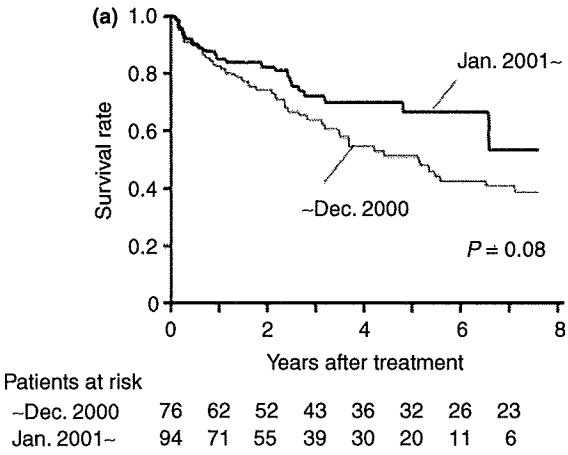

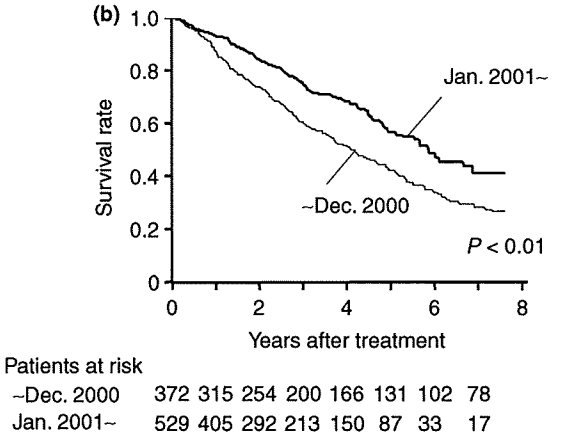

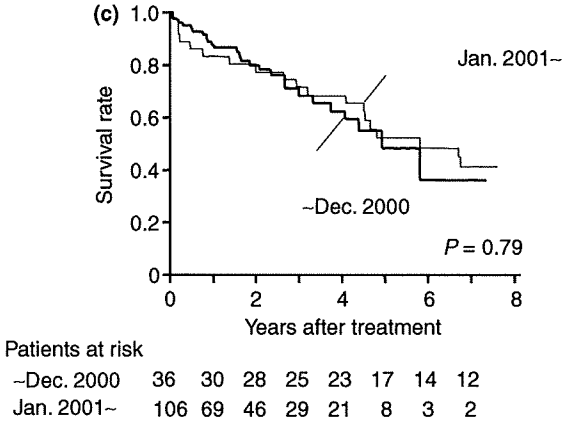

Figure 2. Survival curves of B-HCC (a), C-HCC (b) and NBNC-HCC (c). Note that the survival of C-HCC improved ($P < 0.01$) and a tendency towards improvement was observed in B-HCC ($P = 0.08$); however, no difference was observed for NBNC-HCC ($P = 0.79$). Thin line, HCC patients treated before December 2000: Thick line, HCC patients treated after January 2001.

($n = 391$). Among the patients who received local ablation therapy, PEIT was popular (168/190, 88.4%) before 2000, but RFA was chosen as the standard therapy after 2001 (364/391, 93.1%).

Changes in survival

Overall, survival of the HCC patients was prolonged after 2001. The 3- and 5-year survival rates were 63.0% and 44.2% before 2000 and 74.7% and 57.7% after 2001 respectively ($P < 0.01$). The survival of C-HCC improved ($P < 0.01$) and a tendency towards improvement was observed in B-HCC ($P = 0.08$). However, no difference was observed for NBNC-HCC ($P = 0.79$, Figure 2).

Changes of risk factors for survival

Among the 15 parameters, high T. Bil (>2 mg/dL), low albumin (<3.5 g/dL), high AST (>40 IU/mL), low platelet count ($<10 \times 10^4$), low PT (<80%), the presence of ascites, large tumour size (>3 cm), multiple tumour number and high AFP (>200 ng/mL) were the risk factors for survival before 2000 according to univariate analysis (Table 3). These risk factors were the same as the factors for survival after 2001, except that low platelet count was not selected. In multivariate analysis, low albumin, high AST, the presence of ascites, large tumour size, multiple tumour number and high AFP were the risk factors for survival before 2000, whereas positive HBsAg in addition to low albumin, the presence of ascites, large tumour size, multiple tumour number and high AFP were selected as risk factors for survival after 2001 (Table 4).

DISCUSSION

Many studies have been conducted to elucidate the factors that define the prognosis of HCC.[15–17] The factors can be classified generally into two categories. One is background liver factors such as bilirubin, and albumin, and the other is tumour factors such as the size and number of tumours. The results of this study are comparable with those of previous reports in terms of containing factors belonging to both categories; however, several new insights have emerged by examining the changes in prognostic factors with time.

When we analysed HCC altogether or limited to viral hepatitis-related HCC (B-HCC and C-HCC), we found that they were detected earlier and that the prognosis

Table 4. Multivariate analysis for the prognostic factors of HCC

	~Dec 2000 (n = 504)			Jan 2001~ (n = 746)		
	RR	95% CI	P-value	RR	95% CI	P-value
Age (>65 years old)	1.06	0.80–1.39	0.66	1.22	0.85–1.78	0.27
Gender (male)	1.05	0.78–1.44	0.71	1.36	0.94–2.02	0.10
HCVAb (positive)	1.34	0.82–2.24	0.23	0.74	0.48–1.16	0.18
HBsAg (positive)	1.15	0.68–1.90	0.58	0.39	0.21–0.71	<0.01
Total bilirubin (>2 mg/dL)	1.19	0.68–1.98	0.52	1.46	0.79–2.57	0.21
Albumin (<3.5 g/dL)	1.41	1.03–1.93	0.02	1.94	1.30–2.89	<0.01
AST (>40 IU/L)	1.86	1.13–3.12	0.01	1.59	0.96–2.65	0.06
ALT (>40 IU/L)	0.75	0.53–1.09	0.13	0.73	0.49–1.10	0.13
Platelet (<10 × 10^4/mm^3)	1.10	0.81–1.50	0.51	1.10	0.74–1.62	0.62
Prothrombin time (<80%)	1.29	0.96–1.74	0.08	1.13	0.75–1.68	0.54
Ascites (present)	1.50	1.04–2.13	0.02	1.93	1.28–2.86	<0.01
Alcohol (>90 g/day)	0.89	0.58–1.33	0.58	0.69	0.39–1.15	0.16
Tumour size (>3 cm)	2.27	1.69–3.04	<0.01	3.92	2.79–5.53	<0.01
Tumour (multiple)	2.09	1.58–2.79	<0.01	1.66	1.20–2.32	<0.01
AFP (>200 ng/mL)	1.89	1.33–2.50	<0.01	2.05	1.38–3.01	<0.01

Abbreviations are the same as listed in Table 3.

improved after 2001; however, neither early detection nor the improvement of prognosis was achieved in patients with NBNC-HCC. Hepatitis B or C infections are well-known risk factors for the occurrence of HCC; therefore, these patients were regularly surveyed for HCC.[18] Moreover, nationwide surveillance of hepatitis virus infection was started in 2002 in Japan, and many high-risk patients were identified. It is well known that screening for HCC has a survival benefit.[19, 20] Therefore, HCC was detected at an early stage after 2001 and thus the survival of such patients was prolonged. Nevertheless, surveillance has not been established for patients with NBNC-HCC because the risk factors are not well understood, except for excessive alcoholic drinking and nonalcoholic steatohepatitis.[18] As a result, the prognosis of patients with NBNC-HCC remains poor. The recent increase in metabolic syndrome may increase the likelihood of patients developing nonalcoholic steatohepatitis; therefore, careful follow-up of these patients is necessary to improve patient survival of NBNC-HCC.

In this study, hepatitis B virus infection was a good prognostic factor after 2001, according to multivariate analysis. For patients with HCC, prognosis (including risk of death, metastasis and recurrence after surgery) is reported to be worse in patients with higher serum HBV DNA levels.[21] Lamivudine treatment was started in September 2000 in Japan. In fact, 64.8% of patients with B-HCC were treated with nucleotide analogues after 2001, whereas only 1 patient (1.1%) was treated with Lamivudine before 2000. Nucleotide analogues are known to improve inflammation of the liver caused by hepatitis B virus infection and to prolong survival of patients with B-HCC.[12, 13] The use of nucleotide analogues in addition to the prevalence of surveillance of patients with hepatitis B infection may result in the selection of hepatitis B virus infection as a good prognostic factor after 2001.

Interferon (IFN) has been shown by randomized controlled trials to decrease the late recurrence after curative therapies and has also been proven to improve the survival of patients with C-HCC.[10, 22] However, hepatitis C virus infection was not a good prognostic factor before 2000 or after 2001. In contrast to the nucleotide analogues used for the therapy of hepatitis B virus, IFN has been used for the treatment of hepatitis C virus from the early 90s. The sustained virus response (SVR) rate was quite low for IFN monotherapy, especially for cases in genotype 1b with a high virus titre (2~10%), which is the dominant status of the patients in Japan.[23, 24] Even after combination therapy with peg-interferon and ribavirin for 48 weeks, the SVR rate was about 50%,[25, 26] which is lower than the response rate of lamivudine (90%). Although IFN therapy for HCV infection is similar to the nucleotide analogues used for HBV infection in

terms of being a therapy against the causative virus of HCC, the response rate of IFN therapy may be too low for HCVAb to be a good prognostic factor. In addition, the percentage of candidates for IFN treatment was much lower than that for nucleotide analogues. Many patients with C-HCC are of advanced age and cannot tolerate IFN therapy. In this study population, only 15.5% and 19.8% of C-HCC were treated with IFN before 2000 and after 2001 respectively. With the development of new drugs such as protease inhibitors, the response rate might be improved and the presence of HCVAb might be a good prognostic factor in the next decade.

Although we did not analyse the rate of recurrence or the content of repeat therapies in this study, we nevertheless clearly indicated the changes in prognostic factors of HCC with time. The prognosis of the patients with HCC improved with time. Early detection of B-HCC and C-HCC has been achieved and the presence of HBsAg was found to be a good prognostic factor after 2001. On the contrary, the number of patients with NBNC-HCC has increased with time, and the prognosis of these patients has not changed. Further examination of the risk factors of NBNC-HCC and subsequent establishment of an effective surveillance system for these patients will be necessary to improve the future prognosis of HCC patients.

ACKNOWLEDGEMENT

Declaration of personal and funding interests: None.

REFERENCES

1 Parkin DM. Global cancer statistics in the year 2000. *Lancet Oncol* 2001; 2: 533–43.
2 Bolondi L. Screening for hepatocellular carcinoma in cirrhosis. *J Hepatol* 2003; 39: 1076–84.
3 Adams PC, Arthur MJ, Boyer TD, *et al.* Screening in liver disease: report of an AASLD clinical workshop. *Hepatology* 2004; 39: 1204–12.
4 Trevisani F, Cantarini MC, Labate AM, *et al.* Surveillance for hepatocellular carcinoma in elderly Italian patients with cirrhosis: effects on cancer staging and patient survival. *Am J Gastroenterol* 2004; 99: 1470–6.
5 Ikai I, Arii S, Kojiro M, *et al.* Reevaluation of prognostic factors for survival after liver resection in patients with hepatocellular carcinoma in a Japanese nationwide survey. *Cancer* 2004; 101: 796–802.
6 Bruix J, Sherman M, Llovet JM, *et al.* Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. European Association for the Study of the Liver. *J Hepatol* 2001; 35: 421–30.
7 Llovet JM. Updated treatment approach to hepatocellular carcinoma. *J Gastroenterol* 2005; 40: 225–35.
8 Makuuchi M, Kokudo N. Clinical practice guidelines for hepatocellular carcinoma: the first evidence based guidelines from Japan. *World J Gastroenterol* 2006; 12: 828–9.
9 Yoshida H, Shiratori Y, Moriyama M, *et al.* Interferon therapy reduces the risk for hepatocellular carcinoma: national surveillance program of cirrhotic and noncirrhotic patients with chronic hepatitis C in Japan. IHIT Study Group. Inhibition of Hepatocarcinogenesis by Interferon Therapy. *Ann Intern Med* 1999; 131: 174–81.
10 Kudo M. Impact of interferon therapy after curative treatment of hepatocellular carcinoma. *Oncology* 2008; 75(Suppl 1): 30–41.
11 Liaw YF. Prevention and surveillance of hepatitis B virus-related hepatocellular carcinoma. *Semin Liver Dis* 2005; 25(Suppl 1): 40–7.
12 Matsumoto A, Tanaka E, Rokuhara A, *et al.* Efficacy of lamivudine for preventing hepatocellular carcinoma in chronic hepatitis B: A multicenter retrospective study of 2795 patients. *Hepatol Res* 2005; 32: 173–84.
13 Piao CY, Fujioka S, Iwasaki Y, *et al.* Lamivudine treatment in patients with HBV-related hepatocellular carcinoma–using an untreated, matched control cohort. *Acta Med Okayama* 2005; 59: 217–24.
14 Honda H, Ochiai K, Adachi E, *et al.* Hepatocellular carcinoma: correlation of CT, angiographic, and histopathologic findings. *Radiology* 1993; 189: 857–62.
15 Mann CD, Neal CP, Garcea G, Manson MM, Dennison AR, Berry DP. Prognostic molecular markers in hepatocellular carcinoma: a systematic review. *Eur J Cancer* 2007; 43: 979–92.
16 Sala M, Forner A, Varela M, Bruix J. Prognostic prediction in patients with hepatocellular carcinoma. *Semin Liver Dis* 2005; 25: 171–80.
17 Varela M, Sala M, Llovet JM, Bruix J. Review article: natural history and prognostic prediction of patients with hepatocellular carcinoma. *Aliment Pharmacol Ther* 2003; 17(Suppl 2): 98–102.
18 El-Serag HB, Rudolph KL. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. *Gastroenterology* 2007; 132: 2557–76.
19 Zhang BH, Yang BH, Tang ZY. Randomized controlled trial of screening for hepatocellular carcinoma. *J Cancer Res Clin Oncol* 2004; 130: 417–22.
20 Tanaka H, Nouso K, Kobashi H, *et al.* Surveillance of hepatocellular carcinoma in patients with hepatitis C virus infection may improve patient survival. *Liver Int* 2006; 26: 543–51.
21 Chen CJ, Yang HI, Iloeje UH, REVEAL-HBV Study Group. Hepatitis B virus DNA levels and outcomes in chronic hepatitis B. *Hepatology* 2009; 49: S72–84.
22 Shiratori Y, Shiina S, Teratani T, *et al.* Interferon therapy after tumor ablation improves prognosis in patients with hepatocellular carcinoma associated with hepatitis C virus. *Ann Intern Med* 2003; 138: 299–306.
23 Shiratori Y, Kato N, Yokosuka O, *et al.* Predictors of the efficacy of interferon

therapy in chronic hepatitis C virus infection. Tokyo-Chiba Hepatitis Research Group. *Gastroenterology* 1997; 113: 558–66.

24 Poynard T, Marcellin P, Lee SS, *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. International Hepatitis Interventional Therapy Group (IHIT). *Lancet* 1998; 352: 1426–32.

25 Manns MP, McHutchison JG, Gordon SC, *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 Fried MW, Shiffman ML, Reddy KR, *et al.* Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 2002; 347: 975–82.

Digestive and Liver Disease 42 (2010) 51–54

Contents lists available at ScienceDirect

Digestive and Liver Disease

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

Liver, Pancreas and Biliary Tract

Autoimmune hepatitis with acute presentation in Japan

Y. Miyake[a,b,*], Y. Iwasaki[b], H. Kobashi[b], T. Yasunaka[b], F. Ikeda[a,b], A. Takaki[b], K. Yamamoto[a,b]

[a] Department of Molecular Hepatology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan
[b] Department of Gastroenterology & Hepatology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan

ARTICLE INFO

Article history:
Received 21 November 2008
Accepted 15 April 2009
Available online 26 May 2009

Keywords:
Acute exacerbation
Acute hepatitis
Cirrhosis
Zone 3 necrosis

ABSTRACT

Background: In Caucasians with autoimmune hepatitis, patients with acute presentation have autoimmune thyroiditis and histological zone 3 necrosis more frequently.
Aim: We aimed at investigating clinical features of Japanese autoimmune hepatitis patients with acute presentation.
Methods: Of 176 patients retrospectively reviewed, 53 were diagnosed with acute presentation.
Results: Patients with acute presentation had higher serum levels of bilirubin and transaminase, lower frequencies of autoimmune thyroiditis and antinuclear antibodies of 1:160 or greater, and a higher frequency of zone 3 necrosis. Of the 53 patients with acute presentation, 10 showed histological acute hepatitis; however, advanced staging of fibrosis was found in 13 patients. In patients with acute presentation, those with histological acute hepatitis were younger than those with chronic hepatitis. The cumulative incidental rate of the normalization of serum alanine aminotransferase levels with prednisolone treatment was similar between patients with acute presentation and those with classical presentation.
Conclusions: In line with previous results, zone 3 necrosis is a histological characteristic of autoimmune hepatitis with acute presentation. Autoimmune hepatitis with acute presentation includes not only histological acute hepatitis but also acute exacerbation of pre-existing chronic disease. On the other hand, Japanese patients with acute presentation may also have different clinical features from Caucasian patients.

1. Introduction

Autoimmune hepatitis (AIH), defined as a chronic hepatitis of unknown aetiology, is characterized by circulating autoantibodies, hypergammaglobulinemia, and the presence of interface hepatitis and lymphoplasmacytic infiltration on histological examination [1,2]. The prognosis is generally good with immunosuppressive treatment.

Recently, the number of patients with acute presentation has increased. Czaja and co-workers [3] reported that acute and classical presentation of AIH were indistinguishable from each other by clinical and laboratory features. In contrast, Hofer et al. [4] reported that zone 3 necrosis was a histological characteristic of AIH with acute presentation. Kessler et al. [5] reported that patients with acute presentation had severe hepatitis and autoimmune thyroiditis more frequently than those with classical presentation. The clinical features of AIH with acute presentation have not been fully elucidated.

On the other hand, the clinical features of Japanese AIH patients are different from those of Caucasian patients [2]. These are attributed to differences in human leukocyte antigen (HLA) DR status. In Caucasian patients, HLA DR3 and DR4 are independently susceptible to AIH [6], while DR4 is predominant in Japanese patients; there are few Japanese patients with DR3 [7]. Patients with DR3 are younger and have a higher frequency of treatment failure; however, those with DR4 frequently have concurrent extrahepatic autoimmune disease and respond better to corticosteroid treatment [8]. The clinical features of AIH with acute presentation may differ between Japanese and Caucasian patients. In the present study, we assessed the clinical features of Japanese AIH patients with acute presentation.

2. Patients and methods

One-hundred-and-seventy-six patients with type 1 AIH (153 females, 23 males, median age 55 years) were admitted to the Okayama University Hospital or six affiliated hospitals between March 1989 and April 2008. All patients were seronegative for hepatitis B surface antigen, anti-hepatitis C virus antibody, hepatitis C virus RNA, and anti-mitochondrial antibody, and all underwent liver biopsy. A diagnosis of AIH was based on the revised scoring system proposed by the International Autoimmune Hepatitis

* Corresponding author at: Department of Molecular Hepatology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, 2-5-1, Shikata-cho, Okayama 700-8558, Japan. Tel.: +81 86 235 7219; fax: +81 86 225 5991.
E-mail address: miyakeyasuhiro@hotmail.com (Y. Miyake).

1590-8658/$36.00
doi:10.1016/j.dld.2009.04.009

Group [9]. A definite diagnosis of AIH based on this revised scoring system required a pretreatment score exceeding 15, while a probable diagnosis required a score between 10 and 15. Patients with an overlapping syndrome or a coexistent disease (for example, primary biliary cirrhosis, primary sclerosing cholangitis, nonalcoholic fatty liver disease, and alcohol-induced liver injury) were excluded from this analysis. Of the 176 patients, 136 were diagnosed as definite AIH, and the other 40 were diagnosed as probable AIH.

Forty-two patients (26%) had concurrent autoimmune diseases: 18 had autoimmune thyroiditis; fours had Sjögren's syndrome; three each had systemic lupus erythematosus, Graves' disease, and ulcerative colitis; two each had autoimmune hemolytic anaemia, idiopathic thrombocytopenic purpura, progressive systemic sclerosis, and rheumatoid arthritis; one each had both autoimmune thyroiditis and autoimmune hemolytic anaemia, both systemic lupus erythematosus and Sjögren's syndrome, and both autoimmune thyroiditis and Sjögren's syndrome.

Patients were diagnosed with acute presentation if they had acute onset of symptoms (for example, jaundice and/or fatigue and/or anorexia) in conjunction with serum bilirubin levels ≥5 mg/dL and/or serum alanine aminotransferase (ALT) levels ≥10-fold the upper normal limit without any history of prior liver disease. Fifty-three patients (30%) showed acute presentation. The remaining patients, those with liver function abnormalities upon medical checkup or in their history, or patients without the criteria for acute presentation, were diagnosed as having the classical presentation.

The titers of antinuclear antibodies (ANA) were measured using a standard IIF technique with HEp-2 cells. Smooth muscle antibodies (SMA) were assayed by the IIF technique using rat kidney and stomach cells. A serum titer of 1:40 or greater was positive for ANA or SMA. Antibodies to liver/kidney microsome type 1 (anti-LKM-1) were measured using an enzyme-linked immunosorbent assay using recombinant cytochrome P4502D6 as the antigen, and a serum value of 50.0 index or greater was positive.

Liver biopsy was performed with a Vim–Silverman needle (14-G) under laparoscopy, or with a 17-G needle under ultrasonographic guidance, before or just after commencing the treatment. Liver biopsy specimens were evaluated by two pathologists and diagnosed as having either acute or chronic hepatitis. A diagnosis of acute hepatitis was based on the presence of histologically predominant zone 3 necrosis with minimal lymphoplasmacytic cell infiltration into portal tracts, in the absence of interface hepatitis or portal fibrosis. Liver biopsy specimens diagnosed as chronic hepatitis underwent histological staging based on the classification of Desmet et al. [10]. Histologically, acute hepatitis was shown in 10 patients.

Initial treatment was defined as any therapy that was started within 3 months after the diagnosis of AIH. The treatment was continued until the normalization of serum ALT levels.

To compare the clinicopathological characteristics between patients with acute presentation and those with classical presentation, we analyzed patient age, gender, pretreatment score based on the revised scoring system, concurrent autoimmune disease, laboratory data {albumin, bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), immunoglobulin G (IgG), ANA, SMA, HLA DR4}, and histological features (staging of fibrosis, rosetting of liver cells, zone 3 necrosis). Furthermore, in 53 patients with acute presentation, we compared those with histological acute hepatitis to those with histological chronic hepatitis.

2.1. Statistics

Statistical analysis was performed using the SPSS statistical program (release 11.0.1 J, SPSS, Inc., Chicago, IL).

Continuous variables were expressed as medians and ranges. The Mann–Whitney *U*-test was used to evaluate differences in the continuous variables. Dichotomous variables were compared by the χ^2-test. Cumulative incidental rates were estimated using the log-rank test. *P*-values of less than 0.05 were considered significant.

3. Results

3.1. Clinical and laboratory findings

Patients with acute presentation were indistinguishable from those with classical presentation by age and gender. In contrast, patients with acute presentation showed lower pretreatment scores and a lower frequency of definite diagnosis according to the revised scoring system proposed by the International Autoimmune Hepatitis Group [9]. Autoimmune thyroiditis was less frequently in patients with acute presentation (Table 1). On the other hand, of the 53 patients with acute presentation, the 10 with histological acute hepatitis were younger than the remaining 43 with chronic hepatitis (Table 2).

Serum IgG levels were similar between patients with acute presentation and those with classical presentation (Table 1). In patients with acute presentation, those with acute hepatitis and those with chronic hepatitis were indistinguishable by serum levels of bilirubin, transaminase, and IgG (Table 2).

Table 1
Clinical features of AIH patients with acute presentation compared with those with classical presentation.

	Acute presentation	Classical presentation	*P*-value
Patients (*n*)	53	123	
Age (years)	54 (16–76)	56 (18–79)	0.51
Gender, female (%)	46 (87)	107 (87)	0.97
Criteria of the International Autoimmune Hepatitis Group			
Pretreatment score	17 (10–23)	18 (10–21)	0.01
Definite diagnosis (%)	34 (64)	102 (83)	0.006
Concurrent autoimmune disease, *n* (%)	8 (15)	34 (28)	0.07
Autoimmune thyroiditis, *n* (%)	2 (4)	18 (15)	0.04
Laboratory data			
Albumin (g/dL)	3.6 (2.3–4.7)	3.9 (2.1–5.1)	<0.0001
Bilirubin (mg/dL)	5.0 (0.6–29.2)	0.8 (0.3–5.0)	<0.0001
Bilirubin > 3.0 mg/dL, *n* (%)	38 (72)	5 (4)	<0.0001
AST (IU/L)	753 (197–2330)	109 (28–769)	<0.0001
ALT (IU/L)	939 (109–2161)	129 (23–1027)	<0.0001
IgG (mg/dL)	2430 (724–528)	2568 (1085–562)	0.20
IgG < 2000 mg/dL, *n* (%)	16 (30)	23 (19)	0.09
ANA or ASMA ≥ 1:40, *n* (%)	47 (89)	120 (98)	0.01
HLA DR4, *n* (%)	21/32 (66)	39/55 (71)	0.61
Fibrosis staging, *n* (%)			
Acute hepatitis	10 (19)	0 (0)	<0.0001
Chronic hepatitis			
F1	12 (23)	39 (32)	
F2	18 (34)	35 (28)	
F3	9 (17)	35 (28)	
F4	4 (7)	14 (12)	
F3 + F4	13 (24)	49 (40)	0.05
Rosetting of liver cells, *n* (%)	19 (36)	30 (24)	0.12
Zone 3 necrosis, *n* (%)	28 (53)	24 (20)	<0.0001

Abbreviations: AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; ULN, upper limit of normal; IgG, immunoglobulin G; ANA, antinuclear antibody; ASMA, anti-smooth muscle antibody; HLA, human leukocyte antigen.

Table 2
Clinical features of the 53 patients with acute presentation: comparison between the 10 patients with acute hepatitis and the 43 with chronic hepatitis.

	Acute hepatitis	Chronic hepatitis	*P*-value
Patients, *n*	10	43	
Age (years)	37 (16–66)	58 (19–76)	0.006
Gender, female (%)	8 (80)	38 (88)	0.48
Criteria of the International Autoimmune Hepatitis Group			
Pretreatment score	16 (10–20)	17 (10–23)	0.37
Definite diagnosis (%)	5 (50)	29 (67)	0.30
Concurrent autoimmune disease, *n* (%)	2 (20)	6 (14)	0.63
Autoimmune thyroiditis, *n* (%)	0 (0)	2 (5)	0.49
Laboratory data			
Albumin (g/dL)	3.5 (3.0–4.7)	3.6 (2.3–4.4)	0.96
Bilirubin (mg/dL)	7.4 (1.8–29.2)	5.0 (0.6–25.8)	0.24
Bilirubin > 3.0 mg/dL, *n* (%)	9 (90)	29 (67)	0.15
AST (IU/L)	832 (197–2330)	722 (230–1716)	0.33
ALT (IU/L)	1100 (335–2161)	930 (109–2132)	0.29
IgG (mg/dL)	2630 (1370–3602)	2394 (724–4528)	0.73
IgG < 2000 mg/dL, *n* (%)	4 (40)	12 (30)	0.45
ANA or ASMA ≥ 1:40, *n* (%)	8 (80)	39 (91)	0.34
HLA DR4, *n* (%)	6/8 (75)	15/24 (63)	0.52
Rosetting of liver cells, *n* (%)	5 (50)	14 (33)	0.30
Zone 3 necrosis, *n* (%)	10 (100)	18 (42)	0.0009

Abbreviations: AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; ULN, upper limit of normal; IgG, immunoglobulin G; ANA, antinuclear antibody; ASMA, anti-smooth muscle antibody; HLA, human leukocyte antigen.

3.2. Immunoserologic features

The ANA-positive frequency was similar between patients with acute presentation and those with classical presentation (79% vs. 89%; $P = 0.07$). The SMA-positive frequency was also similar between the groups (58% vs. 65%; $P = 0.42$). None had anti-LKM-1. However, patients with acute presentation less frequently had ANA of high titers (1:160 or greater) than those with classical presentation (49% vs. 72%; $P = 0.005$). On the other hand, in patients with acute presentation, the SMA-positive frequency and the frequency of ANA of high titers were similar between patients with acute hepatitis and those with chronic hepatitis (44% vs. 72%; $P = 0.12$ and 30% vs. 53%; $P = 0.18$).

3.3. HLA DR status

Patients with acute presentation and those with classical presentation had similar frequencies of HLA DR4 (Table 1). None had DR3. In patients with acute presentation, the frequency of DR4 was similar between those with acute hepatitis and those with chronic hepatitis.

3.4. Histological features

Histological acute hepatitis was more frequent in patients with acute presentation. None of those with classical presentation showed acute hepatitis. In contrast, advanced staging of fibrosis (F3 + F4) was less frequent in patients with acute presentation (Table 1).

The frequency of liver cell rosetting was similar in both groups. However, patients with acute presentation showed zone 3 necrosis more frequently (Table 1). Furthermore, in patients with acute presentation, those with acute hepatitis showed zone 3 necrosis more frequently than those with chronic hepatitis (Table 2).

3.5. Response to corticosteroid treatment

As an initial medical treatment, 135 of the 176 patients were treated with prednisolone (PSL) (≥20 mg/day). Of the 135 patients treated with PSL ≥20 mg/day, 15 were transferred to other hospitals without follow-up. Of the remaining 120 patients, 44 showed acute presentation. Patients with acute presentation were treated with higher doses of initial PSL than those with classical presentation {40 (20–60 mg/day) vs. 30 (20–45 mg/day); $P < 0.0001$}. In contrast, after 4-week PSL treatment, patients with acute presentation had a lower cumulative incidental rate of serum ALT normalization (41% vs. 62%; $P = 0.01$). The cumulative incidental rates of serum ALT normalization within 6 months after the introduction of PSL treatment were also similar in both groups (91% vs. 88%; $P = 0.33$). On the other hand, in patients with acute presentation, the cumulative incidental rates of serum ALT normalization after 4-week PSL treatment and those within 6 months after the introduction of PSL treatment were similar between patients with acute hepatitis and those with chronic hepatitis (44% vs. 44%; $P = 0.79$ and 100% vs. 89%; $P = 0.19$).

4. Discussion

In this study, Japanese AIH patients with acute presentation showed histological zone 3 necrosis more frequently than those with classical presentation. This is consistent with the previous reports [4,5]. Furthermore, of patients with acute presentation, all 10 with histological acute hepatitis showed zone 3 necrosis, and the remaining patients with histological chronic hepatitis more frequently showed zone 3 necrosis than those with classical presentation (42% vs. 20%; $P = 0.004$). Thus, zone 3 necrosis is considered a histological characteristic of AIH with acute presentation. On the other hand, all 10 patients with histological acute hepatitis showed acute presentation. In contrast, 24% of patients with acute presentation had advanced staging of fibrosis, although this frequency is lower than that in patients with classical presentation. AIH with acute presentation is considered to include not only histological acute hepatitis but also acute exacerbation of the pre-existing chronic disease.

Hypergammaglobulinemia and the positivity of autoantibodies are essential for a diagnosis of AIH. In Caucasian patients, these two factors are similar between patients with acute presentation and those with classical presentation [3,5]. In contrast, in the present study, patients with acute presentation showed a significantly lower frequency of the positivity for ANA or SMA and a slightly higher frequency of serum IgG levels < 2000 mg/dL. Thus, the frequency of a definite diagnosis according to the revised scoring system proposed by the International Autoimmune Hepatitis Group [9] was significantly lower in patients with acute presentation. Japanese patients with acute presentation may have atypical clinical features frequently, and these features may be different from those of Caucasian patients with acute presentation.

In this study, concurrent extrahepatic autoimmune diseases occurred at similar rates between acute and classical presentation. In contrast, autoimmune thyroiditis occurred less frequently in patients with acute presentation. This result differs from the report by Kessler et al. [5]. Recently, a strong association between autoimmune thyroiditis and HLA DR4 was reported [11]. However, in this study, the frequency of HLA DR4 is similar between patients with acute presentation and those with classical presentation. Thus, factors other than HLA DR4 may contribute to the concurrence of autoimmune thyroiditis.

In this study, of the 53 patients with acute presentation, the 10 with histological acute hepatitis were younger than the remaining patients with chronic hepatitis; however, these two groups were indistinguishable in the other clinical and laboratory findings. Furthermore, those with acute hepatitis were younger than those with

classical presentation ($P = 0.006$). Previously, cases with acute presentation whose initial liver biopsy showed acute hepatitis were reported to show typical AIH by a repeat liver biopsy [12,13]. Some AIH patients are not referred at the time of the first flare-up because they are asymptomatic [14]. We speculate that AIH with acute hepatitis may reflect an early stage of the disease. On the other hand, of 176 patients in this study, the frequency of patients aged ≤40 years was higher in the 10 patients with histological acute presentation than in the 166 with chronic hepatitis (50% vs. 14%; $P = 0.003$). It is well recognized that although AIH activity is remitted during pregnancy, a flare-up often occurs after delivery [15]. Increased production of cortisol, progesterone, and oestrogen during pregnancy suppresses Th1 cytokine production (e.g., IL-12, interferon-γ) and enhances Th2 cytokine production (e.g., IL-4, IL-10) [16]. Hormonal factors may be associated with the pathogenesis of AIH with histological acute hepatitis.

In conclusion, the present results agree with those of previous reports, in that zone 3 necrosis is a histological characteristic of AIH with acute presentation. AIH with acute presentation includes not only histological acute hepatitis but also acute exacerbation of pre-existing chronic disease. On the other hand, Japanese patients with acute presentation may have atypical clinical features frequently, and these features may be different from those of Caucasian patients with acute presentation. Further studies are required in order to confirm these findings.

Conflict of interest statement

None declared.

References

[1] Krawitt EL. Autoimmune hepatitis. N Engl J Med 2006;354:54–66.
[2] Miyake Y, Yamamoto K. Current status of autoimmune hepatitis in Japan. Acta Med Okayama 2008;62:217–26.
[3] Nikias GA, Batts KP, Czaja AJ. The nature and prognostic implications of autoimmune hepatitis with an acute presentation. J Hepatol 1994;21:866–71.
[4] Hofer H, Oesterreicher C, Wrba F, Ferenci P, Penner E. Centrilobular necrosis in autoimmune hepatitis: a histological feature associated with acute clinical presentation. J Clin Pathol 2006;59:246–9.
[5] Kessler WR, Cummings OW, Eckert G, Chalasani N, Lumeng L, Kwo PY. Fulminant hepatic failure as the initial presentation of acute autoimmune hepatitis. Clin Gastroenterol Hepatol 2004;2:625–31.
[6] Donaldson PT, Doherty DG, Hayllar KM, McFarlane IG, Johnson PJ, Williams R. Susceptibility to autoimmune chronic active hepatitis: human leukocyte antigens DR4 and A1-B8-DR3 are independent risk factors. Hepatology 1991;13:701–70.
[7] Toda G, Zeniya M, Watanabe F, Imawari M, Kiyosawa K, Nishioka M, et al. Present status of autoimmune hepatitis in Japan—correlating the characteristics with international criteria in an area with a high rate of HCV infection. Japanese National Study Group of Autoimmune Hepatitis. J Hepatol 1997;26:1207–12.
[8] Czaja AJ, Carpenter HA, Santrach PJ, Moore SB. Significance of HLA DR4 in type 1 autoimmune hepatitis. Gastroenterology 1993;105:1502–7.
[9] Alvarez F, Berg PA, Bianchi FB, Bianchi L, Burroughs AK, Cancado EL, et al. International Autoimmune Hepatitis Group report: review of criteria for diagnosis of autoimmune hepatitis. J Hepatol 1999;31:929–38.
[10] Desmet VJ, Gerber M, Hoofnagle JH, Manns M, Scheuer PJ. Classification of chronic hepatitis: diagnosis, grading and staging. Hepatology 1994;19:1513–20.
[11] Zeitlin AA, Heward JM, Newby PR, Carr-Smith JD, Franklyn JA, Gough SC, et al. Analysis of HLA class II genes in Hashimoto's thyroiditis reveals differences compared to Graves' disease. Genes Immun 2008;9:358–63.
[12] Singh R, Nair S, Farr G, Mason A, Perrillo R. Acute autoimmune hepatitis presenting with centrizonal liver disease: case report and review of the literature. Am J Gastroenterol 2002;97:2670–3.
[13] Pratt DS, Fawaz KA, Rabson A, Dellelis R, Kaplan MM. A novel histological lesion in glucocorticoid-responsive chronic hepatitis. Gastroenterology 1997;113:664–8.
[14] Dufour JF, Zimmermann M, Reichen J. Severe autoimmune hepatitis in patients with previous spontaneous recovery of a flare. J Hepatol 2002;37:748–52.
[15] Buchel E, Van Steenbergen W, Nevens F, Fevery J. Improvement of autoimmune hepatitis during pregnancy followed by flare-up after delivery. Am J Gastroenterol 2002;97:3160–5.
[16] Wilder RL. Hormones, pregnancy, and autoimmune diseases. Ann N Y Acad Sci 1998;840:45–50.

Hepatol Int (2009) 3:556–562
DOI 10.1007/s12072-009-9155-9

ORIGINAL ARTICLE

Efficacy of ursodeoxycholic acid for Japanese patients with autoimmune hepatitis

Yasuhiro Miyake · Yoshiaki Iwasaki · Haruhiko Kobashi · Tetsuya Yasunaka · Fusao Ikeda · Akinobu Takaki · Ryoichi Okamoto · Kouichi Takaguchi · Hiroshi Ikeda · Yasuhiro Makino · Masaharu Ando · Kohsaku Sakaguchi · Kazuhide Yamamoto

Received: 23 February 2009 / Revised: 12 August 2009 / Accepted: 29 September 2009 / Published online: 22 October 2009

Abstract
Purpose This study aimed to investigate the efficacy of ursodeoxycholic acid (UDCA) for Japanese patients with autoimmune hepatitis (AIH).
Methods One hundred forty-seven patients were investigated.
Results As initial treatment, 25 patients received UDCA (300–600 mg/day) monotherapy (UDCA group), 40 received a combination of prednisolone (PSL) (≥20 mg/day) and UDCA (combination group), 68 received PSL monotherapy (PSL group), and 14 received other treatments. During the follow-up, in the UDCA group, PSL was added to 8 of 12 patients failing to achieve the normalization of serum transaminase levels with UDCA monotherapy. Cumulative incidence of the normalization of serum transaminase levels was 64% in the UDCA group, 95% in the combination group, and 94% in the PSL group (log-rank test, $P = 0.0001$). UDCA group required longest periods until the normalization of serum transaminase levels. Eleven patients, who achieved persistent normalization

Y. Miyake (✉) · F. Ikeda
Department of Molecular Hepatology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan
e-mail: miyakeyasuhiro@hotmail.com

F. Ikeda
e-mail: fikeda@md.okayama-u.ac.jp

Y. Iwasaki · H. Kobashi · T. Yasunaka · A. Takaki · K. Sakaguchi · K. Yamamoto
Department of Gastroenterology & Hepatology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, 2-5-1, Shikata-cho, Okayama 700-8558, Japan
e-mail: yiwasaki@cc.okayama-u.ac.jp

H. Kobashi
e-mail: hkobashi@md.okayama-u.ac.jp

T. Yasunaka
e-mail: Yasu0328@gmail.com

A. Takaki
e-mail: akitaka@md.okayama-u.ac.jp

K. Sakaguchi
e-mail: sakaguti@cc.okayama-u.ac.jp

K. Yamamoto
e-mail: kazuhide@md.okayama-u.ac.jp

R. Okamoto
Department of Internal Medicine, Hiroshima City Hospital, Hiroshima, Japan
e-mail: okamoto6488@yahoo.co.jp

K. Takaguchi
Department of Internal Medicine, Kagawa Prefectural Central Hospital, Takamatsu, Japan
e-mail: k.takaguchi@mail.central-hp.pref.kagawa.jp

H. Ikeda
Department of Gastroenterology, Kurashiki Central Hospital, Kurashiki, Japan
e-mail: hi5206@kchnet.or.jp

Y. Makino
Department of Gastroenterology, Iwakuni Clinical Center, Iwakuni, Japan
e-mail: makino@iwakuni-nh.go.jp

M. Ando
Department of Gastroenterology, Mitoyo General Hospital, Kanonji, Japan
e-mail: m-ando@mx8.tiki.ne.jp

of serum transaminase levels with UDCA monotherapy, did not reach liver failure or develop hepatocellular carcinoma for 49.7 (range = 13.4–137.3) months. Meanwhile, during the taper of PSL, doses of PSL at the initial relapse were lower in patients treated with PSL and UDCA than in those treated with PSL monotherapy, and initial relapse occurred earlier in patients treated with PSL monotherapy.
Conclusions UDCA monotherapy is effective for some Japanese AIH patients; however, UDCA monotherapy for patients with either high-grade inflammatory activity or poor residual capacity of liver function is not recommended because they may reach liver failure before achievement of remission. Meanwhile, additional use of UDCA during the taper of corticosteroids may be effective for the prevention of early relapse.

Keywords Autoimmune hepatitis · Ursodeoxycholic acid · Corticosteroid

Introduction

Autoimmune hepatitis (AIH) is generally responsive to immunosuppressive treatment, and corticosteroids are commonly used for initial and maintenance treatment [1]. Approximately 90% of patients can achieve the normalization of serum transaminase levels within 6 months after the introduction of corticosteroid treatment [2] that leads to the improvement of histological activity and staging, even in patients with cirrhosis [3]. With appropriate immunosuppressive treatment, the 10-year survival rate is reported at more than 90% regardless of histological staging [4].

However, prolonged use of corticosteroids leads to the development of adverse events (osteoporosis, type 2 diabetes, hypertension, and Cushing's syndrome). On the other hand, rapid taper of corticosteroids leads to relapse and multiple relapses are associated with hepatic death and liver transplantation [5]. Relapse occurs in approximately 90% of patients within 1 year after immunosuppressive treatment withdrawal, especially in approximately 50% of patients within 3 months. These are dilemmas in clinical practice.

In a previous report [6], ursodeoxycholic acid (UDCA), which is a hydrophilic dihydroxy bile acid and effects the protection of hepatocytes against bile acid-induced apoptosis, stimulation of impaired hepatobiliary secretion, and protection of cholangiocytes against the toxic effects of hydrophobic bile acids [7], improved serum transaminase levels and histological activities in Japanese AIH patients; however, the study population was too small. In another report [8], short-term treatment with UDCA did not facilitate reduction in either the dose of corticosteroids or histological activity in white patients with intractable AIH. It has been controversial whether UDCA has a beneficial therapeutic effect in AIH patients.

In this study, we aimed to investigate the efficacy of UDCA treatment in Japanese AIH patients. We evaluated the normalization of serum alanine aminotransferase (ALT) levels under UDCA treatment and the association between dose reduction of corticosteroids under the additional use of UDCA and relapse of the disease.

Materials and methods

Patients

One hundred seventy-one Japanese AIH patients (148 females, median age 55 [16–79] years) were admitted to the Okayama University Hospital or one of six affiliated hospitals between March 1989 and June 2007. All patients who were seronegative for hepatitis B surface antigen, anti-hepatitis C virus antibody, hepatitis C virus-RNA, and antimitochondrial antibody underwent liver biopsy. A diagnosis of AIH was made according to the revised scoring system proposed by the International Autoimmune Hepatitis Group (IAIHG) [9]. A definite diagnosis of AIH based on this revised scoring system required a pretreatment score exceeding 15, whereas a probable diagnosis required a score between 10 and 15. Patients with an overlapping syndrome or a coexistent liver disease (e.g., primary biliary cirrhosis, primary sclerosing cholangitis, nonalcoholic fatty liver disease, or alcohol-induced liver injury) were excluded from this analysis.

In this study, 24 of the 171 patients (14%) were transferred to other hospitals without follow-up. Thus, 147 patients (86%) were included in the present analysis.

Criteria for acute presentation

An acute presentation was defined by the presence of acute onset of symptoms (e.g., jaundice and/or fatigue and/or anorexia) in conjunction with bilirubin levels of 5 mg/dL or more and/or serum ALT levels higher than 10-fold the upper normal limit.

Histological evaluation

Liver biopsy was performed with a Vim-Silverman needle (14-G) under laparoscopy or with a 17-G needle under ultrasonography guidance, before or just after commencing treatment. Liver biopsy specimens were evaluated by two pathologists and diagnosed as acute or chronic hepatitis. A diagnosis of acute hepatitis was made on the basis of the presence of histologically predominant zone 3 necrosis with minimal lymphocytic and plasma cell infiltration into

portal tracts, in the absence of interface hepatitis or portal fibrosis. Liver biopsy specimens diagnosed as chronic hepatitis underwent histological staging based on the classification of Desmet et al. [10].

Treatment

The standard initial treatment was prednisolone (PSL) (30–40 mg/day) with or without UDCA (300–600 mg/day). In patients with histological low-grade inflammatory activity, the initial treatment was low-dose PSL (20 mg/day) with or without UDCA (300–600 mg/day). Elderly patients with histological low-grade inflammatory activity and comorbidities such as osteoporosis and/or diabetes were treated with UDCA (300–600 mg/day) with or without lower doses of PSL (<20 mg/day). An initial treatment was defined as any therapy that was started within 3 months after the diagnosis of AIH. It was continued until the normalization of serum ALT levels.

After the normalization of serum ALT levels, PSL was tapered by 2.5–5 mg every 1 or 2 weeks to a maintenance dose of 10 mg/day or less. When an incomplete response to initial treatment or relapse was observed, PSL was added or increased or UDCA (300–600 mg/day) and/or azathioprine (50–100 mg/day) were added.

Follow-up

Each patient underwent a comprehensive clinical review and physical examination at each follow-up visit. Conventional laboratory blood tests were performed every 1–3 months.

Table 1 Concurrent autoimmune diseases in 147 patients with autoimmune hepatitis

Disease	Patients (*n*)
Autoimmune thyroiditis	15
Sjögren's syndrome	3
Systemic lupus erythematosus	3
Graves' disease	3
Ulcerative colitis	3
Autoimmune hemolytic anemia	2
Idiopathic thrombocytopenic purpura	2
Progressive systemic sclerosis	2
Rheumatoid arthritis	1
Autoimmune thyroiditis + autoimmune hemolytic anemia	1
Systemic lupus erythematosus + Sjögren's syndrome	1
Autoimmune thyroiditis + Sjögren's syndrome	1

Criteria for the relapse of AIH

Relapse was defined as an increase in serum ALT levels to more than twofold the upper normal limit following the normalization of serum ALT levels with medical treatment.

Statistics

Statistical analysis was performed using the SPSS statistical program (release 11.0.1 J, SPSS, Inc., Chicago, IL).

Continuous variables were expressed as medians and ranges. The Mann–Whitney *U* test was used to evaluate differences in the continuous variables between two groups, and the Kruskal–Wallis test was carried out among three groups. Dichotomous variables were compared by the χ^2 test. Cumulative incidence was estimated by the Kaplan–Meier method, and significance was determined by the log-rank test. The values of $P < 0.05$ were considered significant.

Results

Clinical features of 147 AIH patients

One hundred twenty-seven patients (86%) were female, and the median age was 55 (16–79) years. On the basis of the revised scoring system proposed by IAIHG [9], the median pretreatment score was 18 (10–21). One hundred

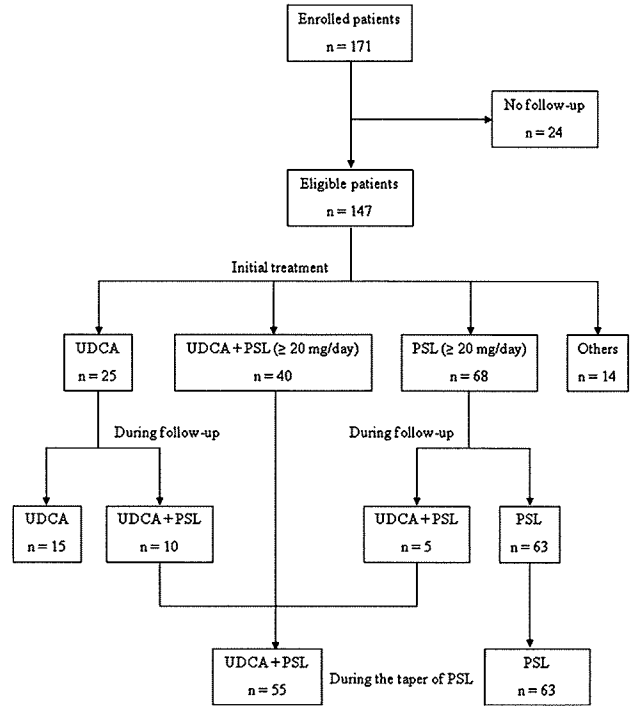

Fig. 1 Clinical course of study population

Table 2 Clinical features of the three groups classified according to initial treatment

	UDCA (n = 25)	Combination (n = 40)	PSL (n = 68)	P
Gender (female), n (%)	22 (88)	33 (83)	59 (87)	0.78
Age (years)	62 (37–77)	52 (18–78)	52 (16–79)	0.03
International diagnostic criteria for the diagnosis of autoimmune hepatitis				
Pretreatment score	17 (10–20)	17 (11–20)	18 (10–21)	0.03
Definite diagnosis, n (%)	17 (68)	33 (83)	54 (79)	0.36
Acute presentation, n (%)	4 (16)	17 (43)	19 (28)	0.07
Concurrent autoimmune disease, n (%)	3 (12)	4 (10)	22 (32)	0.01
Laboratory data				
Bilirubin (mg/dL)	0.6 (0.3–29.2)	1.4 (0.4–24.3)	1.1 (0.4–17.1)	0.0001
AST (IU/L)	96 (33–1,502)	388 (43–1,704)	187 (37–2,330)	0.0005
ALT (IU/L)	98 (28–1,433)	407 (36–1,450)	229 (26–2,161)	0.0004
ALP, ×ULN	1.0 (0.6–2.6)	1.4 (0.5–3.8)	1.1 (0.3–5.1)	0.02
Albumin (g/dL)	4.0 (3.0–5.1)	3.7 (2.4–4.4)	3.8 (2.7–4.7)	0.04
IgG (mg/dL)	2,134 (1,085–3,970)	2,517 (1,300–4,530)	2,663 (1,170–6,562)	0.04
ANA (≥1:40), n (%)	22 (88)	30 (75)	57 (84)	0.35
SMA (≥1:40), n (%)	9/14 (64)	22/31 (71)	38/52 (73)	0.81
HLA DR4, n (%)	6/11 (55)	16/23 (70)	25/34 (74)	0.50
Histological finding				
Staging, n (%)				0.80
Acute hepatitis	1 (4)	4 (10)	5 (7)	
Chronic hepatitis				
F1	7 (28)	11 (28)	16 (24)	
F2	7 (28)	11 (28)	25 (37)	
F3	8 (32)	9 (22)	19 (28)	
F4	2 (8)	5 (12)	3 (4)	
Rosetting of liver cells, n (%)	6 (24)	13 (33)	18 (26)	0.71
Zone 3 necrosis, n (%)	5 (20)	17 (43)	25 (37)	0.17

Abbreviations: *UDCA* ursodeoxycholic acid, *PSL* prednisolone, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *ALP* alkaline phosphatase, *IgG* immunoglobulin G, *ANA* antinuclear antibody, *SMA* smooth muscle antibody, *HLA* human leukocyte antigen, *ULN* upper limit of normal

fifteen patients (78%) were diagnosed as definite AIH patients. Forty-one patients (28%) showed acute presentation. Thirty-seven patients (25%) had concurrent autoimmune diseases (Table 1).

One hundred twenty-two patients (83%) were positive for antinuclear antibodies (ANA) (≥1:40). Of the 109 patients screened, 72 (66%) were positive for smooth muscle antibodies (SMA) (≥1:40). One hundred thirty-nine of 147 patients (95%) were positive for ANA and/or SMA. Of the 70 patients who were screened for HLA DR status, 49 (70%) had DR4. None had DR3.

Histological acute hepatitis and cirrhosis were observed in 10 (7%) and 12 patients (8%), respectively. Rosetting of liver cells and zone 3 necrosis were observed in 38 (26%) and 47 patients (32%), respectively.

As initial treatment, of the 147 patients, 25 received UDCA monotherapy (300–600 mg/day) (UDCA group), 40 received a combination of PSL (≥20 mg/day) and UDCA (300–600 mg/day) (combination group), 68 received PSL monotherapy (≥20 mg/day) (PSL group), and 14 received other treatments (Fig. 1).

Comparison of clinical features among three groups classified according to initial treatment

UDCA group had lowest serum levels of bilirubin, transaminase, and immunoglobulin G (IgG) and highest serum albumin levels among the three groups. However, there were no differences in the frequencies of positivity for ANA or SMA, HLA DR4, and the histological features (Table 2).

The follow-up durations were 73.5 (12.2–149.3) months in the UDCA group, 65.1 (12.2–162.3) months in the combination group, and 79.1 (17.2–204.2) months in the PSL group, respectively (P = 0.14). Cumulative incidence of the normalization of serum ALT levels was 64% in the

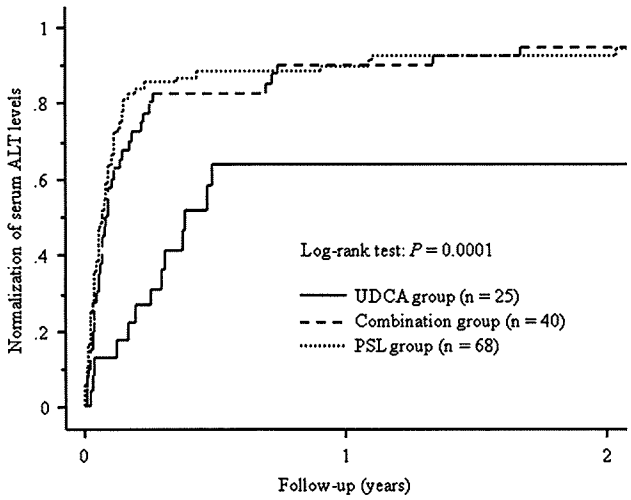

Fig. 2 Cumulative incidence of the normalization of serum ALT levels. The follow-up durations were 73.5 (12.2–149.3) months in 25 patients initially treated with UDCA monotherapy (UDCA group), 65.1 (12.2–162.3) months in 40 patients initially treated with a combination of PSL and UDCA (combination group), and 79.1 (17.2–204.2) months in 68 patients initially treated with PSL monotherapy (PSL group), respectively. Cumulative incidence of the normalization of serum ALT levels was 64% in the UDCA group, 95% in the combination group, and 94% in the PSL group (log-rank test, $P = 0.0001$)

UDCA group, 95% in the combination group, and 94% in the PSL group (Fig. 2).

UDCA monotherpay as initial treatment

Of the UDCA group, 12 patients failed to achieve the normalization of serum ALT levels with UDCA monotherapy during the follow-up and PSL was added to eight patients. On the other hand, during the follow-up, in 2 of the 13 patients who once achieved the normalization of serum ALT levels, serum ALT levels fluctuated and PSL was added. Thus, PSL was added to 10 patients of the UDCA group and UDCA monotherapy was continued in the remaining 15 patients (Fig. 1). Eleven of the 15 patients who continued UDCA monotherapy achieved the normalization of serum ALT levels during the follow-up, and none of the 11 patients reached liver failure or developed hepatocellular carcinoma during 49.7 (13.4–137.3) months.

Efficacy of UDCA during the taper of corticosteroids

During the taper of PSL, UDCA was added to five patients of the PSL group. As mentioned above, PSL was added to 10 patients of the UDCA group (Fig. 1). Thus, during the follow-up, 55 patients were treated with a combination of PSL and UDCA and 63 were treated with PSL monotherapy. There were no differences in clinical features at presentation except a frequency of concurrent autoimmune diseases between the two groups (Table 3). There was no difference in follow-up duration between the two groups (follow-up duration; 71.5 [12.2–186.1] months vs. 77.1 [17.2–204.2] months; $P = 0.31$).

During the follow-up, relapse occurred in 32 of 55 patients (58%) treated with combination treatment and 36 of 63 patients (57%) treated with PSL monotherapy (log-rank test, $P = 0.97$). In patients with relapse, there was no difference in the duration from the initial normalization of serum ALT level to the initial relapse between the two groups (13.3 [3.0–138.5] months in the combination treatment group vs. 17.1 [1.5–129.0] months in the PSL monotherapy group; $P = 0.77$). However, doses of PSL at the initial relapse was lower in patients treated with combination treatment than in those treated with PSL monotherapy (5 [0–10] mg/day vs. 5 [0–15] mg/day; $P = 0.03$) (Fig. 3). During treatment periods with PSL 10 mg/day or more, 7.5 mg/day, and 5 mg/day, relapse occurred in 3, 4, and 21 of 55 patients treated with combination treatment whereas it occurred in 9, 13, and 28 of 63 patients treated with PSL monotherapy (5%, 7%, and 38% vs. 14%, 21%, and 44%; $P = 0.49$, 0.04, and 0.09).

Discussion

UDCA has some degree of immunomodulating effects. Experimentally, UDCA suppresses the secretion of interleukin-2, interleukin-4, and interferon-γ from activated T lymphocytes and immunoglobulin production from B lymphocytes [11]. Recently, in concanavalin A-induced liver injury, the administration of UDCA before concanavalin A injection has been reported to dose dependently reduce plasma levels of tumor necrosis factor-α and interleukin-6 and to inhibit the elevation of plasma transaminase levels and decrease the incidence of liver necrosis [12]. In AIH patients, serum levels of tumor necrosis factor-α and interleukin-6 have been reported to be associated with the disease activity [13, 14]. Furthermore, increased expressions of interleukin-4 and interferon-γ are shown in the liver specimens of AIH patients [15]. Thus, UDCA may control the disease activity of AIH on the basis of these mechanisms.

In this study, 64% of the UDCA group achieved the normalization of serum ALT levels with UDCA monotherapy. So, UDCA monotherapy will be effective in some Japanese AIH patients. However, in this study, patients treated with UDCA monotherapy had lower serum ALT levels at presentation than those treated with PSL. Furthermore, until the normalization of serum ALT levels, longer periods were required in patients treated with UDCA monotherapy than in those treated with PSL. Thus, we consider that UDCA monotherapy for patients with

either high-grade inflammatory activity or poor residual capacity of liver function is not recommended because they may reach liver failure before the achievement of remission.

Does UDCA monotherapy improve the prognosis of AIH patients? To improve the prognosis of AIH, persistent normalization of serum transaminase levels is necessary [16, 17]. In this study, none of 11 patients who achieved the normalization of serum ALT levels with UDCA monotherapy reached liver failure or developed hepatocellular carcinoma during the follow-up period. UDCA monotherapy may improve the prognosis of Japanese AIH patients if persistent normalization of serum ALT levels is achieved.

Once remission is achieved with corticosteroid treatment, doses of corticosteroids are gradually tapered. Histological improvement lags behind laboratory improvement by 3–6 months, so maintenance treatment (PSL 0.1–0.2 mg/kg daily or 5 mg daily) for 1–2 years after the normalization of serum transaminase levels is recommended [18]. However, prolonged use of corticosteroids leads to the development of adverse events. This study suggests that the additional doses of UDCA may be useful for the safer taper of corticosteroids and lead to the decrease in the adverse events due to prolonged use of corticosteroids.

It is unclear when to commence UDCA for Japanese AIH patients in order to prevent relapse during the taper of corticosteroids. In this study, there was no difference in cumulative incidence of the normalization of serum ALT levels after the introduction of initial treatment between the PSL and combination groups. Thus, the addition of UDCA to PSL as an initial treatment modality may not be always necessary. On the other hand, in the PSL group, doses of PSL at the initial relapse were 15 mg/day or less. We

Table 3 Clinical features of patients treated with a combination of PSL and UDCA or PSL monotherapy during the taper of PSL

	Combination (n = 55)	PSL (n = 63)	P
Gender (female), n (%)	47 (85)	54 (86)	0.97
Age (years)	53 (16–78)	52 (16–79)	0.88
International diagnostic criteria for the diagnosis of autoimmune hepatitis			
Pretreatment score	17 (10–20)	18 (10–21)	0.07
Definite diagnosis, n (%)	43 (78)	50 (79)	0.88
Acute presentation, n (%)	19 (35)	17 (27)	0.37
Concurrent autoimmune disease, n (%)	7 (13)	20 (32)	0.01
Laboratory data			
Bilirubin (mg/dL)	1.2 (0.3–24.3)	1.1 (0.4–17.1)	0.34
AST (IU/L)	278 (43–2,330)	179 (37–1,716)	0.46
ALT (IU/L)	280 (28–1,783)	229 (26–2,161)	0.56
ALP, × ULN	1.2 (0.5–3.8)	1.1 (0.3–5.1)	0.30
Albumin (g/dL)	3.8 (2.4–4.8)	3.8 (2.7–4.7)	0.18
IgG (mg/dL)	2,579 (1,300–5,356)	2,624 (1,170–6,562)	0.68
ANA (≥1:40), n (%)	42 (76)	52 (83)	0.41
SMA (≥1:40), n (%)	24/39 (62)	36/49 (73)	0.23
HLA DR4, n (%)	21/31 (68)	23/31 (74)	0.58
Histological finding			
Staging, n (%)			0.82
Acute hepatitis	5 (9)	4 (6)	
Chronic hepatitis			
F1	15 (27)	16 (25)	
F2	17 (31)	22 (35)	
F3	13 (24)	18 (29)	
F4	5 (9)	3 (5)	
Rosetting of liver cells, n (%)	15 (27)	16 (25)	0.82
Zone 3 necrosis, n (%)	21 (38)	22 (35)	0.71

Abbreviations: *UDCA* ursodeoxycholic acid, *PSL* prednisolone, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *ALP* alkaline phosphatase, *IgG* immunoglobulin G, *ANA* antinuclear antibody, *SMA* smooth muscle antibody, *HLA* human leukocyte antigen, *ULN* upper limit of normal

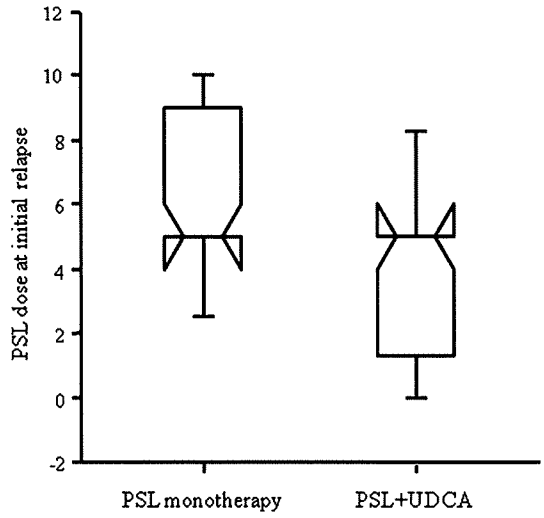

Fig. 3 Box plots indicate the median, interquartile range, and 90 percentile range of maintenance doses of PSL at initial relapse. The dose was smaller in patients treated with a combination of PSL and UDCA than in those treated with PSL monotherapy, respectively (5 [0–10] mg/day vs. 5 [0–15] mg/day; $P = 0.03$)

suggest that the addition of UDCA before the taper of PSL dose to 15 mg/day may be effective for the prevention of relapse.

In conclusion, UDCA monotherapy is effective in some Japanese AIH patients; however, until the normalization of serum transaminase levels, longer periods were required in patients treated with UDCA monotherapy than in those treated with PSL. Thus, we consider that UDCA monotherapy for patients with either high-grade inflammatory activity or poor residual capacity of liver function is not recommended because they may reach liver failure before the achievement of remission. Meanwhile, the additional use of UDCA during the taper of corticosteroid may be effective for the prevention of early relapse. UDCA will be worth using in Japanese AIH patients with both low-grade inflammatory activity and sufficient residual capacity of liver function. To confirm these findings, validation based on randomized trials is required.

References

1. Krawitt EL. Autoimmune hepatitis. N Engl J Med 2006;354: 54–66
2. Toda G, Zeniya M, Watanabe F, Imawari M, Kiyosawa K, Nishioka M, et al. Present status of autoimmune hepatitis in Japan—correlating the characteristics with international criteria in an area with a high rate of HCV infection. Japanese National Study Group of Autoimmune Hepatitis. J Hepatol 1997;26:1207–1212
3. Czaja AJ, Carpenter HA. Decreased fibrosis during corticosteroid therapy of autoimmune hepatitis. J Hepatol 2004;40:646–652
4. Roberts SK, Therneau TM, Czaja AJ. Prognosis of histological cirrhosis in type 1 autoimmune hepatitis. Gastroenterology 1996; 110:848–857
5. Montano-Loza AJ, Carpenter HA, Czaja AJ. Consequences of treatment withdrawal in type 1 autoimmune hepatitis. Liver Int 2007;27:507–515
6. Nakamura K, Yoneda M, Yokohama S, Tamori K, Sato Y, Aso K, et al. Efficacy of ursodeoxycholic acid in Japanese patients with type 1 autoimmune hepatitis. J Gastroenterol Hepatol 1998; 13:490–495
7. Beuers U. Drug insight: mechanisms and sites of action of ursodeoxycholic acid in cholestasis. Nat Clin Pract Gastroenterol Hepatol 2006;3:318–328
8. Czaja AJ, Carpenter HA, Lindor KD. Ursodeoxycholic acid as adjunctive therapy for problematic type 1 autoimmune hepatitis: a randomized placebo-controlled treatment trial. Hepatology 1999;30:1381–1386
9. Alvarez F, Berg PA, Bianchi FB, Bianchi L, Burroughs AK, Cancado EL, et al. International Autoimmune Hepatitis Group Report: review of criteria for diagnosis of autoimmune hepatitis. J Hepatol 1999;31:929–938
10. Desmet VJ, Gerber M, Hoofnagle JH, Manns M, Scheuer PJ. Classification of chronic hepatitis: diagnosis, grading and staging. Hepatology 1994;19:1513–1520
11. Yoshikawa M, Tsujii T, Matsumura K, Yamao J, Matsumura Y, Kubo R, et al. Immunomodulatory effects of ursodeoxycholic acid on immune responses. Hepatology 1992;16:358–364
12. Ishizaki K, Iwaki T, Kinoshita S, Koyama M, Fukunari A, Tanaka H, et al. Ursodeoxycholic acid protects concanavalin A-induced mouse liver injury through inhibition of intrahepatic tumor necrosis factor-alpha and macrophage inflammatory protein-2 production. Eur J Pharmacol 2008;578:57–64
13. Maggiore G, De Benedetti F, Massa M, Pignatti P, Martini A. Circulating levels of interleukin-6, interleukin-8, and tumor necrosis factor-alpha in children with autoimmune hepatitis. J Pediatr Gastroenterol Nutr 1995;20:23–27
14. Durazzo M, Niro G, Premoli A, Morello E, Rizzotto ER, Gambino R, et al. Type 1 autoimmune hepatitis and adipokines: new markers for activity and disease progression? J Gastroenterol 2009;44:476–482
15. Cherñavsky AC, Paladino N, Rubio AE, De Biasio MB, Periolo N, Cuarterolo M, et al. Simultaneous expression of Th1 cytokines and IL-4 confers severe characteristics to type I autoimmune hepatitis in children. Hum Immunol 2004;65:683–691
16. Miyake Y, Iwasaki Y, Terada R, Takagi S, Okamaoto R, Ikeda H, et al. Persistent normalization of serum alanine aminotransferase levels improves the prognosis of type 1 autoimmune hepatitis. J Hepatol 2005;43:951–957
17. Miyake Y, Iwasaki Y, Terada R, Okamaoto R, Ikeda H, Makino Y, et al. Persistent elevation of serum alanine aminotransferase levels leads to poor survival and hepatocellular carcinoma development in type 1 autoimmune hepatitis. Aliment Pharmacol Ther 2006;24:1197–1205
18. Czaja AJ, Freese DK, American Association for the Study of Liver Disease. Diagnosis and treatment of autoimmune hepatitis. Hepatology 2002;36:479–497

Hepatol Int (2009) 3:403–410
DOI 10.1007/s12072-008-9108-8

CASE REPORT

Two cases of development of entecavir resistance during entecavir treatment for nucleoside-naive chronic hepatitis B

Haruhiko Kobashi · Shin-ichi Fujioka · Mitsuhiko Kawaguchi · Hiromitsu Kumada · Osamu Yokosuka · Norio Hayashi · Kazuyuki Suzuki · Takeshi Okanoue · Michio Sata · Hirohito Tsubouchi · Chifumi Sato · Kendo Kiyosawa · Kyuichi Tanikawa · Taku Seriu · Hiroki Ishikawa · Akinobu Takaki · Yoshiaki Iwasaki · Toshiya Osawa · Toshiyuki Takaki · Kosaku Sakaguchi · Yasushi Shiratori · Kazuhide Yamamoto · Daniel J. Tenney · Masao Omata

Received: 27 June 2008 / Accepted: 2 October 2008 / Published online: 9 December 2008

Abstract
Background Entecavir (ETV) is a potent nucleoside analogue against hepatitis B virus (HBV), and emergence of drug resistance is rare in nucleoside-naive patients because development of ETV resistance (ETVr) requires at least three amino acid substitutions in HBV reverse transcriptase. We observed two cases of genotypic ETVr with viral rebound and biochemical breakthrough during

H. Kobashi (✉) · A. Takaki · Y. Iwasaki · K. Sakaguchi · Y. Shiratori · K. Yamamoto
Department of Gastroenterology and Hepatology, Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama University, Okayama, Japan
e-mail: hkobashi@md.okayama-u.ac.jp

S.-i. Fujioka · M. Kawaguchi · T. Osawa · T. Takaki
Department of Medicine, Okayama Saiseikai General Hospital, Okayama, Japan

H. Kumada
Center of Liver Disease, Toranomon Hospital, Kanagawa, Japan

O. Yokosuka
Department of Medicine and Clinical Oncology, Graduate School of Medicine, Chiba University, Chiba, Japan

N. Hayashi
Department of Gastroenterology and Hepatology, Graduate School of Medicine, Osaka University, Osaka, Japan

K. Suzuki
First Department of Internal Medicine, Iwate Medical University, Morioka, Japan

T. Okanoue
Molecular Gastroenterology and Hepatology, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto, Japan

M. Sata
Department of Gastroenterology, Kurume University School of Medicine, Fukuoka, Japan

H. Tsubouchi
Digestive Disease and Life-style Related Disease Health Research, Human and Environmental Sciences, Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima, Japan

C. Sato
Department of Analytical Health Science, Graduate School of Allied Health Sciences, Tokyo Medical and Dental University, Tokyo, Japan

K. Kiyosawa
Division of Hepatology and Gastroenterology, Department of Internal Medicine, Shinshu University School of Medicine, Matsumoto, Japan

K. Tanikawa
International Institute for Liver Research, Kurume University, Kurume, Japan

T. Seriu · H. Ishikawa
Bristol-Myers Squibb Japan, Pharmaceutical Research Institute, Tokyo, Japan

D. J. Tenney
Bristol-Myers Squibb, Research and Development, Wallingford, CT, USA

M. Omata
Department of Gastroenterology, Faculty of Medicine, University of Tokyo, Tokyo, Japan

ETV treatment of nucleoside-naive patients with chronic hepatitis B (CHB).

Results Case 1: A 44-year-old HBeAg-positive man received ETV 0.1 mg/day for 52 weeks and 0.5 mg/day for 96 weeks consecutively. HBV DNA was 10.0 $\log_{10}$ copies/ml at baseline, declined to a nadir of 3.1 at week 100, and rebounded to 4.5 at week 124 and 6.7 at week 148. Alanine aminotransferase (ALT) level increased to 112 IU/l at week 148. Switching to a lamivudine (LVD)/adefovir-dipivoxil combination was effective in decreasing HBV DNA. *Case 2*: A 47-year-old HBeAg-positive man received ETV 0.5 mg/day for 188 weeks. HBV DNA was 8.2 $\log_{10}$ copies/ml at baseline, declined to a nadir of 2.9 at week 124, and then rebounded to 4.7 at week 148 and 6.4 at week 160. ALT level increased to 72 IU/l at week 172. The ETVr-related substitution (S202G), along with LVD-resistance-related substitutions (L180M and M204V), was detected by sequence analysis at week 124 in both case 1 and case 2.

Conclusions ETVr emerged in two Japanese nucleoside-naive CHB patients after prolonged therapy and incomplete suppression and in one patient after <0.5 mg of dosing. ETV patients with detectable HBV DNA or breakthrough after extended therapy should be evaluated for compliance to therapy and potential emergence of resistance.

Keywords Entecavir · HBV · Chronic hepatitis B · Drug resistance · Nucleoside-naive

Introduction

Hepatitis B virus (HBV) infection is a serious health problem because of its high prevalence, estimated to be infecting more than 350 million people worldwide, and its potential for inducing chronic hepatitis, cirrhosis, hepatic decompensation, and hepatocellular carcinoma (HCC) [1, 2]. It has been demonstrated that the most potent risk factor for development of cirrhosis or HCC is serum HBV DNA level [3, 4], and it seems that suppressing serum HBV load is essential for improving the prognosis of HBV carriers. Treatment of chronic hepatitis B (CHB) has evolved markedly with the introduction of nucleoside-analogue antivirals, that is, lamivudine (LVD), adefovir-dipivoxil (ADV), entecavir (ETV), and telbivudine, to clinical practice. LVD, the first approved nucleoside analogue against HBV, was shown to be effective in suppressing HBV DNA replication, improving transaminase levels, improving liver histology, inducing hepatitis B e antigen (HBeAg) seroconversion, and suppressing hepatic insufficiency and hepatocarcinogenesis in CHB and compensated cirrhosis [5, 6]. However, the effectiveness of LVD is limited because of frequent development of drug resistance followed by a hepatitis flare and, occasionally, hepatic failure [7, 8].

ETV, a novel anti-HBV nucleoside analogue, has more than 1,500 times greater potency than LVD in vitro [9]. In clinical trials, ETV administration demonstrated potent anti-HBV activity with a marked decline in serum HBV DNA level and a significant improvement in liver histology than LVD in nucleoside-naive HBeAg-positive and -negative patients [10, 11]. In addition, emergence of ETV resistance (ETVr) or viral rebound was shown in these studies to be rare. From these results, recent treatment guidelines have recommended ETV as the first-line nucleoside analogue for nucleoside-naive CHB patients, including those with cirrhosis [12, 13].

It has been reported that the development of ETVr in nucleoside-naive patients is very rare, even after 4 years of therapy. Recently, however, rare cases of ETVr, which developed in nucleoside-naive patients in clinical studies, have been reported [14–16]. We also observed two patients who developed ETVr-associated HBV reverse transcriptase (RT) substitutions, followed by *virologic rebound*, defined as an elevation in serum HBV DNA of more than 1 $\log_{10}$ copy/ml from nadir, and biochemical breakthrough in long-term ETV treatment of nucleoside-naive CHB patients. In this article, we report these two cases in detail.

Case report

Case 1

A 44-year-old Japanese male CHB patient was positive for hepatitis B surface antigen (HBsAg), HBeAg, serum HBV DNA, and had HBV genotype C, had elevated alanine aminotransferase (ALT) levels, and had no history of nucleoside analogue treatment. The patient had a history of acute appendicitis at age 30, ureteral stone at age 35, and hyperlipidemia at age 43. He had a habit of drinking alcohol (700 ml) daily but did not smoke. At age 27, he was diagnosed for the first time by health screening as an asymptomatic HBV carrier in the immune-tolerant phase, defined by HBsAg positivity and normal liver enzymes, and he was followed up regularly elsewhere with blood tests for liver enzymes. He was found to have ALT elevation. He was referred to our hospital at age 44 and was diagnosed with CHB. Serum HBV DNA level determined by Roche Amplicor™ Monitor PCR assay (lower limit of detection is 2.6 $\log_{10}$ copies/ml = 400 copies/ml; Roche Diagnostics K.K., Tokyo, Japan) [17] was 10.0 $\log_{10}$ copies/ml and serum ALT level was 199 IU/l. Histologic diagnosis by percutaneous liver biopsy at baseline revealed chronic hepatitis with mild fibrosis and mild activity (CH F1/A1, according to the New Inuyama Classification) [18].

Table 1 Baseline characteristics

	Normal range	Unit	Case 1	Case 2
Age	–	–	44 years	47 years
Gender	–	–	Male	Male
T. Bil	0.2–1.0	mg/dl	0.8	0.5
AST	10–40	IU/l	113	48
ALT	5–40	IU/l	199	74
ALP	115–359	IU/l	268	216
BUN	6–20	mg/dl	9.5	15.9
CREA	0.61–1.04	mg/dl	0.95	0.83
ALB	4.0–5.0	g/dl	4.2	4.3
WBC	3500–8500	/μl	6,800	5,650
Hb	13.5–17.0	g/dl	15.7	14.8
PLT	13.1–36.2	10^4/μl	18.9	14.5
Prothrombin time	10–13	second	10.8	11.2
INR		–	1.0	0.9
HBsAg (CLIA)	0–0.05	IU/ml	>100 (positive)	>100 (positive)
anti-HBs (CLIA)	0–10	IU/ml	0 (negative)	0 (negative)
HBeAg (CLIA)	0–1		120 (positive)	190 (positive)
anti-HBe (CLIA)	0–50	%	<35 (negative)	0 (negative)
HBV DNA (PCR)	<2.6	$\log_{10}$ copies/ml	10.0	8.2
HBV genotype			Genotype C	Genotype C
YMDD (sequencing)			YMDD+	YMDD+
			YVDD–	YVDD–
			YIDD–	YIDD–
Liver histology[a]			CH F1/A1	CH F2/A2

[a] Diagnosed according to New Inuyama classification. T. Bil: total bilirubin, AST: aspartate aminotransferase, ALT: alanine aminotransferase, ALP: alkalinephosphatase, BUN: blood urea nitrogen, CREA: serum creatinine, ALB: serum albumin, WBC: white blood cell count, Hb: hemoglobin, PLT: platelet count, INR: international normalized ratio, HBsAg: hepatitis B surface antigen, CLIA: chemiluminescent immunoassay, anti-HBs: antibody to hepatitis B surface antigen, HBeAg: hepatitis B e antigen, anti-HBe: antibody to hepatitis B e antigen, HBV: hepatitis B virus, PCR: polymerase chain reaction, YMDD: tyrosine-methionine-aspartate-aspartate motif, YVDD: tyrosine-valine-aspartate-aspartate motif, YIDD: tyrosine-isoleucine-aspartate-aspartate motif, CH F1/A1: chronic hepatitis with mild fibrosis and mild activity, CH F2/A2: chronic hepatitis with moderate fibrosis and moderate activity

Other baseline characteristics are shown in Table 1. He was enrolled in a phase II clinical trial of ETV and was randomized into 0.1- and 0.5-mg dosage groups. The trial was conducted in Japan in compliance with the ethical principles of the Declaration of Helsinki, Good Clinical Practice guidelines, and Articles/Notifications of the Ministry of Health, Labor and Welfare (H. Kobashi et al., J Gastroenterol Hepatol, in press). He was assigned into the 0.1-mg dosage group and administered ETV at daily dose of 0.1 mg for an initial 52 weeks. Subsequently, he was administered ETV continuously at a daily dose of 0.5 mg for the following 96 weeks. The serum HBV DNA level, which was 10.0 $\log_{10}$ copies/ml at baseline, declined to a nadir of 3.1 $\log_{10}$ copies/ml at week 88 of ETV treatment. Thereafter, HBV DNA level increased from 4.5 $\log_{10}$ copies/ml at week 124 to 6.3 $\log_{10}$ copies/ml at week 140 and 6.7 $\log_{10}$ copies/ml at week 148. ALT levels increased from 28 IU/l at week 144 to 112 IU/l at week 148. The patient discontinued ETV therapy at week 148, and then received a combination therapy of 100 mg of LVD and 10 mg of ADV per day. Afterwards, HBV DNA level dropped to below 2.6 $\log_{10}$ copies/ml and ALT level was normalized after 28 weeks of LVD/ADV dosing (Fig. 1).

HBV DNA sequence analysis was performed using PCR-amplified HBV DNA from preserved serum samples at baseline and at every 24 weeks via HBV DNA polymerase sequence assay (developed at SRL, Inc., Tokyo, Japan). Although sequence analysis of the baseline isolate revealed no substitution in the RT domain of the HBV DNA polymerase gene, analysis of the isolates collected over time revealed the M204I substitution at week 100 and the L180M, S202G, and M204V substitutions at weeks 124 and 144, respectively (Table 2). In addition, a polymorphic residue N238 was found as mixed N238 N/H at week 100 and thereafter. The

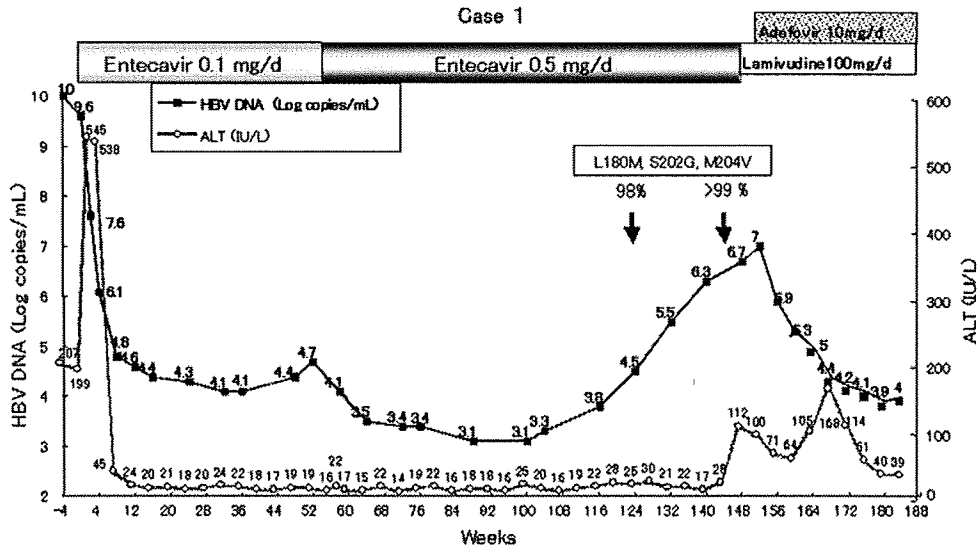

Fig. 1 Clinical course of case 1, a 44-year-old man with nucleoside-naive CHB. ETV treatment reduced ALT levels to below the upper normal limit at week 12 and reduced HBV DNA load to a nadir of 3.1 $\log_{10}$ copies/ml at week 88. However, HBV DNA re-elevated to 4.5 $\log_{10}$ copies/ml at week 124 (virologic breakthrough) and 6.3 $\log_{10}$ copies/ml at week 140, as well as ALT level re-elevated at week 148 (biochemical breakthrough). Sequence analysis of the HBV DNA polymerase gene using serum sample obtained at weeks 124 and 144 revealed the emergence of L180M, M204V (related to LVD resistance), and S202G (related to ETVr) substitutions. SNP-PCR assay revealed that LVDr M204V and ETVr S202G substitutions were detected first at week 124 (98%) and increased at week 148 (>99%). Switching from ETV to LVD/ADV combination treatment at week 148 was successful in reducing HBV DNA load and ALT again

Table 2 Population sequence analysis of isolates from case 1 on ETV therapy

Week	Reverse transcriptase position				
	180	202	204	223	238
0	L	S	M	S/A	N
24	L	S	M	S/A	N
100	L	S	M/I	S/A	N/H
124	M	G	V	S	N/H
144	M	G	V	S	N/H

polymorphic residue S223, which was mixed as S/A at baseline, was found to be only S at weeks 124 and 144.

In addition, preserved serum samples from this patient at baseline and at every 24 weeks were analyzed by an ultrasensitive, single-nucleotide-polymorphism (SNP)-PCR assay, using a method similar to Punia et al. [19] for identification of resistance substitutions, as well as analyzing the sequence of individual clones to determine the genetic linkage of substitutions. SNP-PCR analysis was performed for the two LVD-resistance (LVDr) substitutions, M204V (codon GTG) and M204I (codons ATA and ATT), and the ETVr substitution S202G. Both wild-type and positive control plasmids containing the correct sequence were used at various concentrations to establish the background level as well as the level of detection for each substitution. For clonal analysis, the amplified RT gene from the patient's HBV was cloned into plasmids, as well as 22 to 24 individual clones were selected and sequenced, to determine the genetic linkage of the different substitutions observed.

SNP-PCR analysis for ultrasensitive detection of the resistance substitutions revealed that the LVDr M204V(GTG) and ETVr S202G(GGT) substitutions were not detected (<0.1%) at baseline, week 24, or week 100. The M204I substitution (codon ATA) was detected at low levels at week 24 (0.4%), increased levels at week 100 (6.6%), and was present but at reduced levels at weeks 124 and 148 (0.4% at both time points). The LVDr M204V and ETVr S202G substitutions were detected first at week 124 (98%) and increased levels at week 148 (>99%). The levels of M204I(ATA) were lower at weeks 124 and 144, likely as a result of the dominant M204V/S202G virus (Table 3). Samples at weeks 48 and 76 could not be analyzed conclusively because of low yields of HBV DNA from serum samples.

Clonal analysis revealed that position 223 was a mixture of S and A residues at baseline, the LVDr substitutions L180M and M204V, as well as the ETVr substitution S202G, all emerged simultaneously and were linked in the same virus isolate clones at week 124, isolates that also contained S at position 223. These substitutions did not appear to arise from the LVDr isolates with M204I because the M204I substitution emerged in an isolate with substitution S223A.

Table 3 SNP-PCR analysis of case 1 isolates

Week	M204V		S202G		M204I (ATA)		M204I (ATT)	
	Mut/WT	Ave (%)	Mut/WT	Ave (%)	Mut/WT	Ave (%)	Mut/WT	Ave (%)
0	1/5,424	0.018	1/15,453	0.0065	1/4,199	0.024	1/37,940	0.0026
24	1/5,655	0.018	1/19,000	0.0052	**1/243**	**0.410**	1/46,518	0.0021
100	1/3,846	0.026	1/16,038	0.0062	**1/14**	**6.569**	1/50,456	0.0020
124	**48/1**	**97.973**	**59/1**	**98.327**	**1/265**	**0.377**	1/12,879	0.0078
144	**706/1**	**99.859**	**1,250/1**	**99.920**	**1/237**	**0.421**	1/10,573	0.0095

Cells with bold and underlined font are considered positive (>1/1000 or >0.1% mutant/wild-type)

Mut/WT, mutant/wild type, mean ($N = 3$)

Ave %, average % in total HBV DNA

Case 2

A 47-year-old Japanese male CHB patient was positive for HBsAg, HBeAg, serum HBV DNA, and had HBV genotype C, had elevated ALT levels, and had no history of nucleoside analogue treatment. At age 33, he was diagnosed for the first time as an asymptomatic HBV carrier in the immune-tolerant phase because of positive HBsAg and normal liver enzymes. At age 44, he was found to have ALT elevation, referred to our hospital, and diagnosed with CHB. Histologic diagnosis by percutaneous liver biopsy revealed chronic hepatitis with moderate fibrosis and moderate activity (CH F2/A2 according to the New Inuyama Classification). He was treated with ursodeoxycholic acid at a daily dose of 600 mg orally and glycyrrhizin preparation (stronger Neo-Minophagen C™) 40 ml i.v. thrice per week for 3 months. However, liver enzymes did not normalize. Interferon-α2b administration, three mega units i.m. thrice per week, was started at age 45 and continued for 24 weeks. Although HBV DNA level was reduced transiently to below 3.7 $\log_{10}$ copies/ml at the end of therapy, it rose 9 months after cessation of interferon therapy to 8.2 $\log_{10}$ copies/ml and ALT level increased to 483 IU/l. At age 47, the patient was started on ETV treatment as the subject enrolled in the ETV clinical trial (ETV-053) in Japan at a daily oral dose of 0.5 mg and continued for 188 weeks. A liver biopsy performed 1 month before starting the ETV treatment showed chronic hepatitis with moderate fibrosis and moderate activity (CH F2/A2, according to the New Inuyama Classification). The baseline serum HBV DNA level was 8.2 $\log_{10}$ copies/ml, ALT level was 74 IU/l, and other baseline characteristics were as shown in Table 1. The serum HBV DNA level declined to 3.2 $\log_{10}$ copies/ml and ALT level decreased to below the upper limit of normal at week 32. Liver histology improved to mild-to-moderate fibrosis and mild activity (CH F1-2/A1) at week 48 and chronic hepatitis with mild-to-moderate fibrosis and mild activity (CH F1/A1) at week 148. HBV DNA level was suppressed to a nadir of 2.9 $\log_{10}$ (794) copies/ml at week 124 and rose again to 4.7 $\log_{10}$ copies/ml at week 148, 5.4 $\log_{10}$ copies/ml at week 152, and 6.4 $\log_{10}$ copies/ml at week 160 and 7.0 $\log_{10}$ copies/ml at week 164. ALT level rose to 79 IU/l at week 172 and remained between 40 and 50 IU/l thereafter. ETV at 0.5 mg/day was continued until this time (Fig. 2).

HBV DNA sequence analysis revealed no resistance substitutions in the patient's baseline virus. However, the LVDr-related substitutions L180M and M204V, as well as ETVr-related substitution S202G, were detected at week 124, as a mixed population with wild type, and at week 148, as a pure population (Table 4). In addition, the patient displayed evidence of several polymorphic substitutions at baseline, indicating a mixed quasi-species, which became enriched for those with the resistant virus over time.

SNP-PCR analysis was used to determine the first appearance of the resistance substitutions, using the same method as for case 1. There was no antiviral resistance detected at baseline (<0.1%). The M204V (0.65%) and S202G substitutions were detected first at week 24 but not again until week 124. At weeks 124 and 148, the resistant isolate had become enriched to 43% (M204V) and 98% (M204V), respectively (Table 5).

Clonal analysis was performed to determine the genetic linkage of the various substitutions observed, using the same method as for case 1. The amplified RT gene from the patient's virus was cloned into plasmids, and 24 to 27 individual clones were selected and sequenced. From the clonal analysis, it can be seen that there are three positions that contain mixtures at baseline; position 55 is a mixture of H and R residues, position 221 is a mixture of Y and F residues, and position 269 is a mixture of I and L residues. The substitutions L180M and M204V, as well as the ETVr-related substitution S202G, all emerge simultaneously and in an isolate with H at position 55, Y at position 221, and I at position 269.