ALT and HBcrAg differed significantly between the groups, multivariate analysis showed ALT normalization within 2 years after SC to be the only significant determining factor for this disease ($P = 0.001$). We then assessed the 19 patients whose ALT was normal at 2 years following SC, four of whom developed HBeAg negative hepatitis. Increased levels of HBV DNA ($P = 0.037$) and HBcrAg ($P = 0.033$) were significant factors of potential relevance.

Conclusion: ALT abnormality after 2 years of SC may be evaluated as HBeAg-negative hepatitis. ALT, HBV DNA and HBcrAg levels may be useful in predicting the outcome of patients who achieve HBeAg SC.

Key words: hepatitis B core-related antigen, hepatitis B virus, reactivation, seroconversion

INTRODUCTION

HEPATITIS B VIRUS (HBV) infection is a major health concern with an estimated 350–400 million carriers worldwide. Whereas acute infection in adults is generally self-limiting, that during early childhood develops into persistent infection in most individuals, which can lead to chronic hepatitis and eventually liver cirrhosis and hepatocellular carcinoma (HCC).[1–3] The natural history of chronic HBV infection can be classified into several phases based on levels of alanine aminotransferase (ALT) and HBV DNA, hepatitis B e-antigen (HBeAg) status and estimated immunological status.[4] In the immune tolerance phase, HBeAg is positive, ALT level is normal, histological evidence of hepatitis is absent or minimal, and HBV DNA level is elevated. The chronic hepatitis B phase is characterized by raised ALT and HBV DNA levels. In this phase, the host's immune system initiates a response that results in active hepatitis. In patients who are HBeAg positive, active hepatitis can be prolonged and may result in cirrhosis. However, chronic hepatitis B eventually transitions into an inactive phase with a loss of HBeAg positivity in the majority of patients. Seroconversion (SC) of HBeAg to HBe antibodies and the fall of HBV DNA level result in the disappearance of disease activity despite persisting hepatitis B surface antigen (HBsAg) and low HBV DNA level. The SC of

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HBeAg marks the transition from the hepatitis phase to the inactive carrier phase, which is generally thought to be a benign course for the HBV carrier, although hepatitis can sometimes reactivate spontaneously.[5]

Patients experiencing HBV reactivation undergo another transition characterized by increases in HBV DNA and ALT levels and disease activity without the reappearance of HBeAg. This phase is referred to as HBeAg negative chronic hepatitis B. Occasional severe hepatitis B flare-ups with moderate HBV DNA level occur in this phase.[6,7] It is thought that HBeAg negative chronic hepatitis B is caused by mutant strains of HBV that are unable to produce HBeAg[6,8] and tends to develop into cirrhosis and HCC more frequently than does HBeAg positive chronic hepatitis B.[9–13] Therefore, it is important to identify patients who are likely to develop HBeAg negative hepatitis after HBeAg SC from those who can maintain an inactive carrier phase. In the present study, we evaluated 36 patients with HBeAg SC to examine the effects of host factors and viral factors, including serum quantitative HBsAg, hepatitis B core-related antigen (HBcrAg), HBV DNA, PC (A1896) mutation and BCP mutations (T1762 and A1764) before, during and after SC.

METHODS

Patients

A TOTAL OF 36 patients with sustained HBeAg SC (24 men and 12 women; median age, 38 years [range, 23–65]) were enrolled in this study after meeting the following criteria: (i) follow ups for at least 3 years before and after HBeAg SC; and (ii) serum samples at several time points before, during and after SC available for testing. HBeAg SC was defined as seroclearance of HBeAg with the appearance of anti-HBe that was not followed by HBeAg reversion or loss of anti-HBe. All patients were seen at Shinshu University Hospital from 1985 to 2009. The median follow-up period after SC was 11.6 years (range, 3.2–26.0). HBsAg was confirmed to be positive on two or more occasions at least 6 months apart in all patients. No patients had other liver diseases, such as alcoholic or non-alcoholic fatty liver disease, autoimmune liver disease or drug-induced liver injury. Patients who were complicated with HCC or who showed signs of hepatic failure were excluded from the study. HBV genotype was C in all patients, who were also negative for antibodies to hepatitis C virus and HIV. Nucleoside/nucleotide analog (NUC) therapy was introduced in 14 patients after HBeAg SC on physicians' decision, and then follow up was stopped. No patient was treated with interferon during the study period. ALT, albumin, bilirubin, platelet and other relevant biochemical tests were performed using standard methods.[14] The integration value of ALT after SC was calculated using the method described by Kumada *et al.*[15] (median determination frequency, 4.7/year per person [range, 1.6–13.9]) because a previous study showed integration values to be more meaningful than arithmetic mean values in long-term follow-up cohorts.[16] As guidelines released by the Ministry of Health, Labor and Welfare of Japan advise consideration of antiviral therapy for patients with ALT levels of 31 IU/L or more,[17] an ALT integration value of less than 31 IU/L was defined as normal in this report. Serum samples were stored at −20°C until tested. Liver biopsies were performed by percutaneous sampling of the right lobe with a 14-G needle in eight patients with HBeAg negative hepatitis, as reported previously.[14] All biopsies were 1.5 cm or more in length. Liver histological findings were scored by the histology activity index of Knodell *et al.*[18] The protocol of this study was approved by the ethics committee of our university and was in accordance with the Declaration of Helsinki of 1975. Informed consent was obtained from each patient.

Hepatitis B viral markers

Serological markers for HBV, including HBsAg, HBeAg and anti-HBe, were tested using commercially available enzyme immunoassay kits (Abbott Japan, Tokyo, Japan).[19] Quantitative measurement of HBsAg was done using a chemiluminescence enzyme immunoassay (CLEIA)-based HISCL HBsAg assay manufactured by Sysmex (Kobe, Japan).[20] The assay had a quantitative range of −1.5 to 3.3 log IU/mL. Serum HBcrAg level was measured using a CLEIA HBcrAg assay kit with a fully automated Lumipulse System analyzer (Fujirebio, Tokyo, Japan) as described previously.[21] We expressed HBcrAg level in terms of log U/mL, with a quantitative range set at 3.0–6.8 log U/mL. End titers of HBsAg and HBcrAg were determined by diluting samples with normal human serum when initial results exceeded the upper limit of the assay range. HBV DNA level was measured using an Amplicor monitor assay with a dynamic range of 2.6–7.6 log copies/mL.[22] Six major genotypes (A–F) of HBV were determined using the method reported by Mizokami *et al.*,[23] in which the surface gene sequence amplified by polymerase chain reaction was analyzed by restriction fragment length polymorphism.

The PC and BCP mutations of HBV were assessed as previously described. Briefly, the stop codon mutation in the PC region (A1896) was detected with an enzyme-linked mini-sequence assay kit (Smitest; Roche Diagnostics, Tokyo, Japan) with a sensitivity of 1000 copies/mL. The results were expressed as the percent mutation rate as defined by Aritomi *et al.*[24] The PC mutation was judged to exist when the mutation rate exceeded 50% in the present study because the mutation rate would increase to 100% once surpassing this value.[25] The BCP double mutation was detected using an HBV core promoter detection kit (Smitest; Genome Science Laboratories) with a detection limit of 1000 copies/mL.[24] The BCP mutation was judged to exist for all classifications of mutant in the present study.

Statistical analysis

Clinical factors were compared between patients with and without HBeAg negative hepatitis after SC using the χ^2-test and Fisher's exact test, and group medians were compared using the Mann–Whitney *U*-test. Receiver-operator curves (ROC) with Youden's index were used to decide each cut-off point for predicting HBeAg negative hepatitis after SC. Differences between the analyzed groups were assessed using Kaplan–Meier analysis and the log-rank test. Sex, age at SC, HBcrAg level, ALT level, HBV DNA level, HBsAg level, PC mutation and BCP mutation were all suspected to be associated with ALT elevation after SC. Factors attaining a *P*-value of less than 20% in univariate analysis were used in multivariate analysis that employed a stepwise Cox proportional hazard model. These included level of serum albumin and platelet count at SC, levels of ALT at 0, 1, 2 and 3 years after SC, and levels of HBcrAg at 1, 2 and 3 years after SC. All tests were performed using the IBM SPSS Statistics Desktop for Japan ver. 19.0 (IBM Japan, Tokyo, Japan). *P*-values less than 0.05 were considered to be statistically significant.

RESULTS

Baseline characteristics of patients

ALL 36 PATIENTS enrolled showed abnormal levels of ALT before SC, with the majority showing normalization around the time of SC. We defined ALT normalization as a decrease in ALT level to less than 31 IU/L for at least 1 year. The change in ratio of patients not achieving normalization over time revealed two distinct phases (Fig. 1): the first was a fast decline phase from 2 years before SC to 2 years afterwards, and the second was a slow decline phase from 2 years after SC to the end of follow up. Normalization of ALT during the fast phase was presumed to be associated with HBeAg SC, which was seen in 53% (19/36) of total patients. Based on this, we analyzed the risk factors associated with ALT abnormality after the time point of 2 years from SC by calculating integrated ALT levels (Fig. 2). We defined patients whose integrated ALT level exceeded 30 IU/L as having HBeAg negative hepatitis in the present study. Serum HBV DNA of over 4.0 log copies/mL was observed in all patients with HBeAg negative hepatitis.

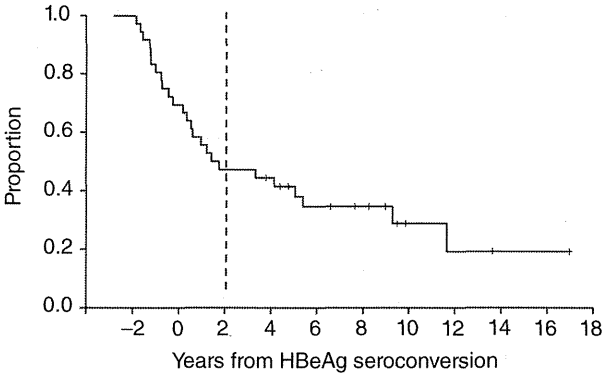

Figure 1 Changes in the proportion of patients with alanine aminotransferase (ALT) abnormality. ALT normalization was defined as ALT level decreasing to lower than 31 IU/L and maintained for at least 1 year. These data reveal two distinct time frames: a fast decline phase around the seroconversion (SC) period until 2 years afterwards, and a slow decline phase from 2 years after SC to the end of follow up. The vertical broken line at 2 years after SC indicates the borderline between the two phases. HBeAg, hepatitis B e-antigen.

Of the 36 patients enrolled, 20 (56%) developed HBeAg negative hepatitis and 16 (44%) did not. ALT normalization within 2 years after SC was significantly less frequent in patients with HBeAg negative hepatitis (Table 1). Median age, sex distribution and follow-up period did not differ between the two groups. Median albumin level tended to be lower in patients with HBeAg negative hepatitis, but only modestly. Eight of 20 HBeAg negative hepatitis patients underwent liver biopsy after SC. All had necroinflammatory activity. Initiation of NUC therapy was more common in the HBeAg negative hepatitis group.

Clinical and virological profiles

Changes in median levels of ALT, HBV DNA, HBsAg and HBcrAg during the course of SC have been compared between patients with and without HBeAg negative

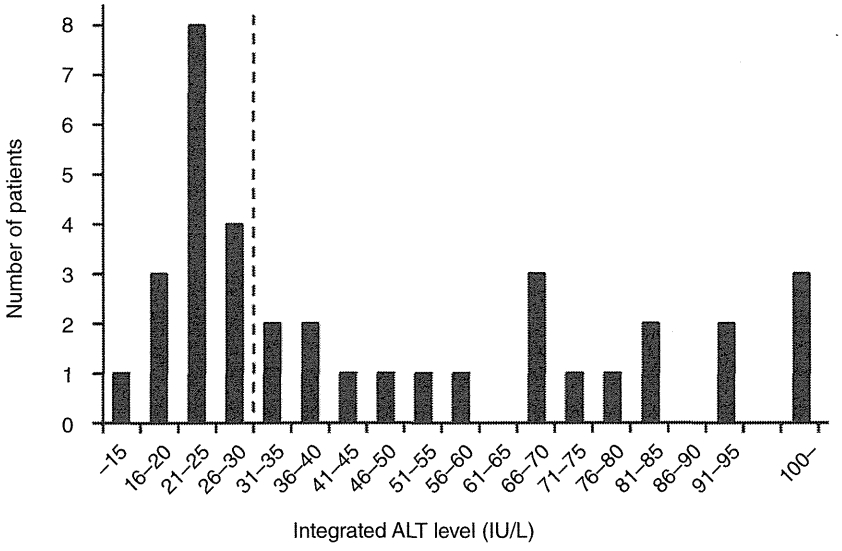

Figure 2 Distribution of integrated alanine aminotransferase (ALT) level from the time point of 2 years after seroconversion (SC) to the end of follow up.

hepatitis in Figure 3. We observed that median ALT level decreased around the time of SC in patients without HBeAg negative hepatitis, but did not in the other group. Overall, median ALT differed significantly between the two groups at the time of SC (43.0 vs 21.5 IU/L; $P = 0.009$) and at 1 (67.0 vs 15.0 IU/L; $P = 0.001$), 2 (52.0 vs 14.5 IU/L; $P < 0.001$) and 3 years (41.5 vs 15.0 IU/L; $P < 0.001$) afterwards (Fig. 3a). Median HBV DNA level decreased similarly in both groups around the time of SC (Fig. 3b). Median HBsAg level was unchanged or minimally decreased in both groups around the time of SC, but was significantly lower in patients with HBeAg negative hepatitis at 1 (3.9 vs 3.2 log IU/mL; $P = 0.025$) and 2 years (3.9 vs 3.2 log IU/mL; $P = 0.045$) before SC and at 2 years (3.7 vs 3.0 log IU/mL; $P = 0.023$) after SC (Fig. 3c). Median HBcrAg level decreased in both groups around the time of SC, but this decline was more gradual in patients with HBeAg negative hepatitis, becoming significantly higher at 1 (5.2 vs 3.9 log U/mL; $P = 0.011$), 2 (4.6 vs 3.5 log

Table 1 Comparison of host and viral factors between patients with and without HBeAg negative hepatitis among total patients

Clinical characteristics	HBeAg negative hepatitis		*P*
	Present ($n = 20$)	Absent ($n = 16$)	
Age at SC (years)†	40 (23–64)	38 (24–65)	0.504
Sex (male : female)	15:5	9:7	0.298
Follow-up period (years)†	10.6 (3.8–26.0)	12.4 (3.2–23.1)	0.610
Laboratory data at SC			
Albumin (g/dL)†	4.1 (3.6–4.6)	4.3 (3.7–4.8)	0.030
Bilirubin (mg/dL)†	1.0 (0.4–2.6)	0.8 (0.5–1.3)	0.319
Platelets (/μL)†	13.9 (8.5–24.3)	18.1 (9.6–22.9)	0.187
ALT normalization within 2 years after SC‡	4 (20)	15 (94)	<0.001
Events during follow-up period			
Initiation of NUC therapy‡	12 (60)	2 (13)	0.006
Development of HCC‡	2 (10)	1 (6)	1.000

†Data are expressed as median (range).
‡Data are expressed as number of patients (%).
ALT, alanine aminotransferase; HBeAg, hepatitis B e-antigen; HCC, hepatocellular carcinoma; NUC, nucleoside/nucleotide analog; SC, seroconversion.

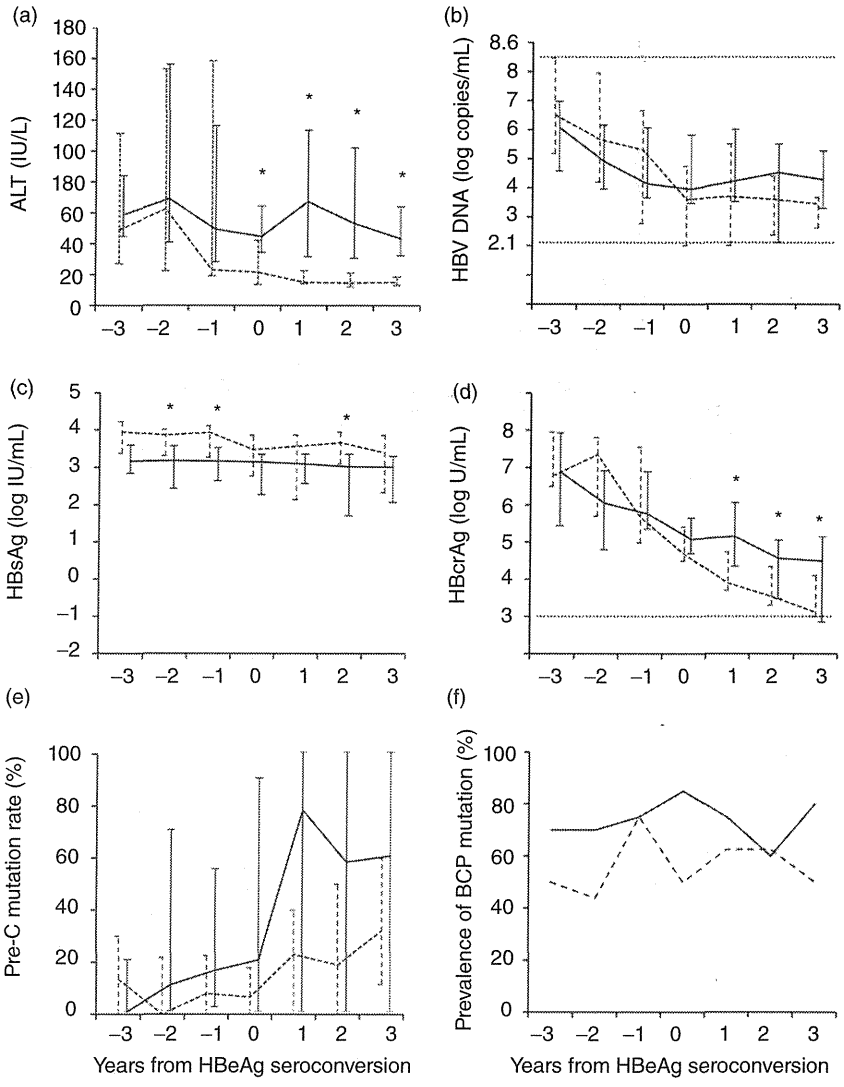

Figure 3 Changes in median levels of serum alanine aminotransferase (ALT) (a), hepatitis B virus (HBV) DNA (b), hepatitis B surface antigen (HBsAg) (c), hepatitis B core-related antigen (HBcrAg) (d) and PC mutation rate (e) are compared between patients with and without the occurrence of hepatitis B e-antigen (HBeAg) negative hepatitis. A similar comparison is made for prevalence of patients with BCP mutations (f). Solid lines indicate patients with HBeAg negative hepatitis ($n = 20$) and broken lines indicate those without ($n = 16$). Data are shown as median values with 25% and 75% ranges at each point for (a–e). Horizontal broken lines in (b) and (d) indicate the upper and lower detection limits of the corresponding markers. $^*P < 0.05$.

U/mL; $P = 0.041$) and 3 years (4.6 vs 3.1 log U/mL; $P = 0.016$) after SC (Fig. 3d). PC mutation rate increased similarly in both groups during the course of SC (Fig. 3e), and the prevalence of BCP mutation positive patients remained comparatively high in both groups throughout the study period (Fig. 3f).

All factors that were associated with the occurrence of HBeAg negative hepatitis were evaluated for independence by multivariate analysis. We found that only abnormal level of ALT (≥31 IU/L) at 2 years after SC (odds ratio, 42.0; 95% confidence interval, 4.3–405.4; $P = 0.001$) was an independent predictive factor. Therefore, we examined for factors associated with the occurrence of HBeAg negative hepatitis in the 19 patients whose ALT level had normalized by 2 years after SC. Four (21%) of these patients developed HBeAg negative hepatitis and the remaining 15 (79%) did not. We found no significant differences between the two groups with regard to age at SC, sex or laboratory data (Table 2). We next analyzed HBV DNA, HBsAg and HBcrAg levels at 2 years after SC to see if these factors could discriminate between patients with and without the development of HBeAg negative hepatitis. Cut-off values for each factor were determined by ROC analysis. As shown in Figure 4, serum levels of HBV DNA (7% vs 60%; $P = 0.037$) and HBcrAg (0% vs 44%; $P = 0.033$) were significant factors indicating susceptibility, but HBsAg was not.

Table 2 Comparison of host and viral factors between patients with and without HBeAg negative hepatitis in 19 patients whose ALT levels were normal at 2 years after SC

Clinical characteristics	HBeAg negative hepatitis		*P*
	Present ($n = 4$)	Absent ($n = 15$)	
Age at SC (years)†	41 (30–43)	37 (23–65)	0.549
Sex (male : female)	2:2	8:7	1.000
Follow-up period (years)†	9.1 (8.3–14.1)	12.2 (3.2–23.1)	0.610
Laboratory data at SC			
Albumin (g/dL)†	4.3 (3.8–4.3)	4.3 (3.7–4.7)	0.364
Bilirubin (mg/dL)†	1.0 (1.0–1.3)	0.8 (0.5–1.3)	0.083
Platelets (/μL)†	14.9 (13.3–16.4)	16.9 (9.6–22.5)	0.667
Events during follow-up period			
Initiation of NUC therapy‡	3 (75)	2 (13)	0.037
Development of HCC‡	1 (25)	1 (7)	0.386

†Data are expressed as median (range).
‡Data are expressed as number of patients (%).
ALT, alanine aminotransferase; HBeAg, hepatitis B e-antigen; HCC, hepatocellular carcinoma; NUC, nucleoside/nucleotide analog; SC, seroconversion.

DISCUSSION

ALTHOUGH ACTIVE HEPATITIS usually subsides following HBeAg SC, it recurs in a considerable proportion of patients several years afterwards. Hsu *et al.*[5] followed 283 patients with HBeAg SC for a median follow-up period of 8.6 years and observed that ALT elevation of over twice the upper limit of normal occurred in 94 patients (33%). Of these, 68 (72%) were considered to have HBeAg negative hepatitis B because HBV DNA was detectable without the reappearance of HBeAg at the time of ALT elevation. HBeAg negative hepatitis is a major health concern because its occurrence is closely associated with progression to cirrhosis and development of HCC,[9–12] and thus prediction of its onset is important. Hsu *et al.*[5] found that patients with more frequent acute exacerbations of hepatitis before HBeAg SC and those with cirrhosis at the time of HBeAg SC had a higher risk of developing HBeAg negative hepatitis. Although significant, these factors were insufficient to accurately predict the occurrence of the disease.[26–30] Therefore, we analyzed several additional factors, including HBV DNA, HBsAg and HBcrAg levels, as well as viral mutations that halt HBeAg production.

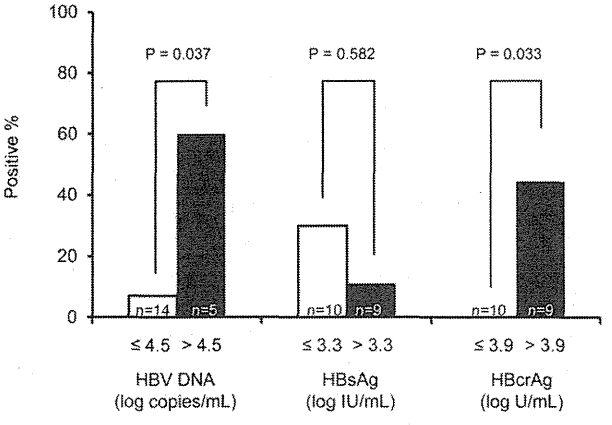

Figure 4 Occurrence of hepatitis B e-antigen (HBeAg) negative hepatitis is compared among patients using higher and lower levels of corresponding markers at 2 years after seroconversion (SC). The cut-off value for each marker was determined by receiver–operator curve analysis. HBcrAg, hepatitis B core-related antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus.

In the present study, we found that the majority of patients with HBeAg SC achieved normalization of ALT within 2 years following SC, after which such normalization became relatively rare. Abnormal ALT was determined using the distribution of integrated ALT level from 2 years after SC to the end of follow up, which clearly showed the existence of two groups. We defined patients with an abnormal integrated level of ALT as having HBeAg negative hepatitis because this abnormality tended to persist and was preceded by HBV DNA elevation. Our result also conferred the important realization that ALT abnormality within 2 years after SC may not necessarily indicate the occurrence of HBeAg negative hepatitis, which has a poor prognosis. NUC

therapy was not available for patients with chronic hepatitis B in Japan when our subjects began follow up. Hence, the natural history of SC has been evaluated in this cohort. Follow up stopped in this study when NUC therapy was commenced. Currently, we perform NUC therapy on patients with HBe negative hepatitis based on age and ALT activity, as advised by the Ministry of Health, Labor and Welfare.[17]

Many host and viral factors were also analyzed to predict the occurrence of HBeAg negative hepatitis in the current study. Host factors, including age and sex, did not differ between the groups with and without HBeAg negative hepatitis, but changes in median ALT level around SC clearly differed between the two groups. Specifically, ALT level did not decrease even after SC in patients with HBeAg negative hepatitis, while it normalized during the SC period in those without. Viral factors were analyzed at several time points around SC. Among them, median HBcrAg level clearly differed between the groups; HBcrAg showed a steep decrease around the SC period in patients without HBeAg negative hepatitis, while it exhibited a significantly slower decline in those with. Similarly to earlier reports, median levels of HBV DNA and HBsAg showed some differences between the two groups, but these were not remarkable when analyzed chronologically. Negative results were also seen in the analyses of PC and BCP mutations. Multivariate analysis showed that abnormal ALT level at 2 years after SC was the only significant factor to predict the occurrence of HBeAg negative hepatitis among the factors analyzed. Because patients with normal ALT had maintained that level for at least 1 year, this result may indicate that continuous normalization of ALT is rare in patients with HBeAg negative hepatitis after SC and that ALT abnormality is associated with higher levels of HBcrAg and HBV DNA.

Because ALT level was closely related to the occurrence of HBeAg negative hepatitis, we next analyzed for predictive factors in patients whose ALT level was normal (<31 IU/L) at 2 years after SC. We observed that increased HBV DNA and HBcrAg levels at 2 years after SC were significant factors for predicting the occurrence of HBeAg negative hepatitis, but that HBsAg level was not. Single or combined monitoring use of HBV DNA and HBcrAg levels may therefore be useful to predict the recurrence of hepatitis in patients whose ALT level normalizes following HBeAg SC. However, further studies are required to verify this in the clinical setting.

Whereas HBsAg is a serum marker commonly used for the diagnosis of HBV infection, HBcrAg assays measure serum levels of HBc, HBe and the 22-kDa precore antigens simultaneously using monoclonal antibodies that recognize the common epitopes of these three denatured antigens.[31] Because the latter assay measures all antigens transcribed from the precore/core gene, it is regarded as core-related.[21] It has been suggested that viral antigen levels, including those of HBsAg and HBcrAg, are differently associated with HBV activity from HBV DNA and ALT levels, and thus are useful for predicting the future activity of hepatitis B. For example, HBcrAg level was seen to predict hepatitis relapse after discontinuation of NUC therapy,[32,33] and HBsAg level has been reportedly associated with the response to pegylated interferon therapy differently from HBV DNA.[34,35] Both antigen levels are believed to be related to intracellular levels of HBV cccDNA. However, it is possible that levels of HBsAg and HBcrAg have different roles in monitoring viral activity because the transcription of these two antigens is regulated by alternative enhancer–promoter systems in the HBV genome.[1] The serum level of HBcrAg was more useful than that of HBsAg to predict the occurrence of HBeAg negative hepatitis in the present study. This difference may be attributed to the fact that the production of all antigens that constitute HBcrAg is regulated by the same system as that of HBeAg, while the production of HBsAg is not.

Lastly, it is reasonable to presume that the PC and BCP mutations which halt HBeAg production are associated with integrated values of ALT elevation because the disease is essentially caused by HBV containing these mutations.[8,10] However, the prevalence of either mutation did not differ between the groups at any time point during the study. Our results showed that almost all patients had PC and/or BCP mutations, especially after SC, and implied that the existence of these mutations alone was not sufficient for developing ALT elevation. HBV genotype is also closely associated with HBeAg SC,[36] but we could not include genotype as a factor because our entire cohort was genotype C.

A recent review by Papatheodoridis *et al.*[37] showed that histologically significant liver disease is rare in HBeAg negative patients with persistently normal ALT based on stringent criteria and serum HBV DNA of 20 000 IU/mL or less. They suggest that such individuals can be considered as true inactive HBV carriers, who require continued follow up rather than liver biopsy or immediate therapy. On the contrary, liver biopsy samples obtained from eight of our patients with HBeAg negative hepatitis having elevated ALT levels after SC revealed necroinflammatory activity. Hence, it remains controversial if histological findings are important for diagnosis of HBeAg negative hepatitis.

This study has the main limitations of a retrospective design and a small cohort size. However, our findings from careful extended follow up indicate that ALT abnormality after 2 years from SC can be considered to be HBeAg negative hepatitis, and that HBcrAg and HBV DNA levels may be useful for predicting the long-term outcome of patients who achieve HBeAg SC and ALT normalization.

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ORIGINAL ARTICLE

Safety and Effectiveness of Low-Dose Propofol Sedation During and After Esophagogastroduodenoscopy in Child A and B Cirrhotic Patients

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Abstract
Background Effective and safe sedation for patients with liver cirrhosis is problematic.
Aim To examine the safety and effectiveness of low-dose propofol sedation during and after esophagogastroduodenoscopy (EGD) in cirrhotic patients.
Methods Study 1 was a prospective study in cirrhotic patients who underwent diagnostic EGD under propofol sedation. Propofol was given by bolus injection with an age-adjusted standard protocol consisting of 40 mg for patients <70 years, 30 mg for patients aged 70–89 years; additional injections of 20 mg propofol were given up to a maximum of 120 mg. The principal parameter was the occurrence of adverse events within 24 h after EGD. Secondary parameters included successful procedures, complications, and full recovery within 60 min. In Study 2, the residual effects of propofol were evaluated using a driving simulator and blood propofol concentrations in a subset of cirrhotic patients undergoing EGD and compared with healthy individuals. The principal parameter was driving ability.
Results Study 1: Consecutive cirrhotic patients were entered and all 163 successfully completed EGD. The mean dose of propofol was 46 mg (range 30–120 mg). No complications occurred. Full recovery had occurred in 100 % 60 min after the procedure. No adverse events occurred within 24 h after EGD. Study 2: There were no significant differences in blood propofol levels between cirrhotic patients ($n = 21$) and healthy individuals ($n = 20$) after sedation. In cirrhotic patients, there was no deterioration in driving ability as compared with healthy individuals.
Conclusion Low-dose propofol sedation provided safe and effective sedation for EGD in cirrhotic patients with rapid recovery.

Keywords Esophagogastroduodenoscopy · EGD · Liver cirrhosis · Propofol · Sedation

Abbreviation
EGD Esophagogastroduodenoscopy

Introduction

Patients with liver cirrhosis are frequently referred for esophagogastroduodenoscopy (EGD) for diagnostic assessment of esophageal varices and portal hypertensive gastropathy. Sedation with benzodiazepines, such as midazolam and diazepam, is generally used to improve procedure acceptability. However, benzodiazepines may result in increased risks of sedation-related complications, such as respiratory

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depression, delayed recovery, and overt hepatic encephalopathy in patients with liver cirrhosis [1–4]. For example, sedation with midazolam has been shown to exacerbate subclinical hepatic encephalopathy in patients with Child A or B cirrhosis [1].

Propofol is a hypnotic agent widely used as the induction and maintenance of general anesthesia and sedation and is believed to promote γ-aminobutyric acid activity in the brain. Propofol has a favorable pharmacokinetic profile compared to benzodiazepine with regard to rapid induction and recovery and equivalent levels of amnesia [5–9]. Propofol is quickly metabolized and no adjustments in dosage are needed in patients with liver dysfunction, theoretically making it a good candidate for conscious sedation in cirrhotic patients. However, the safety and effectiveness of sedation with propofol has not been investigated within 24 h after EGD in cirrhotic patients.

We previously reported the safety and effectiveness of age-adjusted low-dose propofol injection as endoscopic sedation in healthy adults [10–13] and in patients aged 90 years and older [14]. Here, we assessed the safety and effectiveness of low-dose propofol sedation during and after EGD including the recovery in driving ability in cirrhotic patients.

Patients and Methods

Two prospective studies were approved by the ethical committee of Showa Inan General Hospital and adhere to the principles of the Declaration of Helsinki. Verbal and written informed consent was obtained from all subjects.

Participants

The study was done at the Showa Inan General Hospital and included outpatients having liver cirrhosis who underwent diagnostic EGD. Emergency procedures were excluded. The diagnosis of cirrhosis was made by histological findings or imaging findings (e.g., liver surface irregularity, swelling of the left lobe and caudal lobe, the presence of splenomegaly, and the absence of portal vein stenosis/obstruction) [15, 16]. Fifteen of the patients enrolled in Study 1 were diagnosed as liver cirrhosis using histologic criteria. The exclusion criteria at entry were Child C cirrhosis and to avoid unexpected complications due to poor general condition, the presence of past history of overt hepatic encephalopathy, poor performance status, American Society of Anesthesiologists class III and IV, pregnancy, and the presence of severe underlying diseases including cardiac, pulmonary, renal, or hematological disease. No patients enrolled in this study took non-selective beta-blocking agents.

In Study 2, a subset of cirrhotic patients undergoing EGD was selected. The healthy individuals who underwent EGD for annual health check-up at Showa Inan General Hospital were enrolled as controls. The inclusion criteria of Study 2 included age over 20 years and a valid driver's license holder, and subjects who were scheduled to drive themselves home after EGD.

The clinical course and a detailed past history were recorded. All cirrhotic patients were subjected to physical, radiological, and blood examinations at least every 3 months. Routine blood tests, such as complete blood counts and chemistries, were obtained in a fasting state and determined using standard methods [17].

Propofol Sedation Procedure and EGD

Propofol was administered for endoscopic sedation by nurses supervised by endoscopists. Both the nurses and endoscopists had advanced cardiac life support (ACLS) certification. Training of nurses and endoscopists involved advanced airway training, didactic training on propofol, observation of cases, and supervised administration of propofol by an anesthesiologist before beginning propofol administration supervised by the endoscopist. The training period typically lasts about 2 weeks.

The endoscopic team consisted of 3 medical staff: an endoscopist, a nurse administering drugs and responsible for the subject, and a second nurse assisting the endoscopist. EGD was performed in the left lateral decubitus position. Subjects received topical pharyngeal anesthesia with lidocaine spray (AstraZeneca, Osaka, Japan). No pretreatment agents for controlling bowel movement were used. Propofol (Duprivan®; AstraZeneca) was administered by bolus injection according to the protocol previously reported (i.e., 40 mg for subjects <70 years old and 30 mg for ≥70 years old). Adequate sedation was generally achieved when the subject passed through the following sequence: eyes closing, one or two yawns, and cessation of body movements. The degree of sedation was assessed by a nurse using the American Society of Anesthesiologists criteria of sedation (minimal = responds readily to name; moderate = responds only when called loudly and/or repeatedly, or only after mild prodding and/or shaking; and deep = does not respond to mild prodding and/or shaking). The target level of sedation was moderate sedation. If subjects were not adequately sedated, an additional injection of 20 mg of propofol was given with a total maximum dose of 120 mg.

Monitoring during EGD included continuous assessment of peripheral oxygen saturation (SpO_2), heart rate, and blood pressure. Clinical assessment of the subject, including measurement of respiratory effort by visual assessment and by palpation of the chest wall and abdominal excursion

and/or palpation of exhaled breath, was performed routinely. A fall in SpO_2 to <90 % was regarded as respiratory depression associated with the sedation and a standard chin lift maneuver was performed promptly by the nurse. When SpO_2 was <90 % for more than 20 s, oxygen supplementation was commenced. Monitoring and complications were recorded by the registered nurse.

After the EGD, the subjects were moved to the waiting room. Full recovery, including consciousness and psychomotor function was assessed using the following 3 criteria: (1) level of consciousness (fully awake and responding to questions from the recovery room nurse), (2) ability to stand on one foot, and (3) ability to walk in a straight line for 5 m without instability. The nurses reconfirmed the absence of reemerging sedative effects and finally permitted the subjects to leave the endoscopic unit.

Study Design

Study 1

The principal outcome parameter was the occurrence of adverse events within 24 h after EGD. Adverse events were defined as any unpleasant incidents which occurred within 24 h after EGD [12]. We used a questionnaire to identify unpleasant incidents occurring within 1 month after the procedure at a visit based on patients' memory. In addition, the presence or absence of problems was confirmed by a family member. Secondary parameters included successful procedures, complications such as respiratory depression, aspiration pneumonia, overt encephalopathy and variceal bleeding, and full recovery within 60 min of the procedure. Based on our previous studies [10–14], full recovery was evaluated at 60 min after the procedure.

Study 2

A subset of cirrhotic patients undergoing EGD was compared with healthy individuals where the residual effects of propofol was tested using a driving simulator and blood propofol concentrations. The principal parameter was driving ability. Driving ability was assessed before sedation and 30, 60, and 90 min after EGD using a driving simulator. Also, blood was taken at 30, 60, and 90 min after EGD to measure blood propofol levels.

Driving Simulation

Driving ability is a sensitive indicator of residual drug effects [10, 13, 18]. All subjects performed a 10-min divided attention driving simulation test (DADST) after a 5-min practice session using a commercially available simulator (DS-20; Mitsubishi Precision, Tokyo, Japan) that was located within the endoscopy unit. The subjects sat in front of a monitor and used a steering wheel, accelerator, and brakes to control the vehicle. The road scene display changed in accordance with the subject's actions. The object of the test was to steer an image of a car bonnet down the center of a winding road as accurately as possible (measuring the ability to track) using a steering wheel. During the test, crossing pedestrians appeared randomly on the screen and often attempted to cross the road of the computer screen. To test vigilance and reaction time, the subjects were required to properly identify and respond to the behavior of pedestrians. The results of the DADST were expressed as accelerating reaction time (average time respond to pedestrians) and braking reaction time (average time respond to pedestrians). In addition, changes in accelerating/braking reaction time (% initial) were calculated as the ratio of reaction time at the indicated time point to that before injection.

Blood Concentrations of Propofol

The measurement of blood concentration of propofol was performed according to previously described methods [10, 13, 19]. For the measurement of propofol, acetnitrile and an internal standard were added to a plasma sample and vortexed for 1 min. After centrifugation at 13,000 rpm for 5 min, 50-mL aliquots of the supernatant were directly injected into the HPLC system involving a C18 reversed-phase column. Propofol and the internal standard (thymol) were quantified using a coulometric electrochemical detection.

Statistical Analysis

Clinical parameters were expressed as a number (%) or mean ± SD. Comparisons were made using the χ^2 test for categorical variables and the Student's t test for continuous variables. All P values were based on a two-sided test and a P value of <0.05 was considered to be statistically significant. Statistical analyses were performed using SPSS software v.11.0 for Windows (SPSS, Chicago, IL, USA).

Results

Study 1: Safety and Effectiveness of Low-Dose Propofol Sedation for EGD During and After EGD in Cirrhotic Patients

Cirrhotic patients who underwent EGD between January 2008 and December 2011 were enrolled. For this study period, 163 patients successfully completed EGD based on a standard protocol of age-adjusted doses of propofol

(Table 1). The patients' mean age was 67 ± 13 years (range 42–82 years). The mean venous ammonia levels in the enrolled subjects were 62 ± 20 mg/dL. The mean dose of propofol used was 46.9 ± 16 mg. One hundred and twenty-seven subjects (78 %) completed the diagnostic EGD following a single bolus injection of propofol. The single bolus injection of propofol (30–40 mg) induced deep sedation in 16 of the 36 subjects (22 %) who had deep sedation. The majority of subjects who had deep sedation were women older than 70 years. Eleven subjects (7 %) showed a paradoxical response and a total dose of 80–120 mg of propofol was required to obtain adequate sedation. All 11 were the relatively younger male subjects (40–60 years). Mean procedure time was 5.6 min (range 3–8 min). A biopsy was taken in 15 subjects (9.2 %). There was no difference in the results between alcoholic cirrhotic patients and other subjects. Oxygen desaturation requiring supplemental oxygen did not occur in any subjects and mask ventilation or endotracheal intubation was not required in any case. Full recovery within 60 min after the procedure was present in 100 % (Table 1). Other complications such as aspiration pneumonia, overt encephalopathy, and variceal bleeding did not occur after EGD. No adverse events within 24 h after EGD occurred.

Table 1 Clinical characteristics and outcomes of liver cirrhotic patients enrolled in Study 1

Parameters	Patients (n = 163)
Gender: male	93 (57)
Age (years) (range)	67 ± 13 (42–82)
Body weight (kg)	57 ± 12
Etiology of liver cirrhosis	
HBV	34 (21)
HCV	46 (28)
Alcohol	78 (48)
Unknown	5 (3)
Child–Pugh classification	
A	64 (39)
B	99 (61)
C	0(0)
Mean propofol dose (mg)	46 ± 16
Propofol dose (mg)	
30–40	127 (78)
50–60	25 (15)
80–120	11(7)
Successful procedure	163 (100)
Procedure time (min)	5.6 ± 1.8
Degree of sedation	
Minimal	0(0)
Moderate	127(78)
Deep	36 (22)
Full recovery 60 min after the procedure	163 (100)
Complications during EGD	
Oxygen administered	0
Mask ventilation required	0
Heart rate <50 beats/min	0
Complications after EGD	
Aspiration pneumonia	0
Overt encephalopathy	0
Variceal bleeding	0
Adverse events within 24 h after EGD	0

Data are expressed as number (%) or mean ± SD

EGD esophagogastroduodenoscopym, *HBV* hepatitis B virus, *HCV* hepatitis C virus

Study 2: Comparison of Residual Effects of Low-Dose Propofol Sedation Between Cirrhotic Subjects and Healthy Individuals

Cirrhotic subjects and healthy individuals who underwent EGD between January 2008 and December 2010 were enrolled. Twenty-one cirrhotic subjects (14 men, mean age 65 years) were compared with 20 healthy individuals (11 men, mean age 55 years). Their clinical features were shown in Table 2. Cirrhotic subjects were significantly older than healthy individuals ($P < 0.001$). There were no significant differences in the degree of SpO_2 depression during EGD and the sedation level at the end of EGD between cirrhotic subjects and healthy individuals. Nobody required oxygen supplementation during EGD. Other complications such as aspiration pneumonia, overt encephalopathy, and bleeding did not occur after EGD (Table 3).

There were no significant differences in blood propofol levels between cirrhotic subjects and healthy individuals (Table 4). Braking reaction time and accelerating reaction time both recovered to the basal levels within 60 min of administration in all subjects receiving propofol. A significant prolongation of these reaction times was not found in cirrhotic subjects as compared with healthy individuals (Table 4). Although there were statistical differences in braking reaction time before and 60 min after injection between the two groups, these were because the basal values of braking reaction time in healthy individuals were faster than those of cirrhotic subjects.

Discussion

No adverse incidents occurred within 24 h after EGD under low-dose propofol sedation in Child group A or B cirrhotic patients. Delayed recovery and appearance of overt hepatic encephalopathy were also not observed. Furthermore, blood propofol concentrations and driving abilities in a

Table 2 Clinical characteristics of both healthy individuals and cirrhotic patients enrolled in Study 2

	Healthy individuals ($n = 20$)	Cirrhotic patients ($n = 21$)	P
Age (years)	55 ± 8	65 ± 9	<0.001
Male	11 (55 %)	14 (67 %)	0.444
Etiology of liver cirrhosis			
HBV	6 (29 %)		
HCV	11 (52 %)		
Alcohol	3 (14 %)		
NASH	1 (5 %)		
BMI (kg/m^2)	22.7 ± 2.5	23.8 ± 4.5	0.332
Platelet (×10^4/μL)	20.9 ± 3.8	9.0 ± 3.9	<0.001
Albumin (g/dL)	4.4 ± 0.2	3.6 ± 0.4	<0.001
Bilirubin (mg/dL)	0.8 ± 0.2	1.6 ± 1.1	0.004
AST (U/L)	24 ± 8	46 ± 20	<0.001
ALT (U/L)	25 ± 14	31 ± 14	0.132
Cholinesterase (U/L) not measured	185 ± 62		
Total cholesterol (mg/dL)	208 ± 34	123 ± 28	<0.001
Ammonia (μg/dL)	Not measured	55 ± 23	
Prothrombin time (%)	Not measured	66 ± 16	
Child–Pugh classification			
A		16 (76 %)	
B		5 (24 %)	
C		0 (0 %)	

Data are expressed as number (%) or mean ± SD

HBV hepatitis B virus, *HCV* hepatitis C virus, *NASH* nonalcoholic steatohepatitis, *BMI* body mass index, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase

Table 3 Comparison of EGD between healthy individuals and cirrhotic patients in Study 2

	Healthy individuals ($n = 20$)	Cirrhosis patients ($n = 21$)	P
Propofol administered (mg)	40 ± 0	39 ± 12	0.707
During EGD			
Initial SpO_2 (%)	95.8 ± 2.3	95.4 ± 1.7	0.518
Lowest SpO_2 (%)	95.3 ± 2.1	94.9 ± 1.8	0.516
SpO_2 change (%)	−0.5 ± 0.9	−0.5 ± 1.6	0.950
Oxygen administered	0 (0 %)	0 (0 %)	
HR < 50 beats/min	0 (0 %)	0 (0 %)	
SBP < 70 mmHg	0 (0 %)	0 (0 %)	
At the end of EGD			
Degree of sedation			
Minimal	0 (0 %)	0 (0 %)	
Moderate	20 (100 %)	21 (100 %)	
Deep	0 (0 %)	0 (0 %)	
Complications after EGD			
Overt encephalopathy	0 (0 %)	0 (0 %)	
Bleeding	0 (0 %)	0 (0 %)	
Aspiration pneumonia	0 (0 %)	0 (0 %)	

Data are expressed as number (%) or mean ± SD

EGD esophagogastroduodenoscopy, SpO_2 peripheral oxygen saturation, *HR* heart rate, *SBP* systolic blood pressure

subset of cirrhosis patients were similar to those in healthy individuals after propofol sedation, and subjects were able to safely drive home after diagnostic EGD using propofol sedation.

It has been stated that: "Patients should be advised prior to the administration of sedatives that a prolonged period of impaired cognition may occur. They should be instructed to make plans not to drive, operate heavy or potentially harmful machinery, or make legally binding decisions for 24 h. When sedatives are administered, a competent companion for discharge must accompany patients from the recovery area." Byrne [20], and this is thought to be a common practice in many endoscopy units. The goal of Study 1 was to examine the risk of adverse events during and within 24 h after EGD under propofol sedation among patients with Child A or B cirrhosis.

MacGilchrist et al. [4] reported that half-life of midazolam was twofold–threefold longer and clearance rate was lower in cirrhotic patients compared with controls (3.9 ± 0.8 vs. 1.6 ± 0.3 h for elimination half life and 5.4 ± 1.0 vs. 10.4 ± 1.3 mL/min/kg for clearance, respectively) after 0.075 mg/kg of bolus injection. The delayed clearance of midazolam in cirrhotic patients is thought to be related to reduced conjugation ability of the liver and/or decreased portal blood flow. Additionally, many of the patients having cirrhosis were older and increased age is also a risk factor for delayed clearance of midazolam [6]. In this study, blood concentrations of propofol in cirrhotic patients were similar to those in healthy adults at 30, 60, and 90 min after propofol injection, which is in agreement with the previous data showing that continuous infusion of propofol to cirrhotic patients showed similar pharmacokinetics to healthy controls [21]. These data are consistent with the notion that low-dose bolus propofol injection for cirrhotic patients may be superior to midazolam in terms of clearance and depth of sedation.

Hepatic encephalopathy is a serious complication of cirrhosis. Although the mechanisms to develop hepatic encephalopathy have not been fully clarified, hepatic encephalopathy is considered to be associated with overfunction of endogenous benzodiazepine receptors [3, 22]. As such delayed clearance of midazolam in cirrhotic patients can possibly trigger overt hepatic encephalopathy in previously compensated cirrhotic patients. As noted

Table 4 Comparison of blood propofol concentrations and driving performance between healthy individuals and cirrhotic patients in Study 2

	Healthy individuals ($n = 20$)	Cirrhotic patients ($n = 21$)	P
Blood propofol concentrations (ng/mL)			
30 min after injection	122.5 ± 41.7	122.9 ± 79.3	0.839
60 min after injection	64.6 ± 21.3	73.3 ± 72.1	0.496
90 min after injection	36.5 ± 11.7	45.1 ± 18.7	0.088
Accelerating RT (s)			
Before injection	0.56 ± 0.21	0.70 ± 0.39	0.167
30 min after injection	0.66 ± 0.17	0.69 ± 0.23	0.644
60 min after injection	0.58 ± 0.12	0.67 ± 0.33	0.170
90 min after injection	0.57 ± 0.12	0.63 ± 0.27	0.374
Changes in accelerating RT (% initial)			
30 min after injection	132.7 ± 61.1	108.7 ± 40.1	0.164
60 min after injection	112.2 ± 31.6	97.9 ± 26.0	0.133
90 min after injection	115.9 ± 50.7	92.9 ± 36.8	0.117
Braking RT (s)			
Before injection	0.58 ± 0.14	0.80 ± 0.24	0.002
30 min after injection	0.63 ± 0.10	0.72 ± 0.16	0.053
60 min after injection	0.45 ± 0.09	0.67 ± 0.21	<0.001
90 min after injection	0.55 ± 0.18	0.62 ± 0.18	0.179
Changes in braking RT (% initial)			
30 min after injection	115.1 ± 28.9	95.2 ± 26.8	0.032
60 min after injection	83.3 ± 24.9	85.2 ± 18.7	0.790
90 min after injection	97.6 ± 29.8	81.7 ± 21.3	0.064

Changes in reaction time (RT) were calculated as the ratio of RT at the indicated time point to that before injection. Data are expressed as mean ± SD

above, propofol is theoretically provides sedation with a low risk of inducing hepatic encephalopathy and it has been previously been shown that propofol did not worsen number connection test results among cirrhotic patients with minimal hepatic encephalopathy [23]. In addition, propofol sedation did not induce deterioration in the results of number connection test or critical flicker frequency in patients with Child C cirrhosis [24]. Finally, deep propofol sedation by continuous infusion was shown not to aggravate critical flicker frequency results in patients previously diagnosed as having minimal hepatic encephalopathy [25]. In this study, driving ability recovered to the basal levels within 60 min in cirrhotic patients as well as in the healthy individuals, consistent with the notion that recovery of cognitive and motor function in cirrhotic patients is similar to that of healthy individuals after low-dose propofol sedation.

Rapid recovery and mild sedation of propofol may be advantageous for diagnostic EGD in cirrhotic patients. However, 36 patients (22 %) experienced deep levels of sedation but without incident. The majority with deep sedation were women older than 70 years, and additional studies will be needed to confirm that this is higher risk group and which sedation would be best. In addition, it remains unclear whether propofol is more suitable than midazolam for therapeutic endoscopic procedures, such as endoscopic variceal ligation, endoscopic variceal injection sclerotherapy, and endoscopic mucosal resection. The ideal protocol for propofol administration (e.g., continuous infusion or periodic bolus infusion) also needs to be clarified in such circumstances.

Although there is an antagonist, flumazenil, for midazolam, there is no reversal agent for propofol. Because propofol can produce respiratory depression, nurses and endoscopists receive training before beginning to use propofol, including advanced airway training, didactic training on propofol, and supervised administration of propofol by an anesthesiologist. Although sedation may be influenced by the intake of alcohol, no marked differences in propofol sedation were found between cirrhosis induced by viral infection compared to cirrhosis related to alcohol ingestion.

Our study has some limitations. In addition to the small sample size, there was neither a control arm nor a benzodiazepine/narcotic arm. Theoretically, the study would also have been stronger if there had been control groups that were unsedated and/or sedated with the usual benzodiazepine/narcotic combination. However, the majority of subjects prefer sedation, and the adverse effects of benzodiazepine/narcotic combination has been well demonstrated previously in comparison to propofol sedation. In addition, Child C patients were excluded in this preliminary study. The excellent results suggest that subsequent studies should include Child C patients and those with previous episodes of encephalopathy.

In conclusion, low-dose propofol sedation was safe and practical during and after EGD, and the recovery including driving ability was within 60 min in cirrhotic patients.

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Conflict of interest None.

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KIR, *HLA*, and *IL28B* Variant Predict Response to Antiviral Therapy in Genotype 1 Chronic Hepatitis C Patients in Japan

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Abstract

Natural killer cell responses play a crucial role in virus clearance by the innate immune system. Although the killer immunoglobulin-like receptor (KIR) in combination with its cognate human leukocyte antigen (HLA) ligand, especially *KIR2DL3-HLA-C1*, is associated with both treatment-induced and spontaneous clearance of hepatitis C virus (HCV) infection in Caucasians, these innate immunity genes have not been fully clarified in Japanese patients. We therefore investigated 16 KIR genotypes along with *HLA-B* and *-C* ligands and a genetic variant of interleukin (IL) 28B (rs8099917) in 115 chronic hepatitis C genotype 1 patients who underwent pegylated-interferon-α2b (PEG-IFN) and ribavirin therapy. *HLA-Bw4* was significantly associated with a sustained virological response (SVR) to treatment (P = 0.017; odds ratio [OR] = 2.50,), as was the centromeric A/A haplotype of *KIR* (P = 0.015; OR 3.37). In contrast, SVR rates were significantly decreased in patients with *KIR2DL2* or *KIR2DS2* (P = 0.015; OR = 0.30, and P = 0.025; OR = 0.32, respectively). Multivariate logistic regression analysis subsequently identified the *IL28B* TT genotype (P = 0.00009; OR = 6.87, 95% confidence interval [CI] = 2.62 - 18.01), *KIR2DL2/HLA-C1* (P = 0.014; OR = 0.24, 95% CI = 0.08 - 0.75), *KIR3DL1/HLA-Bw4* (P = 0.008, OR = 3.32, 95% CI = 1.37 - 8.05), and white blood cell count at baseline (P = 0.009; OR = 3.32, 95% CI = 1.35 - 8.16) as independent predictive factors of an SVR. We observed a significant association between the combination of *IL28B* TT genotype and *KIR3DL1-HLA-Bw4* in responders (P = 0.0019), whereas *IL28B* TT along with *KIR2DL2-HLA-C1* was related to a non-response (P = 0.0067). In conclusion, combinations of *KIR3DL1/HLA-Bw4*, *KIR2DL2/HLA-C1*, and a genetic variant of the *IL28B* gene are predictive of the response to PEG-IFN and ribavirin therapy in Japanese patients infected with genotype 1b HCV.

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Introduction

Hepatitis C virus (HCV) infection is a major cause of chronic liver disease worldwide. Chronic HCV infection often develops into chronic hepatitis, which may progress to liver cirrhosis and/or hepatocellular carcinoma (HCC)[1]. HCC is a leading cause of death from malignant neoplasms in Japan[2]. Since approximately 70% of Japanese HCC patients are infected with HCV, the successful eradication of this virus, defined as a sustained virological response (SVR), is considered important to decrease the incidence of HCC.

Natural killer (NK) cells are key components of the innate antiviral immune response that are controlled by a balance of activation and inhibitory receptors. NK cell activation receptors include C-type lectin-like receptors (NKG2C, NKG2D, and NKG2E), natural cytotoxicity receptors (NKp30, NKp44, and NKp46), and CD16, while known inhibitory receptors include killer cell immunoglobulin-like receptors (KIRs) and the CD94/NKG2 family, which also contains a C-type lectin-like receptor (NKG2A) [3,4]. Sixteen *KIR* genes and pseudogenes have been identified that are encoded by a family of genes located on human chromosome 19q13.4. One particular feature of *KIRs* is their substantial genetic diversity. Some inhibitory *KIRs*