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Interferon in HDV infection

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Abstract

Chronic hepatitis D is a usually severe and progressive liver disease due to infection with the hepatitis delta virus, a unique RNA virus requiring the hepatitis B virus helper function to exert its pathogenic potential. Alpha IFN is at present the treatment of choice for chronic viral hepatitis, but the results obtained in chronic hepatitis D are far from being satisfactory. Available data show that IFN is more likely to be effective if administered to patients with a recent infection (lasting less than 1 year) at high doses (9–10 MU thrice in a week) and for a prolonged length of time (at least 12 months). The optimal timing of IFN treatment remains to be addressed: apart from the clearance of HBsAg and seroconversion to anti-HBs (an event often occurring months to years after completion of a successfull IFN treatment) no other early biochemical or virological events can predict a sustained response. Better therapeutic options are therefore needed. Unfortunately, antiviral agents, such as Ribavirin, active against HDV in cell cultures, have failed to confirm their attitude in the clinical setting. In vitro and in vivo evidence points to HBV as a possible target for antiviral therapy in chronic hepatitis D, providing the rationale for trying new deoxynucleotide analogues also in this severe form of hepatitis.

Key words: Interferon; Hepatitis delta virus; Cirrhosis; Chronic Hepatitis

The Hepatitis delta virus (HDV) is a subviral satellite of hepatitis B virus (HBV). Although self-cleaving, it is distinct from the satellites that self-cleave and although forming highly base-paired structures, it is distinct from viroids in its size, in containing coding sequences and in being a satellite. HDV thus seems to define a type of

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satellite of which it is the sole representative (Mayo, 1993). The virus, which appears as a 32 nm spherical particle at electron microscopy, contains a 1.7 kb single-stranded circular RNA genome (HDV-RNA) and the delta antigen (HDAg). These elements are encapsidated by a lipid envelope in which HBV surface antigens proteins are embedded. There are two isoforms of the delta antigen (Bergmann and Gerin, 1986; Bonino et al., 1986). This heterogeneity arises from an unidirectional mutation at a single nucleotide in the termination codon for delta antigen (codon 196: UAG→UGG) which occurs during replication (Luo et al., 1990). Thus, while small delta antigen (HDAg-p24) is 195 amino acids long, the large delta antigen (HDAg-p27) is identical in sequence except that it contains 19 additional amino acids at its carboxy terminus. Both forms of delta antigen contain the same nuclear localization signals and RNA-binding domain and yet they display dramatically different roles. The small form is required for replication, while the large form is a potent *trans*-dominant inhibitor. The large antigen is sufficient for assembly of secretable viral-like particles with HBV surface antigens, while the small antigen is not. An additional property of the small delta antigen in cell cultures is cytotoxicity, an unusual characteristic for a virus which is known to cause persistent infection. In contrast, the large antigen has no cytotoxic effect (Cole et al., 1993). These experimental evidences support the hypothesis that HDAg-p27 expression favours the establishment of a persistent infection, as proteins expressed during persistence are usually non cytotoxic. During the acute phase HDAg-p24 may represent the bulk of HDAg. If some HDV-infected hepatocytes survive the cytotoxic action of the HDAg-p24, natural mutant genomes may be produced that have the capacity to express the non cytotoxic HDAg-p27, which in turn reduces the level of HDV-RNA replication and leads to persistent infection.

The conventional interpretation of HDV disease is based on the postulate that expression of HDV depends on the expression of HBV. Recent studies in chimpanzees and liver transplants alert to the possibility that hepatitis D may also result from activation of a latent HDV infection by superinfection with the HBV (Ottobrelli et al., 1991). This inverted pathogenic mechanism has not so far been substantiated in the ordinary epidemiologic setting. There are therefore now three recognized patterns of HDV infection: coinfection, superinfection and latent infection.

In coinfection the synthesis of HBV is transient and the expression of HDV can be only short-lived. HDV coinfections seem to have no major impact on the natural history of acute HBsAg positive hepatitis in endemic areas, where they usually run a benign acute course, rarely progressing to chronicity. Coinfection, however, for factors at present not understood can induce severe and fulminant hepatitis in drug addicts.

In superinfection of HBAg carriers, the long-standing HBV infection offers to the defective virus the unique substrate upon which it can establish its own persistent infection, leading to chronicity in over 90% of istances (Rizzetto and Verme, 1985).

The medical sequelae of HDV infection appear to encompass the whole spectrum of clinical manifestations. Although HDV was previously reputed invariably pathogenic and rapidly leading to cirrhosis, liver failure and death, this view has been changed by identification in some areas around the world of healthy carriers of

HDV (Hadziyannis et al., 1991; Hadler et al., 1991). The natural history of hepatitis D has been described as bimodal, with disease rapidly progressing to liver failure in about 15% of patients and running an asymptomatic course in the remaining patients (Bonino et al., 1987). The first course is typical of drug-addicts with an underlying active HBV and HDV infection (Smedile et al., 1991). The second pattern is typical of patients without overt risk factors and in areas where HDV is endemic; the accompanying HBV infection is inactive and HDV infection is usually less florid than in the former group. Also in this group cirrhosis is reached in a matter of few years, but this cirrhotic status represents a clinically stable condition compatible with a prolonged survival (Rizzetto et al., 1992).

Changes in the epidemiology and in medical manifestations of HDV infection have been occurred over the last 5 years, fostered by improvement of social conditions and hygiene in endemic areas, by the enforcement of measures to prevent AIDS epidemic and large-scale introduction of HBV vaccination. Recent reports from several Italian authors strongly indicate a decrease of HBV endemicity in children. Data from a study on military recruits recently performed in Italy indicate a dramatic decrease in the HBV endemicity level in young adults (D'Amelio et al., 1992). This decline of HBV enables to forecast a decrease in HDV endemicity in the near future, as suggested by some clinicians now finding HDV-related acute and chronic hepatitis less frequently than in the past. As a consequence of the reduced risk of HDV infection we are now seeing less of early stages of HBV/HDV infections characterized by extensive necroinflammatory involvement of the liver parenchyma.

From the discovery of the etiologic agent in 1977 to the early '80s the treatment of chronic hepatitis D rested on immunosuppressive and immunostimulant drugs (Rizzetto et al., 1983; Arrigoni et al., 1983). The poor results obtained with these compounds, the availability of large doses of molecular engineered interferon (IFN), the demonstrated efficacy of this cytokine against DNA and RNA viruses prompted the mid' 80s investigators to try alpha IFN in chronic HDV hepatitis.

A retrospective study and a pilot trial showed that IFN-treated patients experienced amelioration of the disease with marked decrease of aminotranferases (ALT)

Table 1
Interferon treatment in chronic hepatitis D: Retrospective and pilot studies

Reference	Pt No.	IFN type and schedule	End of treatment		End of follow-up	
			HDV-RNA neg.ve	ALT Normal	HDV-RNA neg.ve	ALT Normal
Thomas* 1987	5	Lymphoblastoid t.i.w. 2.5–7.5 MU/m^2 × 2–12 weeks	4 (80%)	0 (0%)	4 (80%)	0 (0%)
Hoofnagle 1987	5	Alpha 2b rec daily 5 MU × 4 mo	3 (60%)	3 (60%)	0 (0%)	0 (0%)

rec.: recombinant, t.i.w.: thrice in a week, mo: months, *: retrospective study.

and improvement in liver histology in about 50% of cases. Testing for HDV markers showed that improvement in disease was associated with decrease or disappearance of HDV-RNA and HDAg in serum and of HDAg in the liver, despite the persistence of HBsAg and of the antibody to HDV (anti-HD). However, when IFN was stopped, most of the patients relapsed with reappearence of HDV-RNA, aminotransferases elevation and worsening of liver histology (Rosina and Rizzetto 1989) (Table 1).

These promising results led to randomized controlled trials, in which higher doses of IFN were administered for more prolonged periods (Table 2) and a pletora of

Table 2
Interferon treatment in chronic hepatitis D: Controlled trials

Reference	Pt No.	IFN type and schedule	End of treatment		End of follow-up	
			HDV-RNA neg.ve	ALT Normal	HDV-RNA neg.ve	ALT Normal
Rosina 1989	12	Alpha 2b rec t.i.w. 5 MU/m^2 × 3 mo	4 (33%)	4 (33%)	1 (8%)	1 (8%)
	12	Untreated	2 (17%)	0 (0%)	2 (17%)	0 (0%)
Rosina 1991	31	Alpha 2b rec t.i.w. 5 MU/m^2 × 4 mo 3 MU/m^2 × 8 mo	14 (45%)	8 (25%)	14 (45%)	1 (3%)
	30	Untreated	8 (27%)	0 (0%)	10 (33%)	0 (0%)
Farci 1994	14	Alpha 2a rec t.i.w. 9 MU × 12 mo	10 (71%)	10 (71%)	0 (0%)	5 (36%)
	14	Alpha 2a rec t.i.w. 3 MU × 12 mo	5 (36%)	4 (28%)	0 (0%)	0 (0%)
	14	Untreated	1 (7%)	1 (7%)	0 (0%)	0 (0%)
Gaudin 1993	11	Alpha 2b rec t.i.w. 5 MU/m^2 × 4 mo 3 MU/m^2 × 8 mo	7 (66%)	7 (66%)	1 (9%)	1 (9%)
	11	Untreated	4 (36%)	2 (18%)	?	?
Cotonat 1992	12	Alpha 2a rec t.i.w. 18 MU × 6 mo 9 MU × 1 mo 6 MU × 1 mo 3 MU × 4 mo	6 (50%)	4 (37%)	0 (0%)	0 (0%)
	14	Alpha 2a rec daily 3 MU × 12 mo	3 (19%)	1 (7%)	0 (0%)	0 (0%)

rec.: recombinant, t.i.w.: thrice in a week, mo: months, ?: data not available.

small uncontrolled trials often dealing with different subsets of patients (Table 3).

The trials showed that the rate of response was proportional to IFN doses: patients treated with 9MU responded better than did patients treated with 3MU (Farci et al., 1994); lowering the dose from 5MU/m^2 to 3MU/m^2 was usually followed by a

Table 3
Interferon treatment in chronic hepatitis D: Uncontrolled trials

Reference	Pt No.	IFN type and schedule	End of treatment		End of follow-up	
			HDV-RNA neg.ve	ALT Normal	HDV-RNA neg.ve	ALT Normal
Buti 1989	4	Alpha 2a rec t.i.w. 9 MU × 6 mo	3 (75%)	3 (75%)	?	?
Taillan 1989	8	Alpha 2b rec t.i.w. 5 MU × 6 mo	?	2 (25%)	?	?
Di Bisceglie 1990	12	Alpha 2b rec daily 5 MU × 4–36 mo	5 (42%)	0 (0%)	?	?
Marinucci 1991	8	Alpha 2a rec t.i.w. 6 MU × 12 mo 1 month stop 1 MU × 12 mo	3 (37%)	1 (12%)	1 (12%)	1 (12%)
Craxi 1991	10	Alpha 2b rec t.i.w. 5 MU/m^2 × 4 mo 3 MU/m^2 × 8 mo	7 (70%)	?	2 (20%)	?
Berk 1991	10	Lymphoblastoid daily 5 MU × 4 mo Acyclovir 2 gr daily × 2 weeks × 2 cycles	?	5 (50%)	?	2 (20%)
Marzano 1992	7	Lymphoblastoid t.i.w. 5 MU/m^2 × 4 mo 3 MU/m^2 × 8 mo	5 (71%)	5 (71%)	3 (43%)	3 (43%)
Johnson 1993	6	Alpha 2b rec daily 10 MU × 5 days t.i.w. 10 MU × 1 mo. 5 MU × 2 mo. 3 MU × 9 mo.	n.p	4 (66%)	?	?

rec.: recombinant, t.i.w.: thrice in a week, mo: months, ?: data not available, n.p.: not performed.

"break through" and rise in aminotransferases toward pre-treatment levels. (Rosina et al., 1991; Gaudin et al., 1992). Daily administration at 5MU dose did reduce the burden of side-effects but also the likelihood of recovery (Di Bisceglie et al., 1990). More complex schedules and combination of IFN with other antiviral drugs, e.g., acyclovir, have been no more successful and again reactivation during follow-up was the rule (Marinucci et al., 1991; Cotonat et al., 1992; Berk et al., 1991).

At histology, a significant decrease of hepatic injury and HDAg staining was often seen in patients who had responded to IFN (Kleiner et al., 1990; Farci et al., 1994).

Reported side-effects in the above trials can largely be ascribed to IFN (flu-like symptoms, fatigue and weight loss), were seen in almost all patients and their severity was usually depending on the IFN dose and sometimes on the presence of a previous history of intravenous drug abuse. In the latter patients compliance is often poor and intermittent use of IFN increases the incidence and severity of side-effects from the drug. Furthermore, the necessity to self-inject IFN presents particular psychological stresses in patients who have overcome a past addiction, often leading to intermittent re-use of addictive drugs (Johnson et al., 1993). One such patient committed suicide during the eleventh month of treatment, and another attempted suicide shortly after the discontinuation of treatment (Gaudin et al., 1993).

Two patients have shown a severe hepatitic episode while on full dose IFN (Rosina et al., 1991; Marinucci et al., 1991): anti-liver-kidney microsomes antibodies were present in the serum of one of these patients (Todros et al., 1991).

Unlike in chronic hepatitis B, no factors have been identified which can predict the outcome of alpha IFN therapy. Demographic, clinical, serological, biochemical and histological findings do not appear to be associated with an increased or decreased probability of response to IFN. The only possible exception seems to be the duration of the disease. Encouraging therapeutic results have often been obtained in patients with a history of intravenous drug abuse, highly likely to have acquired their HBV and HDV infection through sharing needles (Marzano et al., 1992; Johnson et al., 1993). These patients have probably harboured their infection for a relatively short period of time, in contrast with the patients of the endemic areas who mostly acquire the infection early in life. Furthermore, studies in drug addicts, with multiple blood-borne infections, have shown a response in anti-HIV seropositive patients if immunocompetence is preserved (Buti et al., 1989; Taillan et al., 1988).

Many adults and children with a concomitant active HBV replication cleared HBV-DNA and HBeAg from serum and often seroconverted to anti-HBe; this event occurred without much change in liver chemistry and was usually not followed by normalization of ALT levels (Rosina et al., 1991; Gaudin et al., 1992; Craxi' et al., 1992). However, in view of the worse prognosis of patients with active replication of both viruses (Smedile et al., 1991), HBV inhibition has to be considered a positive event in the long-term run.

Although a patient with anti-LKM autoantibodies had a severe bout of hepatitis, that prompted interruption of therapy, ten of these patients have in different trials completed cycles of IFN therapy without complications (Todros et al., 1991).

A young age may have an adverse influence on therapy of hepatitis D, in the same way that it does for IFN treatment of chronic hepatitis B. None of the 10 children treated by Craxi' et al. (1990) improved clinically or histologically after a year of treatment. IFN has to be used cautiously in young patients cured of pediatric malignancies: 2/9 such patients with compensated cirrhosis developed on IFN a severe biochemical exacerbation leading to liver failure, which was fatal in one (Rossetti et al., 1991).

Mechanisms of action of IFN in chronic hepatitis D are poorly understood. The HDV genome is capable of extensive intramolecular base pairing to form double-stranded RNA (dsRNA) (Kuo et al., 1988). Such RNAs are strong inducers of interferon (IFN) and activate two IFN-inducible enzymes involved in the antiviral response: 2′,5′ A synthetase and the dsRNA-dependent proteine kinase (DAI). Since HDV frequently gives rise to chronic infection and replicates to a very high copy number in infected cells, it appears to be able to overcome these cellular antiviral mechanism. This hypothesis was tested in two in vitro experiments using different hepatoma cell lines (HepG2 and Huh-7), both transfected with a plasmid containing a trimer of HDV and capable of stably expressing delta virus RNA and antigen. The results of these studies indicated that the transcriptional response of IFN inducible genes, including the 2′,5′A synthetase and DAI was normal in these cells. Expression of 2′,5′A synthetase in the presence of dsRNA should have activated endogenous ribonuclease leading to the breakdown of RNA. Since the level of HDV-RNA was not affected by treatment with alpha IFN, it appeared that either the action of the synthetase or ribonuclease was inhibited in the cell lines or the HDV-RNA did not exist in a form capable of activating 2′,5′ A synthetase. The HDV-RNA may not be double-stranded in vivo or it may be masked perhaps by binding to the delta antigen (McNair et al., 1993; Ilan et al., 1993).

In vitro experiments apparently contrast with the results obtained in patients, where response to IFN is characterized by concomitant decrease of HD viremia and ALT levels, suggesting a direct antiviral effect of the cytokine against HDV. A major difference between these two models is that patients are always coinfected with HBV, which could represent the primary target of IFN, in keeping with the frequent clearance of serum HBsAg and seroconversion to anti-HBs in long-term responders. In these patients HDV-RNA and HDAg are no longer detectable in serum and liver (Rosina et al., 1990; Hoofnagle et al., 1993; Johnson et al., 1993). Other patients, however, enter a prolonged phase of biochemical and histological remission, despite persistence of active HDV replication and latent HBV infection (Farci et al., 1994).

It is therefore conceivable that IFN activity against HBV may play a crucial role. Although HDV inhibits per se HBV replication, some degree of HBV activity is required by HDV to exert its pathogenic potential. If HBV is further suppressed by IFN, HDV could be cleared itself or enter a non pathogenic phase, possibily sustained by over-expression of the non cytotoxic HDAg-p27, analogous to that observed in the liver transplant model (Ottobrelli et al., 1991).

Alpha IFN represents at present the only choice of treatment for chronic hepatitis D. Available data show that patients respond to IFN if adequate doses (i.e., 9–10

MU three times weekly) are administered for a sufficient length of time. Since HDV superinfection runs a chronic course in over 90% of cases and since early administration of IFN may improve the response rate, treatment should be started in patients with acute hepatitis progressing to chronicity as soon as the acute phase of the disease is over. Patients with no history of overt acute hepatitis and of decompensanted liver cirrhosis should be treated as soon as the diagnosis is made.

Unfortunately the "timing" of IFN treatment remains an open question. Parameters predictive of response are are still lacking. Furthermore, whereas in chronic HBV and HCV hepatitis response to IFN occurs within 1–3 months from the beginning of the treatment, in HDV infection response can take up to 10 months. Thus IFN needs to be administered for at least a year before defining a patient as a non responder.

Another major challenge is deciding when to stop therapy in a patient with a good initial response. Loss of serum HDV-RNA and HDAg does not accurately reflect the clearance of the virus, as patients who respond to treatment will loose these viral markers but they may still relapse once the treatment is stopped. Treatment, however, can be safely interrupted if serum HBsAg has disappeared. If this event does not occur within 1 year of effective therapy (i.e., 1 year of undetectable HDV-RNA and normal ALT), the therapeutic approach should be guided by histological and immunohistochemical findings. In patients with undetectable liver HDAg, IFN should be stopped and the patients monitored for a potential relapse. If HDAg is still detectable, but liver histology has improved, IFN should be continued possibly until it disappears. Careful medical supervision of patients treated for a long time is mandatory for early detection and management of major medical and psychiatric complications.

Better therapies for chronic hepatitis D are needed. Few other antiviral agents have been assessed in this form of hepatitis. Suramin and ribavirin have been found effective against HDV-infected primary woodchuck hepatocytes, whereas foscarnet and acyclovir have an enhancing effect on HDV replication (Rasshofer et al., 1991). Suramin is too toxic to be acceptable for long-term use in humans. Ribavirin, a nucleotide analogue effective in HCV chronic hepatitis, has been tried in two small trials without any virological or biochemical success (Garripoli et al., 1993; Buti et al., 1993). Other antiviral agents have not been evaluated because most of them are effective against DNA viruses. However, in vitro and in vivo evidence points to HBV as a possible target for IFN in chronic HDV infection. This line of reasoning provides the rationale for trying deoxynucleotide analogues.

The many basic advances in understanding the replicative cycle, the genomic and protein structure of HDV promises to provide new approaches to treat this severe form of hepatitis.

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