Chronic hepatitis C: new diagnostic tools and therapeutic strategies
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FORMATION OF ANTIBODIES TO FACTOR VIII IN PATIENTS WITH HEMOPHILIA A WHO ARE TREATED WITH INTERFERON FOR CHRONIC HEPATITIS C

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Objective: To evaluate the risk for development of antibodies to factor VIII (factor VIII inhibitors) during and after interferon therapy in patients with hemophilia A and chronic hepatitis C infection.

Design: Patients were divided into two treatment groups and an untreated control group. Test results from the two treatment groups were compared with those from the control group.

Setting: 3 clinical centers in the Netherlands.

Patients: 35 men with hemophilia A who had acquired hepatitis C through the use of plasma-derived clotting factor concentrates.

Measurements: Patients were tested for factor VIII inhibitors before the start of interferon therapy and every six months thereafter.

Results: 21 patients with hemophilia A received interferon therapy for chronic hepatitis C infection for a mean of 19.5 months (range 0.5 to 36 months). In 2 patients, inhibitors were detected on one occasion (maximum titer, 1.2 Bethesda units/mL) during interferon therapy. In 3 patients who were known to have had inhibitors before interferon therapy, no anamnestic reaction was seen during treatment. In 3 of 14 untreated controls who were followed for a mean of 28 months (range, 18 to 40 months), inhibitors were detected on one occasion (maximum titer, 2.3 Bethesda units/mL).

Conclusion: Long-term interferon therapy in patients with hemophilia did not increase the risk for development of factor VIII inhibitors.
INTRODUCTION

In most developed countries, most patients with hemophilia A and B are infected with hepatitis C virus (HCV) through the use of clotting products. In the Netherlands, 64% of patients with hemophilia have chronic HCV infection (1). Interferon therapy (3 to 5 MU 3 times per week for 6 months) results in the sustained normalization of liver function and the disappearance of HCV RNA in only 15 to 25% of patients (2). However, interferon can cause numerous side effects, including formation of autoantibodies against human tissue, which may provoke autoimmune diseases (3). Another side effect that may result from autoimmune dysfunction has recently been reported: the development of antibodies against factor VIII (factor VIII inhibitors) (4,5). These inhibitors rapidly neutralize the activity of the infused factor VIII; they are classified as persistent (type A) or transient (type B) (6). Development of these inhibitors is one of the most severe complications of treatment for hemophilia; thus, the benefits of interferon therapy should be carefully weighted against the risk for inhibitor development.

We started a pilot study in 1990 (7) to analyze the rate of response to long-term (3 years) interferon therapy and to monitor possible side effects of this treatment. In 1991, Bresters (8) began a randomized, controlled trial to compare long-term interferon therapy with no therapy in patients with hemophilia and chronic HCV infection. In both of these studies, patients were regularly tested for inhibitors.

We did the present evaluation to estimate the risk for development of factor VIII inhibitors during and after interferon treatment and to compare this risk with that seen in untreated patients.

METHODS

In cooperation with the Department of Hepatology at the Academic Medical Center, the Amsterdam Red Cross Bloodbank and the Central Laboratory of The Netherlands Red Cross Blood Transfusion Service, we designed two studies to examine the treatment of HCV infection with interferon-alpha 2b (Schering-Plough, Amstelveen, the Netherlands).

Patient Selection

Men with hemophilia A and chronic hepatitis C (positive for both antibodies to HCV and HCV RNA) who visited the National Hemophilia Center were enrolled. All patients had acquired hepatitis C through the use of blood-derived clotting factor concentrates. None were positive for the human immunodeficiency virus or had major illnesses other than hemophilia and chronic hepatitis C. In all patients, results of liver enzyme tests done at the beginning of interferon therapy showed an increase to at least 1.5 times the upper limit of normal. Before the trial, patients had not received any other treatment for HCV infection. Patients were divided into 3 groups. Group A comprised 8 patients who were participating in a pilot study and received 5 MU of interferon daily for 2 weeks, followed by 2.5 MU of interferon daily for 4 weeks and then 1.5 MU of interferon 3 times a week for 16 weeks (7). Group B was composed of 13 patients who entered a randomized, controlled trial and received 3 x 5 MU of interferon per week for 8 weeks, followed by 2.5 MU of interferon 3 times per week for 16 weeks. In groups A and B, interferon therapy was discontinued after 26 weeks in patients who had had a complete response (defined as the disappearance of
HCV RNA). In non-responders and transient responders, interferon therapy was continued (5 MU of interferon 3 times per week for 36 months). Group C was a control group comprising 14 patients who participated in the randomized, controlled trial but did not receive interferon therapy.

**Laboratory Assays**

Inhibitor titers were measured using the method as described by Kasper and coworkers (9). A titer of 1.0 Bethesda unit/mL or more was considered positive. Assays for factor VIII inhibitors were done using the one stage method (10). Factor VIII recovery was measured before and 15 minutes after infusion of factor VIII (11). All samples were analyzed at the coagulation laboratory of the University Hospital Utrecht.

**Follow-up**

Before the start of therapy and every 6 months thereafter, blood samples were obtained for factor VIII inhibitor tests. All patients were tested at least once during the course of interferon therapy and within 6 months after cessation of treatment.

**Table 1.**

Interferon therapy and incidence of development of antibodies to factor VIII.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients in the pilot study group (n=8)</th>
<th>Patients in the randomized trial</th>
<th>Patients in the interferon group (n=13)</th>
<th>Controls (n=14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at start of trial, y*</td>
<td>36.8 (27-47)</td>
<td>32 (21-54)</td>
<td>37.5 (23-54)</td>
<td></td>
</tr>
<tr>
<td>Duration of interferon therapy or follow-up, mo*</td>
<td>18.6 (0.5-36)</td>
<td>20.1 (6-36)</td>
<td>28.1 (18-40)</td>
<td></td>
</tr>
<tr>
<td>Known presence of antibodies to factor VIII before interferon therapy, n</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Newly developed antibodies to factor VIII, n</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Range of maximum titers of antibodies to factor VIII, Bethesda units/mL</td>
<td>-</td>
<td>1.0-1.2</td>
<td>1.2-2.3</td>
<td></td>
</tr>
</tbody>
</table>

*Values are mean (range)
Both studies were approved by the Medical Ethical Committee of the Wilhelmin Children's Hospital in Utrecht, the Netherlands.

RESULTS

Data on interferon therapy and development of factor VIII inhibitors are shown in Table 1. One patient, who was known to have type A inhibitors at the start of interferon therapy and two patients who were known to have had type A inhibitors in the past had no anamnestic reaction or recurrence of inhibitory activity during the course of interferon therapy. Positive test results were obtained on one occasion in 2 of 21 patients treated with interferon and in 3 of 14 controls. Four of the 5 patients with positive results had no clinical evidence of inhibitors and normal factor VIII recovery.

In one patient who tested positive for inhibitors and was receiving interferon therapy, the clinical response to factor VIII was less pronounced than it had been in the past. This patient, who had severe hemophilia A (factor VIII concentration <1%) was treated with monoclonally purified factor VIII two to four times per month. After 21 months of interferon therapy, he developed a subcutaneous and muscular bleeding that did not respond well to the normal dosage of 30 units of factor VIII per kg of body weight twice daily. Factor VIII concentrations were measured, and tests for inhibitors were done. Six hours after the patient had received factor VIII, 30 units/kg, the factor VIII concentration was 1%, indicating that the half-life of factor VIII was shortened. The inhibitor titer was 1.2 Bethesda units/mL. Unfortunately, the blood sample obtained 15 minutes after infusion of factor VIII was lost. Factor VIII therapy was continued at the same dosage; the next day, we found only a slightly lower-than-expected concentration of factor VIII and a normal recovery. The inhibitor titer was 0.4 Bethesda units/mL. Two days after interferon therapy was discontinued, factor VIII recovery was normal on repeated tests and the inhibitor titer remained less than 1.0 Bethesda unit/mL.

This patient subsequently had surgery without increased bleeding tendency and required only normal amounts of factor VIII.

DISCUSSION

We did a randomized, controlled study to evaluate the development of factor VIII inhibitors during interferon therapy in patients with hemophilia A and chronic hepatitis C (21 patients received interferon, and 14 were not treated). In 4 patients already known to have had inhibitors, no negative effects of interferon were seen. Of 5 patients who developed transient inhibitors during the trial (2 treated patients and 3 controls), 1 treated patient had clinical evidence of inhibitors; interferon therapy in this patient was stopped as a preventive measure.

Interferons, which are cytokines, influence parts of the immune response. Most of these effects can be attributed to the enhancement of the expression of lymphocyte surface antigens, such as MHC, by interferon. This upregulation of cell-mediated immunity is one of the mechanisms of interferon's antiviral activity, but it may also produce side-effects. For example, in recipients of renal transplants, the rate of organ rejection was increased by systemic use of interferon (12). Furthermore, interferon can enhance production of antibodies by directly affecting the B-cells, especially when the drug is added at the same time as the antigen (13). The role of interferon in the formation of interferon-neutralizing
antibodies and antibodies against thyroid has been described (3, 12).
Two dissimilar cases of inhibitor development in relation to interferon therapy have been reported (5, 6). In one patient, who had mild hemophilia and had rarely been treated with factor VIII, interferon therapy had been discontinued for 2 months before inhibitors were detected. The other patient, who was still receiving interferon therapy when inhibitors were detected, did not have hemophilia but did have a multiple myeloma; the inhibitors had developed after autologous bone marrow transplantation. Factor VIII inhibitors may be associated with lymphadenopathy, and we should therefore be cautious about concluding that interferon therapy caused the development of this inhibitor in this patient.

We show that the risk for development of inhibitors is not increased in patients receiving interferon compared with untreated patients. Inhibitor testing is usually done once or twice per year. The mean incidence of factor VIII inhibitors in our overall patient population is 29 per 1000 patient years (14). Most of these inhibitors are transient and produce no clinical complications; the cause of inhibitor development is usually unclear. We cannot exclude the possibility that the presence of inhibitors in our patients was caused by complications of interferon treatment. However, in our randomized, controlled trial - although the number of patients studied was limited - we found no evidence of enhanced inhibitor formation during interferon therapy.

REFERENCES