ORIGINAL ARTICLE

Biochemical Rather than Virologic Response to Interferon Therapy may be More Closely Associated with Decrease of Hepatocellular Carcinoma Incidence in Patients with Chronic Hepatitis B

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Background/Aims: The authors examined whether the response to interferon (IFN) therapy can affect the development of hepatocellular carcinoma (HCC) in chronic hepatitis B (CHB) patients. **Methods:** Out of 353 biopsy-proven CHB patients, 229 (65%) were treated with IFN- α for 6 to 12 months. They were followed for a median period of 75 months (range, 6-120). In patients treated with IFN, biochemical and virologic responses were evaluated at the end of treatment (EOT). The cumulative incidence rates of HCC were calculated and analyzed in relation to baseline characteristics as well as biochemical and virologic responses to IFN therapy. **Results:** The overall cumulative incidence of HCC was 0%, 0.8%, 3.7% and 5.5% at 3, 5, 7 and 8 years, respectively. Age, serum AFP levels and the stage of fibrosis were significantly associated with the occurrence of HCC. As a whole, IFN therapy did not affect the occurrence of HCC. Among the patients treated with IFN, biochemical responders had low HCC incidence rates compared with non-responders ($p=0.018$). However, the HCC incidence rates of virologic responders were not different from non-responders ($p=0.203$). **Conclusions:** Biochemical rather than virologic response to IFN therapy may be more closely associated with decrease of HCC incidence in CHB patients. (*Gut and Liver* 2007;1:49-55)

Key Words: Chronic hepatitis B; Hepatocellular carcinoma; Interferon; Biochemical response; Virologic response

INTRODUCTION

HBV infection is one of the most common causes of the chronic hepatitis (CH), liver cirrhosis (LC), and hepatocellular carcinoma (HCC).¹⁻³ In general, 3 to 4% of patients with CH have been reported to progress to LC every year. Also, HCC develops in 2 to 3% of LC patients, hepatic decompensation occurs in 4 to 5% of LC patients annually.^{2,3} Thus, the long-term therapeutic goals of CHB patients were retardation of the progression to LC and prevention of HCC occurrence.^{4,5} To achieve these purposes antiviral agents including interferons and oral nucleoside analogues have been tried in patients with CHB. However, the effects of antiviral therapy on the prevention of HCC occurrence are still controversial.

Therefore, in this study, the authors intended to examine whether interferon (IFN) therapy can reduce the occurrence of HCC, and to determine whether the biochemical or virologic response to IFN therapy can affect the development of HCC in patients with CHB. The authors also aimed to determine the factors associated with the decrease of HCC incidence in CHB patients treated with IFN.

MATERIALS AND METHODS

1. Subjects

In this study, a total of 353 patients who had serum hepatitis B surface antigen (HBsAg) for at least 6 months and were diagnosed as CHB histologically from June 1996 to June 2001 at Asan Medical Center were included. Out

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of these 353 patients, 229 (65%) were treated with interferon-alpha (IFN) for 6 to 12 months; 81 (23%) with lamivudine (LMV) (Fig. 1). IFN or LMV was administered to the patients whose serum HBV-DNA titer $>10^5$ copies/ml and ALT levels >2 upper limit of normal (ULN). IFN- α was administered subcutaneously at a dose of 5 million units, three times a week for 6 to 12 months. LMV was given orally at a dose of 100 mg per day.

All the patients were followed at 3-6 month intervals for a median period of 75 months (range, 6-120). At every visit, history of alcohol abuse, hematology, serum biochemistry, alpha-fetoprotein (AFP) level, HBeAg/anti-HBe and HBV-DNA titers were determined. Also, abdominal ultrasonography (US) or CT scanning was performed at 6-12 month intervals to detect hepatic masses. Diagnosis of HCC was based on either histopathological finding obtained by fine-needle biopsy or radiologically hypervascular hepatic masses with serum AFP level exceeding 400 ng/mL.^{10,11}

2. Methods

Histological features including stage of fibrosis and degree of necro-inflammation were analyzed according to the new histological classification of chronic hepatitis proposed by the Korean Study Group for the Pathology of Digestive Diseases.^{8,9} The degree of fibrosis and necro-inflammation were analyzed semiquantitatively and scored based on five scoring system; none, minimal, mild, moderate and severe. Serologic markers for hepatitis B virus (HBV) such as HBsAg, anti-HBs, HBeAg, and anti-HBe were tested by radioimmunoassay and serum HBV-DNA titer was determined by solution hybridization method (lower limit of detection: 1×10^5 copies/mL).

In patients treated with IFN, biochemical and virologic responses were assessed at the end of treatment (EOT). The cumulative incidence rates of HCC were also calculated and compared in relation to the baseline characteristics as well as biochemical and virologic responses to IFN therapy. Biochemical response was defined as continuous decrease of serum ALT level during treatment period and finally serum ALT normalization (≤ 40 IU/L) at

Fig. 1. Treatment modalities in the patients with chronic hepatitis B (n=353).

LAM, lamivudine; IFN, interferon; Tx, treatment.

EOT. Virologic response was defined as negative serum HBV-DNA by solution hybridization method at EOT.

3. Statistical analysis

Of all baseline characteristics, categorical variables such as gender, history of alcohol abuse, or positivity of HBeAg or anti-HBe were expressed as percentage. And continuous variables such as age, serum ALT level, albumin level, total bilirubin level, CBC, AFP level, or HBV-DNA level were expressed as median (range) using descriptive statistics. Age and serum AFP level were classified to two groups on the basis of 40 years and ULN (20 ng/mL), respectively, and serum ALT level was classified to three groups ($\leq 2 \times \text{ULN}$ / $2-5 \times \text{ULN}$ / $> 5 \times \text{ULN}$). We also divided fibrosis, lobular activity and porto-periportal activity into two groups, group 0 to 2 and group 3 to 4. The development of HCC between two or three groups according to each variable was compared using Kaplan-Meier method and the log rank test. The overall cumulative incidence of HCC was plotted using the Kaplan-Meier method. In order to determine the independent factors for occurrence of HCC, Cox's regression method was applied. Statistical analysis was performed using SPSS version 12.0. A p-value of less than 0.05 was considered statistically significant.

RESULTS

1. Baseline characteristics of subjects

The median age of 353 CHB patients was 32 years

Table 1. Baseline Characteristics of Patients with Chronic Hepatitis B (n=353)

Variables	
Follow-up period, months*	75 (6-120)
Age, years*	32 (18-64)
Male [†]	278 (79)
History of alcohol abuse [†]	156 (44)
serum HBV-DNA, pg/mL*	218 (0-14600)
HBeAg positivity [†]	313 (89)
Anti-HBe positivity [†]	76 (22)
serum AFP, ng/mL*	7.6 (1-1290)
WBC, $\times 10^3/\text{mm}^3$ *	5.5 (2.7-13.4)
Hemoglobin, g/dL*	14 (7-17)
Platelet, $\times 10^3/\text{mm}^3$ *	186 (64-363)
Prothrombin Time, INR*	1.10 (0.88-1.64)
Albumin, g/dL*	4.0 (1.1-5.0)
Total bilirubin, mg/dL*	0.8 (0.1-25.2)
Serum ALT, IU/L*	204 (12-2427)

*median (range).

[†]numbers (%); HBV, hepatitis B virus; HBeAg, hepatitis B e antigen; Anti-HBe, Anti-hepatitis B e antibody; AFP, alpha-fetoprotein; WBC, white blood cells; ALT, alanine aminotransferase.

(range, 18-64 years) and 79% was male. Eighty nine percent of patients had HBeAg in their sera and 22% were positive for anti-HBe. The median serum HBV-DNA titer, AFP level, and ALT level were 218 pg/mL (range, 0-14,600 pg/mL), 7.6 ng/mL (range 1-1,290 ng/mL), and 204 IU/L (range, 12-2427 IU/L), respectively (Table 1). Histopathological scores regarding the degree of fibrosis, lobular activity or porto-periportal activity were more common in groups 0 to 2 than in groups 3 to 4 (Fig. 2).

2. Cumulative incidence of HCC in patients with CHB

During the follow-up period, total of eight patients were newly diagnosed as HCC. The median period from the liver biopsy to development of HCC was 74 months (range, 6-114 months). The overall cumulative incidence of HCC was 0%, 0.8%, 3.7%, and 5.5% at 3, 5, 7, and 8 years

Fig. 2. Histopathological scores of the patients with chronic hepatitis B.

Fig. 3. Overall cumulative incidence of hepatocellular carcinoma in patients with chronic hepatitis B.

years, respectively, in patients with CHB (Fig. 3).

3. Effects of antiviral therapy on HCC occurrence in patients with CHB

There was no significant difference in the rate of HCC occurrence between IFN-treated and untreated group ($p=0.173$). Also, the HCC occurrence rates were not different each between IFN-treated and LMV-treated group (Fig. 4).

4. Effects of response to IFN therapy on HCC occurrence in patients with CHB

Out of 229 IFN-treated patients, 152 (67%) showed biochemical response and 151 (66%) achieved virologic re-

Fig. 4. Cumulative incidence of hepatocellular carcinoma (HCC) in relation to treatment modalities. There is no significant difference in HCC development between interferon-alpha (IFN) treated group and untreated group ($p=0.173$). There is significant relation in HCC development between lamivudine (LAM) treated group and untreated group ($p=0.199$).

Fig. 5. Development of hepatocellular carcinoma (HCC) in relation to biochemical response at end-of-treatment in patients treated with interferon-alpha.

sponse at EOT. Among 212 HBeAg-positive patients treated with IFN- α , 55 (26%) lost their serum HBeAg at EOT.

HCC occurred in 5 patients among 229 patients treated with IFN- α . Among IFN-treated patients, biochemical responders had significant lower incidence of HCC rather than non-responders ($p=0.018$) (Fig. 5). On the contrary, there was no significant difference in HCC occurrence rate in relation to virologic response ($p=0.203$) (Fig. 6). Also, HBeAg loss by IFN therapy did not affect the rate of HCC occurrence ($p=0.86$) (Fig. 7).

5. Factors associated with HCC occurrence in patients with CHB

In patients with CHB, the HCC occurrence rates were

Fig. 6. Development of hepatocellular carcinoma (HCC) in relation to virologic response at end-of-treatment in patients treated with interferon-alpha.

Fig. 7. Development of hepatocellular carcinoma (HCC) in relation to serum hepatitis B e antigen (HBeAg) loss at end-of-treatment in patients treated with interferon-alpha.

analyzed in relation to age, gender, history of alcohol abuse, baseline serum AFP, ALT, HBV-DNA level, HBeAg positivity, stage of fibrosis, and grade of lobular or porto-periportal activity. As the results, age, baseline serum AFP level, and degree of fibrosis revealed to be associated with HCC occurrence. Older patients had significantly

Fig. 8. Development of hepatocellular carcinoma (HCC) in relation to (A) age, (B) serum alpha-fetoprotein (AFP) level, and (C) stage of fibrosis in patients with chronic hepatitis B.

Fig. 9. Cumulative incidence of hepatocellular carcinoma (HCC) in relation to gender (A), history of alcohol abuse (B), serum ALT level (C), serum HBeAg positivity (D), serum HBV-DNA positivity (E), lobular activity (F), and porto-periportal activity (G) in patients with chronic hepatitis B.

higher incidence of HCC than younger patients (<40 ($n=275$) vs. ≥ 40 ($n=78$), $p=0.036$). Similarly, the patients with high serum AFP level at baseline developed HCC more often than the patients with normal serum AFP level [<20 ng/mL ($n=227$) vs. ≥ 20 ng/mL ($n=94$), $p=0.039$]. Also, in patients with higher fibrosis scores, the risk of HCC occurrence was much higher than in the patients with lower fibrosis scores [F_{0-2} ($n=214$) vs. F_{3-4} ($n=139$), $p=0.013$], (Fig. 8). However, the development of HCC was not associated with gender, history of alcohol abuse, serum ALT level, HBV-DNA titer, HBeAg positivity, and grade of lobular or porto-periportal activity (Fig. 9).

DISCUSSION

Although many investigators have studied the effects of IFN- α therapy on the development of HCC in patients with CHB, the results are not consistent and thus the

therapeutic efficacies of IFN therapy in reducing HCC occurrence are still controversial.^{6,7,9,16-19} A study reported that the cumulative incidence rate of HCC was significantly lower in IFN- α treated patients with CHB than in untreated patients.⁶ On the other hand, a different study showed that there was no significant difference in occurrence rate of HCC between IFN-treated patient and untreated patient.⁷ In Korea, Choi et al¹⁹ reported that sustained response to IFN- α treatment could suppress the development of HCC in patients with HBeAg positive CHB. However, Yeon et al¹⁸ could not observe any significant difference in the development of HCC in responders to IFN therapy compared with non-responders. In the present study, we also could not find statistically significant difference in cumulative incidence of HCC between IFN-treated group and untreated group. However, the statistical insignificance can be partly caused by the small size of subjects, especially the patients in untreated group ($n=43$). Also, the difference of HCC occurrence be-

Fig. 9. Continued.

came statistically insignificant in this study because IFN-treated group also included the patients without any response in addition to good responders to IFN therapy. Therefore, in order to evaluate the therapeutic efficacies of IFN therapy in reducing HCC occurrence exactly, we need to examine whether the response to interferon (IFN) therapy can affect the development of hepatocellular carcinoma (HCC) in chronic hepatitis B (CHB)

patients.

Previously, many investigators have defined the responses to IFN therapy as negative conversion of serum HBV-DNA or HBeAg and serum ALT normalization. In the present study, we also investigated the effects of response to IFN therapy, in terms of negative conversion of serum HBV-DNA, loss of serum HBeAg and serum ALT normalization at EOT, on the incidence of HCC in CHB patients treated with IFN- α . As the results, biochemical responders who had normal serum ALT at EOT showed significantly lower HCC occurrence rate compared with biochemical non-responders. However, virologic response, which was defined as negative HBV-DNA at EOT, did not affect the occurrence rate of HCC. Also, we could not find significant difference in the occurrence rate of HCC in relation to serum HBeAg loss at EOT. With these results, we can speculate that early control of hepatic inflammation may reduce the development of HCC more effectively rather than the suppression of viral load in CHB patients treated with IFN.

Seropositivity for HBsAg and HBeAg have been known as the important risk factors.^{1,5} The study of Yang et al⁵ showed that the relative risk of HCC was 9.6 among men who were positive for HBsAg alone and 60.2 among those who were positive for both HBsAg and HBeAg, as compared with men who were negative for both. Furthermore, Chen et al¹ reported that higher serum HBV-DNA titer ($\geq 10,000$ copies/mL) is a strong risk factor of HCC independent of serum HBeAg, ALT level or association with liver cirrhosis. These data indicate that HBV infection itself and higher replicative activity of HBV may be the most important risk factors for HCC occurrence in patients with CHB.

Many studies have suggested that, in addition to HBV infection itself, age, gender, history of alcohol abuse or smoking, hormonal factors, and genetic factors also could be the risk factors of HBV-associated HCC.^{1,5} Park et al⁹ suggested that the biochemical activity during follow-up period may be the independent prognostic factor for the development of cirrhosis and HCC in CHB, and that effective treatment decreasing necro-inflammatory activity may reduce the occurrence of LC and HCC. In our results, the incidence of HCC was significantly high in patients with older age (≥ 40 years), advanced stage of fibrosis (stage 3 and 4) and elevated serum AFP level ($\geq$ ULN) at baseline. Since age, advanced stage of fibrosis and higher serum AFP level indicate longer duration of HBV infection with advanced histopathology, HBV infection for longer duration resulting in advance histopathology may accelerate the process of HCC development in patients with CHB.

In conclusion, our study suggests that biochemical rather than virologic response to IFN therapy at the end of treatment may be associated with decrease of HCC incidence in patients with biopsy-proven CHB.

REFERENCES

- Chen CJ, Yang HI, Su J, et al. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. *JAMA* 2006;295:65-73.
- Ganem D, Prince AM. Hepatitis B virus infection-natural history and clinical consequences. *N Engl J Med* 2004;350:1118-1129.
- Park NH, Song IH, Chung YH. Chronic hepatitis B in hepatocarcinogenesis. *Postgrad Med J* 2006;82:507-515.
- Osborn MK, Lok AS. Antiviral options for the treatment of chronic hepatitis B. *J Antimicrob Chemother* 2006;57:1030-1034.
- Yang HI, Lu SN, Liaw YF, et al. Hepatitis B e antigen and the risk of hepatocellular carcinoma. *N Engl J Med* 2002;347:168-174.
- Lin SM, Sheen IS, Chien RN, Chu CM, Liaw YF. Long-term beneficial effect of interferon therapy in patients with chronic hepatitis B virus infection. *Hepatology* 1999;29:971-975.
- Fattovich G, Giustina G, Realdi G, R Corrocher R, Schalm SW, European Concerted Action on Viral Hepatitis (EUROHEP). Long-term outcome of hepatitis B e antigen-positive patients with compensated cirrhosis treated with interferon alfa. *Hepatology* 1997;26:1338-1342.
- Park YN, Kim HK, Chon CY, et al. Histologic grading and staging of chronic hepatitis - standardized guideline proposed by the Korean study group for the pathology of digestive disease. *Korean J Pathol* 1999;33:337-346.
- Park BK, Park YN, Han KH, et al. Long-term outcome of chronic hepatitis B according to the new histological classification, *Korean J Hepatol* 2000;6:237-300.
- Kim CY, Kim JW, Lee HS, Yoon YB, Song IS. Natural history and survival rate of chronic liver diseases in Korea - 20 years prospective analysis. *Korean J Internal Med* 1994;46:217-229.
- Lee KJ, Han KH, Chun JY, et al. Natural history of chronic hepatitis type B throughout long-term follow-up. *Korean J Gastroenterology* 1997;29:343-351.
- Yoo BC, Kim HJ, Do JH, Park SM. Relationship between core gene mutations of hepatitis B virus and response to alpha interferon therapy in chronic hepatitis B. *Korean J Hepatol* 2002;8:381-388.
- Greenberg HB, Pollard RB, Lutwick LI, Gregory PB, Robinson WS, Merigan TC. Effect of human leukocyte interferon on hepatitis B virus infection in patients with chronic active hepatitis. *N Engl J Med* 1976;295:517-522.
- Pignatelli M, Waters J, Brown D, et al. HLA class I antigens on the hepatocyte membrane during recovery from acute hepatitis B virus infection and during interferon therapy in chronic hepatitis B virus infection. *Hepatology* 1986;6:349-353.
- Mondelli M, Vergani GM, Alberti A, et al. Specificity of T lymphocyte cytotoxicity to autologous hepatocytes in chronic hepatitis B viral infection: evidence that T cells are directed against HBV core antigen expressed on hepatocytes. *J Immunol* 1982;129:2773-2778.
- Camma C, Giunta M, Andreone P, Craxi A. Interferon and prevention of hepatocellular carcinoma in viral cirrhosis: an evidence-based approach. *J Hepatology* 2001;34:593-602.
- Zonneveld M, Honkoop P, Hansen BE, et al. Long-term follow-up of alpha-Interferon treatment of patients with chronic hepatitis B. *Hepatology* 2004;39:804-810.
- Yeon JE, Seo YS, Kim YH, et al. Long-term follow-up of patients treated with interferon alfa for chronic hepatitis B. *Korean J Hepatol* 1999;5:12-21.
- Choi DR, Jang MK, Kim SM, et al. Long-term follow-up of interferon alpha treatment in HBeAg positive chronic hepatitis B: focused on prevalence of hepatocellular carcinoma. *Korean J Hepatol* 2004;10(suppl 3):S56.