Recombinant human interleukin 2 treatment in patients with chronic hepatitis B virus infection

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[Summary]

A preliminary study of recombinant human interleukin 2 therapy was made in 8 patients with chronic hepatitis B. They received 250 to 1,000 units per day of interleukin 2 for 28 consecutive days. Fever, fatigue, and anorexia were the major side effects, although all of them were resolved after interleukin 2 administration was discontinued. Serum deoxyribonucleic acid (DNA) polymerase activity decreased in all patients during the administration of interleukin 2. There was an abrupt rise in serum alanine aminotransferase level associated with a fall in serum DNA polymerase level in 5 of the patients. One patient had sustained losses of hepatitis B e antigen and DNA polymerase. Recombinant interleukin 2 therapy may be of benefit in some hepatitis B virus carriers, although further development of this approach and additional patient follow-ups are required.

Key words: interleukin 2, chronic hepatitis, treatment, HBe Ag, DNA polymerase
I. Introduction

Accumulating evidence has suggested that cellular immunity may play an essential role in the pathogenesis of viral hepatitis B as well as in virus elimination. The immunity against the latter is thought to be reduced or deficient in chronic carriers of hepatitis B virus antigens. Since these carriers often develop severe liver diseases including hepatocellular carcinoma, many attempts have been made to potentiate specific immunity against virus antigens with immunostimulants. However, no promising results have so far been obtained. Interleukin 2, originally called the T cell growth factor, is a lymphokine produced by T cells when they are activated with lectins or alloantigens. It has been demonstrated to be a critical factor for the growth of T cells, and it is able to augment various T cell functions as well as natural killer (NK) cell activity. Moreover, the recent availability of purified recombinant interleukin 2 from Escherichia coli expressing the gene for human interleukin 2 has allowed its evaluation as a therapeutic reagent in patients. In the present study, we examined the efficacy and toxicity of recombinant interleukin 2 in a pilot study of interleukin 2 therapy in patients with chronic hepatitis B.

II. Materials and methods

1. Materials

Eight patients with chronic hepatitis B were studied (Table 1). All had been positive to hepatitis B e antigen (HBe Ag) and negative to hepatitis B e antibody (HBe Ab) for 3 months. Patients receiving the following drugs for the indicated number of days before the study were excluded: adenine arabinoside (30 days), interferon (180 days), prednisone (60 days), and cianidanol (60 days). Patients with symptomatic ischemic heart disease and active neurological diseases, and pregnant or lactating women were also excluded. All details of the protocol were approved by the Japanese Ministry of Health and Welfare (February 2, 1985), and a fully informed written consent was obtained from all patients.

2. Interleukin 2

Interleukin 2 was produced by recombinant DNA techniques and supplied by Takeda Chemical Industries Inc. Osaka, Japan. This purified protein was homogenous on sodium dodecyl sulfate polyacrylamide gel. The material was >95% pure, and the specific activity of the purified recombinant interleukin 2 was 3.5×10^4 units/mg. One unit of interleukin 2 was defined as the amount of activity present in a 48 hour cultured conditioned medium of human peripheral blood lymphocytes (5×10^6 cells/ml) in the presence of TPA (15 ng/ml, 12-O-tetradecanoyl-phor-
Table 1 Clinical features of 8 patients with chronic hepatitis B treated with interleukin 2.

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age</th>
<th>Sex</th>
<th>DNAp (cpm/0.2 ml) Before treatment</th>
<th>Liver biopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>29</td>
<td>♂</td>
<td>198</td>
<td>severe CAH</td>
</tr>
<tr>
<td>2</td>
<td>38</td>
<td>♂</td>
<td>3,890</td>
<td>severe CAH</td>
</tr>
<tr>
<td>3</td>
<td>37</td>
<td>♂</td>
<td>200</td>
<td>Not done</td>
</tr>
<tr>
<td>4</td>
<td>27</td>
<td>♂</td>
<td>1,112</td>
<td>mild CAH</td>
</tr>
<tr>
<td>5</td>
<td>41</td>
<td>♂</td>
<td>394</td>
<td>Not done</td>
</tr>
<tr>
<td>6</td>
<td>39</td>
<td>♂</td>
<td>2,524</td>
<td>CPH</td>
</tr>
<tr>
<td>7</td>
<td>36</td>
<td>♂</td>
<td>660</td>
<td>CPH</td>
</tr>
<tr>
<td>8</td>
<td>23</td>
<td>♂</td>
<td>660</td>
<td>mild CAH</td>
</tr>
</tbody>
</table>

Fig. 1 Treatment protocol of 8 patients with chronic hepatitis B treated with recombinant human interleukin 2 (rIL 2).

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The patients were initially admitted to the Third Department of Internal Medicine, Osaka City University Hospital for medical evaluation and liver biopsy. Bol-13-acetate (20 μg/ml) and concanavalin A (20 μg/ml) were used. It corresponded to 1.2 × 10^7 units/mg, calculated on the basis of the Biological Response Modifier Program reference reagent human interleukin 2 prepared from the JURKAT cell line. Various in vitro biological activities of the recombinant interleukin 2 were comparable to those of natural interleukin 2 on the basis of protein weight.

3. Protocol

The patients were initially admitted to the Third Department of Internal Medicine, Osaka City University Hospital for medical evaluation and liver biopsy. They received 250 to 1,000 units per day of interleukin 2 for 28 consecutive days (Fig. 1). Recombinant interleukin 2 was diluted in 250 ml of normal saline, and this was infused intravenously over a 30 minute period every day at 1:00 p.m. The patients were discharged from the hospital 4 weeks after treatment and were seen in the outpatient clinic every one to 2 weeks. Pretreatment liver biopsy was performed in all patients, and in 5 patients, it was also performed after treatment.

4. Methods

For safety, blood samples were obtained weekly
during therapy and every 2 to 4 weeks thereafter. Each blood sample was tested for complete blood counts, routine serum biochemical test, and hepatitis B virus markers. The serum biochemical test included serum bilirubin, albumin, globulin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, blood glucose, blood urea nitrogen, uric acid, lactate dehydrogenase, creatinine, urinalysis, and prothrombin time. HBsAg and antibody (anti-HBe, HBeAb) were assayed by solid phase immunoassay (Abbot Laboratories, North Chicago, Illinois, U.S.A.). Deoxyribonucleic acid (DNA) polymerase activity was determined by $^{3}H$ thymidine incorporation according to the modification of the method of Kaplan et al. and the results were expressed as counts per minute per 0.2 ml of serum (negative > 30). Liver biopsy specimens were obtained before and within 2 weeks after treatment. The two biopsy specimens were compared, and the degree of hepatitis and the presence or absence of cirrhosis were determined.

III. Results

1. Antiviral activity
To assess the interleukin 2-induced response of DNA polymerase activity, percent reduction of DNA polymerase during treatment was calculated (Fig. 2). Serum DNA polymerase activity decreased in all the patients during interleukin 2 administration. In one patient (Patient No. 7), HBeAg became negative 2 weeks after treatment, but he did not develop HBeAb after therapy (Fig. 3). In 2 cases, serum DNA polymerase activity returned to pretreatment level within 2 weeks after treatment. Thus, interleukin 2 led to a temporary inhibition of serum DNA polymerase activity in most patients, and the enzyme level increased once therapy was stopped in some cases.

2. Liver function test
An elevation in serum ALT level was observed during treatment in 5 cases. The peak of this enzyme was noted in the second or third week (Fig. 4). The typical ALT peak does not appear to be due to dose-related interleukin 2 hepatotoxicity. In patient No. 3, 4, and 7, serum ALT level markedly increased during treatment, which occurred simultaneously with or just before the decrease in serum DNA polymerase activity (Fig. 2 and 4). As shown in Fig. 3, a slight increase in serum bilirubin level was observed during treatment in only one case (Patient No. 7). The elevated level of serum ALT returned to pretreatment level within 2 weeks after treatment.

3. Side effects
Fever, chilliness, "influenza-like" symptoms, myalgia, headache, and fatigue occurred during treatment, irrespective of the dose given (Table 2). Pulmonary, hematologic, and renal toxicity and...
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Fig. 3 Changes in serum alanine aminotransferase (ALT), total bilirubin, DNA polymerase activity, and hepatitis B e antigen (HBe Ag) before, during, and after interleukin 2 therapy in patient No. 7.

Fig. 4 Changes in serum alanine aminotransferase (ALT) before, during, and after interleukin 2 therapy in patients with chronic hepatitis B.

thrombocytopenia were not seen during and after treatment.

4. Liver biopsies

Post-treatment biopsy specimens were obtained from 5 of the patients within 2 weeks after the end of treatment (Table 1). Four of the biopsy specimens did not show any changes compared to pre-treatment biopsy specimens, but one case (Patient No. 7) became worse after treatment. In this case, although the lobular architecture remained almost intact, many of the portal tracts were enlarged with round cell infiltration and portal fibrosis in the pretreatment biopsy specimen. Piecemeal necrosis was also identified. Single cell necrosis and focal liver cell necrosis were seen in some parts of the liver lobules. A slight proliferation of the sinusoidal lining cells including Kupffer cells was also noted. In the findings of the post-treatment biopsy in this case, zonal necrosis was partially revealed in the lobules. Hepatocellular degeneration, proliferation of sinusoidal lining cells, focal necrosis, and
single cell necrosis were more prominent after interleukin 2 therapy.

IV. Discussion

Interleukin 2 is a lymphokine that plays an important role in a variety of immune reactions. This lymphokine is produced by T cells stimulated with mitogens or antigens, and modulates the proliferation and/or differentiation of T, B, and NK cells through interaction with highly specific receptors on their cell surfaces. On the basis of these facts, it has been presumed that interleukin 2 might be therapeutically useful to augment immune responses in vivo. Recently, the production of human interleukin 2 from Escherichia coli by recombinant DNA technology has increased the supply and purity of this lymphokine and acquired immunodeficiency syndrome (AIDS). However, the efficacy and toxicity of purified recombinant interleukin 2 in chronic hepatitis B are unknown. In this study, we investigated the clinical and adverse effects and the antiviral activity of such a preparation in a pilot study of interleukin 2 therapy in patients with chronic hepatitis B.

DNA polymerase activity decreased soon after the start of interleukin 2 treatment in all patients, but viral replication returned to pretreatment level by the end of treatment in 2 patients. The timing of the ALT peak in relation to recombinant interleukin 2 treatment suggests that the exacerbation of chronic hepatitis and subsequent decrease in DNA polymerase activity was not spontaneous. The increased level of serum ALT returned to pretreatment level during or just after treatment.

One patient (Patient No. 7) became seronegative during treatment with an increased level of serum ALT. The histopathological findings of the liver became worse after treatment in this case. A slight increase in serum bilirubin level was also seen during treatment. These phenomena observed in this case have not been described in previous reports.

Adverse reactions to recombinant interleukin 2 were similar to those previously reported with various types of interferons and included fever, headache, anorexia, and myalgia. These symptoms did not necessitate adjustment of dosage. Low grade fever of 37°C to 38°C occurred in most cases. There was, however, no correlation between the severity of the symptoms and increased interleukin 2 dose. In addition, no neutropenia nor thrombocytopenia were seen in any patient during treatment.

Recombinant interleukin 2 therapy may be of benefit in certain carriers, if they can be precisely identified. In view of the promising outcome obtained in a percentage of the patients, prospective randomized trials of interleukin 2 are warranted. These studies should be focused toward daily regimens of low-dose, long-term interleukin 2 therapy.
References


