Therapy of Chronic Hepatitis B with Recombinant Human Alpha and Gamma Interferon

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Eight patients with chronic hepatitis B entered a pilot study of gamma interferon and alpha interferon in combination. Gamma interferon alone had minimal inhibitory effects on serum levels of hepatitis B virus as monitored by serum HBV DNA and DNA-polymerase activity. The drug also gave troublesome side effects. In contrast, alpha interferon had more potent inhibitory effects on serum HBV levels and fewer side effects. When combined, the two interferons showed no additive or synergistic effects in inhibiting serum levels of HBV DNA or DNA polymerase. These findings indicate that the addition of gamma interferon to alpha interferon provides no additional antiviral effects but contributes significantly to side effects. (HEPATOLOGY 1990;11:266-270.)

Chronic HBV infection may be complicated by the development of chronic hepatitis, cirrhosis or HCC (1). Therapy is generally aimed at eliminating viral replication in the hope of preventing these complications (2). There is no standard form of therapy for chronic type-B hepatitis; among the various agents tried, alpha interferon is the most promising. Approximately 30% to 40% of patients respond to this therapy (3, 4).

In an attempt to increase the response rate, alpha interferon has been used in combination with other antiviral or immunomodulatory agents, such as corticosteroids (5) or adenine arabinoside 5′ monophosphate (6). In this study, we treated chronic hepatitis B patients with gamma and alpha interferon (separately at first and then in combination) to determine whether this combination is more effective against HBV than alpha interferon alone.

PATIENTS AND METHODS

The study included eight white men aged 28 to 41 yr (mean = 35) who were known to have had HBsAg in serum for 0.4 to 10 yr (mean = 4.6). All had chronic hepatitis with persistently elevated serum ALT levels between 96 and 450 U/L (mean = 209). They also showed HBeAg, HBV-associated DNA polymerase activity and HBV DNA in serum. Liver histological studies showed chronic active hepatitis in four patients and chronic persistent hepatitis in three patients (liver biopsy was not done in one case). Antibody to human immunodeficiency virus (anti-HIV) was present in the sera of two cases although neither had any clinical features of AIDS. Hepatitis delta virus (HDV) infection was excluded by the absence of anti-HDV. Three of the eight patients had been treated previously but had not responded to alpha interferon more than 1 yr before the current study.

Patients were treated with recombinant human gamma interferon (SCH 36850, Schering-Plough Corp., Kenilworth, NJ) and recombinant human interferon alfa 2B (Intron A, Schering-Plough Corp.). The patients self-administered interferon with subcutaneous injections. The schedule of administration of the two interferons is shown in Figure 1.

Gamma interferon was given first; the dosage was increased in a stepwise fashion (0.1, 0.2, 0.5 and 1 million units daily) every 2 wk for 8 wk. After a 4-wk rest period, alpha interferon dosage was begun at a fivefold higher dosage and increased in a similar stepwise fashion every 2 wk (0.5, 1, 2 and, 5 million units daily) for 8 wk. After a second 4-wk rest period, gamma and alpha interferon were given in combination for 8 wk (0.1 and 1 million units, respectively, daily for 4 wk and 0.2 and 2 million units daily for 4 wk). The treatment period was extended and the dosage increased thereafter for another 4 wk in selected patients.

All details of the treatment protocol were approved by the Institutional Clinical Research Subcommittee of the National Institute of Diabetes and Digestive and Kidney Diseases; patients gave written informed consent.

Patients were seen in the outpatient department at weekly intervals during therapy and at monthly intervals thereafter for 1 yr. On each occasion, blood was drawn and tested for complete blood counts, for ALT and AST levels and for selected markers of HBV infection including HBsAg, HBeAg, HBV-associated DNA polymerase activity and HBV DNA. HBsAg, HBeAg, anti-HDV and anti-HIV were assayed by commercial RIA or by enzyme immunoassay (Abbott Laboratories, North Chicago, IL). DNA polymerase was quantitated by measurement of incorporation of tritiated thymidine in virus particles purified from serum and expressed as cpm/0.2 ml (7). HBV DNA was detected by molecular hybridization using a ^{32}P-labeled HBV DNA probe and quantified by densitometry with comparison to standards with known levels (8).

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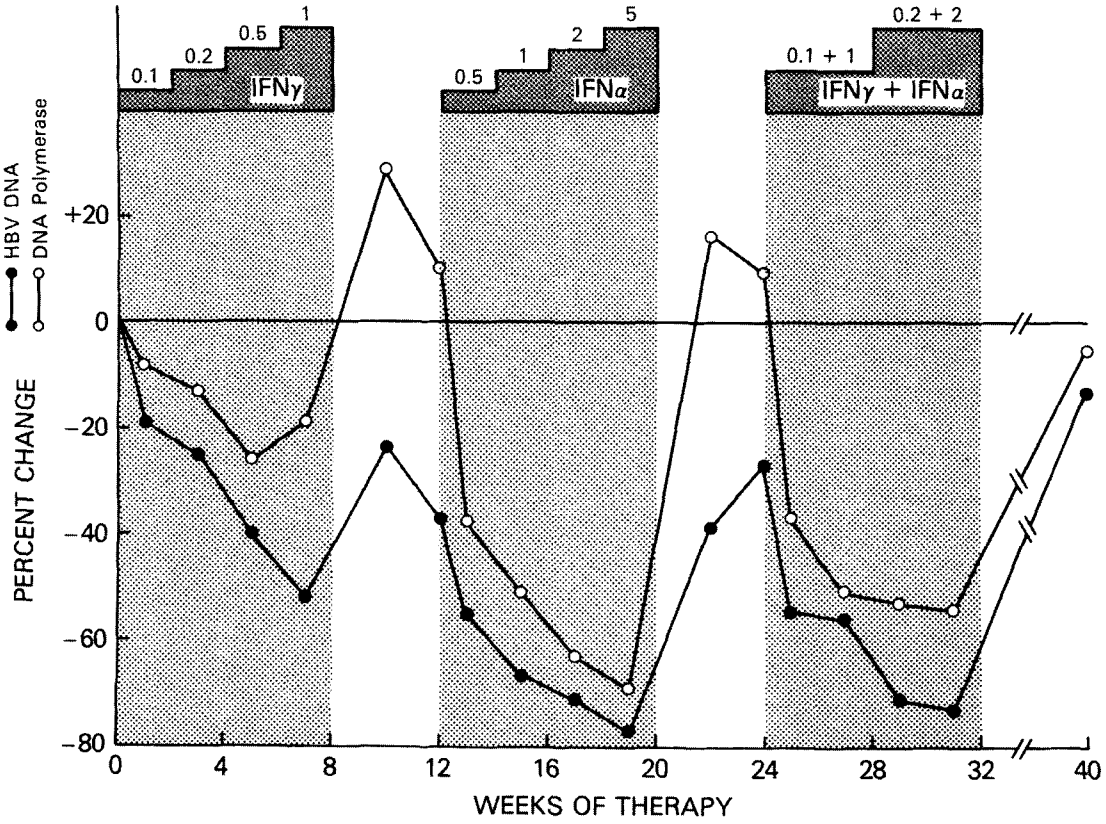

FIG. 1. Dosage schedule for alpha *(IFNα)* and gamma *(IFNγ)* interferon and changes in serum levels of HBV DNA and DNA polymerase activity. Changes expressed as percentage of change from baseline (pretreatment) levels.

Pharmacokinetic studies were done in each patient at the beginning of each new dose of gamma interferon and at the start of the second dose of alpha interferon (1 million units daily). The purpose of these studies was to determine whether the presence of liver disease affected the persistence of interferon in serum. We also compared the half-life of the two interferons in case one of the agents showed prolonged inhibition of HBV replication.

Patients had blood taken to measure serum interferon levels before and 1, 2, 4, 6, 12 and 24 hr after the first dose. Interferon levels were measured by RIA (Centocor Gamma Interferon Radioimmunoassay, Centocor, Malvern, PA and NK2 Interferon Alpha Immunoradiometric Assay, Celltech, Slough, England).

Changes in serum levels of HBV DNA and DNA polymerase were expressed as percentage of change. Group means were compared using Student's *t* test. Changes in serum aminotransferase activities and HBV markers were analyzed by paired Student's *t* tests and the Wilcoxon one-sample test, respectively.

RESULTS

During gamma interferon therapy, serum levels of DNA polymerase and HBV DNA decreased minimally. The decrease was maximal during the eighth week of treatment at a dose of 1 million units daily (19% decrease in DNA polymerase and 52% decrease in HBV DNA) (Fig. 1). The decrease in these viral markers was statistically significant at the end of therapy ($p < 0.01$, Wilcoxon one-sample test). There was also an apparent dose-dependent increase in serum ALT and AST levels (Fig. 2) that was statistically significant. The maximal decrease in serum HBV DNA and DNA polymerase occurred at a time of maximal increase in serum ALT and AST (mean = 250% and 280%, respectively).

During alpha interferon therapy, serum DNA polymerase and HBV DNA were inhibited in an apparent dose-dependent fashion to a greater extent than with gamma interferon alone (Fig. 1). For DNA polymerase activity, the inhibition averaged 35% at 0.5 million units, 48% at 1 million units, 56% at 2 million units and 70% at 5 million units daily. In one patient, serum HBV DNA and DNA polymerase became undetectable during treatment. The clearance of HBV DNA was followed by a loss of serum HBeAg and a striking improvement in aminotransferase activities. This patient completed the course of alpha interferon and was not treated with the combination of the two interferons. However, 2 mo after the discontinuation of alpha interferon, HBeAg reappeared in the serum. Subsequently, HBV DNA, DNA polymerase activity and elevations in aminotransferases appeared.

During therapy with the combination of alpha and gamma interferon, serum HBV DNA and DNA polymerase activity decreased in all patients. However, the

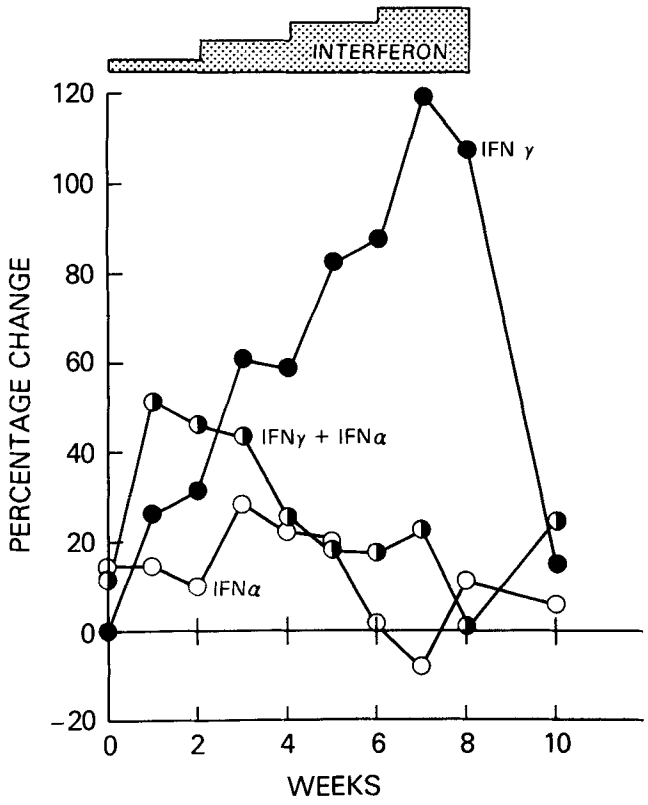

FIG. 2. Changes in serum levels of ALT activities during therapy with alpha (IFNα) and gamma (IFNγ) interferon. Changes expressed as percentage of change from baseline levels.

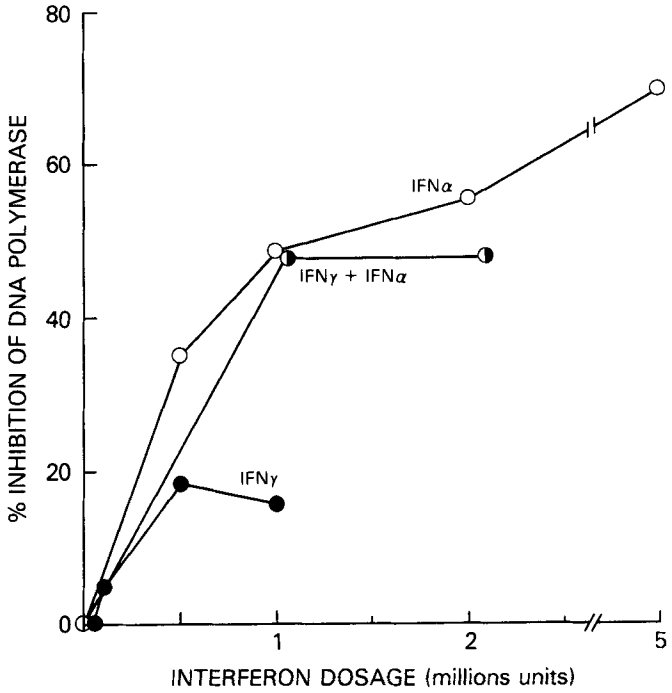

FIG. 3. Percentage of inhibition of hepatitis B virus DNA polymerase activity in serum as function of interferon dose administered. For each dose studied, there was less inhibition of DNA polymerase activity with gamma (IFNγ) than with alpha (IFNα) interferon. Furthermore, the combination of alpha and gamma interferon did not yield evidence for a synergistic or even additive effect on DNA polymerase levels.

amount of inhibition of these markers was the same with the combination of 0.1 million units of gamma with 1 million units of alpha interferon, as was noted with 1 million units of alpha interferon alone (Fig. 3). Increasing the dose of gamma interferon to 0.2 million units daily and of alpha interferon to 2 million units daily was not followed by a further decrease in serum levels of HBV markers.

Combination therapy was continued in six patients for a further 4 wk at varying doses of gamma interferon (0.2 to 0.4 million units daily) and alpha interferon (2 to 4 million units daily), depending on patient tolerance of side effects. No patient became HBV DNA-negative or lost HBeAg or HBsAg during therapy or the 4 mo after discontinuation of treatment.

Pharmacokinetic studies showed that gamma interferon was undetectable in serum at doses of 0.1 or 0.2 million units daily. Peak levels of 2 and 29 U/ml, respectively, were achieved at 6 to 8 hr after doses of 0.5 and 1 million units of gamma interferon. Similar studies during alpha interferon therapy revealed high levels of alpha interferon before the index dose in two patients and peak levels of alpha interferon of 20 to 30 U/L 4 to 6 hr after the injection in the remaining six patients.

Side effects seemed to be greater during gamma interferon therapy than during alpha interferon therapy despite the fivefold lower dose of gamma interferon. The side effects during gamma interferon therapy appeared to be cumulative; patients felt worse after 2 wk of each dose of gamma interferon as opposed to feeling better near the end of each dose schedule, which often occurred with alpha interferon. Two patients had troublesome depression and difficulty in concentrating during gamma interferon therapy but had no recurrence of these conditions during alpha interferon treatment. Gamma interferon was often associated with pain and erythema at the injection site lasting up to 5 days. Only mild erythema without pain occurred after alpha interferon injections. One patient had diarrhea during both types of interferon treatment. The dose of gamma interferon had to be decreased from 1 million units to 0.5 million units daily in one patient because of depression and mental slowing. In no patient did the dose of alpha interferon have to be modified because of side effects. All patients had mild decreases in peripheral RBC, WBC and platelet counts. The relative changes in blood counts were proportionally greater with gamma interferon than with alpha interferon treatment. Thus the average decrease in total WBC count was 20% during treatment with 1 million units

of gamma interferon daily as opposed to 14% during treatment with 1 million units of alpha interferon daily.

DISCUSSION

The interferons have been shown to have antiviral action against a wide variety of human and animal viruses, both *in vitro* and *in vivo*. Three types of interferon are recognized—alpha, beta and gamma. These are all antigenically and biochemically distinct but have a variety of similar actions, including antiviral, antiproliferative and immunomodulatory effects (9). The mechanism of antiviral action is uncertain but may be related to the intracellular production of oligonucleotides, which activate ribonucleases in the presence of viral RNAs. The antiviral effects of gamma interferon may also be partly caused by the indirect action of stimulating alpha or beta interferon production.

The interferons also have potent immunomodulatory actions that may be important in inhibiting viral replication. For example, in HBV infection alpha interferon has been shown to increase the production of virus-specific antibody (antiHBc) *in vitro* (10). Both interferons are known to increase cell surface expression of major histocompatibility complex (MHC) antigens: alpha interferon stimulates class I and gamma interferon stimulates both class I and class II antigen expression (9). Increases in MHC antigen expression probably have major effects on the immune recognition and response to viral agents. Thus alpha and gamma interferon may have major effects on HBV infection through their antiviral or immunomodulatory actions.

Interferon was first noted to have some effect against HBV in 1976 (11), but large clinical trials have only become possible since recombinant interferons became readily available. To date, alpha interferon has shown the most promise against HBV, although gamma interferon and beta interferon have also been tested (12, 13). The rationale for using these agents in combination includes the fact that gamma interferon and alpha interferon both have potent but different immunomodulatory actions *in vitro* (14). When used in combination *in vitro*, they also seem to have a synergistic antiviral effect (15). A previous study has suggested a modest benefit from combined alpha and gamma interferon given twice weekly for 6 mo in chronic hepatitis B when compared to alpha interferon given alone. No differences in side effects were observed in the two groups despite the generally lower doses used in the current study (13).

Although there is no clear explanation for this apparent disparity, it is important to note that we have concentrated on studying the direct antiviral effect of these two agents by measuring HBV DNA and DNA polymerase inhibition in serum. Gomez et al. (13), however, focused on ultimate loss of markers of viral replication (HBeAg and HBV DNA). The small number of patients in their study makes it difficult to interpret. The difference in reported side effects remains unexplained, but could be related to different commercial preparations of gamma interferon used in the two studies.

Our data showed that alpha interferon is more effective in inhibiting serum levels of HBV than gamma interferon at comparable doses. The mechanism by which gamma interferon reduces HBV replication is uncertain. It is noteworthy that the slight decrease in viral levels was accompanied by marked increases in serum aminotransferase activities. Thus a temporary gamma interferon–induced exacerbation of hepatitis may have accounted for the mild decreases in serum HBV levels. Gamma interferon therapy was not associated with clearance of HBeAg from serum in any patient, whereas one patient responded temporarily to alpha interferon.

In contrast to gamma interferon, alpha interferon was effective in decreasing serum HBV levels even at low doses, and a stepwise dose-dependent inhibitory effect was noted. When gamma interferon and alpha interferon were administered in combination, HBV DNA and DNA polymerase were inhibited to the same extent with alpha interferon alone at the same dose. Neither an additive nor synergistic antiviral effect was noted *in vivo* against HBV.

The pharmacokinetic studies of gamma interferon were interesting but difficult to interpret because they were done at each new dose the day after the previous dose was stopped. Thus a low level of interferon was usually found in serum before the injection of the test dose, which precluded an accurate calculation of the serum half-life. The fact that gamma interferon levels persisted in serum for more than 24 hr is in keeping with the observation that patients often experienced side effects for several days after each injection and that the side effects appeared to be cumulative.

Two of the eight patients in this study had high serum levels of alpha interferon before starting treatment. Previous studies have shown that most patients with chronic hepatitis do not have detectable serum levels of alpha interferon (16). Even in the presence of acute type-B hepatitis, only low levels (often <20 U/ml) are found. In fact, it has been suggested that patients with chronic hepatitis B have deficient production of alpha interferon (17). The reason for the high circulating alpha interferon levels in these two patients is therefore unknown. Their responses to interferon were no different from any other patient's, and neither lost HBeAg from serum. Both were homosexual men, but neither had anti-HIV.

In conclusion, short courses of gamma interferon had minimal apparent antiviral activity against HBV *in vivo*. Furthermore, gamma interferon had no apparent additive or synergistic effects when combined with alpha interferon. The dose of gamma interferon that could be used in our study was limited because of

possible side effects. The effectiveness of alpha interferon in inhibiting HBV was confirmed in this study. Although a potential additive effect of gamma and alpha interferon cannot be excluded entirely, the use of alpha interferon in combination with other antiviral agents may be more rewarding.

REFERENCES

1. Hoofnagle JH, Alter HJ. Chronic viral hepatitis. In: Vyas GN, Dienstag JL, Hoofnagle JH, eds. Viral hepatitis and liver disease. Orlando, FL: Grune & Stratton, 1984:97-113.
2. Alexander G, Williams R. Antiviral treatment in chronic infection with hepatitis B virus. Br Med J 1986;292:915-197.
3. Dusheiko G, Di Bisceglie A, Bowyer S, Sachs E, Ritchie M, Schoub B, Kew MC. Recombinant leucocyte interferon treatment of chronic hepatitis B. HEPATOLOGY 1985;5:556-560.
4. Alexander GJ, Brahm J, Fagan EA, Smith HM, Daniels HM, Eddleston ALWF, Williams R. Loss of HBsAg with interferon therapy in chronic hepatitis B virus infection. Lancet 1987;2:66-68.
5. Perrillo RP, Regenstein FG, Peters MG, DeSchryrer-Keoskemeti K, Bodicky CJ, Campbell CR, Kuhns MC. Prednisone withdrawal followed by recombinant alpha interferon in the treatment of chronic type B hepatitis. Ann Intern Med 1988;109:95-100.
6. Hoofnagle JH. Therapy of chronic type B hepatitis with adenine arabinoside and adenine arabinoside monophosphate. J Hepatol 1986;3(suppl 2):573-580.
7. Kaplan PN, Greenman RL, Gerin JL, Purcell RH, Robinson WS. DNA polymerase associated with human hepatitis B antigen. J Virol 1983;12:995-1002.
8. Di Bisceglie AM, Waggoner JG, Hoofnagle JH. Hepatitis B virus deoxyribonucleic acid in the liver of chronic carriers. Gastroenterology 1987;93:1236-1241.
9. Peters M, Davis G, Dooley JS, Hoofnagle JH. The interferon system in acute and chronic viral hepatitis. Prog Liver Dis 1986;8:453-467.
10. Kinoyama S, Rich S, Perrillo RP, Hoofnagle JH, Zheleznyak A, Peters MG. Effects of therapy on anti-HBc production by mononuclear cells from patients with chronic type B hepatitis [Abstract]. HEPATOLOGY 1987;7:1024.
11. Greenberg HB, Pollard RB, Lutwick LI, Gregory PB, Robinson WS, Merigan TC. Effect of human leucocyte interferon on hepatitis B virus infection in patients with chronic active hepatitis. N Engl J Med 1976;295:517-522.
12. Bissett J, Eisenberg M, Gregory P, Robinson WS, Merigan TC. Recombinant fibroblast interferon and immune interferon for treating chronic hepatitis B virus infection: patients' tolerance and the effect on viral markers. J Infect Dis 1988;157:1076-1080.
13. Gomez C, LaBanda F, Porres JC, Mora I, Andres A, Bartolomé J, Quirogia JA, et al. Combined recombinant alpha and gamma interferon treatment of chronic hepatitis B virus infection. In: Zuckerman AJ, ed. Viral hepatitis and liver disease. New York: Alan R. Liss, 1988:372-874.
14. De Maeyer-Guignard J, De Maeyer E. Immunomodulation by interferons: recent developments. In: Gresser I, ed. Interferon 6. London: Academic Press, 1985:69-91.
15. Czarniecki CW, Fennie CW, Powers DB, Estell DA. Synergistic antiviral and antiproliferative activities of *Escherichia coli*–derived human alpha, beta and gamma interferons. J Virol 1984;49:490-496.
16. Poitrine A, Chousterman S, Chousterman M, Naveau S, Thang MN, Chaput J-C. Lack of *in vivo* activation of the interferon system in HBsAg-positive chronic active hepatitis. HEPATOLOGY 1985;5:171-174.
17. Davis GL, Grimley PM, Hoofnagle JH. Alpha and gamma interferon in patients with chronic viral hepatitis. Antiviral Res 1984;1:133-135.