Table 2 Single nucleotide polymorphisms within human leukocyte antigen loci associated with outcomes of hepatitis C virus infection

Ethnic group	Outcome	No. of cohorts	HLA locus	SNP	Odds	95%CI	Haplotype	Odds	Ref.
Japanese	HCC	721 HCC *vs* 2890 HCV-negative controls	MICA	rs2596542	1.34	1.16-1.53			[30]
Japanese	Cirrhosis	682 cirrhosis *vs* 1045 Chronic hepatitis	C6orf10	rs910049	1.73	1.40-2.15			[31]
			No gene	rs3135363	1.58	1.32-1.90			
							DQA1*0601	2.80	
							DPB1*0405	1.45	

SNP: Single nucleotide polymorphism; HCC: Hepatocellular carcinoma; HLA: Human leukocyte antigen; HCV: Hepatitis C virus.

independently associated with the risk of HCC in Han Chinese populations[14]. There was a moderate association between the genotype of rs9275319 SNPs with *HLA-DQB1*0401* and *HLA-DQA1*0303*. On the other hand, there was no significant association between HCC development by HBV infection and *HLA* alleles in Korean or Japanese populations[26]. It thus remains unclear whether specific HLA loci play important roles in hepatocarcinogenesis in patients with HBV.

HCV infection

It is globally recognized that interleukin-28B (IL-28B) gene polymorphisms originally detected by GWAS are associated with spontaneous clearance of HCV, as well as with the response to combination therapy with pegylated interferon and ribavirin in patients with HCV[27,28]. However, this SNP is not located in HLA loci. A recent study identified rs4273729 SNP near *HLA DQB1*0301* as a candidate allele for spontaneous clearance of HCV in populations with European and African ancestry[29]. *HLA DQB1*0301* and *IL28B* are independently associated with spontaneous resolution of HCV infection.

Comparisons between cohorts with and without HCC showed that rs2596542 SNP at the 5' flanking region of *MICA* in *HLA* class III was significantly associated with HCC development in Japanese patients with HCV[30]. Soluble MICA levels in serum were significantly lower in AA genotype of rs2596542 and were associated with a high risk of HCC progression. The same group identified 2 SNPs in the *MHC* region that were associated with progression from chronic hepatitis to cirrhosis. These SNPs were located at rs910049 and rs3135363 on chromosome 6p21.3[31]. Imputation-based association analysis showed that *HLA-DQA1*0601* and *HLA-DPB1*0405* were associated with progression of cirrhosis (Table 2).

FUTURE DIRECTIONS

Ongoing association studies are evaluating the effects of genetic variations on the outcomes of hepatitis virus infection in large groups of patients. However, most SNPs identified by association studies did not link to phenotype, and many other SNPs remained untyped. Imputation-based association analysis exploits information on patterns of multi-marker correlation ("linkage disequilibrium") from publically available databases to estimate ("impute") patient genotypes associated with identified SNPs and thereby assess the relations of such genotypes to phenotypes[32,33]. Owing to this method, the relations between SNPs and *HLA* haplotypes associated with the outcomes of HBV or HCV infection are becoming clearer.

In HBV infection, conventional genotyping showed that *HLA* class II, DR and DQ haplotypes were the most important regions of host genetic factors for outcomes. However, GWAS showed that rs3077 SNP near *HLA-DPA1* gene and rs9277535 SNP near *HLA-DPB1* gene were associated with persistent HBV infection in Asian populations[7,9,12,13]. *HLA-DPA1* and *DPB1* have also been associated with responsiveness to HB vaccination[10,34,35]. To date, however, few studies have focused on *HLA-DP* because polymorphisms of *HLA-DP* haplotype do not vary greatly as compared with other loci of *HLA*[36]. The structures of *HLA-DP* and *HLA-DP* molecules are similar to those of other *HLA* class II molecules. Therefore, similar to the functions of other *HLA* class II molecules, *HLA-DP* and *HLA-DP* molecules might affect the ability of *HLA* class II molecules to present antigens to CD4-positive helper T cells and result in immune response to HBV. Recently, *HLA-DPA1* and *HLA-DPB1* mRNA expressions in normal liver were respectively associated with SNP types rs3077 and rs9277535 in European populations. The mRNA expressions of *HLA-DPA1* and *HLA-DPB1* were low in genotypes rs3077-G and 9277535-G, which were associated with a high risk of persistent HBV infection[37]. However, another study in European- and African-Americans showed that rs9277534 of the *HLA-DPB1* allele (496-A/G) was a novel variant associated with persistent HBV infection[38]. In contrast to the former study, the 496-GG genotype was associated with both higher mRNA expression of *HLA-DP* and persistent HBV infection.

Inconsistent results have been obtained for the association between *HLA* alleles and HCC in patients with HBV infection. In Han Chinese populations, several SNPs in *HLA* class II have been associated with progression of HCC. However, no common SNP was confirmed by independent researchers. In addition, SNPs in chromosomes 1p36.22, 2q32.2, and 21q21.3, were also associated with HBV-related HCC[39]. Further examinations are definitely required to elucidate the role of *HLA* loci on the progression of HCC in patients with HBV.

GWAS indicated that *HLA* loci are related to important host factors involved in several aspects of HCV

infection. First, *HLA DQB1*0301* was reported to be independently associated with spontaneous clearance of HCV infection. Previous HLA haplotype analysis showed that *HLA DQB1*0301* was associated with HCV clearance in French females, African-Americans, and Italian populations[40-42]. Thus, GWAS confirmed the results of previous results. However, the mechanism by which such alleles affect HCV clearance remains undetermined.

In the Japanese population, rs4273729 SNP near *HLA DQB1*0301* and *MICA* SNP in - *HLA* class Ⅲ were respectively associated with progression of hepatitis to cirrhosis[31] and HCC[30] in patients with HCV. This is attractive information for the prediction of clinical course, but several issues remain to be defined. First, HCC most frequently develops in cirrhotic patients infected with HCV. It is not known why different SNPs are identified in continuous pathological conditions such as HCC and hepatic cirrhosis in patients with HCV. Next, an intronic SNP in the *DEPDC-5* gene, without an *HLA* locus, was also associated with HCC development in the Japanese population[43]. In European populations, several SNPs without *HLA* loci were associated with the progression of hepatic fibrosis[44]. The progression of chronic hepatitis C has been confirmed to depend on multiple factors, including age, gender, infection period, obesity, alcohol intake, and treatment[45]. It is suspected that the effects of *HLA* loci on fibrosis progression or the development of HCC (or both) differ in the each population studied.

In conclusion, genome association analysis of large numbers of cohorts indicated that *HLA* loci are one of the most important host determinants of the clinical characteristics of HBV and HCV infections, acting in conjunction with factors such as viral load, viral genotype, age, alcohol intake, and hepatic fibrosis. However, it is necessary to validate reported SNPs on *HLA* loci in global populations and to elucidate *HLA*-allele-regulated molecular responses to hepatitis virus infection.

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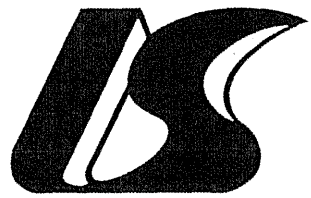

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ISSN 1007-9327

J Gastroenterol (2013) 48:397–404
DOI 10.1007/s00535-012-0645-5

ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Entecavir and interferon-α sequential therapy in Japanese patients with hepatitis B e antigen-*positive* chronic hepatitis B

Masaru Enomoto · Shuhei Nishiguchi · Akihiro Tamori · Sawako Kobayashi · Hiroki Sakaguchi · Susumu Shiomi · Soo Ryang Kim · Hirayuki Enomoto · Masaki Saito · Hiroyasu Imanishi · Norifumi Kawada

Received: 8 April 2012 / Accepted: 5 July 2012 / Published online: 2 August 2012

Abstract
Background The outcomes of sequential therapy with lamivudine followed by interferon have been unsatisfactory in Japanese patients with hepatitis B envelope antigen (HBeAg)-*positive* chronic hepatitis B. However, the efficacy of sequential therapy with entecavir and interferon remains unclear.
Methods Twenty-four HBeAg-*positive* patients (23 men and 1 woman; mean age 39 ± 7 years) received entecavir 0.5 mg alone for 36–52 weeks, followed by entecavir plus interferon-α for 4 weeks, and lastly by interferon-α alone for 20 weeks. Twenty-three patients had genotype C infection, and one had genotype A infection.
Results No entecavir-resistant mutant variants emerged in any patient. Hepatitis flare occurred in three patients during interferon-α treatment after the withdrawal of entecavir, but none had hepatic decompensation. Serum hepatitis B surface antigen levels did not change during or after therapy. Serum hepatitis B core-related antigen levels were significantly decreased at the start ($P < 0.0001$) and at the end of interferon-α treatment ($P < 0.0001$), but returned to baseline levels after treatment. Twenty-four weeks after the completion of the sequential therapy, a sustained biochemical, virological, and serological response was achieved in 5 (21 %) patients. The proportion of patients in whom HBeAg was lost during entecavir treatment was significantly higher among those with a sustained response than among those with no response ($P = 0.015$).
Conclusions The rate of response to sequential therapy with entecavir and interferon-α in Japanese patients with HBeAg-*positive* chronic hepatitis B was not higher than the rate in previous studies of lamivudine followed by interferon.

Keywords Chronic hepatitis B · Genotypes · Interferon-α · Entecavir · Sequential therapy

For the B-SHOT Study Group.
Other members of the B-SHOT Study Group are listed in the Appendix.

M. Enomoto · A. Tamori · S. Kobayashi · H. Sakaguchi · N. Kawada (✉)
Department of Hepatology, Osaka City University Graduate School of Medicine, 1-4-3 Asahimachi, Abeno-ku, Osaka 545-8585, Japan
e-mail: kawadanori@med.osaka-cu.ac.jp

S. Nishiguchi · H. Enomoto · M. Saito · H. Imanishi
Department of Internal Medicine, Hyogo College of Medicine, Hyogo, Japan

S. Shiomi
Department of Nuclear Medicine, Osaka City University Graduate School of Medicine, Osaka, Japan

S. R. Kim
Department of Gastroenterology, Kobe Asahi Hospital, Hyogo, Japan

Introduction

Infection with hepatitis B virus (HBV) remains an important public health problem and a leading cause of liver-related morbidity worldwide [1, 2]. The natural course of chronic HBV infection acquired perinatally or during infancy consists of three distinct phases: 'immune tolerant', 'immune reactive', and 'inactive carrier'. During the immune-reactive phase, rises in alanine aminotransferase (ALT) are attributable to the host's immune response to HBV, and the occurrence of hepatitis will eventually be followed by spontaneous seroconversion from hepatitis B

envelope antigen (HBeAg) to anti-HBe. HBeAg seroconversion usually results in clinical remission and a life-long inactive state; however, patients with persistently detectable HBeAg and high HBV DNA levels who have recurrent hepatitis flares are at increased risk of developing cirrhosis and hepatocellular carcinoma [3, 4].

Currently available antiviral treatment for chronic hepatitis B includes nucleos(t)ide analogues such as lamivudine, adefovir, entecavir, and tenofovir, and the immunomodulator interferon [5–7]. The direct, potent antiviral effects of nucleos(t)ide analogues induce biochemical and virological responses in most patients, but viral relapse and exacerbations of hepatitis commonly occur after discontinuation of treatment. Long-term use of nucleos(t)ide analogues is associated with the emergence of drug-resistant variants possessing mutations in the HBV polymerase gene. In contrast, interferon-induced remission of chronic hepatitis B is durable, but is achieved in only a minority of patients. In randomized controlled trials, concomitant treatment with lamivudine and interferon-α has offered little clinical benefit, in terms of the rates of sustained therapeutic response, as compared with interferon-α alone [8, 9].

Serfaty et al. [10] reported that sequential therapy with lamivudine followed by interferon-α was effective in patients with chronic hepatitis B. In their pilot study in France, sustained virological and biochemical response was achieved in 8 (57 %) of the 14 patients who received lamivudine 100 mg alone for 20 weeks, followed by interferon-α 5 MU 3 times/week plus lamivudine for 4 weeks, and lastly by interferon-α alone for 24 weeks [10]. Some other groups have studied similar protocols for sequential therapy, but results have been conflicting [11–17]. The inconsistent results may have been caused, at least in part, by differences in the included HBV genotypes among studies, because HBV genotypes have specific geographic distributions and can affect the response to interferon [18, 19]. In our previous study [14], the rate of response to sequential therapy with lamivudine and interferon in 24 Japanese HBeAg-*positive* patients with chronic HBV genotype C infection was 29 %, considerably lower than the rate reported by Serfaty et al. [10].

Randomized controlled trials have shown that entecavir has higher antiviral activity against HBV than lamivudine [20, 21]. Among licensed nucleos(t)ide analogues, entecavir is used as a first-line treatment of choice for chronic hepatitis B, similar to tenofovir disoproxil fumarate [22]. Use of a potent nucleoside analogue before the initiation of interferon may improve the outcomes of sequential therapy.

In this study, we evaluated the efficacy of sequential therapy with entecavir and interferon-α in Japanese patients with HBeAg-*positive* chronic hepatitis B. In addition to the monitoring of serum HBeAg and HBV DNA levels, serum hepatitis B surface antigen (HBsAg) and hepatitis B core-related antigen (HBcrAg) [23, 24] levels were monitored during and after sequential therapy. The clinical characteristics of patients who had a sustained response to the sequential therapy were compared with those of patients who had no response.

Patients and methods

Patients

The subjects were 24 Japanese patients with HBeAg-*positive* chronic hepatitis B (23 men and 1 woman; mean age 39 ± 7 years) who had received sequential therapy with entecavir alone and then entecavir plus interferon-α followed by interferon-α alone between September 2006 and August 2011. The inclusion criteria were as follows: (1) persistent or fluctuating elevations of serum ALT levels for at least 6 months before the start of therapy; (2) presence of HBsAg in serum; (3) presence of HBeAg and absence of anti-HBe; (4) presence of HBV DNA $>10^5$ copies/mL (equivalent to 20,000 IU/mL); (5) no use of corticosteroids or immunomodulatory drugs, including interferon, within 1 year before the start of therapy; (6) no use of nucleos(t)ide analogues, such as lamivudine, within 1 year before the start of therapy; (7) absence of resistance to nucleos(t)ide analogues; (8) absence of antibodies to hepatitis C virus and other likely causes of chronic liver disease; and (9) no clinical signs of decompensated cirrhosis or hepatocellular carcinoma. The study procedures were in accordance with the Helsinki Declaration of 1975 (1983 revision) and were approved by the ethics committee of each participating center. Written informed consent was obtained from each patient. This study was registered in the UMIN Clinical Trials Registry (registration ID number, UMIN000000808).

Treatment

Patients were treated with entecavir alone for 36–52 weeks, followed immediately by both entecavir and interferon-α for 4 weeks, and lastly by interferon-α alone for 20 weeks. Entecavir (Baraclude; Bristol-Myers, Tokyo, Japan) was given orally at a dose of 0.5 mg once daily. Natural interferon-α (Otsuka Pharmaceutical, Tokyo, Japan) was given by intramuscular injection, at a dose of 5 MU, three times a week for 24 weeks (a protocol commonly used in Japan during the study period). All patients were followed up for at least 24 weeks after the completion of treatment, and responses to therapy were assessed as follows: *biochemical response* was defined as a decrease in

serum ALT levels to within the normal range; *virological response* was defined as a decrease in serum HBV DNA to $<10^4$ copies/mL; and a *serological response* was defined as loss of serum HBeAg. A sustained response was defined as fulfillment of the criteria for combined biochemical, virological, and serological responses 24 weeks after the end of therapy.

Assays

The following variables were determined for all enrolled patients: complete blood counts; serum ALT level; HBsAg, HBeAg, anti-HBe, HBcrAg, and HBV DNA levels; HBV genotypes; proportion of mutants in the precore and basal core promoter regions of HBV DNA; and drug-resistant mutations in the HBV polymerase gene.

Complete blood counts and serum ALT (upper limit of normal, 30 IU/L) were determined by standard procedures. HBsAg was measured with a chemiluminescent microparticle immunoassay (Architect HBsAg QT; Abbott Japan, Tokyo, Japan) as described elsewhere [25]. HBeAg and anti-HBe were detected with chemiluminescence enzyme immunoassays. HBcrAg was also detected with a chemiluminescence enzyme immunoassay (Fuji-Rebio, Tokyo, Japan) [23]. HBV DNA was measured with a real-time polymerase chain reaction (PCR) assay (COBAS TaqMan HBV Test v2.0; Roche Diagnostics, Tokyo, Japan) [26]. Genotypes of HBV were identified by enzyme-linked immunosorbent assay with monoclonal antibodies to type-specific epitopes in the preS2-region (Institute of Immunology, Tokyo, Japan) [27]. Mutations at nucleotide (*nt*) 1896 in the precore region and at *nt* 1762 and *nt* 1764 in the basal core promoter region of HBV DNA were found by means of an enzyme-linked minisequence assay (Genome Science Laboratory, Tokyo, Japan). Drug-resistant mutations (at codons 180, 181, 184, 202, 204, 236, and 250 of the HBV reverse transcriptase domain) were detected by PCR-Invader technology (BML, Tokyo, Japan) [28].

Histopathology

When informed consent had been obtained, a liver biopsy was performed before the patient started therapy. Histopathological findings were assessed by grading inflammatory activity and staging fibrosis according to the METAVIR scoring system [29]. An experienced pathologist blinded to the clinical data performed these evaluations.

Statistical analysis

Statistical analysis was performed with SAS, version 9.2 for Windows (SAS Institute, Cary, NC, USA). Distributions of continuous variables were analyzed with the non-parametric Mann–Whitney *U*-test. Differences in proportions were tested by Fisher's exact test. The significance of changes in values between two time points was evaluated by the Wilcoxon signed-rank test. A two-tailed *P* value of less than 0.05 was considered to indicate statistical significance.

Results

Rate of response to therapy

Although common interferon-α-related side effects included pyrexia, fatigue, headache, and myalgia, the therapy was well tolerated, and all patients completed the treatment according to the protocol. The proportions of patients with biochemical, virological, and serological responses during and after sequential therapy with entecavir and interferon-α are shown in Fig. 1. Drug-resistant mutant variants did not emerge in any patient during entecavir treatment. At the start of interferon-α treatment (about 1 year after the start of the entecavir treatment), most patients had normal ALT levels and serum HBV DNA levels of $<10^4$ copies/mL

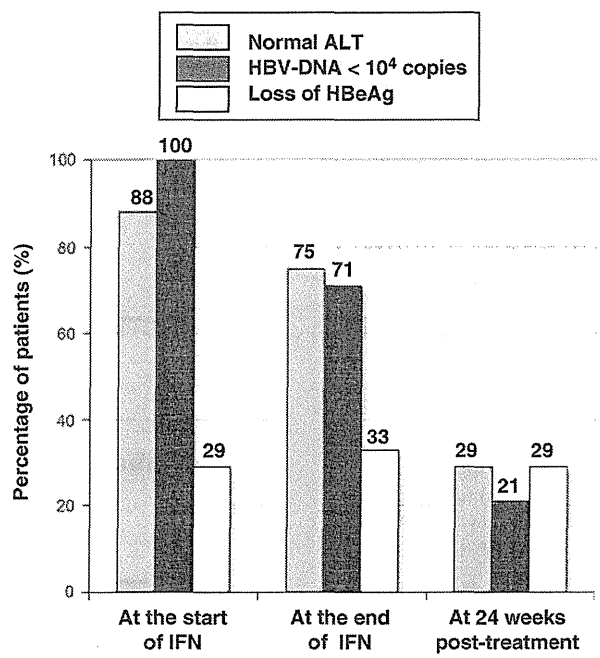

Fig. 1 Rate of biochemical, virological, and serological responses during and after sequential therapy with entecavir and interferon-α. Combined sustained biochemical, virological, and serological response was achieved in 5 (21 %) of the 24 enrolled patients 24 weeks after completion of the sequential therapy. *ALT* Alanine aminotransferase, *HBeAg* hepatitis B envelope antigen, *HBV* hepatitis B virus, *IFN* interferon

(88 and 100 %, respectively). However, loss of HBeAg was achieved in a minority of patients (29 %) during the entecavir treatment.

In most patients without HBeAg loss at the end of the entecavir treatment, serum ALT and HBV DNA levels increased even during the interferon-α treatment. Hepatitis flare (defined as a rise in ALT equivalent to 10 times higher than the upper limit of normal and more than twice the baseline value) occurred in 3 patients after the withdrawal of entecavir. Although peak ALT levels in these patients were 693, 721, and 876 IU/L, respectively, none had jaundice or hepatic decompensation. At the end of the interferon-α treatment, the percentages of patients with normal ALT, HBV DNA $<10^4$ copies/mL, and loss of HBeAg were 75, 71, and 33 %, respectively.

Lastly, 24 weeks after the completion of the sequential therapy, a sustained biochemical, virological, and serological response was achieved in 5 (21 %) of the 24 patients. No patient had loss of serum HBsAg in response to the sequential therapy.

Changes in HBsAg and HBcrAg during and after sequential therapy

Changes in serum HBsAg and HBcrAg levels during and after the sequential therapy with entecavir and interferon-α are shown in Fig. 2. The serum HBsAg level did not change significantly during or after the therapy (Fig. 2a). The serum HBcrAg levels were significantly decreased at the start ($P < 0.0001$) and at the end of interferon-α treatment ($P < 0.0001$), but returned to baseline levels after completion of the sequential treatment (Fig. 2b). The serum HBsAg level did not differ significantly between patients with a sustained response and those with no response (Fig. 2c). In contrast, the serum HBcrAg level was significantly lower in patients with a sustained response than in those with no response at the end of the interferon-α therapy ($P = 0.013$) and 24 weeks post-treatment ($P = 0.031$) (Fig. 2d).

Characteristics of patients at the start of entecavir treatment

The baseline demographic, biochemical, virological, and histological characteristics of patients at the start of entecavir treatment, classified according to the response to sequential therapy, are listed in Table 1. The mean age of patients with a sustained response was more than 10 years less than that of the patients with no response, but this difference did not reach statistical significance ($P = 0.102$). There were no significant differences between the two groups with respect to sex ratio, proportion of patients with a history of interferon treatment, ALT level, HBV DNA level, ratios of HBV genotypes, ratios of precore or basal core promoter mutants, or histopathological findings in the liver.

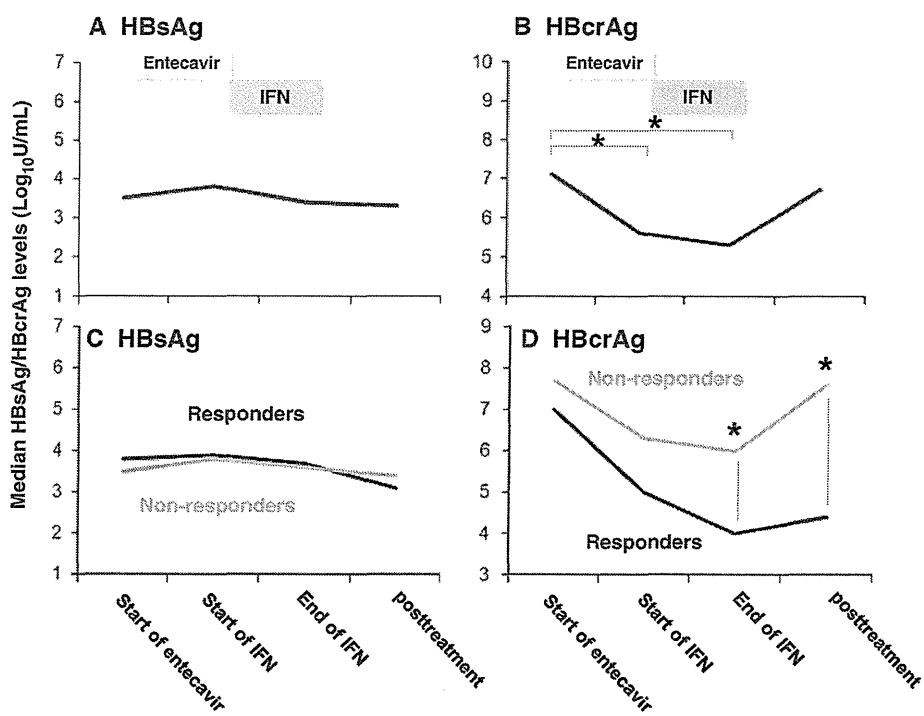

Fig. 2 Changes in serum levels of hepatitis B surface antigen (*HBsAg*) and hepatitis B core-related antigen (*HBcrAg*) during and after sequential therapy with entecavir and interferon-α. Serum HBsAg levels did not change during or after therapy (**a**). As compared with the baseline value, the serum HBcrAg level was significantly decreased at the start ($P < 0.0001$) and at the end of interferon-α treatment ($P < 0.0001$) (*asterisks*) (**b**). When sustained responders were compared with non-responders, there was no significant difference in the serum HBsAg level (**c**). In contrast, the serum HBcrAg level was significantly lower in sustained responders than in non-responders at the end of interferon-α therapy ($P = 0.013$) and 24 weeks post-treatment ($P = 0.031$) (*asterisks*) (**d**)

Table 1 Baseline characteristics of patients at the start of entecavir treatment

Characteristics	Sustained responders ($n = 5$)	Non-responders ($n = 19$)	P values
Age (years)	29 ± 6	41 ± 5	0.10
Male sex (%)	5 (100 %)	18 (95 %)	0.99
History of interferon treatment (%)	3 (60 %)	12 (63 %)	0.99
ALT (IU/L)	85 (65, 322)	210 (79, 531)	0.37
HBV DNA ($\log_{10}$ copies/mL)	7.7 ± 0.4	7.8 ± 0.8	0.31
Genotype (A/B/C/D)	0/0/5/0	1/0/18/0	0.99
Precore (wild/mixed/mutant)	0/4/1	9/9/1	0.12
Basal core promoter (wild/mixed/mutant)	1/0/4	5/8/6	0.070
Grade of inflammation (mild/moderate/severe)	2/3/0	9/7/2	0.60
Stage of fibrosis (mild/moderate/severe/cirrhosis)	2/2/0/1	10/5/3/0	0.19

Values are means ± SDs for normally distributed variables, and medians (with the interquartile range) for non-normally distributed variables

ALT alanine aminotransferase, *HBV* hepatitis B virus

Table 2 Characteristics of patients at the start of interferon-α treatment

Characteristics	Sustained responders ($n = 5$)	Non-responders ($n = 19$)	P values
ALT (IU/L)	24 (23, 35)	20 (15, 32)	0.27
ALT normal (%)	5 (100 %)	16 (84 %)	0.99
HBV DNA ($\log_{10}$ copies/mL)	2.1 ± 0.3	2.3 ± 0.4	0.18
HBV DNA negative (%)	3 (60 %)	6 (32 %)	0.33
HBeAg loss (%)	4 (80 %)	3 (16 %)	0.015

Values are means ± SDs for normally distributed variables, and medians (with the interquartile range) for non-normally distributed variables

HBeAg hepatitis B envelope antigen

Characteristics of patients at the start of interferon-α treatment

The characteristics of the patients at the start of interferon-α treatment, classified according to the response to sequential therapy, are shown in Table 2. The responders and non-responders did not differ significantly with respect to ALT level or HBV DNA level at the start of interferon-α treatment. The proportion of patients in whom HBeAg was lost during entecavir treatment was significantly higher among those with a sustained response than among those with no response ($P = 0.015$). In another comparison, a sustained response was achieved in 4 (57 %) of the 7 patients with loss of HBeAg during entecavir treatment, as compared with 1 (5.9 %) of the 17 patients without loss of HBeAg during treatment; this difference was also statistically significant ($P = 0.015$).

Case presentation

A 24-year-old man with no response to previous treatment with interferon-α was referred to us (Fig. 3). His ALT level

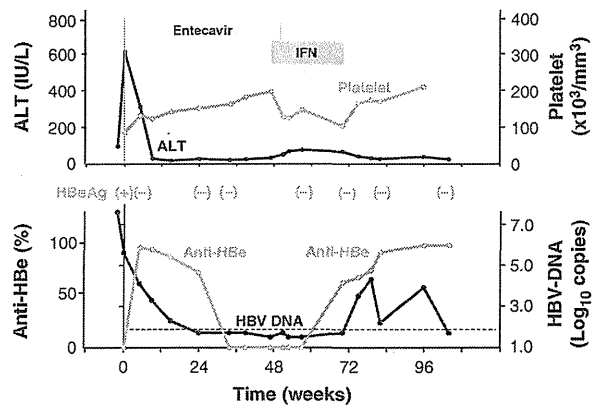

Fig. 3 Changes in platelet count, ALT, HBeAg, anti-HBe, and HBV DNA in a 24-year-old man with sustained response to sequential therapy with entecavir and interferon-α. In the *upper panel*, the changes in ALT levels (*filled circles*) and platelet counts (*open circles*) are shown. In the *lower panel*, the changes in HBV DNA (*filled circles*) and anti-HBe (*open circles*) titers are shown. During 1 year of entecavir treatment, the platelet count rose from 87,000 to 199,000/mm^3. After the patient was switched to interferon-α, his anti-HBe antibody titer increased. At the most recent hospital visit, the patient's ALT level was normal, HBeAg was negative, and HBV DNA was negative; the patient has remained drug-free since the completion of treatment

was 617 IU/L, HBV DNA level was 7.6 $\log_{10}$ copies/mL, and HBV genotype was C. A precore stop codon mutation at *nt* 1896 and basal core promoter mutations at *nt* 1762 and *nt* 1764 were detected. A liver biopsy showed moderate inflammation and cirrhosis. Although the patient was young, interferon-α was not indicated because of a low platelet count and concern about exacerbation of hepatitis. However, during 1 year of entecavir treatment, his ALT level became normal, and his platelet count rose from 87,000 to 199,000/mm^3. After switching to interferon-α, his HBV DNA rose transiently, but his anti-HBe antibody titer increased. At the most recent hospital visit (up to 35 weeks after the completion of treatment), his ALT level was normal and HBeAg and HBV DNA were negative; the

patient has remained drug-free since the completion of treatment.

Discussion

Several groups have evaluated protocols for sequential therapy with lamivudine and interferon-α, and their protocols were similar to that originally described by Serfaty et al. [10]. Manesis et al. [11], from Greece, where HBV genotype D is predominant, found that in HBeAg-*negative* patients, the rate of sustained biochemical and virological response was 22 %, which did not differ from that obtained in an age/sex-matched historical control group treated with interferon-α alone. In another report from Greece [12], sequential combination therapy significantly prevented the emergence of resistance to lamivudine, but the rate of sustained virological response was only 17 % among HBeAg-*negative* patients. A group from China, where genotype B or C is predominant, reported very similar results [13]. To date, only the study by Moucari et al. [17] has used adefovir dipivoxil instead of lamivudine. Sustained virological response was achieved in 50 % of their subjects, although only 20 HBeAg-*negative* patients were included.

In Japan and other countries in East Asia, genotype C is the most prevalent type of HBV [18, 19], and most patients with chronic hepatitis B acquire the virus perinatally or in early childhood [7]. The rates of response to interferon are thus lower than those reported in Europe and the United States. In our previous study [14], using a sequential therapy protocol similar to that described by Serfaty et al. [10], we found that the rate of sustained response was only 29 % among 24 HBeAg-*positive* patients. The patients with a sustained response were significantly younger and had a significantly lower HBV DNA level at the start of interferon than did those with no response. The rate of HBeAg loss during lamivudine treatment was slightly but not significantly higher among sustained responders than among non-responders. Minami and Okanoue [15] also found that patients who lost HBeAg during lamivudine treatment were more likely to have a sustained response to sequential therapy. Okuse et al. [16] reported that sequential therapy was effective for patients with acute exacerbations of chronic hepatitis B, particularly those in whom HBeAg had become negative during lamivudine treatment.

One objective of sequential therapy is to lower the viral load by the use of a nucleos(t)ide analogue, thereby restoring sensitivity to interferon treatment. In clinical studies, a low HBV DNA level is predictive of a favorable response to interferon-α [30, 31]. In basic studies, a high viral load is associated with T-cell hyporesponsiveness [32], and treatment with nucleos(t)ide analogues restores cellular immune response in chronic HBV infection [33]. Although lamivudine had been administered for about half a year before the start of interferon administration in previous studies (including ours) [10–16], we administered entecavir, a more potent antiviral agent, for about 1 year in the present study. Treatment with entecavir was given for a longer period because it has been reported in previous studies that patients in whom HBeAg and HBV DNA levels were lowered by lamivudine were more likely to have a sustained response and because few entecavir-resistant variants emerge within the first few years [34]. However, the use of entecavir for a longer duration did not raise the rate of off-treatment sustained response to sequential therapy in the present study, although the rate of on-treatment biochemical and virological responses was higher with entecavir than that obtained with lamivudine in our previous study [14].

Another objective of sequential therapy is to prevent the relapse of hepatitis after discontinuation of the nucleos(t)ide analogue through the use of interferon-α. Nucleos(t)ide analogues rapidly decrease serum HBV DNA levels by suppressing the reverse transcription of pregenomic HBV RNA, but viral relapse commonly occurs after the cessation of treatment. This high risk of viral relapse may be attributed to the persistence of HBV replicative intermediate covalently closed circular DNA (cccDNA) in the liver even during nucleos(t)ide treatment. The measurement of HBV antigens in serum is thus clinically important as a surrogate marker of intrahepatic cccDNA. In particular, a decline in serum levels of HBsAg is strongly associated with response to interferon-α [35]. The HBcrAg assay measures serum levels of all antigens transcribed from the precore/core gene, including hepatitis B core and e antigens, by using monoclonal antibodies that recognize common epitopes of the denatured antigens [23, 24]. Matsumoto et al. [36] recently proposed a model for predicting relapse of hepatitis after discontinuation of nucleos(t)ide analogue administration, in which cut-off values were set at 1.9–2.9 $\log_{10}$ IU/mL of HBsAg and 3.0–4.0 $\log_{10}$ U/mL of HBcrAg at the withdrawal of treatment. In our study, only one patient had a decrease in HBsAg to between 1.9 and 2.9 $\log_{10}$ IU/mL and another one had a decrease in HBcrAg to between 3.0 and 4.0 $\log_{10}$ U/mL at the withdrawal of entecavir (data not shown), probably because of an insufficient duration of entecavir treatment in our protocol. The finding that at least 21 % of our patients with insufficient HBsAg and HBcrAg decline during entecavir treatment achieved a sustained response to sequential therapy suggests that switching to interferon-α contributes to the safe termination of nucleos(t)ide analogue treatment in some patients.

The major advantages of interferon-α include a finite course of treatment, the opportunity to obtain an off-treatment durable response to therapy, and absence of drug resistance. The advantages of nucleos(t)ide analogues include good tolerance and potent antiviral activity associated with high rates of on-treatment response to therapy. Guidelines proposed by the Japanese Study Group of the Standardization of Treatment of Viral Hepatitis basically recommend interferon-α as the first-line treatment for patients with chronic hepatitis B who are younger than 35 years, to attain a 'drug-free state'; and entecavir for patients who are 35 years or older, to persistently suppress HBV DNA [37]. Consistent with the findings of previous studies [14–16], our results show that sequential therapy is best indicated for patients who have lost HBeAg during nucleoside analogue treatment, because such patients have a higher probability of a sustained response. As shown in Fig. 3, patients who are young but have exacerbation of hepatitis, cirrhosis, or both, were also good candidates for sequential therapy, because interferon-α is generally not recommended for such patients because of concern about hepatic decompensation, and the preceding use of a nucleos(t)ide analogue can reduce such risk.

Our study had several limitations. First, it was not a randomized controlled trial. The reported rate of HBeAg seroconversion obtained by 6-month interferon-α monotherapy among Japanese patients was about 20 % [38], which is similar to the rate obtained by the sequential therapy used in our study (21 %). As compared with our previous study of lamivudine [14], the rate of sustained response in our present study of entecavir did not differ significantly (21 % in the entecavir group vs. 29 % in the lamivudine group). Although the patients were not randomly assigned to treatment, the baseline characteristics of the subjects did not differ between those in our previous study of lamivudine and those in the present study of entecavir with respect to mean age, sex ratio, ALT level, HBV DNA level, ratios of HBV genotypes, ratios of precore or basal core promoter mutants, or histopathological findings (data not shown). Thus, we cannot conclude that sequential therapy with entecavir and interferon-α is more effective than interferon-α monotherapy or sequential therapy with lamivudine and interferon-α. Second, we gave patients non-pegylated interferon-α for 6 months, because pegylated interferon-α had not been approved for the treatment of chronic hepatitis B by the Japanese medical insurance system during the study period. Further studies are thus needed to evaluate the efficacy of sequential therapy with entecavir and pegylated interferon-α.

To our knowledge, this is the first study to report on the response to sequential therapy with entecavir and interferon-α in patients with chronic hepatitis B. In summary, an off-treatment sustained response to sequential therapy with entecavir and interferon-α was achieved in 21 % of HBeAg-*positive* patients with chronic hepatitis B in Japan, where genotype C is predominant. This rate of response was not higher than that in our previous study using lamivudine [14]. Patients who had loss of HBeAg during entecavir treatment were more likely to have a sustained response to sequential therapy.

Acknowledgments The authors are grateful to Ms. Sanae Deguchi, Ms. Rie Yasuda, and Ms. Ami Saito for their technical assistance. This work was supported in part by a grant from the Ministry of Health, Labour and Welfare, Japan.

Conflict of interest Dr. Shuhei Nishiguchi has received research grants from Bristol-Myers K.K. and Otsuka Pharmaceutical Co., Ltd. Dr. Norifumi Kawada has received research grants from Bristol-Myers K.K. and Otsuka Pharmaceutical Co., Ltd.

Appendix

In addition to the authors, the B-SHOT Study Group includes the following persons: Kohshun Kim, Motoharu Tanaka (Higashi Sumiyoshi Morimoto Hospital, Osaka, Japan); Hideji Nakamura, Hiroko Iijima, Soji Shimomura, Noritoshi Koh, Yoshinori Iwata, Yoshiyuki Sakai, Tomoyuki Takashima (Hyogo College of Medicine, Nishinomiya, Japan); Tetsuo Yamamoto (Kita-Osaka Police Hospital, Osaka); Hirokazu Kadoya (Izumiotsu Municipal Hospital, Osaka); Chika Kawamura (Ohno Memorial Hospital, Osaka); Kiyohide Kioka, Yasuko Kawasaki (Osaka City General Hospital, Osaka); Osamu Kurai, Hiroko Oka (Osaka City Juso Hospital, Osaka); Daiki Habu, Hiroyasu Morikawa, Shuji Iwai, Hideki Fujii (Osaka City University Medical School, Osaka); Katsuhiko Fukuda (PL Hospital, Osaka); Machiko Shintani (Sumiyoshi Municipal Hospital, Osaka); and Hisato Jomura (Wakakokai Hospital, Osaka).

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J Gastroenterol (2013) 48:999–1005
DOI 10.1007/s00535-012-0742-5

REVIEW

Combination therapy with a nucleos(t)ide analogue and interferon for chronic hepatitis B: simultaneous or sequential

Masaru Enomoto · Akihiro Tamori · Shuhei Nishiguchi · Norifumi Kawada

Received: 2 October 2012 / Accepted: 11 December 2012 / Published online: 22 January 2013

Abstract Currently available antiviral treatment for chronic hepatitis B virus infection can be divided into two classes of therapeutic agents: nucleos(t)ide analogues (NAs) and interferon (IFN). The major advantages of NAs are good tolerance and potent antiviral activity associated with high rates of on-treatment response to therapy; the advantages of IFN include a finite course of treatment, absence of drug resistance, and an opportunity to obtain a post-treatment durable response to therapy. The use of these two antiviral agents with different mechanisms of action in combination is theoretically an attractive approach for treatment. Here, we have reviewed previous reports of either simultaneous or sequential combination therapy with NA and IFN for chronic hepatitis B patients. In previous studies comparing the lamivudine/IFN combination and lamivudine monotherapy in a finite course, combination therapy was associated with higher rates of sustained post-treatment response and lower rates of drug resistance than lamivudine monotherapy. However, NAs such as lamivudine are generally administered indefinitely because of high rates of post-treatment relapse. In addition, concern for drug resistance has decreased significantly with newer, high-potency NAs even when administered alone. In previous studies comparing the lamivudine/IFN combination and IFN monotherapy, the combination therapy showed greater on-treatment viral suppression, but no difference was observed in the post-treatment sustained response. Thus, whether combination therapy confers an additional benefit compared to monotherapy for treating chronic hepatitis B remains unclear. The efficacy of IFN in combination with a more potent NA, such as entecavir or tenofovir, remains to be comprehensively evaluated.

Keywords Chronic hepatitis B · Lamivudine · Adefovir · Entecavir · Interferon · Nucleos(t)ide analogue

Abbreviations

ALT	Alanine aminotransferase
CccDNA	Covalently closed circular DNA
HBcrAg	Hepatitis B core-related antigen
HBeAg	Hepatitis B e antigen
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
IFN	Interferon
NA	Nucleos(t)ide analogue

Introduction

More than 350 million people worldwide are infected with hepatitis B virus (HBV) which is a leading cause of liver-related morbidity that accounts for 1 million deaths annually [1, 2]. Currently available antiviral treatment for chronic hepatitis B can be divided into two classes of therapeutic agents: nucleos(t)ide analogues (NAs) and interferon (IFN). Nucleosides include lamivudine [3, 4], telbivudine [5], and entecavir [6, 7]; nucleotides include adefovir [8, 9] and tenofovir [10]. The direct, potent antiviral effects of NAs induce an on-treatment response in

M. Enomoto (✉) · A. Tamori · N. Kawada
Department of Hepatology,
Osaka City University Graduate School of Medicine, 1-4-3 Asahimachi, Abeno-ku, Osaka 545-8585, Japan
e-mail: enomoto-m@med.osaka-cu.ac.jp

S. Nishiguchi
Department of Internal Medicine,
Hyogo College of Medicine, Nishinomiya, Hyogo, Japan

most patients, but post-treatment relapse commonly occurs after treatment discontinuation. Long-term use of NAs triggers the emergence of drug-resistant variants possessing mutations in the hepatitis B virus (HBV) polymerase gene. Among the NAs currently available, entecavir or tenofovir is recommended as the first-line treatment because of the low rate of drug resistance. In contrast, IFN has both antiviral and immunomodulatory actions [11, 12]. The major advantages of IFN include a finite course of treatment, absence of drug resistance, and an opportunity to obtain a post-treatment durable response to therapy; however, a response to IFN is achieved in only a minority of patients with chronic hepatitis B.

In this article, we have reviewed previous reports on combination therapy with NA and IFN for chronic hepatitis B. Regimens of combination therapy can be classified into two main groups: (1) simultaneous therapy with the drugs in the combination and (2) sequential combination therapy in which treatment with one drug follows that of the previously administered one. To compare the results of previous trials, we noted that age/sex of the included subjects, HBV genotypes, and mode of vial transmission varies among the different studies. These differences may affect the results and their interpretations as older age, male sex, HBV genotypes C and D (vs. A and B), and vertical transmission are associated with a poor response to IFN therapy [13–15]. In particular, HBV genotypes have specific geographic distributions, with genotype A being prevalent in Northwest Europe, North America, and Central Africa, genotypes B and C being common in Southeast Asia, China, Japan, and Oceania, and genotype D being prevalent in Southern Europe, the Middle East, and India, although it has a nearly worldwide distribution.

Theoretical background

Nucleos(t)ide analogues directly inhibit viral replication by targeting at least one of the three replication steps: priming of HBV DNA polymerase, reverse transcription of negative-strand HBV DNA from pregenomic RNA, and synthesis of positive-strand HBV DNA. IFN also possesses antiviral activity but does not act directly on the virus or replication complex. Instead, it acts by inducing IFN-stimulated genes to establish a non-virus-specific antiviral state within the cell. In addition to their role as antivirals, IFNs are important immunomodulators that interact with the adaptive and innate immune responses. Combining NA and IFN, with their different mechanisms of action, in a therapeutic regimen is theoretically an attractive approach for treating chronic hepatitis B.

The action of NAs has little or no effect on the decrease in the intrahepatic HBV replicative intermediate, covalently closed circular DNA (cccDNA). In the experimental woodchuck hepatitis virus system, cccDNA persisted even when viral production was strongly reduced by NA treatment [16, 17]. To reduce the level of intrahepatic cccDNA, the immunomodulatory activity of IFN, which presumably induces cytotoxic T cell activity for immune clearance of infected cells, may be required. However, a high HBV DNA load is associated with an inefficient T cell response to HBV-related antigens, such as hepatitis B surface antigen (HBsAg) [18]. Several studies have shown that a decreased viral load induced by NA treatment can result in the subsequent restoration of CD4 followed by CD8 cellular immune response against HBV [19, 20]. The rationale for combination therapy is based on the concept that suppression of viral replication by NA can decrease viral protein synthesis on the surface of hepatocytes, which may restore the immune response and optimize the immunomodulatory effects of IFN for clearing infected cells.

Simultaneous combination with NA and IFN

Table 1 shows a summary of previous studies examining simultaneous combination therapy with NA and IFN for chronic hepatitis B. The first trial was reported by Mutimer et al. [21] in the UK. Since this study was designed to assess the safety and tolerability of combination treatment, the duration of treatment was only 16 weeks, and few patients showed sustained seroconversion from hepatitis B e antigen (HBeAg) to anti-HBe (antibody to HBe) by this short-term therapy with lamivudine and IFN-α. Barbaro et al. [22] reported the results of a randomized trial conducted in Italy where the 24-week combination with lamivudine and IFN-α increased the rate of sustained HBeAg seroconversion compared to the 52-week lamivudine monotherapy (33 vs. 15 %; $P = 0.014$). Tatulli et al. [23] in Italy found that the 52-week combination with lamivudine and IFN-α resulted in a sustained loss of serum HBV DNA, based on the results of a solution hybridization assay, and normalization of alanine aminotransferase (ALT) in only 14 % of HBeAg-negative patients, but drug-resistant mutation variants did not emerge in any patients. However, from these previous studies, it is still unclear whether combination therapy with lamivudine and IFN confers an additional benefit compared to IFN monotherapy.

Three randomized controlled trials (2 in HBeAg-positive patients [24, 25] and 1 in HBeAg-negative patients [26]) did not show that 1-year combination therapy with lamivudine and pegylated IFN-α was superior to monotherapy with pegylated IFN-α in terms of the rate of sustained response. The results of these globally conducted

Table 1 Simultaneous combination therapy with nucleos(t)ide analogues and interferon

Reference (first author)	HBeAg	*n* (genotype)	Age (years)	Male (%)	Regimens	Biochemical response (%)	Virologic response (%)
Mutimer [21]	+	20 (N.D.)	39 ± 11[a]	95	LAM + IFN for 12–16 weeks	0	5
Barbaro [22]	+	76 (N.D.)	42 (33–50)[b]	84	LAM + IFN for 24 weeks	37	33
Tatulli [23]	−	29 (N.D.)	44 (27–64)[b]	90	LAM + IFN for 52 weeks	14	14
Janssen [24]	+	130 (A43/B11/C18/D52)	34 ± 12[a]	75	LAM + PEG for 52 weeks	35	35
Lau [25]	+	271 (A18/B82/C156/D11)	32 ± 10[a]	77	LAM + PEG for 48 weeks	39	28
Marcellin [26]	−	179 (N.D.)	41 ± 11[a]	82	LAM + PEG for 48 weeks	60	44
Wursthorn [29]	±	26 (A8/B0/C1/D14)	34 (19–55)[b]	77	ADV + PEG for 48 weeks	N.D.	N.D.
Takkenberg [30]	±	40 (A20/B2/C2/D9)	40 ± 10[a]	88	ADV + PEG for 48 weeks	N.D.	50

HBeAg hepatitis B e antigen, *LAM* lamivudine, *ADV* adefovir dipivoxil, *IFN* interferon, *PEG* pegylated interferon, *N.D.* not described

[a] Mean (± standard deviation, SD)

[b] Median (range)

trials, which included many patients with various HBV genotypes, appear to be reliable. All studies found that the combination therapy had greater on-treatment viral suppression and higher rates of sustained post-treatment response than therapy with lamivudine alone, but no difference was observed in the sustained post-treatment virologic response compared to that with pegylated IFN-α alone. Janssen et al. [24], for example, found that more patients in the patient group receiving the 52-week pegylated IFN-α + lamivudine combination than in the group receiving the 52-week pegylated IFN-α monotherapy showed a response, as assessed by serum HBeAg loss at the end of treatment (44 vs. 29 %; $P = 0.01$). However, this difference was not sustained; 35 % of the combination-therapy group and 36 % of the monotherapy group showed a sustained HBeAg loss at the end of follow-up ($P = 0.91$). The trial also showed that pegylated IFN-α therapy improves liver histology, particularly in responders to therapy, but that the addition of lamivudine to therapy with pegylated IFN-α did not further improve histological outcome [27] and that genotypes C and D are associated with a lower rate of response to IFN than genotypes A and B.

To date, few studies have examined the combination of IFN and other NAs that are more potent than lamivudine. The telbivudine + IFN combination is prohibited because of the high risk of severe polyneuropathy [28]. Interestingly, Wursthorn et al. [29] from Germany found that 48-week combination therapy with adefovir dipivoxil and pegylated IFN-α led to marked decreases in cccDNA in the liver, which has been correlated with reduced HBsAg in serum. However, the rate of the post-treatment sustained response was not reported in this study. Another group from the Netherlands [30] showed that intrahepatic cccDNA levels at the end of 48-week treatment with adefovir dipivoxil and pegylated IFN-α were predictive of a sustained response defined as HBV DNA <2,000 IU/mL and normal ALT. The efficacy of combining IFN and other NAs, such as entecavir or tenofovir, remains to be elucidated.

Sequential combination starting with IFN followed by NA

Table 2 shows a summary of previous reports concerning sequential combination therapy starting with IFN followed by NAs for chronic hepatitis B. Hasan et al. reported that the rate of sustained HBeAg seroconversion was only 6.2 % in patients in Kuwait receiving IFN-α alone for 4 weeks, followed by the IFN-α + lamivudine combination for 12 weeks, and lastly by lamivudine alone for 36 weeks; this rate was similar to that observed in patients receiving lamivudine alone for 48 weeks [31].

In contrast, a randomized trial by Chan et al. in China [32] showed that the rate of sustained virologic response, defined as HBeAg seroconversion and a HBV DNA level of <500,000 copies/mL, was 36 % in patients receiving pegylated IFN-α alone for 8 weeks, followed by the pegylated IFN-α + lamivudine combination for 24 weeks, and lastly by lamivudine alone for 28 weeks; this rate was

Table 2 Sequential combination therapy starting with IFN followed by nucleos(t)ide analogues

Reference (first author)	HBeAg	*n* (genotype)	Age (years)	Male (%)	Regimens	Biochemical response (%)	Virologic response (%)
Hasan [31]	+	32 (N.D.)	32 (17–63)[b]	88	IFN for 4 weeks, IFN + LAM for 12 weeks, and then LAM for 36 weeks	9.3	6.2
Chan [32]	+	50 (A0/B18/C35/D0)	32 (19–57)[b]	62	PEG for 8 weeks, PEG + LAM for 24 weeks, and then LAM for 28 weeks	50	36

[a] Mean (range)

[b] Median (range)

significantly higher than that observed in patients receiving lamivudine alone for 52 weeks (14 %; $P = 0.011$). At the end of the treatment period, 21 % of patients in the sequential combination treatment group developed a lamivudine-resistant mutant, compared to 40 % of patients in the lamivudine monotherapy group. Follow-up of this study demonstrated that sequential combination with pegylated IFN-α followed by lamivudine maintained a higher long-term virologic response than lamivudine monotherapy for up to 3 years [33]. However, this study did not include a study arm of pegylated IFN-α alone.

Sequential combination starting with NA followed by IFN

Table 3 shows a summary of previous studies which examined sequential combination therapy starting with NAs followed by IFN for chronic hepatitis B. In a pilot study [34] by Serfaty et al. in France, sustained responses, defined as serum HBV DNA clearance based on the results of a branched DNA assay and ALT normalization, were achieved in 57 % of patients who received lamivudine alone for 20 weeks followed by the lamivudine + IFN-α combination for 4 weeks, and lastly by IFN-α alone for 24 weeks.

Some groups have studied similar protocols for sequential therapy, but the results have been conflicting. Consistent with the results reported by Serfaty et al. [34], Sarin et al. [35] in India reported that the addition of 4-week lamivudine before starting 24-week pegylated IFN-α therapy resulted in a significantly higher rate of sustained HBeAg clearance (39 %) than that with 24-week pegylated IFN-α monotherapy (14 %; $P = 0.05$). In contrast, Manesis et al. [36] found that in HBeAg-negative patients in Greece, where genotype D is predominant, the rate of sustained response to sequential therapy, defined as HBV DNA of ≤30,000 copies/mL and normal ALT, was only 22 %, which did not differ from that obtained in age/sex-matched historical controls treated with IFN-α alone for 12 months (14 %; $P = 0.36$). In another report in Greece [37], sequential therapy did not raise the rate of sustained virologic response, defined as HBV DNA levels of <400 copies/mL, in HBeAg-negative patients compared to lamivudine monotherapy for a median duration of 25 months (33 vs. 17 %; $P = 0.40$), although no patients in the sequential therapy group showed emerging resistance to lamivudine. A group in China, where genotype B or C is predominant, reported very similar results [38].

In Japan and other East Asian countries, genotype C is the most prevalent HBV type [39, 40], and most patients with chronic hepatitis B acquire the virus perinatally [13]. Thus, response rates to IFN-based therapy in these countries are lower than those reported in Europe and the USA. In our previous study [41] using sequential therapy with lamivudine alone for 16–32 weeks, followed by the lamivudine + IFN combination for 4 weeks and lastly by IFN alone for 20 weeks, the rate of sustained loss of HBeAg was only 29 %. The rate of HBeAg loss during lamivudine treatment was higher among sustained responders than that among non-responders. In a multicenter trial, Minami et al. [42] found that patients who lost HBeAg during lamivudine treatment were more likely to show a sustained response to sequential therapy. Okuse et al. [43] reported that sequential therapy was effective for patients with acute exacerbations of chronic hepatitis B, particularly those in whom HBeAg had become negative during lamivudine treatment.

To date, a small study by Moucari et al. [44] from France has been the only one to evaluate the efficacy of sequential therapy with adefovir dipivoxil followed by IFN-α. Sustained virologic response, defined as serum HBV DNA of <10,000 copies/mL, was achieved in 50 % of patients, but only 20 HBeAg-negative patients were included in this study.

We recently reported the outcomes of sequential therapy with entecavir followed by IFN-α [45]. Among the 24 patients receiving entecavir alone for 36–52 weeks, followed by the entecavir + IFN-α combination for 4 weeks, and lastly by IFN-α alone for 20 weeks, the rate of sustained response, defined by HBeAg loss, HBV DNA of <10,000 copies/mL, and normal ALT, was 21 %; this was not higher than the rate found in our previous study using lamivudine [41]. In the study carried out in China, Chen

Table 3 Sequential combination therapy starting with a nucleos(t)ide analogue followed by IFN

Reference (first author)	HBeAg	*n* (genotype)	Age (years)	Male (%)	Regimens	Biochemical response (%)	Virologic response (%)
Serfaty [34]	±	14 (A6/B0/C1/D4)	40 (30–57)[a]	100	LAM for 20 weeks, followed by LAM + IFN for 4 weeks, and then IFN for 24 weeks	57	57
Sarin [35]	+	36 (N.D.)	33 ± 11[b]	93	LAM for 4 weeks, followed by PEG for 24 weeks	36	39
Manesis [36]	−	36 (N.D.)	55 (46–66)[a]	69	LAM for 6 months, followed by LAM + IFN for 6 months, and then IFN for 6 months	39	22
Vassiliadis [37]	−	18 (N.D.)	42 (19–63)[a]	83	LAM for 3 months, followed by LAM + PEG for 3 months, and then by PEG for 9 months	72	33
Shi [38]	−	64 (N.D.)	35 (21–56)[a]	60	LAM for 20 weeks, followed by LAM + IFN for 4 weeks, and then IFN for 24 weeks	53	14
Enomoto [41]	+	24 (C)	37 ± 11[b]	88	LAM for 16–32 weeks, followed by LAM + IFN for 4 weeks, and then IFN for 20 weeks	46	29
Minami [42]	±	37 (N.D.)	N.D.	N.D.	LAM for 20 weeks, followed by LAM + IFN for 4 weeks, and then IFN for 20 weeks	46	35
Okuse [43]	±	12 (C)	32 ± 8[b]	83	LAM for 20 weeks, followed by LAM + IFN for 4 weeks, and then IFN for 20 weeks	N.D.	58
Moucari [44]	−	20 (A5/B3/C1/D9)	44 (41–52)[a]	85	ADV for 20 weeks, followed by ADV + PEG for 4 weeks, and then PEG for 44 weeks	50	50
Enomoto [45]	+	24 (A1/B0/C23/D0)	39 ± 7[b]	96	ETV for 36–52 weeks, followed by ETV + IFN for 4 weeks, and then IFN for 20 weeks	29	21
Chen [46]	±	32 (A0/B23/C9/D0)	35 ± 5[b]	72	ETV for 12–26 days, followed by ETV + PEG for 2 weeks, and then PEG for 22–46 weeks	61	74

[a] Median (range)

[b] Mean (± SD)

ETV Entecavir

et al. [46] included only patients with acute exacerbation (ALT >10-fold the upper limit of normal) who were treated with entecavir alone for 12–26 days before the ALT had declined to five- to ten-fold the upper limit of normal, followed by the entecavir + pegylated IFN-α combination for 2 weeks, and then by pegylated IFN-α alone for 22–46 weeks. Sustained virologic response, defined as HBV DNA of <10,000 copies/mL, was obtained in 69 % of HBeAg-positive and in 80 % of HBeAg-negative patients with acute exacerbation of chronic hepatitis B.

One objective of sequential therapy starting with NA is to lower the viral load before IFN therapy is initiated, thereby restoring treatment sensitivity as low HBV DNA levels are associated with a favorable response to IFN. Another objective of sequential therapy is to prevent the relapse of hepatitis following the discontinuation of NA therapy through the use of IFN. The high risk of viral relapse after treatment may be attributed to the persistence of cccDNA in the liver, which is correlated with HBV antigen levels in the serum. Using HBsAg and hepatitis B core-related antigen (HBcrAg) levels, Matsumoto et al. [47] proposed a model for predicting relapse after the discontinuation of NA therapy. In our study of sequential therapy using entecavir [45], few patients showed a decrease in HBsAg or HBcrAg to the level meeting the criteria of safe discontinuation of NA. Taken together with the fact that at least 21 % of our patients achieved a sustained response, we suggest that the switch to IFN-α contributes to the safe termination of NA therapy in some patients [48].

Combination with NA and IFN in the guidelines

Combination therapy with NA and IFN is not recommended in the guidelines proposed by the Asian–Pacific Association for the Study of the Liver (updated in 2008) [13] and the American Association for the Study of Liver Diseases (updated in 2009) [14] because there has been no large clinical trial to confirm the benefits of combination therapy over monotherapy in inducing a higher rate of sustained response. The most recently updated guidelines proposed by the European Association for the Study of the Liver (updated in July 2012) [15] also does not recommend combination therapy of IFN with lamivudine or telbivudine. However, the limited information currently available on the efficacy and safety of combining IFN with other NAs has raised an unresolved issue of assessing the safety and efficacy of combining IFN with a more potent NA, such as entecavir or tenofovir.

Guidelines proposed by the Japanese Study Group of the Standardization of Treatment of Viral Hepatitis [49] basically recommend IFN as the first-line treatment for chronic hepatitis B patients aged <35 years to attain a "drug-free state" and entecavir for patients aged ≥35 years to persistently suppress HBV DNA (as tenofovir disoproxil fumarate has not been licensed in Japan to date). In patients aged <35 years and harboring HBV DNA in titers of ≥7 log copies/mL, sequential treatment with entecavir followed by IFN is recommended as the first-line therapy if HBeAg is negative and as the second-line therapy (next to IFN monotherapy) if HBeAg is positive. In patients aged ≥35 years and harboring HBV DNA of ≥7 log copies/mL, sequential treatment is recommended as the second-line therapy (next to entecavir) if the HBeAg is positive.

Conclusions

It remains unclear whether combination therapy is superior to monotherapy for treating chronic hepatitis B. Consequently, controlled trials comparing combination and monotherapy are necessary. In previous studies comparing the lamivudine + IFN combination and lamivudine monotherapy in a finite course, combination therapy was associated with higher rates of sustained post-treatment response and lower rates of drug resistance than lamivudine monotherapy. However, NAs are generally administered indefinitely due to high rates of post-treatment relapse. Additionally, even when NAs are administered alone, concern for drug resistance has significantly decreased (≤1.2 % in 3–5 years [50, 51]) with the development of newer high-potency NAs, such as entecavir and tenofovir. In previous studies comparing the lamivudine + IFN combination and IFN monotherapy, combination therapy showed greater on-treatment viral suppression, but no difference in the post-treatment sustained response was observed when compared to therapy with IFN alone. The efficacy of combining IFN with a more potent NA remains to be evaluated. Further studies are needed to determine whether switching to IFN contributes to the safe discontinuation of therapy, particularly in patients with decreased HBsAg and/or HBcrAg levels during long-term NA treatment [52].

Conflict of interest Dr. S. Nishiguchi has received research grants from Bristol-Myers K.K., MSD K.K., and Chugai Pharmaceutical Co., Ltd. Dr. N. Kawada has received grants from Bristol-Myers K.K., MSD K.K., and Chugai Pharmaceutical Co., Ltd.

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