Neutralizing interferon \( \beta \) antibodies in melanoma patients treated with recombinant and natural interferon \( \beta \)

Gerhard Fierlbeck\(^1\), Tilmann Schreiner\(^1\), Bernd Schaber\(^1\), Anton Walser\(^2\), Gernot Rassner\(^1\)

\(^1\) Department of Dermatology, University of Tübingen, Liebermeisterstrasse 25, 72076 Tübingen, Germany
\(^2\) Research Department, Rentschler GmbH, Laupheim, Germany

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Abstract. The incidence and clinical significance of therapy-induced neutralizing interferon \( \beta \) (IFN\( \beta \)) antibodies was studied in a group of 21 melanoma patients treated with natural IFN\( \beta \) and 7 patients treated with recombinant IFN\( \beta \). They were treated subcutaneously with \( 3 \times 10^6 \) IU three times per week in an adjuvant open trial for 24 weeks after surgical removal of all detectable metastases. Of the 21 patients treated with natural IFN\( \beta \), 95% developed significant levels of neutralizing antibodies after 24 weeks. In comparison, 28% of the 7 patients treated with recombinant IFN\( \beta \) developed neutralizing IFN\( \beta \) antibodies. Cross-reactivity of the antibodies could be demonstrated. Persistence of antibody titers was seen in 80% of the patients 24 weeks after cessation of treatment with natural IFN\( \beta \). No correlation between the maximum antibody titers and the antibody persistence after cessation of therapy could be established. We detected a clear correlation between the formation of neutralizing antibodies and the decrease in \( \beta \)-2-microglobulin and 2',5'-oligoadenylate synthetase and therefore the drop in biological activity. In this adjuvant trial there was no difference in relapse rate and time until relapse between antibody-positive and antibody-negative patients. No difference in clinical outcome could be established between the patients treated with natural IFN\( \beta \) and recombinant IFN\( \beta \).

Key words: Melanoma - Interferon \( \beta \) - Antibodies - Adjuvant trial

Introduction

It is well established that patients can develop binding (non-neutralizing) and neutralizing antibodies towards genetically engineered protein products as well as natural proteins.

This phenomenon was observed as far back as 1956 when antibodies against thyroxine were first demonstrated [20]. In 1970 antibodies against insulin were first observed [7] and, more recently, antibodies against parathyroid hormone, interleukin-2 and erythropoetin have been found [22, 25]. It is, therefore, not surprising that the formation of antibodies against interferons (IFN) has also been demonstrated [3, 10, 11, 17, 21, 23, 24].

The role of neutralizing antibodies against natural and recombinant IFN\( \alpha \) in hematological disorders has been well studied [18, 23]. If a certain titer of serum antibodies is reached, the therapeutic response is markedly reduced or no further therapeutic response can be observed. The same phenomenon has been observed in one study on patients with hypernephroma treated with recombinant IFN\( \alpha \) [18] and one study on patients with malignant carcinoid tumors treated with recombinant IFN\( \alpha \)2b [15]. Responders who developed antibodies against IFN\( \alpha \) had a markedly reduced remission time compared to responders without antibodies, whereas a larger adjuvant study on patients treated with recombinant IFN\( \alpha \) for hypernephroma could not establish any difference in clinical outcome between patients with neutralizing antibodies and those without [16].

Antibody formation has been demonstrated against all IFN [11, 17, 23, 24]. The induction of antibody formation depends on the type of IFN, the route of administration (s.c., i.m. or i.v.), the cumulative dose, the duration of treatment, the age of the patient and the underlying disease [1, 3, 5, 10, 23]. Methodological aspects, for example the type of antibody assay or the time when patients’ blood is drawn, may possibly play a role as well [27].

The role of IFN in the treatment of metastatic malignant melanoma has been studied extensively, and response rates range from 3% to 25% [9]. Established chemotherapy regimes produce similar response rates. These relatively disappointing results in the treatment of metastatic malignant melanoma show the necessity to develop adjuvant therapeutic regimes.

We performed a trial with IFN\( \beta \) in stage II melanoma patients (UICC 1979) in which all detectable metastases had been removed surgically before enrollment. Patients were
Table 1. Chemical comparison between natural interferon \(\beta\) (nIFN\(\beta\)) and recombinant (r) IFN\(\beta\)

<table>
<thead>
<tr>
<th>Glycosylation pattern</th>
<th>nIFN(\beta) (mol/100 mol)</th>
<th>rIFN(\beta) (mol/100 mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biantennary structure</td>
<td>80–90</td>
<td>65–85</td>
</tr>
<tr>
<td>Triantennary structure</td>
<td>10–20</td>
<td>15–20</td>
</tr>
<tr>
<td>Triantennary + one report of lactosamine</td>
<td>&lt;1</td>
<td>3–6</td>
</tr>
<tr>
<td>Sialic acid content</td>
<td>60–80</td>
<td>&gt;90</td>
</tr>
</tbody>
</table>

Patients were closely monitored for the development of neutralizing IFN\(\beta\) antibodies before, during and after therapy. We also measured \(\beta\)2-microglobulin and \(2',5'\)-oligoadenylate synthetase levels to assess the biological response. Owing to the adjuvant trial design, relapse rate and time until relapse were used to evaluate the efficacy of this treatment.

Materials and methods

Patients and study design

A group of 28 patients with stage II malignant melanoma, according to the 1979 TNM classification, were enrolled. The study was approved by the Ethics Commission of the university of Tübingen. All patients gave written consent after they had been thoroughly informed about the study. Clinical stage II includes patients with satellite, in transit or lymph node metastases. Before enrollment in the adjuvant study all clinically detectable metastases had been removed surgically, and treatment started within 4 weeks after surgery. IFN was given three times per week for a treatment period of 24 weeks; 21 patients were treated with natural IFN\(\beta\), 7 patients were treated with recombinant IFN\(\beta\).

Interferons

We used natural IFN\(\beta\) (Fiblaferon, Rentschler, Laupheim, Germany) and recombinant IFN\(\beta\) (Betaferon, Rentschler, Laupheim, Germany). The source of natural IFN\(\beta\) was fibroblasts; recombinant IFN\(\beta\) was produced in Chinese hamster ovary cells [19]. The differences and similarities between natural IFN\(\beta\) and recombinant IFN\(\beta\) are shown in Table 1 [2].

Assessment of relapse

After a complete staging, patients were enrolled in the study. Staging included chest X-ray, abdominal and regional lymph-node ultrasonography, computed tomography of the abdomen, thorax and brain and a bone scan. Every 4 weeks patients were seen and a detailed history as well as a complete physical examination were performed. In the case of doubtful findings further technical examinations were performed.

After the 24-week treatment period the complete staging procedure was repeated.

Patients’ sera

Patients were seen during the morning and were told to inject the IFN\(\beta\) 24 h before the hospital visit. All blood samples were therefore drawn at a fairly similar time and at a similar interval after the last injection.

Determination of \(2',5'\)-oligoadenylate synthetase and \(\beta\)2 microglobulin

\(2',5'\)-Oligoadenylate synthetase is an interferon induced enzyme. It is part of the \(2',5'\)-oligoadenylate system, which represents one of the effector mechanisms of interferon’s antiviral action. It may also be involved in its antiproliferative and immunoregulative actions. The commercially available radioimmunoassay of EIKEN (Immunological Laboratory Kita-Ku, Tokyo, Japan), provided by Mecconti, Bad Homburg, Germany, was used.

\(\beta\)2-microglobulin is a protein found on the surface of most nucleated cells, where it is one of the components of the HLA antigen complex. It is also found in the serum and can be induced by interferons. The \(\beta\)2-microglobulin enzyme immunoassay kit (Serex Inc., Maywood, N. J., USA) was used for its determination.

Determination of neutralizing antibodies against interferon-\(\beta\)

Antiviral assay. To determine neutralizing antibodies against IFN\(\beta\), serum was diluted and incubated with defined amounts of the cytokine. This mixture was then spread out on microchambers containing FS-4 fibroblasts. After incubation for 16 h, cells were infected with the encephalomyocarditis virus. After 24 h the remaining interferon was assessed and this amount was subtracted from the original amount of interferon. The difference was similar to the amount of neutralizing antibodies, as one unit of IFN is neutralized by one unit of neutralizing antibodies. This result was then multiplied by the dilution factor, leading to the titer of neutralizing antibodies in undiluted serum.

Antiproliferative assay. We used the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test to measure indirectly the proliferating cells in a sample as described before [14]. IFN plus pretreatment serum (controls) or IFN plus serum containing neutralizing antibodies obtained during IFN\(\beta\) therapy was added to wells containing melanoma cells. MTT solution was added. Cells were then lysed with an equal volume if isopropanol/HCl and the absorbance was measured at 540 nm within 30 min.

The test wells were assayed for percentage proliferation in relation to the control, which was defined as 100% proliferation. Tests were performed eight times.

To assess cross-reactivity between recombinant and natural IFN\(\beta\), serum containing antibodies against recombinant IFN\(\beta\) was incubated with natural IFN\(\beta\) and vice versa.

Results

Neutralizing antibodies

Therapy with IFN\(\beta\) was associated with the development of neutralizing antibodies (Table 2). No IFN\(\beta\) antibodies were present in the 28 patients before therapy. Antibodies were first observed after 8 weeks of therapy in those patients treated with natural IFN\(\beta\). After 12 weeks of therapy, 62% of the patients and, after 24 weeks of therapy, 95% of the patients had developed neutralizing antibodies against IFN\(\beta\). Two patients had antibodies titers exceeding 50 000 units of IFN\(\beta\)-neutralizing antibodies/ml. In contrast only 2 out of 7 (28%) patients treated with recombinant IFN\(\beta\) had developed neutralizing antibodies after 24 weeks of therapy: one patient developed antibodies after 16 weeks, the other after 24 weeks. Levels were markedly lower in these patients compared to those treated with natural IFN\(\beta\) (Tables 3, 4).