Interferon Alpha-2b in the Treatment of Chronic Hepatitis C: Early Experience

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The antiviral and immunomodulatory effects of interferon were assessed in the treatment of chronic Hepatitis C in multi-ethnic patients to prevent viral replication and chronic liver damage. Three million units of recombinant interferon alpha-2b were administered three times a week for 48 weeks to a group of 9 active Hepatitis C patients. A clinical response was defined as normalization of serum ALT values. Serum was frozen and stored for Hepatitis C viral assays. Four patients normalized their liver functions. When viral levels were measured only two patients had unmeasurable levels of HCV RNA after treatment. Therapeutic results were observed and much work needs to be done to improve therapy because a serious epidemic is predicted for the future.

Introduction

The Federal Drug Adminstration approved Interferon alpha-2b for the treatment of chronic Hepatitis C in February 1991. The Hepatitis C antigen was identified in 1989 and an antibody test developed soon thereafter; prior to this, Hepatitis C was referred to as Non-A Non-B Hepatitis. Interferon has been shown currently to be the most effective therapy in approximately 30 to 40% of chronic Hepatitis C patients[1-3] because of its antiviral and immunomodulatory effects. The reports leading to FDA approval looked promising so interferon therapy was utilized for our patients soon after FDA approval and forms the basis for this report of our early experiences. Our group was referred the Hepatitis C Virus (HCV) cases for treatment because of our experience in a research project to treat chronic Hepatitis B patients.[4]

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Chronic Hepatitis B is a major public health problem in Hawaii because of the large immigrant population from Asia and the Pacific Basin. Exposure to Hepatitis B virus (HBV) often results in chronic Hepatitis that can significantly increase the risk of developing cirrhosis and hepatocellular carcinoma. Hepatitis C can develop into these same fatal complications. The recent NIH Consensus Conference on Hepatitis C notes that 4 million people in the United States are currently infected with HCV with about 30,000 new cases a year with the numbers to double or triple in the next 20 to 30 years.[5] It will be a major public health problem until an effective therapy and vaccine can be developed.

Materials and Methods

Patient Population

Patients testing positive for the first generation HCV antibody test (Ortho Diagnostics, New Jersey) and negative for Hepatitis B antigens or antibodies were referred for evaluation for treatment. Patients with elevated liver function tests, primarily ALT >=1.5X high normal level for over 6 months, were then selected for treatment according to the FDA approved protocol. Patients were excluded if they showed evidence of cirrhosis as reflected in their alkaline phosphatase levels. Informed consent was obtained.

Interferon Therapy

Chronic active Hepatitis C patients were treated with recombinant interferon alpha-2b (Intron-A, Schering-Plough Corporation, Kenilworth, NJ). Three million units were administered subcutaneously 3 times per week for 48 weeks. If there was no clinical reponse, another 48 week course was offered . Patients received their injections in the Ambulatory Oncology Clinic or chose to voluntarily self-administer their medication after training by the Oncology Nursing Staff. The dose was reduced to 1-2 million units when platelets were <100,000 or granulocytes were <1000.

Evaluation During Therapy

Patients were evaluated during therapy for hematological and biochemical profiles. Blood was collected for complete blood and platelet counts and liver function tests (LFTs) including serum alanine and aspartate aminotransferase and gamma glutamyl transpeptidase activities (ALT, AST, GGPT) prior to therapy, after 2 weeks, monthly during therapy and 2-3 months post therapy. Liver function tests were performed by Immunoassay (EIA) (Abbott Laboratories, Abbott Park, IL). All evaluations were performed by the same Clinical Laboratory. A clincal response was defined as normalization of ALT levels.

Hepatitis C Virus (HCV) Assay

Aliquots of serial serum samples from each patient were drawn at baseline, 2-3 month intervals during therapy, the end of therapy and 2-3 months following therapy and stored at -70° C for analysis when more specific viral tests were available. The first generation test for HCV antibody was performed in the clinical laboratory. Sera drawn during therapy were frozen for later batch analysis for HCV-RNA by reverse transcription-polymerase reaction (RT-PCR) by Lawrence Lumeng MD, Department of Gastroenterology, Indiana University School of Medicine. RT-PCR analysis for the 256BP and 157BP regions confirmed the diagnosis of HCV but it was only semi-quantitative.

Branched HCV RNA analysis was performed by Reference Laboratory Alliance (Pittsburgh, PA).[6] HCV-RNA is quantifiable at levels >3.5X10E+5 Eq/ml but is not FDA cleared for diagnostic use and may not constitute the sole basis for patient diagnosis. HCV-RNA in a patient's sample is captured and hybridized to several target probes corresponding to the conserved 5' nontranslated region of HCV. Amplification of signal from the hybridizations is achieved by addition of branched DNA molecules which can bind multiple copies of enzyme emitted and measured by a luminometer. Concentrations of viral target in individual specimens were determined by comparison with a standard curve.

HCV genotype determination was performed on the baseline samples by RT-PCR at Reference Laboratory Alliance (Pittsburgh, PA). The INNO-LIPA (line probe assay) is a reverse hybridization for the differentiation of the various HCV genotypes. DNA representing a sequence from the 5' nontranslated region was amplified using biotinylated primers. Amplified DNA was hybridized to specific oligonucleotide-probes immobilized on membrane strips. Hybridizations were visualized by reaction of alkaline phosphatase, bound to amplified DNA, with chromogenic substrate. The pattern of reactivity of a simplified fragment with one or more lines upon the test strip allows recognition of five major HCV genotypes (Genotype 1-5) and 6 subtypes (1a, 1b. 2a, 2b, 3a,3b).

Statistical Analysis

Results are expressed as arithmetic mean ± SD except where noted. Data was analyzed with the Sigma Stat program (Jandel Scientific, San Rafael, CA). Continuous variables were analyzed by linear regression or Analysis of Variation (ANOVA) techniques. The IBM 55SX -PS2 computer was used for the analysis.

Results

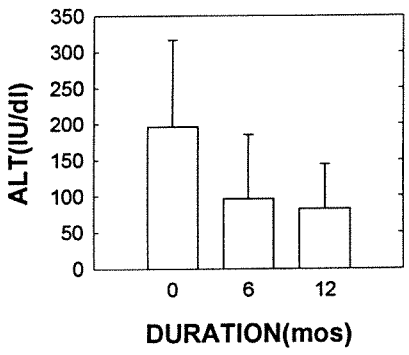
Study Population

Eleven patients were referred for evaluation for treatment; one patient did not qualify because of associated Hepatitis B involvement and evidence of cirrhosis. One of the remaining ten patients was a young patient from a drug rehabilitation program who ran away from his program and did not return for further treatment after his first injection. The remaining nine patients treated ranged in ages of 35-69 years; 6 of the 9 were males. Baseline liver function tests (ALT, AST and GGPT) were increased in the patients: ALT = 195 ± 121 IU/L, AST = 111 ± 64 IU/L, GGPT = 115 ± 109 IU/L. The risk factors identified for Hepatitis C were blood or blood products transfusion (3) and confessed unspecified drug usage (2); no history of any other recognized risk factor was obtained from the remaining 4 patients. There was only one foreign born patient (Korea); the remaining patients were from Hawaii, Guam or the mainland US.

Toxicity

Patients initially experienced flu-like symptoms, including myalgia, headache and fever which generally improved after the first week or two of therapy; one patient (#905) had severe constitutional symptoms which responded to dose reduction. The nine patients tolerated their regimens and completed therapy. One patient (#934) required dose reductions due to decrease in wbc's and platelets.

Fig 1.— Changes in ALT levels of chronic active Hepatitis C patients on interferon therapy, X±SD. Elevated levels at baseline decreased but not to normal levels in all patients.



Biochemical Liver Function Tests

ALT levels decreased in all patients during treatment, P<.05 (Fig 1), but only 4 patients normalized their ALT levels. One patient, #905, who was infected after blood transfusion had normalized her liver function tests after the first 6 months of treatment; she flared to exceptionally high levels of ALT and was started on another course of treatment. She responded to another 6 months of therapy with continued normal liver function tests thereafter. The patients who did not normalize had continued elevated levels or increased after cessation of therapy.

Hepatitis C Virus (HCV) Assay

All patients were confirmed for the presence of HCV by PCR analysis. Serial analysis during treatment of the patients only demonstrated the disappearance of both BP256 and BP157 markers in one patient, again #905, demonstrating eradication of the virus. This patient had flared her LFTs and required a second course of treatment.

Baseline levels of branched HCV RNA analysis ranged from 8.4-862.7 10E+5 Eq/ml. The 4 patients who normalized their LFTs had baseline levels of 8.4, 26.6, 66.9 and 108.2 10E+5 Eq/ml. Only two of the four patients developed unmeasurable levels of HCV RNA after therapy whose baseline levels were 8.4 and 26.6 respectively. The lower baseline levels of HCV RNA may possibly predict a better response.

HCV genotype analysis demonstrated a preponderance of genotype 1, 7/9 patients. There was only single incidences of type 2 and

type 3 genotype groups. Three of the four patients with a clinical response were type 1 or 1b and the fourth was type 3a; the complete responder was type 1b. The relationship of branched chain HCV RNA levels and genotype classification and clinical response is summarized in Table 1.

Discussion

We were able to achieve a clinical response in four out of nine patients completing therapy in this early utilization of Interferon alpha-2b therapy for Hepatitis C infection which is consistent with the results of other trials. However, the antiviral effect of interferon did not correspond to the clinical response; only two patients achieved unmeasurable levels of HCV RNA and only one of these two had no trace of HCV with the sensitive PCR analysis. Grecht et al[6] report that high viremia titers were associated with advanced stages of the disease. Low baseline HCV RNA levels are reported by other investigators[7,8] to be predictors of successful therapy, which may also be seen in some of our responders to interferon treatment.

In the United States, genotype 1 accounts for about 75% of chronic HCV infections with half belonging to the 1a subtype and half to the 1b subtype. Genotype 2 accounts for 10-20% of isolates in the US and genotype 3 for another 5% of isolates. The distribution of HCV genotype in our study group follows these reports. Genotypes 4, 5 and 6 are rarely seen in the USA and when identified usually represents infection acquired abroad. Studies have documented higher rates of long-term response to alpha interferon in patients infected with genotypes 2a, 2b, or 3a compared with genotype 1.[8-11] Chemello and Alberti[12] reports only 29% long term response for type 1, versus 52% for type 2 and 74% for type 3 patients. The predominanace of type 1 virus in Hawaii demonstrates a lowered chance of successful therapy in our patients. In contrast type 2 predominates in the Japanese patients (69%) with 18 (%) Type 3; this gives them a greater probability of successful therapy than our population.[13] One type 3 patient had a clinical response and developed unmeasurable levels of HCV RNA. The one complete responder to all three viral measurements was genotype 1b; three of four clinical responders were serotype 1b or 1. In this study the genotype of HCV did not aid in identifying probable reponders except for one type 3 patient who had a low level of HCV RNA (8.4) at baseline.

Table 1.–Viral Genotype, HCV-RNA and Clinical Response

Patient No.	Sex	Genotype	Clinical Response	HCV RNA** Baseline	HCV RNA End
904	F	1	+	108.2	104.3
905*	F	1b	+	26.6	<3.4
910	M	1a	-	862.7	109.4
913	M	1	-	113.1	140.4
934*	M	1b	-	45.6	44.9
961	F	3a	+	8.4	<3.4
962	M	1a	-	186.9	19.4
963	M	1b	+	66.9	42.3
964	M	2b	-	113.1	34

*—Dose Reduction ** 10E + 5Eq/ml

Interferon still remains the only effective treatment for Hepatitis C infection at present. The optimal duration and dose may still need to be determined. Early trials suggested a better response at higher doses; it has been recommended that patients unresponsive to the standard dose be treated with higher doses.[14] Bellary et al[15] utilized a dose of 5 million units three times a week for 6 months and achieved a 59% reponse rate, but 50% of those with a total reponse had a relapse. Lindsay et al[16] evaluated response rates of 3,5, or 10 million units given thrice weekly for 12 weeks; those not responding after 12 weeks were then randomized to additional therapy at either the same or higher dose for an additional 12 to 36 weeks. They concluded that the initial response to interferon was not increased by treatment with higher doses of the drug; although marginal, the additional higher doses may still be worth the risk of intolerance to the medication. Vogel et al[17] and Ferenci P et al[18] in Austria reported improved response to doses up to 10 million units but there was varying intolerance to the medication. The treatment may also need to be extended for longer periods as well as an increase in dosage. The toxicity and expense of such regimens must be considered if this is comtemplated. Poynard et al[19] extended the interferon treatment randomly for another 12 months after their patients had been treated for 6 months. Those receiving the same dose for an additional 12 months demonstrated a higher percentage with complete ALT and liver histologic reponse.

This early trial reveals only a 4/9 clinical response to Interferon alpha-2b and only 2/9 developed unmeasurable levels of the Hepatitis C virus. Further trials are required since interferon is the only effective treatment at this time.

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