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Meta-analysis: the effect of interferon on development of hepatocellular carcinoma in patients with chronic hepatitis B virus infection

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Abstract

Purpose In patients chronically infected with hepatitis B virus, interferon has been used for the purpose of viral suppression by loss of hepatitis B e antigen (HBeAg) with or without seroconversion to antibody to HBeAg (anti-HBe). However, discussion about the effect of interferon on the development of hepatocellular carcinoma (HCC) has been controversial.

Methods We conducted a meta-analysis of published studies. Eight studies were retrieved (1,303 patients), including two randomized controlled trials (RCTs) and six non-RCTs (553 patients received interferon treatment).

Results The pooled estimate of the preventive effect of treatment was significantly in favor of interferon (risk difference −5.0%; 95% CI −9.4 to −0.5, $P = 0.028$). By subgroup analyses, the preventive effect of interferon treatment was shown in the Asian population (risk difference −8.5%; 95%CI −13.6 to −3.6, $P = 0.0012$), the population with the incidental rate of HCC ≥10% if untreated with interferon (risk difference −9.4%; 95%CI −14.2 to −4.6, $P = 0.0001$), and the population with the proportion of HBeAg-positive patients to the study population ≥70% (RD −6.0%; 95%CI −11.8 to −0.2, $P = 0.043$). However, the preventive effect of interferon was not shown in the European population, the population with a lower incidental rate of HCC if untreated with interferon, and the population with the lower proportion of HBeAg-positive patients to the study population. An evaluation using the Begg method indicated no evidence of publication bias.

Conclusions Interferon treatment is considered to restrain HCC development in patients with chronic hepatitis B virus infection, especially in HBeAg-positive Asians.

Keywords Hepatitis B e antigen · Seroconversion · Prognosis

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Introduction

Worldwide, more than 350 million people are chronic carriers of the hepatitis B virus (HBV) [1]. The distribution of HBV varies throughout the world. Areas with high prevalence include Southeast Asia, China, and Africa, where approximately 10% of the population are chronic carriers [2]. North America, Western Europe, and Australia have low levels of endemicity. Chronic HBV infection is a leading cause of hepatocellular carcinoma (HCC). A recent study has revealed that half of HCC cases are associated with HBV infection and that worldwide 328,000 people die of HBV-related HCC every year [3]. Patients positive for hepatitis B surface antigen (HBsAg) have been reported to have higher hazard ratios for liver cancer mortality (22.4; 95% CI, 15.2–32.9), and liver cancer mortality ranged from 72.8 per 100,000 persons per year for patients with HBV DNA levels less than 300 copies/ml to 815.6 per 100,000 persons per year for those with HBV DNA levels of 1 million copies/ml or greater [4].

Antiviral treatment with the oral nucleoside analogue lamivudine (LMV) is reported to be effective in reducing HCC risk in Asian patients with advanced chronic hepatitis B [5]. The tolerability and safety of LMV are excellent; however, viral resistance, which is usually followed by loss of the clinical response, the rise of aminotransferase levels, and the worsening of hepatic histology, is related to mutations in the YMDD motif that occurred in 70–80% of patients treated continuously for 4–5 years [6]. Furthermore, concerning long-term LMV treatment, teratogenicity has not been adequately evaluated in humans. In contrast, discussion of the effect of interferon (IFN) on the development of HCC has been controversial. Two randomized controlled trials (RCTs) from Taiwan reported a decrease in the development of HCC among hepatitis B patients who received IFN treatment compared to untreated controls [7, 8]. On the other hand, in 2001, a meta-analysis by Camma and colleagues [9], which included patients with co-infection of HBV and the hepatitis C virus (HCV), reported that IFN did not affect the rate of HCC in HBV-related cirrhosis. IFN prevents the development of HCC in patients with HCV-related cirrhosis [10].

Seroconversion from the hepatitis B e antigen (HBeAg) to antibody to HBeAg (anti-HBe) marks the transition from chronic hepatitis B to the inactive HBsAg carrier state with low or undetectable serum HBV-DNA and normal aminotransferases, and confers a favorable long-term outcome with very low risk of HCC. In 2007, Lin and colleagues [11] reported that IFN-treatment improved the HBeAg seroconversion rate and reduced the development of HCC. Furthermore, after 2001, several studies of the effect of IFN on the development of HCC in patients with chronic HBV infection were reported [11–14]. Thus, we considered the effect of IFN on the development of HCC in patients with chronic HBV infection to be worth investigating by another meta-analysis.

Methods

Literature search

We searched PubMed for the medical literature published by using the following key words: *hepatitis B, interferon, and hepatocellular carcinoma*. We limited our search to original, English-language articles published between 1 January 1998 and 31 December 2007; earlier studies were excluded because these studies included patients with a positive test for antibody to HCV (anti-HCV). Studies were included if they met all of the following criteria: they consisted of a clinical trial comparing IFN-treated and untreated patients; they excluded patients who had tested positive for anti-HCV or the human immunodeficiency

virus; they assessed HCC as an outcome of the treatment's effect; they had one or more cases with the development of HCC in IFN-untreated patients. However, studies in which HCV-positive patients could be accurately excluded from the study population were included.

Data collection

Two investigators (Y.M., H·K.) trained in hepatology independently reviewed the included studies by using a standardized protocol and data collection form. Discrepancies among reviewers were solved with discussion. We collected data on patient characteristics, study characteristics, IFN treatment, and outcomes. Our primary goal was to investigate the effect of IFN treatment on the development of HCC in patients with chronic HBV infection.

Statistical analysis

Crude rates of HCC were assessed as the only measure of IFN's potential effect. The evaluation of preventive effectiveness was performed with an intention-to-treat method. In order to combine results from individual studies, we used the crude rate of HCC regardless of when HCC was observed. The risk difference (RD) was calculated for each trial.

In order to obtain an overall measure of the treatment efficacy, we calculated RD and 95% CI for HCC development, according to the DerSimonian and Laird method [15]. Heterogeneity was evaluated by a chi-square test. We considered statistical significance at a P value less than 0.05. When statistically significant heterogeneity of treatment effects was observed, the studies were partitioned (for example, RCTs vs. non-RCTs) to decrease the heterogeneity of studies in a particular partition and increase the differences among the partitions. Subgroup analyses were also performed to evaluate the difference in RD between trials of Europeans and those of Asians, between trials with the rate of HCC in untreated patients below 10% and those above 10%, and between the trials with the rate of HBeAg-positive patients below 70% and those above 70%. We examined publication bias by using the Begg method with funnel plot analysis [16].

Results

Literature searches of PubMed by using the three keywords yielded 347 studies. Of the 347 studies, 336 did not fulfill the inclusion criteria (42 in languages other than English, 5 case reports, 171 review articles, 14 concerning chronic hepatitis C, 88 not concerning the effect of IFN on HCC development, 3 that included HCV-positive patients in which HCV-positive patients could not be accurately

excluded from the study population, 6 concerning the second prevention of HBV-related HCC, 4 without untreated patients, and 3 without the development of HCC in IFN-untreated patients). Eleven studies fulfilled the inclusion criteria. However, these 11 studies included 3 studies reported by Lin and colleagues [7, 8, 11] and 2 studies reported by Ikeda and colleagues [17, 18]. One study each [8, 17] was selected from these researchers. Thus, eight studies in total were selected, and included 1,303 patients, 553 of whom received IFN treatment. The main features of the studies evaluated by meta-analysis are shown in Table 1. One study [19] included seven HCV-positive patients, and these seven patients, who did not develop HCC, were excluded from the study population. Among the reviewed studies, 19 and 28 were RCTs, and 6 [12–14, 17, 20, 21] were non-RCTs (NRCTs). All eight studies have been reported as full papers. Three studies [13, 19, 21] were performed in Europe, three [14, 17, 20] in Japan, and one each in Thailand [12] and Taiwan [8]. The sample size of each study varied greatly, ranging from 52 [20] to 404 patients [13]. Variable IFN schedules were used, both for total dose and for length of treatment. IFN was administered in various fixed, variable, and adjusted doses.

The effect of IFN on the development of HCC is shown in Fig. 1. IFN seemingly decreased the incidental rate of HCC in six studies, and a statistically significant decrease was observed in two. The pooled estimate of the preventive effect of treatment was significantly in favor of IFN (RD −5.0%; 95% CI −9.4 to −0.5, $P = 0.028$) without a significant heterogeneity ($\chi^2 = 10.91$, $P = 0.14$).

A first subgroup analysis was carried out in relation to the ethnic origin of the patients (Europeans vs. Asians). From Asian reports, the pooled estimate of the preventive effect of IFN treatment was significant (RD −8.5%; 95% CI −13.6 to −3.6, $P = 0.0012$) without a significant heterogeneity ($\chi^2 = 4.47$, P = 0.35). On the other hand, from European reports, no preventive effect was shown (RD −0.5%; 95% CI −4.9 to 4.0, $P = 0.84$) without a significant heterogeneity ($\chi^2 = 0.42$, $P = 0.81$).

A second subgroup analysis was performed in relation to the incidental rate of HCC in untreated patients. In the reports with the incidental rate of HCC ≥10% in untreated

Table 1 Eight clinical trials assessing the effect of interferon on the development of hepatocellular carcinoma in patients with chronic hepatitis B virus infection

Study (reference)	Year	Type of study	Sample size	Male (%)	Age (years)	HBeAg-positive (%)	Interferon regimens	Treatment duration (months)	Follow-up (years)	Rate of HCC (%)
Ikeda [17]	1998	NRCT	T 94	83	41	66	α; 6 MU twice a week, or	10 (6–60)	T 6.8	T 11
			C 219	77	44	46	α-2b; 6 MU twice a week, or		C 7.0	C 23
							β; 6 MU twice a week			
IIHCSG [21]	1998	NRCT	T 49	NR	NR	NR	α; 3 MU thrice a week, or	≥3	≥3[a]	T 16
			C 97				α-2a; 3 MU thrice a week, or			C 19
							α-2b; 3 MU thrice a week			
Mazzella [19]	1999	RCT	T 28	76	36	100	α; 6 MU/m^2 thrice a week	6	T 7.2	T 4
			C 29	81	41	100			C 6.6	C 7
Lin [8]	1999	RCT	T 67	100	32	100	α; 4–6 MU/m2 thrice a week	3	T 7.4	T 2
			C 34	100	32	100			C 6.5	C 12
Fujisawa [20]	2000	NRCT	T 16	53[a]	7[a]	100	α; 0.1 MU/kg daily	1	11[a]	T 6
			C 36			100				C 3
Papatheodoridis [13]	2001	NRCT	T 209	83	47	0	α; 3 MU thrice a week	6 or 12	T 6.0	T 8
			C 195	82	49	0			C 6.1	C 8
Tangkijvanich [12]	2001	NRCT	T 67	79	37	100	α-2b; 3–5 MU thrice a week, or	20–24	T 5.0	T 3
			C 72	65	40	100	α-2a; 3–6 MU thrice a week, or		C 5.0	C 13
							α; 3–5 MU thrice a week			
Mahmood [14]	2005	NRCT	T 23	69[a]	49[a]	36[a]	α; 6 MU daily, or	1	>7[a]	T 22
			C 68				β; 6 MU daily			C 26

References are arranged by publication year

HBeAg hepatitis B e antigen, *HCC* hepatocellular carcinoma, *RCT* randomized controlled trial, *NRCT* non-randomized controlled trial, *T* treated, *C* controls, *NR* not reported

[a] Value for the total study population

Fig. 1 Meta-analysis of the eight studies of IFN for prevention of HCC in patients with chronic HBV infection. Relative risk and 95% CIs for each study are plotted on the graph. Studies are arranged by publication year
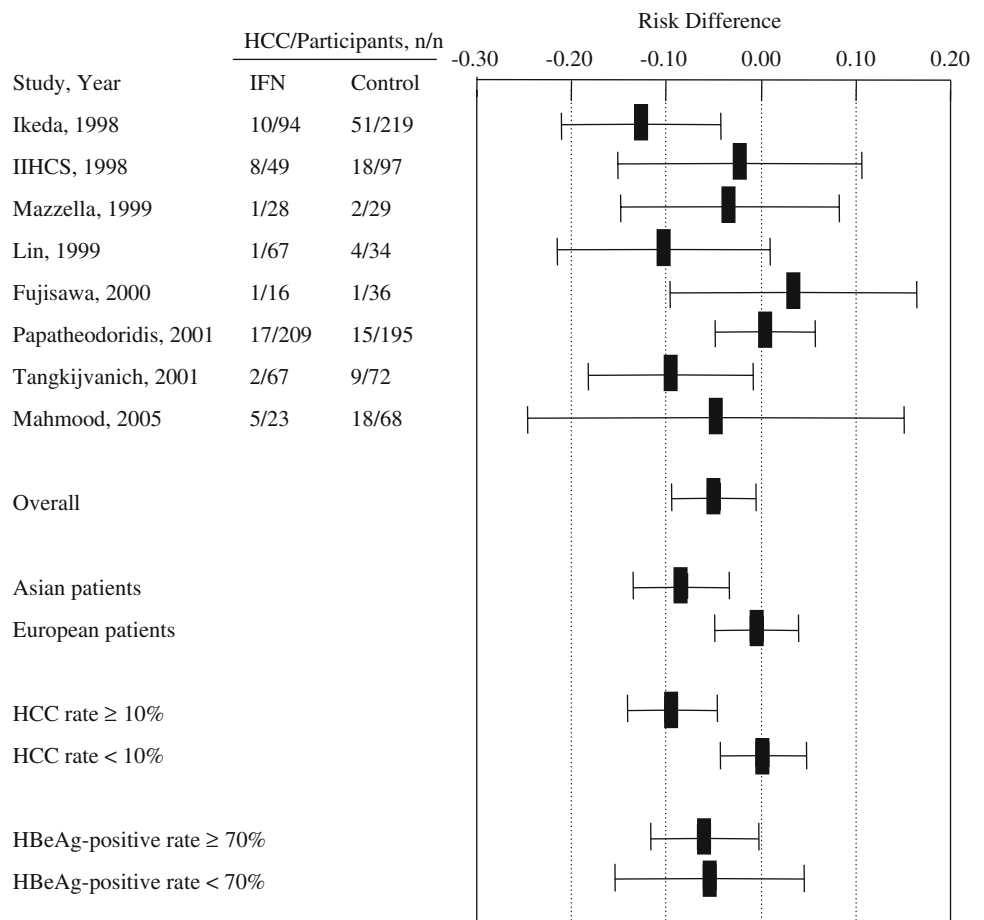
patients, the pooled estimate of the preventive effect of IFN treatment was significant (RD −9.4%; 95% CI −14.2 to −4.6, $P = 0.0001$) without a significant heterogeneity ($\chi^2 = 2.00$, $P = 0.74$). On the other hand, in the reports with the incidental rate of HCC <10% in untreated patients, no preventive effect was shown (RD −0.2%; 95% CI −4.3 to 4.7, $P = 0.92$) without a significant heterogeneity ($\chi^2 = 0.61$, $P = 0.74$).

A last subgroup analysis was performed in relation to the proportion of HBeAg-positive patients to the study population. In the reports with the proportion of HBeAg-positive patients to the study population ≥70%, the pooled estimate of the preventive effect of IFN treatment was significant (RD −6.0%; 95% CI −11.8 to −0.2, $P = 0.043$) without a significant heterogeneity ($\chi^2 = 3.43$, $P = 0.33$). On the other hand, in the reports with the proportion of HBeAg-positive patients to the study population <70%, no preventive effect was shown (RD −5.4; 95% CI −15.4 to 4.6, $P = 0.29$) with a significant heterogeneity ($\chi^2 = 6.74$, $P = 0.034$).

An evaluation using the Begg method suggested no evidence of publication bias visually or statistically ($z = 0.99$, $P = 0.32$).

Discussion

In patients with chronic hepatitis B, IFN has been used for the purpose of viral suppression by the loss of HBeAg with or without seroconversion to anti-HBe and a decrease of the serum HBV DNA levels. In patients with HBeAg seroconversion, the intrahepatic HBV-DNA level also decreases largely [22]. In fact, meta-analyses of RCT have shown the benefit of IFN treatment [23–25]. Wong and colleagues [23] reported that, by a meta-analysis, the HBeAg and HBsAg loss rates were 33 versus 12% and 7.8 versus 1.8%, respectively, in patients treated with IFN versus a placebo group. HBeAg seroconversion leads to the improvement of long-term prognosis. For patients with compensated liver cirrhosis, the 5-year survival rates have been reported to be 72% for HBeAg-positive cirrhosis and 97% for HBeAg-negative cirrhosis [26]. Furthermore, the 10-year survival rate is 98% in HBeAg-positive patients treated with IFN compared to 85% in those untreated [8]. The relative risk for HCC has been reported to be 9.6 (95% CI, 6.0–15.2) for men positive for HBsAg alone and 60.2 (95% CI, 35.5–102.1) for men positive for both HBsAg and HBeAg [27]. Hepatic fibrosis improves in patients with HBeAg

seroconversion [28]. In this study, the effectiveness of IFN treatment on HCC development was shown in populations mainly including HBeAg-positive patients, but not in populations mainly including HBeAg-negative patients. Thus, IFN treatment is speculated to mainly restrain HCC development through HBeAg seroconversion.

Recently, peginterferon has replaced the use of standard interferon in the treatment of chronic hepatitis B. Peginterferon is superior in efficacy to standard interferon in chronic hepatitis B based on the clearance of HBeAg; however, there are no differences in the frequency and severity of adverse events between the two groups [29, 30]. After 1-year therapy, clearance of HBeAg occurred in 30–36% of patients treated with peginterferon compared to 4–12% of placebo recipients, and the serum HBV DNA levels became undetectable by PCR-based assays in 25% of treated patients [31–35]. Furthermore, peginterferon is effective in approximately one-third of patients who failed to respond to previous standard IFN treatment [36]. Even in patients with advanced fibrosis, peginterferon has been reported to be effective and safe [37]. The introduction of peginterferon is expected to decrease the rate of HCC development even further.

In this study, the incidental rate of HCC in untreated patients was associated with the effectiveness of IFN treatment on HCC development, and four of five studies with the incidental rate of HCC in untreated patients above 10% were performed in Asians. The incidental rate of HCC is higher in Asia than in the United States and Europe [2]. On the other hand, recently, HBV genotypes have been reported to be associated with HCC development. Eight genotypes of A–H have been distinguished. Asian patients are mainly infected with genotypes B and C, while European patients are mainly infected with genotypes A and D [38]. In Asian patients, genotype C is a risk factor for HCC development [39]. Furthermore, the HBV genotype is associated with the response to IFN treatment. Genotype B is associated with a better response to IFN treatment compared with genotype C, whereas patients with genotype D have a lower response rate to IFN treatment compared with genotype A [40–43]. In HBeAg-positive patients, HBsAg clearance by peginterferon treatment is associated with genotype. Patients with genotype A tend to achieve HBsAg clearance most [43]. This study indicates that Asian patients may get a more favorable effect from IFN treatment on HCC development than European patients. The difference in the effect of IFN treatment on HCC development may be associated with HBV genotype.

Does IFN treatment prevent HCC development in HBeAg-negative patients? In this study, standard interferon did not prevent HCC development in HBeAg-negative patients. On the other hand, after 1-year treatment with peginterferon, normalization of

serum alanine aminotransferase levels and the lack of detectable HBV DNA occurred in 38 and 63%, respectively, of HBeAg-negative patients [44]. The strong relation between serum HBV DNA levels and HCC development has been reported [45, 46]. Active hepatitis after HBeAg seroconversion is a risk factor for HCC development [47]. Thus, peginterferon treatment may suppress HCC development in HBeAg-negative patients. In order to assess this effect of peginterferon treatment, a study with a longer follow-up period and in a larger study population may be required.

In conclusion, IFN treatment prevented HCC development in patients with chronic hepatitis B infection, especially in Asian patients, patients with the rate of HCC in untreated patients above 10%, and HBeAg-positive patients. However, in this study, associations between a preventive effect of IFN on HCC development and HBV genotypes or serum HBV DNA levels could not be assessed. Furthermore, this meta-analysis included only two RCTs. Further studies are necessary to confirm a preventive effect of IFN on HCC development.

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