Randomized, double-blind, placebo-controlled trial of oromucosal low-dose interferon following prednisone withdrawal for chronic hepatitis B infection in Filipino patients

Thelma E. Tupasi,(1) Vilma M. Co,(1) Ma. Socorro M. Clarin,(1) Evelyn T. Alesna,(1) Ella Mae S. Divinagracia(1) and Nellie V. Mangubat(1)

Objective: To evaluate the efficacy and safety of oromucosal low-dose human lymphoblastoid interferon alpha (IFN-α-nl [INS]) following steroid withdrawal in Filipino patients with chronic replicative hepatitis B virus (HBV) infection.

Study design: Randomized, double blind, placebo-controlled trial on IFN-α-n1 [INS], two tablets of 200 IU each or placebo, given sublingually once daily for eight months following steroid or placebo priming and withdrawal.

Results: A statistically significant clearance of hepatitis B e antigen (HBeAg) (50%) and seroconversion to positive antibody to HBeAg (anti-HBe) (42.9%) was noted in those given IFN-α-n1 [INS] compared with the placebo group. Clearance of serum HBV-DNA was not significantly different and none cleared HBsAg in both groups. More patients (57%) had normalization of ALT on IFN-α-n1 [INS] compared with controls (31.3%). Oromucosal ITN-α-n1 [INS] was devoid of any evidence of toxicity.

Conclusion: This study conducted on a limited number of patients demonstrates the potential efficacy of oromucosal IFN-α-n1 [INS] in chronic HBV infection with therapeutic benefit equal to parenterally administered interferon alpha (IFNα) but without the side effects of myelosupression. Owing to the small population studied, we are unable to extrapolate these findings to the general population of patients with chronic HBV infection. A large-scale study is needed to confirm these findings.

Int J Infect Dis 2002; 6: 37-41

Interferon alpha (IFN-α) has been the standard treatment of chronic HBV infection in most countries.1 The response to interferon among Asian patients has been poorer compared with Caucasians.2-3 Steroid priming and withdrawal preceding IFN-α has been reported to enhance the efficacy of IFN-α and the cumulative sustained response on long-term follow up among Chinese patients was significantly higher compared to those on IFN-α alone.4,5

Parenterally administered IFN(α) is associated with significant dose related side effects.6 In contrast, oromucosal low-dose human lymphoblastoid interferon alpha (IFN-α-n1 [INS]) has been reported to have no associated adverse effect.7-8

This randomized, double blind, placebo-controlled trial was undertaken to determine the efficacy and safety of oromucosal low-dose human lymphoblastoid interferon alpha (IFN-α-n1 [INS]) following prednisone withdrawal in Filipino patients with chronic HBV infection.

PATIENTS AND METHODS

The estimated sample size of 44 (22 allocated per treatment arm) would have a power of 0.8, at a 95% confidence level, to detect a statistically significant difference between a response rate of 40% in the treatment arm, assuming a 7% spontaneous clearance of HBeAg in the placebo control arm.

Patients with chronic replicative HBV infection with alanine aminotransferase (ALT) at least >1.5 of the upper limit of normal were randomly allocated into each arm after written informed consent. Excluded were patients who were positive for HIV, hepatitis C virus, hepatitis delta antibody, or those with evidence of decompensated liver disease, elevated alpha fetoprotein or any serious psychiatric or medical condition.

The patients were primed with two capsules of prednisone, 5 mg/capsule, or matching placebo capsules, thrice daily for three weeks, then one capsule thrice daily during the fourth week. Two weeks after steroid or
placebo withdrawal, the patients were given two tablets once daily of 200 IU IFN-α-n1 [INS] or identical placebo tablets sublingually until they were completely dissolved, allowing nothing by mouth for 30 minutes before and after the medication.

HBsAg, HBeAg, and anti-HBe by radioimmunoassay or enzyme-linked immunoassay (ELISA), and HBV-DNA by hybridization assay (HA) with the commercial ABBOTT immunoblot test or by polymerase chain reaction (PCR) were obtained at baseline, at the end of steroid withdrawal, at the end of treatment, and at two months post-treatment. PCR was done at the Royal Alfred Hospital, Sydney, Australia using Digene HBV-DNA kit. Alanine transaminase (ALT) and aspartate transaminase (AST) were done monthly. Serum bilirubin, serum albumin, alkaline phosphatase, prothrombin time, fasting blood glucose, urea, creatinine, sodium, and potassium were done at baseline.

The primary outcome variables were clearance of HBeAg and seroconversion to positive anti-HBe. The secondary outcome variables included normalization of ALT, loss of serum HBV-DNA, loss of HBsAg, and seroconversion to positive anti-HBs. To assess for safety, drug toxicity was scored by using symptoms and mean values of hemoglobin; neutrophil and platelet count were determined monthly.

All statistical tests were done with the Statistical Package for the Social Sciences for Windows (SPSS for Windows Version 7.5). In-group analysis of parametric data was performed using paired t-test whereas between-group comparison was carried out with unpaired t-test. Analysis of covariance adjusted for baseline value was applied to the data to compare laboratory results of the two groups. Fisher’s exact probability test was used to compare the primary and secondary outcome variables. The clinical trial was approved by the International Review Board of the Makati Medical Centre-Tropical Disease Foundation in accordance with the Helsinki Declaration.

RESULTS

Study population

Of 459 patients with chronic HBV infection screened, 100 (22%) had replicative disease and 44 (10%) had evidence of necroinflammatory disease. Thirty-six patients were enrolled in the study. Enrollment was terminated after a 5-year patient accrual, since most patients screened had no replicative disease or evidence of necroinflammatory liver involvement.

Table 2. Efficacy of IFN-α-n1 [INS] vs placebo: primary analysis (n=14 vs n=16) and intent to treat analysis (n=17 vs n=19) based on primary and secondary outcome variables

<table>
<thead>
<tr>
<th>Outcome variables</th>
<th>Primary analysis</th>
<th></th>
<th></th>
<th>Intent to treat analysis</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary:</td>
<td>IFN-α-n1 [INS]</td>
<td>Placebo</td>
<td></td>
<td>IFN-α-n1 [INS]</td>
<td>Placebo</td>
<td></td>
</tr>
<tr>
<td>Clearance of HBeAg</td>
<td>N=14</td>
<td>N=16</td>
<td>P value</td>
<td>N=17</td>
<td>N=19</td>
<td>P value</td>
</tr>
<tr>
<td>Serocconversion to anti-HBe</td>
<td>7 (50)</td>
<td>6 (42.9)</td>
<td>0.002</td>
<td>7 (42.2)</td>
<td>0</td>
<td>0.002</td>
</tr>
<tr>
<td>Secondary:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normalization of ALT</td>
<td>8 (57.1)</td>
<td>5 (31.3)</td>
<td>0.15</td>
<td>7 (41.2)</td>
<td>4 (41.2)</td>
<td>0.34</td>
</tr>
<tr>
<td>Clearance of HBV-DNA*</td>
<td>7 (50)</td>
<td>6 (37.5)</td>
<td>0.49</td>
<td>7 (41.2)</td>
<td>7 (36.8)</td>
<td>0.94</td>
</tr>
<tr>
<td>Clearance of HBsAg</td>
<td>0</td>
<td>0</td>
<td>1.0</td>
<td>0</td>
<td>0</td>
<td>1.0</td>
</tr>
<tr>
<td>Serocconversion to anti-HBs</td>
<td>0</td>
<td>0</td>
<td>1.0</td>
<td>0</td>
<td>0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

*Loss of serum HBV-DNA at any time during the period of observation
Seventeen patients were randomized to IFN-α-n1 [INS] and 19 to placebo (Table 1). There were three patients in each treatment arm who could not be evaluated; three on IFN-α-n1 [INS] and two on placebo were lost to follow up, and one on placebo had early withdrawal and was switched to IFN-α-n1 [INS] for HBV-related glomerulonephritis. Except for this patient, all were asymptomatic. The proportion of females in each treatment group, the mean age, baseline mean serum HBV-DNA, ALT, and AST levels showed no statistically significant differences between the two treatment groups at randomization, or between those who could and could not be evaluated in each treatment arm, or between those who could be evaluated in both treatment arms.

Virological response

Clearance of HBeAg was noted in seven (50.0%) patients on IFN-α-n1 [INS] compared to none on placebo (P=0.002 by Fishers exact test) (Table 2). Six (42.2%) of those who cleared HBeAg on IFN-α-n1 [INS] had seroconversion to positive anti-HBe while none on placebo did (P=0.05). This had a power of 0.9 at a 95% confidence level, to detect a statistically significant difference.

Clearance of serum HBV-DNA at some point during the observation period in seven (50.0%) on IFN-α-n1 [INS] (including five of those who cleared HBeAg and seroconverted to antiHBe) was not significantly different compared to six (37.5%) on placebo. None had clearance of HBsAg or seroconversion to positive anti-HBs.

Normalization of ALT in eight (57.1%) on IFN-α-n1 [INS] compared to five (31.3%) on placebo was not significantly different. The mean ALT of those on placebo remained generally the same throughout the observation course (Figure 1). In contrast, a flare of the ALT mean levels was seen in the patients on IFN-α-n1 [INS] after steroid withdrawal and at one month after the start of IFN-α-n1 [INS] therapy.

Intent to treat analysis

Clearance of HBeAg in seven (41.2%) and seroconversion to positive anti-HBe in six (35.3%) in the treatment arm were significantly greater than those in the placebo arm on intent to treat analysis (P=0.002 and P=0.006, respectively). The secondary outcome variables were not different in the two groups.

Safety assessments

Except for one patient who developed a drug-induced hypersensitivity skin reaction, no adverse reaction necessitating drug discontinuation or dosage adjustment was observed. Figure 2 shows the: a) mean hemoglobin levels and b) white blood cell count in patients on either treatment arm at various points of their treatment course. No statistically significant difference of these parameters was noted in the two groups.

DISCUSSION

The Philippines is a mesoendemic area for HBV infection with HbsAg-positive prevalence of 12%.
Transmission is primarily vertical and most infections are therefore acquired early in life. While most patients with HBV infection progress to chronicity, however, only 22% have replicative disease, and only 10% had evidence of necroinflammatory liver disease with low levels of aminotransferase elevation as shown in those patients screened in this study. Treatment is therefore indicated only in a minority of patients.

The response rate with oromucosal low-dose IFN-α-n1 [INS] in these Filipino patients studied was 50.0% and is similar to those reported with the use of parenteral interferon among Caucasian as well as Asian patients. Because post-treatment follow up in this study was limited to only two months after end of therapy, the delayed long-term beneficial effects of IFN-α was not ascertained. The encouraging results reported herein, however, should be interpreted with caution, as definite conclusions cannot be drawn from this small number of patients studied.

The evident flare in ALT levels noted at steroid withdrawal and one month after start of IFN-α-n1 [INS] is similar to what has been observed with parenteral IFN-α and has previously been reported with oromucosal IFN-α lozenges in patients with chronic viral hepatitis B infection. The subsequent decline in the mean levels of ALT in those on IFN-α-n1 [INS] suggests an amelioration of the necroinflammatory liver disease. This was more evident in those with HBeAg clearance, all of whom showed a return to normal ALT values that was sustained up to two months post-treatment.

Other than one case of drug-induced perivascular dermatitis, signs and symptoms of toxicity were absent.

This is consistent with earlier reports in animal models in which antitumor and antiviral effects of IFN-α-n1 [INS] were noted without the associated signs of myelosuppression. From these studies, Tovey and co-workers postulated a novel mechanism of action that is different from that of parenteral interferon. They proposed that oromucosal INF-α binds to high affinity cell surface receptors present in the lymphoid tissues in the oral cavity leading to the phosphorylation and activation of signal transducers and activators of transcription (STAT) proteins. This binding, they claim, modulates the transcription of interferon-sensitive genes resulting in immune stimulation mediated by the neo-synthesis of a novel soluble factor in conjunction with CD4+ T lymphocytes with a Th 1 phenotype. The enhanced Th 1 cell-mediated immunity is hypothesized to be responsible for the biological and clinical activities of IFN-α-n1 [INS]. Alternatively, it has also been proposed that the IFN-alpha receptor binding may activate specific cell populations; presumably the intra-epithelial γδ T cells, which enter the circulation to mediate the antitumor and antiviral effects of oromucosal IFNα.

The absence of adverse drug effects and the lower cost of the drug would make it a more cost effective alternative to parenteral interferon. Excluding the cost of steroid priming, the cost incurred with IFN-α-n1 [INS] treatment for an eight-month course based on year 2000 price of US$5.24 per tablet in the Philippines is US$1174.63 compared to US$2127–US$3190 cost incurred for parenteral interferon given five million IU thrice weekly for 16–24 weeks based on a corresponding unit cost of US$44.31 for the parenteral formulation. This cost saving would be an important consideration particularly for countries with limited resources like the Philippines, where HBV infection continues to be a significant public health problem. The therapeutic benefits of oromucosal IFN-α-n1 [INS] therapy need to be confirmed in a large scale study before definite recommendations for its use in chronic HBV infection can be made.

**ACKNOWLEDGMENTS**

We acknowledge with thanks the help of Ms. Jocelyn M. Lazo, Ms. Helen June Lorenzana, and Ms. Victoria Perez in performing the laboratory examinations, Ms. Miriam Davis in the preparation of the manuscript and Dr. Troy Gepte and Ms. Mercy Parazo in the statistical analysis of the data.

**REFERENCES**

2. Wong DKH, Cheung AM, O’Rourke K, Naylor CD, Detisky AS and Heathcote J. Effect of alpha interferon


