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Interferon therapy for the anti-HBe positive form of chronic hepatitis B[1]

Alfredo Alberti*, Giovanna Fattovich

Clinica Medica II°, University of Padova, Via Giustiniani, 2, 35100 Padova, Italy

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Abstract

Two distinct serologic types of chronic hepatitis B have been identified, namely the "classical" HBeAg positive form and the "atypical" HBeAg negative, anti-HBe positive variant which is due to infection by a mutant HBV having a pre-core stop codon that makes the virus unable to produce HBeAg. The anti-HBe positive form is currently the prevalent type of chronic hepatitis B in the Mediterranean area, being associated with a more severe clinical course compared to HBeAg positive cases. The response to interferon therapy in patients with anti-HBe positive chronic hepatitis B has been recently investigated in control trials. These studies have shown that normalization of ALT with efficient suppression of virus activity can be achieved in 50–80% of patients while treated with interferon alpha indicating that also the pre-care mutant of HBV is sensitive to the antiviral effect of interferon. However, reactivation of hepatitis occurs in a variable percentage of initial responders when interferon is stopped. The probability of reactivation increases when the disease is of long duration, when cirrhosis is present and particularly if the pre-core mutant of HBV has become the predominant type of circulating virus, indicating that this HBV variant is more resistant to immunoclearance compared to wild type HBV.

Key words: Interferon; Anti-hepatitis Be antigen; Chronic hepatitis B; Pre-core mutants of HBV

*Corresponding author. Fax +39 049 8754179.

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1. Introduction

Chronic infection caused by hepatitis B virus (HBV) is one of the leading causes of liver disease in most parts of the world. The spectrum of clinical conditions observed in HBV carriers is extremely heterogeneous as to signs, symptoms and severity of hepatic and extrahepatic involvement. The majority of infected individuals are asymptomatic and have a mild form of liver disease but progression to cirrhosis and eventually to hepatocellular carcinoma occurs in a significant number of cases when followed up for decades (Hoofnagle et al., 1987). The variable activity and outcome of chronic HBV infection have been thought to be due mainly to variations in the host immune response against the virus and the infected hepatocytes. In the absence of a direct cythopatic effect of HBV (Alberti et al., 1983) the more severe and evolutive liver lesions were attributed to a vigorous immune response responsible for extensive destruction of infected cells, while unable to eradicate the infection. On the other side, chronic HBV carriage with minimal or no liver damage was interpreted as a tolerant state, with a spectrum of intermediate situations in between. Although most of these concepts are still valid, recent data, derived from the application of molecular biology techniques to the study of HBV sequences, indicate that the virus itself may be directly and primarily involved in determining the severity and course of liver disease (Alberti et al., 1990). Indeed, a number of variants of HBV have been identified and some of these have been incontrovertibly shown to associate with the clinical expression of the disease (Bonino et al., 1991; Carman and Thomas, 1992). The most important example is the evidence which links genomic mutations in the pre-core and core gene of HBV with a clinically well characterised subtype of HBV related liver disease, namely the HBeAg negative and anti-HBe positive form of chronic hepatitis B.

2. Pre-core mutants of HBV

The C gene of HBV DNA encodes two proteins, a nonstructural secretory protein (HBeAg) and the nucleocapsid protein of HBV or hepatitis B core antigen (HBcAg) (Ganem and Varmus, 1987). Translation of the C gene can start at two different codons (ATGs) separated by 87 nucleotides encoding a 29 amino acid fragment (pre-core region). If translation starts at the first ATG (Pre-C) the precursor protein of HBeAg is produced. Soluble HBeAg derives from cleavage of the leader peptide (pre C) and the carboxyterminal sequence of this precursor protein. If translation begins at the second (C) ATG a shorter polypeptide of 183 amino acids is produced, corresponding to the major nucleocapsid protein or core antigen. HBeAg seropositivity is classically linked to productive virus infection with high levels of virus replication and of viraemia, while HBeAg clearance with anti-HBe seroconversion usually identifies termination of virus replication (Realdi et al., 1980; Alberti et al., 1986). However, there are patients with chronic hepatitis who are HBeAg negative and anti-HBe positive and have evidence of ongoing virus replication (Bonino et al., 1986; Fattovich et al., 1988).

Following the serological and clinical recognition of this new subtype of patients, the reasons for the lack of HBeAg expression in serum were extensively explored leading to the demonstration that these patients are frequently infected with a mutant form of HBV which is unable to secrete HBeAg (Brunetto et al., 1989; Carman et al., 1989). Indeed, a number of different HBV strains with mutation in the pre-core sequence have been described in patients with anti-HBe positive chronic hepatitis B (Gunther et al., 1992). A translationally functional pre-core is necessary for HBeAg production but not for virus production and replication (Chang et al., 1987). In patients with anti-HBe positive chronic hepatitis consistent evidence has been obtained of the existence of one major mutation in the pre-core sequence with a single base substitution (guanine to adenine) in codon 28 at nucleotide 1896 (A1896). This point mutation converts codon 28 from tryptophan (TGG) to a stop codon (TAG) thus preventing the expression of the pre-core sequence and hampering secretion of HBeAg (Bonino et al., 1991). This A1896 mutation has been more frequently detected in anti-HBe positive sera obtained from patients with chronic hepatitis in different parts of the world and is considered the "molecular" marker of this patient population. Other genomic defects that predictably prevent HBeAg expression have been described including point mutations of the ATG initiation codon of the pre C region or sequence variations leading to frameshifts but much less frequently that the more typical A1896 point mutation and stop codon.

3. Anti-HBe positive chronic hepatitis: clinical features and pathogenetic mechanisms

This form of chronic hepatitis is characterised by a HBsAg carrier state with HBeAg seronegativity and anti-HBe seropositivity. HBV-DNA is detected in serum and HBcAg is found in liver to testify ongoing activity of virus replication. These forms of chronic hepatitis B are frequently observed in the Mediterranean area and in the Far East and in these regions their prevalence among all forms of chronic hepatitis B has been progressively increasing in recent years, so that they have taken the lead over the classical forms of HBeAg positive chronic hepatitis. The clinical profile of anti-HBe positive chronic hepatitis differs from that seen in patients with HBeAg positive forms of the disease (Brunetto et al., 1989b; Fattovich et al., 1988). Although the wide spectrum of histologic activity, from no or minimal periportal activity, to severe piecemeal necrosis or lobular activity, as seen in HBeAg positive forms may be present also in anti-HBe positive cases, they differ from the former in relation to: (i) presence of large fluctuations over time in virus replication and disease activity with recurrent relapses alternate to transient remission phases; (ii) higher and faster rate of progression to cirrhosis; (iii) much lower rate of sustained spontaneous remission. Most of these patients are infected by pre-core mutants of HBV that have been suggested to be directly linked to the pathogenesis of severe liver disease. However, the point mutation of the pre-core region does not invariably associate with a severe liver disease and has been detected also in anti-HBe positive HBV carriers with normal transaminases and no histologic liver lesions (Okamoto et al., 1990). Thus, 2 two distinct subgroups of pre-core mutant carriers may exist, the

first with low levels of serum HBV-DNA and minimal liver disease, and the second showing high levels of viraemia and severe liver disease. The reason for these differences may relate to efficiency of the host immune response or to be balance between wild-type and mutant HBV or may be linked to the emerging of other mutations along the pre-core and core HBV-DNA sequence. Indeed evidence has been provided that severe liver damage in chronic hepatitis B is related to the clustering of missense mutations along the core and particularly in the region of codons 84–101 thought to control the production of major CTLs epitopes (Chuang, 1993). Pre-core mutants may be present in low amounts since the early stage of infection or alternatively they may emerge by spontaneous mutation during the turbulent virus replication of the HBeAg positive phase. Once they have developed, they may be progressively selected over wild type HBV due to negative selection effect of HBeAg acting as a major target of the immune response which clears infected cells. These concepts on pathogenetic mechanisms involved in anti-HBe positive chronic hepatitis B may be relevant to the understanding of the peculiar type of response seen when the disease is treated with interferon.

4. Interferon therapy in anti-HBe positive, HBV-DNA positive chronic hepatitis B

Seeing the severe course of anti-HBe positive chronic hepatitis and the rarity of sustained spontaneous remission and encouraged by the results obtained with alpha interferon therapy in HBeAg positive patients, we and other authors have decided to evaluate the effect of interferon therapy in anti-HBe positive patients, when HBV-DNA was detectable in serum and there was no evidence of coinfection or superinfection by other hepatotropic agents like hepatitis Delta and C viruses. The number of published trials, however, is still limited, as is the number of patients included, and the results have been somehow conflicting, not allowing to reach consensus on the use of interferon in this category of patients. The first report appeared in 1989 and was a pilot, not randomised, study with rather disappointing conclusions as to the effectiveness of IFN in the treatment of the disease (Brunetto et al., 1986). These authors treated 12 patients with recombinant human alpha interferon 2a using a dose of 9MU given 3 times weekly for 16 months. Patients remaining viraemic after this period received 3 MU 3 times weekly for additional 8 weeks. The initial response was rather good and 8 patients became HBV-DNA negative and normalised transaminases during therapy. However, most of them relapsed after cessation of IFN and a sustained response was observed only in 3 patients.

This rate of cure was better than in untreated controls, none of whom normalised ALT or cleared HBV-DNA, but was nevertheless disappointingly low. A few years later we conducted in Padova the first randomized trial and 30 patients with HBV-DNA and anti-HBe positive chronic hepatitis B were treated with 5 MU/msq of human lymphoblastoid interferon alpha given 3 times a week for 6 months and were compared to 30 patients left untreated (Fattovich et al., 1992). The results obtained in this study were appreciably better than those of Brunetto et al. (1989b). At the end of therapy, around 60% of the patients were HBV-DNA nega-

tive with normal ALT and the response was maintained 1 year after therapy in most of them. The rate of sustained response in treated patients (53%) was significantly higher that in 30 untreated controls (17% $P<0.01$). Thus, reactivation after interferon was in our patients lower than that observed in Torino and this was the major determinant of the different rate in sustained response. Recently, our patients have been revaluated after a prolonged follow-up and, although some cases of late reactivation were observed, the difference between treated and untreated patients remained significant. In the attempt of understanding the reasons of the different response, we then compared the patients population and therapy schedule in the two studies.

The patients treated by Brunetto et al. (1989b) were older, had a longer duration of disease and half were already cirrhotics before therapy, compared to only 10% in our trial (Fattovich et al., 1992). These data would suggest that our patients were in an earlier stage of disease and this might favour a better response to therapy. Since it has been proposed that pre-core mutants progressively overweight and substitute wild type HBV with time in the course of chronic infection, these conclusions are coherent with the concept that pre-core mutant are more resistant to eradication by interferon than wild type HBV. The type of interferon used in the two studies was also different as was the schedule and duration of therapy. Interestingly, several patients in the Padova study, but none in the Torino study, developed a peak in ALT during interferon, before they achieved a sustained response, as typically seen in HBeAg positive patients (Alexander et al., 1987). These observations would suggest that interferon acted essentially by direct antiviral effect in the patients treated by Brunetto et al. (1989b), with efficient suppression of virus replication but no eradication of infected cells, allowing reactivation after cessation of therapy. On the other hand, the ALT peaks often observed in our patients testify that immunoclearance of infected cells was enhanced, leading to successful eradication of virus from the liver in several cases. Apart from different capacity in immunopotentiation which may exist between lymphoblastoid and recombinant interferons, yet to be demonstrated, the most logical explanation of these findings might be that our patients were virologically more similar to HBeAg positive patients, in the sense that their liver was still infected mainly by wild type HBV, rather than by mutant HBV, due to the earlier phase of disease. This hypothesis has been confirmed recently when patients included in these trials were analysed by molecular techniques to define the type and heterogeneity of the infecting virus. In collaboration with Dr. W. Carman in Glasgow and with Prof. H. Thomas in London, we analysed by direct sequencing HBV in pretreatment sera from 26 of the 30 anti-HBe positive patients of our trial and 27% of them were found infected by wild type HBV, while 73% had pre-core mutant virus (Carman et al., 1993). In patients infected by wild type HBV strains, exacerbation of hepatitis during interferon therapy often occurred in parallel with selection of mutants, but this event did not affect the long term response to treatment. In the series published by Brunetto et al. (1989b) 95.5% of anti-HBe positive patients had prevalent pre-core mutant HBV populations pretreatment and 64% had exclusively mutant HBV (Brunetto et al., 1993). When the relative prevalence of wild type and pre-core mutant in baseline viraemia in individual cases

was analysed in relation to response to interferon, no differences were observed as to suppression of virus replication during therapy. However, a sustained response to treatment was observed in 47.3% of patients with prevalent wild type HBV but only in 19% of those with prevalent mutant HBV. These results, while confirming that wild type and pre-core mutant HBV are similarly sensitive to the direct antiviral effect of interferon, indicate that pre-core mutants are resistant to interferon mediated eradication, most likely as the consequence of their natural ability to escape immunoclearance. Similar data have been reported also by Santantonio et al. (1993) who treated 11 anti-HBe positive patients with lymphoblastoid interferon at a dose of 10 MU given 3 times weekly for 6 months. Before therapy 8 of these patients were infected predominantly or exclusively with pre-core mutants and the other 3 had a mixture of wildtype and pre-core mutants. 10 out of 11 cases became HBV-DNA negative and developed normal ALT at the end of therapy but all then relapsed during follow up after discontinuing interferon. Another randomized controlled trial in anti-HBe and HBV-DNA positive chronic hepatitis has been conducted in Greece (Hadziyannis et al., 1990). These authors treated 25 patients with 3 MU of recombinant interferon alpha 2b given 3 times weekly for 14–16 weeks. Also with these rather low doses of interferon, virus replication was inhibited during therapy in 60% of the cases, while a sustained response was observed in one third of treated patients. No information is available on the type of HBV infecting these patients, but their mean age and prevalence of cirrhosis were very similar to those of the patients treated by Brunetto et al. (1989b) as was the rate of long term response. Table 1 summarizes the main data of the published trials of IFN therapy in anti-HBe positive chronic hepatitis. Virus replication was always efficiently suppressed during therapy and the rate of primary response was usually high, while the

Table 1
Published trials of interferon therapy in anti-HBe positive chronic hepatitis B

	Brunetto (1989b)	Hadziyannis (1990)	Pastore (1992)	Fattovich (1992)
Patients treated				
N.	12	25	10	30
mean age (y)	46	49	34	33
duration of disease (y)	7	6	7	4
% cirrhotics	50	40	10	10
% with precore mutant HBV	95	n.t.	100	75
Therapy				
type of IFN	R-2a	R-2b	L	L
total dose (IU)	430–500	125–145	360/msq	360/msq
duration (wks)	16–24	14–16	24	24
% Response				
during IFN	67	60	90	63
long-term	25	28	10	50

R, recombinant interferon; L, lymphoblastoid interferon; n.d., not described; msq, meter square.

probability that primary response could be maintained as sustained response after therapy withdrawal was dependent mainly on duration of disease, and, on the type of prevalent HBV forms, the presence of pre-core mutant behaving as a negative predictive marker.

5. Effects of interferon on liver histology

Brunetto et al. (1989b) found a significant improvement in liver histology in patients showing sustained suppression of virus replication and described an overall decrease in periportal activity in their anti-HBe positive patients treated with interferon, independently of the biochemical and serologic outcome. In the two studies where lymphoblastoid interferon was used with a more aggressive schedule, improvement between pretreatment and post-treatment liver biopsies was observed in 50% of cases by Fattovich et al. (1992) and in 40% of patients by Pastore et al. (1992). Thus, interferon therapy appeared to improve the histologic pattern of chronic hepatitis also in anti-HBe positive patients as already described in HBeAg positive chronic hepatitis B, but it remains to be established which effect, if any, these changes in inflammatory activity may have on the long term outcome of the disease.

6. Predictors of response to interferon

Available data indicate that the response to interferon therapy in anti-HBe positive chronic hepatitis cannot be predicted by conventional clinical, biochemical and serological tests, at variance with what is seen in HBeAg positive cases in whom pretreatment high ALT values and low HBV-DNA levels are associated with a better response. Recent data from Brunetto et al. (1993) indicate that the prevalence of pre-core mutant HBV at baseline influences the response and that a cut-off value of 20% pre-core mutants may be used to discriminate patients responding ($<20\%$) or not responding ($>20\%$) to interferon with permanent eradication of replicating virions.

7. Conclusions and perspectives

Chronic hepatitis B with anti-HBe and HBV-DNA seropositivity is a well defined clinical entity and represents the most frequent forms of HBV related liver disease in the Mediterranean area. The disease may be particularly active and progressive, with low propensity to spontaneous healing. There is therefore an urgent need for effective therapy and the interest is obviously focused on interferons. Published trials, however, indicate that the response to interferon is different and less sadisfactory than in HBeAg positive cases. In fact, anti-HBe positive chronic hepatitis behaves more as chronic hepatitis C when treated with interferon. Half to 70% of patients

respond during therapy but several of them relapse after cessation of treatment, leaving around 20–30% of cured patients. Reactivation appears to be dependent on the presence of HBV pre-core mutants which are sensitive to the antiviral effects of interferon but escape eradication by the immune response. Anti-HBe positive patients with no or low levels of pre-core mutants often respond to interferon as the HBeAg positive cases while patients with prevalent pre-core mutant virus usually show a transient response followed by relapse. Anti-HBe positive patients should be therefore treated as soon as they are identified, even when liver disease is not particularly severe, because the earliest therapy is started the highest is the chance of promoting a sustained response. Patients with severe and advanced disease are unlikely to benefit from interferon, at least using conventional schedules. In these patients therapy should be tried under close monitoring as the relapse after therapy may deteriorate liver function. There is clearly a need for further studies on the possibility of better treating these patients with different schedules of interferon, or in combination with other antiviral agents. Unfortunately, the ability of pre-core mutants to escape immunoclearance makes the identification of the ideal regimen highly problematic.

References

Alberti, A., Tremolada, F., Fattovich, G. and Realdi, G. (1983) Virus replication and liver disease in chronic hepatitis B virus infection. Dig. Dis. Sci. 28, 962–966.

Alberti, A., Pontisso, P., Fattovich, G., Schiavone, E., Chemello, L., Bortolotti, F. and Tremolada, F. (1986) Changes in serum hepatitis B virus (HBV) DNA positivity in chronic HBV infection: results of a long-term follow-up of 138 patients. J. Infect. Dis. 154, 562–569.

Alberti, A. (1990) Do single nucleotide mutations result in clinically significant changes in hepatitis B virus pathogenicity? J. Hepatol. 10, 268–270.

Alexander, G.J.M., Brahm, J., Fagan, E.A., Smith, H.M., Daniels, H.M., Eddleston, A.L.W.F. and Williams, R. (1987) Loss of HBsAg with interferon therapy in chronic hepatitis B virus infection. Lancet ii, 66–69.

Bonino, F., Rosina, F., Rizzetto, M., Rizzi, R., Chiaberge, E., Tardanico, R., Callea, F. and Verme, G. (1986) Chronic hepatitis in HbsAg carriers with serum HBV-DNA and anti-HBe. Gastroenterology 90, 1268–1273.

Bonino, F., Brunetto, M.R., Rizzetto, M. and Will, H. (1991) Hepatitis B virus unable to secrete e antigen. Gastroenterology 100, 1138–1141.

Brunetto, M.R., Stemler, M., Schodel, F., Will, H., Ottobrelli, A., Rizzetto, M., Verme, G. and Bonino, F. (1989) Identification of HBV variants which cannot produce pre-core derived HBeAg and may be responsible for severe hepatitis. Ital. J. Gastroenterol. 21, 151–154.

Brunetto, M.R., Oliver, F., Rocca, G., Criscuolo, D., Chiaberge, E., Capalbo, M., David, E., Verme, G. and Bonino, F. (1989) Natural course and response to interferon of chronic hepatitis B accompained by antibody to hepatitis B e antigen. Hepatology 10, 198–202.

Brunetto, M.R., Giarin, M., Saracco, G., Oliveri, F., Calvo, P., Capra, G., Randone, A., Abate, M.L., Manzini, P., Capalbo, M., Piantino, P., Verme, G. and Bonino, F. (1993) Gastroenterology 105, 845–850.

Carman, W.F., Jacyna, M.R., Hadziyannis, S., Karayannis, P., McGarvey, M.J., Makris, A. and Thomas, J. (1989) Mutation preventing formation of hepatitis B e antigen in patients with chronic hepatitis B infection. Lancet 588–590.

Carman, W.F. and Thomas, H.C. (1992) Genetic Variation in hepatitis B virus. Gastroenterology 102, 711–719.

Carman, W.F., Fattovich, G., McIntyre, G., Alberti, A., Thomas, H.C. (1994) Hepatitis B virus pre-core variation and interferon therapy. Hepatology, in press.

Chang, C., Enders, G., Sprengel, R., Peters, N., Varmus, H.E., Ganem, D. (1987) Expression of the pre-core region of an avian hepatitis B virus is not required for viral replication. J. Virol. 61, 3322–3325.

Chuang, W.L., Omata, M., Ehata, T., Yokosuka, O., Ito, Y., Imazeki, F., Lu, S.N., Chang, W.Y. and Ohto, M. (1993) Pre-core mutations and core clustering mutations in chronic hepatitis B virus infection. Gatroenterology 104, 263–271.

Fattovich, G., Brollo, L., Alberti, A., Pontisso, P., Giustina, G. and Realdi, G. (1988) Long-term follow-up of anti-HBe positive chronic active hepatitis B. Hepatology 8, 1651–1655.

Fattovich, G., Farci, P., Brollo, L., Mandas, A., Pontisso, P., Giustina, G., Lai, M.E., Belussi, F., Busatto, G., Balestrieri, A., Ruol, A. and Alberti, A. (1992) A randomized controlled trial of lymphoblastoid interferon-alpha in patients with chronic hepatitis B lacking HbeAg. Hepatology 15, 4, 584–589.

Ganem, D. and Varmus, H.E. (1987) The molecular biology of hepatitis viruses. Annu. Rev. Biochem. 56, 651–693.

Gunther, S., Meisel, H., Reip, A., Miska, S., Kruger, D.H. and Will, H. (1992) Frequent and rapid emergence of mutated pre-c sequences in HBV from e-antigen positive carriers who seroconvert to anti-HBe during interferon treatment. Virology 187, 271–279.

Hadziyannis, S., Bramou, T., Makris, A., Moussoulis, G., Zignego, L. and Papaioannou, C. (1990) Interferon alfa-2b treatment of HBeAg negative/serum HBV-DNA positive chronic active hepatitis type B. J. Hepatol. 11, 5133–5136.

Hoofnagle, J.H., Shafritz, D.A. and Popper, H. (1987) Chronic type B hepatitis and the "healthy" HBsAg carrier state. Hepatology 7, 758–763.

Okamoto, H., Yotsumoto, S., Akahane, Y., Yamanaka, T., Miyazaki, Y., Sugai, Y., Tsuda, F., Tanaka, T., Miyakawa, Y. and Mayumi, M. (1990) Hepatitis B viruses with pre-core region effects prevail in persistently infected hosts along with seroconversion to the antibody against e antigen. J. Virol. 64, 1298–1303.

Pastore, G., Santantonio, T., Milella, M., Monno, L., Mariano, N., Moschetta, R. and Pollice, L. (1992) Anti-HBe positive chronic hepatitis B with HBV-DNA in the serum response to a 6 months course of lymphoblastoid interferon. J. Hepatol. 14, 221–225.

Realdi, G., Alberti, A., Rugge, M., Bortolotti, F., Rigoli, A.M., Tremolada, F. and Ruol, A. (1980) Seroconversion from hepatitis B e antigen to anti-HBe in chronic hepatitis B virus infection. Gastroenterology 79, 195–199.

Santantonio, T., Jung, M.C., Monno, l, Milella, M., Iacovazzi, T., Pape, G., Pastore, G. and Will, H. (1993) Long-term response to interferon therapy in chronic hepatitis B: importance of hepatitis B virus heterogeneity, Int. Symp. "Viral hepatitis and liver disease", Tokyo. 1993, p. 151.