Clinical trial

Randomised controlled trial of lymphoblastoid interferon for chronic active hepatitis B

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SUMMARY Thirty male patients (27 homosexual) with biopsy proven chronic active hepatitis B were randomised to receive lymphoblastoid interferon (Wellferon) or no treatment. All patients were HBeAg positive and had continuing viral replication. Patients receiving treatment were given a single daily intramuscular injection of interferon for 28 days at a starting dose of 2-5 MU/m² increasing to a maximum of 7-5 MU/m²/day. Transient side effects of malaise and influenza like symptoms occurred in all patients and resolved rapidly after treatment. Hepatitis B viral replication was suppressed during interferon treatment in all patients but the effect was limited to the period of therapy. After one year there was no appreciable difference in viral markers between the two groups of patients and this treatment schedule appears less effective than the thrice weekly, three month regimes recently reported from other centres.

There is considerable interest in the use of antiviral drugs for the treatment of chronic active hepatitis B during the phase of active viral replication. The observation that the hepatic inflammation may subside when viral replication ceases has encouraged the trial assessment of a number of antiviral agents. The interferons, a family of glycoproteins, have both widespread immunomodulatory and antiviral effects. Lymphoblastoid interferon (Wellferon), containing at least eight polypeptides, has been shown to have an antiviral effect against hepatitis B (2) and we undertook a randomised controlled trial of this agent versus no treatment for chronic active hepatitis B.

Methods

Patients
Thirty four consecutive outpatients with documented chronic hepatitis B and satisfying the entry criteria were offered entry to the study and 30 accepted. Their characteristics are shown in the Table. All were adult men known to have carried the hepatitis B virus (HBV) for more than six months and to be positive in serum for HBeAg. HBV DNA, or its associated polymerase (HBV DNAp), was detected in all patients, (HBV DNAp was present in 27 of 30 patients; HBV DNA was present in all 27 patients tested including the three who were negative for HBV DNAp). All had undergone liver biopsy within six months of entry showing chronic active hepatitis and in six of these patients active cirrhosis was also seen. No patient had received previous antiviral therapy, nor any immunomodulatory agents within the last year. Homosexual and heterosexual men were randomised separately. Treated patients underwent a further liver biopsy one year after entry.

Patients randomised to the treatment group were admitted to hospital for the first three days of treatment and thereafter attended daily for the remainder of the treatment period. They received a single daily intramuscular injection of lymphoblastoid interferon (Wellferon, Wellcome Research Laboratories) for 28 consecutive days. The initial daily dose of 2.5 MU/m² was increased in increments to a maximum of 7.5 MU/m² if tolerated. Paracetamol was given as an antipyretic when required. Both
Table: Characteristics of the two patient groups.

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NT = not tested. * = heterosexual. All other patients were homosexual; † = died shortly after entry; ‡ = these patients were all HBV DNA polymerase positive. Serum aspartate transaminase (AST) measured in IU/l (normal range <40). The value of AST shown during treatment is the maximum attained at any time whilst receiving interferon. Post-treatment assessment of HBV DNA in liver and serum was carried out one year after entry.

Groups of patients were seen regularly in outpatients throughout the duration of the study. The protocol for this study was approved by the local ethical committee and all patients gave informed written consent.

During treatment the full blood count, platelet count, urea and electrolytes, bilirubin, total protein, albumin, alkaline phosphatase and serum aminotransferases were measured weekly. The presence of hepatitis B surface antigen (HBsAg) was detected in serum samples by ELISA (Organon Teknica). Antibodies to HBsAg (anti-HBs) and to hepatitis B core antigen (anti-HBc) were detected by radioimmunoassay ('AUSAB' and 'CORAB', Abbott Laboratories). The presence of HBeAg and anti-HBe was determined by ELISA. Hepatitis B virus associated DNA polymerase activity (HBV DNAP) was assayed using 3H thymidine incorporation as previously described. Where sufficient liver tissue was obtained a portion of the biopsy was snap frozen in liquid nitrogen and later analysed for the presence of free and/or integrated HBV DNA. The presence of HBV DNA in serum was detected using a non-quantitative dot hybridisation method. Stored sera obtained at entry to the study and one year later was also tested by radioimmunoassay for the presence of antibody to human T cell lymphotropic virus type 3 (anti-HTLV3)* and by radioimmunoassay for antibody to the delta agent (IgG anti-delta).

Results

Fourteen patients were randomised to the treatment group and 16 to the control group; 28 patients completed the study. One patient in the treatment group was withdrawn after four days when his liver

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*Since the submission of this paper the international nomenclature for HTLV3 has been changed to HIV.
function tests showed that immediately before treat-
ment he had developed an anicteric non-A non-B
hepatitis and treatment was stopped in patient 10
after three weeks when he developed a diarrhoeal
illness associated with the isolation of Salmonella sp
from his stools. One patient in the control group died
from gastrointestinal haemorrhage within six weeks
of entry.

The two groups were similar in relation to age and
known duration of HBV carriage. Twenty seven
patients were homosexual. Two homosexual patients
were from Mediterranean countries and the remain-
ing 28 patients were all Caucasian and born in Great
Britain.

One patient in each group was positive in serum for
antibody to the delta agent. A total of eight patients,
five treated and three controls, were positive for anti-
HTLV3 at both entry and exit from the study. No
patient became anti-HTLV3 positive within one year
of entry into the study. Among the anti-HTLV3
positive patients, one treated patient developed
persistent generalised lymphadenopathy three
months after entry, two control patients developed
persistent generalised lymphadenopathy during the
study and one treated patient developed the acquired
immunodeficiency syndrome nearly three years after
entry to the study.

The side effects of interferon treatment were
mainly malaise, which reversed rapidly after the
completion of therapy, and influenza like symptoms
to which tolerance developed despite the continua-
tion of therapy. These systemic effects were most
marked in older patients. Reversible myelosuppres-
sion was seen in several patients and where the
granulocyte count fell below $1 \times 10^9/l$ the dose of
interferon was reduced or treatment stopped until
the granulocyte count exceeded this figure. Using this
treatment regime almost all patients were readily
able to tolerate a daily dose of 5 MU/m² but greater
dosages were likely to lead to an eventual inter-
ruption in therapy.

During interferon treatment, viral replication as
measured by HBV DNA polymerase was reduced in
all patients and became transiently negative in seven
patients at some stage during their treatment. There
was, however, no apparent long term effect on viral
replication. In two treated patients HBV DNA and
HBV DNAp became negative during follow up and
both developed anti-HBe, five and 18 months respec-
tively after treatment. One untreated patient also lost
HBV DNA and HBV DNAp but was still HBeAg
positive 12 months after entry to the study when he
was lost to follow up.

Twelve of the treated patients underwent repeat
liver biopsy one year after entry. In patient 14 the
latter biopsy was classified as chronic persistent
hepatitis while in the remaining patients the histol-
ogical findings were similar, or the inflammatory
infiltrate was more marked, than at entry to the
study.

In two of the treated patients qualitative changes
were observed in the post-treatment liver biopsies by
nucleic acid hybridisation studies using an HBV
genome probe. In one patient, no HBV DNA
sequences could be observed in the hepatocytes in a
post-treatment biopsy which was taken immediately
before seroconversion to anti-HBe. In the second,
integrated HBV DNA sequences, as well as replicat-
ing viral DNA were detected in the post-treatment
biopsy, where only replicative forms had been detec-
ted pretreatment. This patient also seroconverted
to anti-HBe shortly after the second biopsy was carried
out. In both these patients, examination of the
pretreatment biopsies revealed very low levels of
viral replication and we cannot determine whether
their seroconversion was attributable to interferon
treatment or the natural progression of their disease.
Interestingly, in the control group, where no anti-
HBe seroconversions occurred, all pretreatment
biopsies examined had relatively high levels of
replicating HBV DNA.

**Discussion**

Initial studies of interferon in chronic hepatitis B
were hampered by limited availability of the drug.
IFN-B appeared relatively ineffective and although
IFN-A was shown to have a transient effect on HBV
DNA polymerase there was no apparent benefit
over a longer period. Higher dosages of IFN-A,
given alone or in combination with adenine arabin-
side, produced long term suppression of viral replica-
tion with loss of infectivity in a proportion of
patients but these studies were uncontrolled. An-
other uncontrolled study, from Japan and using low
doses of IFN-A, produced an apparently high rate of
response.

Since the start of our study, thrice weekly rather
than daily lymphoblastoid interferon has been shown
to be effective in inhibiting hepatitis B virus replica-
tion and appears to be more effective when given in
three month courses. Recombinant IFN-A has also been
shown to suppress viral replication and courses
longer than those used in our study were again more
effective. These different responses to treatment
may reflect differences in interferon dosages and
duration of treatment as well as variation in the rate
of spontaneous cessation of viral replication and
seroconversion to anti-HBe positivity seen in differ-
ent groups of chronic hepatitis B carriers. Male
homosexuals, who are likely to form the largest
group of chronic hepatitis B carriers in Western
countries suitable for antiviral treatment, may show altered immune responsiveness" and also appear to respond less well to treatment.17

Interferons may have immunosuppressive effects when given in high dosage and this may be important if viral clearance of HBV depends on the alterations to the immune system as well as the direct antiviral effects. The dosages in this study were immunosuppressive as shown by the effects on natural killer (NK) cell activity (unpublished observations). Studies using different dosage regimes are currently in progress and may help to clarify the relative importance of the immunomodulatory and antiviral effects of the interferons in chronic HBV.

At the time of this study HTLV3 infection was already present amongst the male homosexual population in London and the prior exposure to this agent in eight of our study patients might have been expected to alter their response to treatment.1 We found no obvious difference, however, in response between anti-HTLV3 positive and negative patients. In future controlled studies in different ethnic groups, allowing both for homosexuality and HTLV3 status, will be required to assess the efficacy of the various interferons.

Toxicity of lymphoblastoid interferon was not a major problem during this study and the side effects were both predictable and reversible. These unwanted effects appear to be less pronounced using an intermittent regime which preserves the antiviral efficacy. During this study patients attended daily for treatment with considerable interruption of their daily lives. During subsequent studies we have found that almost all patients can be taught to give their own injections subcutaneously and absorption from this route appears to be equally effective.

This study shows that a one month course of lymphoblastoid interferon appears to have no long term benefit on chronic hepatitis B virus replication, but longer courses of this agent given thrice weekly are showing promise.

We are grateful to Dr B Evans and the department of genitourinary medicine for referring many of the patients and to Dr R Williams for permission to include some of the patients under his care. Dr J Coleman and Mr R Dayton provided valuable assistance with virological assays. MGA was supported by the Bernard Sunley Charitable Foundation. Wellcome Research Laboratories supplied the Wellferon and provided financial support towards the study. Results from this study were presented in part at an international meeting on the treatment of chronic hepatitis B held at the Royal Society, London, in October 1985.

References