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Inhibition of the Growth of Cultured Human Meningioma Cells by Recombinant Interferon-α

Jan W. Koper, Ellen C. Zwarthoff, Anne Hagemeijer, Reinder Braakman, Cees J.J. Avezaat, Mats Bergström and Steven W.J. Lamberts

In this paper the results of investigations on the effect of interferon-α (IFN-α) on the growth of meningioma cells in culture is reported. A consecutive series of six meningiomas and one meningioma/neurofibroma derived from a patient with neurofibromatosis type 2 was investigated and it was found that the growth of all seven tumours in response to mitotic stimuli (fetal bovine serum or epidermal growth factor) is strongly inhibited by IFN-α. Maximal response varied between 100% and 70% inhibition of the incorporation of tritiated thymidine. In some cases an inhibitory response was obtained already at very low doses (~10 U of IFN-(U per ml). These results indicate that further clinical investigation of the application of IFN-α to the treatment of meningioma is warranted. Eur J Cancer, Vol. 27, No. 4, pp. 416-419, 1991
Interferons (IFNs) are a group of proteins that were first recognised by their ability to protect the cell against viral infections [see ref. 14 for review]. The ability of IFNs to influence cell-proliferation and differentiation has only become apparent during the last two decades [15, 16]. Since then it has become clear that the IFNs can be of clinical use in treatment of a number of haematological and solid tumours [17].

We have previously reported preliminary results of the treatment of meningioma patients with interferon-α (IFN-α) [13]. Here we report the results of experiments on the response of cultured human meningioma cells to recombinant human IFN-α. In addition to this we investigated the responsiveness of meningioma cells to IFN-α from a meningioma/neurofibroma removed from a patient with neurofibromatosis type 2. The data presented here show that the mitogen-stimulated growth of meningioma cells in vitro and of the meningioma/neurofibroma cells can be reduced significantly by IFN-α in concentrations below 1000 U/ml.

**MATERIALS AND METHODS**

**Meningioma tissues and cell culture**

Tumour specimens were obtained within 30 min after surgery. They were brought into culture as described previously [9]. After two or three weeks, when the cells had grown to confluency, and dead cells, blood cells and debris resulting from the isolation procedure had been washed away by the medium changes, the cells were harvested as described [9]. The cells were collected by centrifugation, washed and resuspended in fresh culture medium and seeded into 24-well tissue culture plates (Costar Europe, Badhoevedorp, The Netherlands) at 4 × 10⁴ cells/well in 1 ml of medium.

**Cytogenetic analysis of cultured cells**

Colcemid (75-ng/ml) was added to early passages (0-3) of meningioma monolayer cell cultures in logarithmic growth. After 6 h the mitotic cells were detached into the supernatant by vigorous shaking. The supernatant was centrifuged, washed, resuspended in fresh medium and seeded into 24-well tissue culture plate at 4 × 10⁴ cells/well in 1 ml of medium.

**Mitogen-stimulated incorporation of tritiated thymidine**

When the cells in 24-well-plates had grown to confluency, the culture medium was removed and replaced by serum-free medium as described previously [9]. The cells were maintained in this medium for 3 days, after which the medium was replaced by fresh serum-free medium, and the experiment was started by addition of epidermal growth factor (EGF, 10 ng/ml) or fetal bovine serum (FBS, 10%) and IFN-α (0, 10, 100 or 1000 U/ml). All incubations were carried out in triplicate. 20 h later, 3.7 × 10⁻¹⁴ Bq of [methyl-³H]thymidine was added to each well (2.2-3.3 × 10⁻¹⁴ Bq mmol, Amersham Nederland, Houten, The Netherlands). The cells were then incubated for 4 h, the medium was removed, the cells were washed twice with cold 0.15 mol/l NaCl, solubilised with 1 mol/l NaOH and transferred to vials for scintillation counting of the incorporated radioactivity.

**Mitogen-stimulated growth**

Cells were grown to semi-confluency in Dulbecco's modified Eagle's medium (DMEM) with 10% FBS in 24-well tissue culture plates. The medium was replaced by serum-free medium plus EGF (10 ng/ml) or by fresh DMEM/10% FBS and IFN-α was added at concentrations of 0, 10, 100 or 1000 U/ml. The cells were then cultured under these conditions for 7 days, with daily replacement of the medium and all the factors added. At the end of the experiment, the cultures were washed once with 0.15 mol/l NaCl and lysed in 125 μl 1 mol/l NaOH, 0.2% "Triton-X-100". The lysates were sonicated (1 s in an MSE Soniprep 150 at half-maximal output) and diluted with 1 ml 10 mmol HEPES buffer with 100 mmol/l NaCl and 10 mmol/l EDTA (pH 7.0). The DNA-content was then measured in the samples using the bisbenzimide fluorescent dye (Behring Diagnostics, La Jolla, USA) as described [21].

**Materials**

Human EGF (recombinant) was obtained from Boehringer Mannheim, Almere, The Netherlands. Human IFN-α (recombinant, Roferon®) was obtained from Hoffmann-La Roche, Basel, Switzerland.

**Statistics**

The statistical significance of the differences between mean values was determined using one-way analysis of variance (ANOVA). When significant overall effects were obtained by ANOVA, multiple comparisons were made using the Newman–Keuls test. All data are expressed as mean (S.D.) percentages, relative to the control values.

**RESULTS**

Table 1 gives the clinical and histological data of the tumour samples used in this study. As can be seen in the table several different histological types of meningioma were used.

**Cytogenetic analysis**

Cytogenetic analysis was performed on cultured tumour cells from 6 of the 7 cases. This analysis revealed that at least 4 of the 7 tumour samples (3 meningiomas and the meningiomatous/neurofibroma) showed changes with regard to chromosome 22 that are characteristic of meningioma and neurofibromatosis type 2 (Table 1). In all cases these changes consisted of the loss of one entire copy of chromosome 22. 2 cultured meningiomas had a normal karyotype upon examination. This may indicate that the changes in these meningiomas were too small for detection at cytogenetic level (e.g. minor deletions or point mutations). For one meningioma sample, insufficient material was available for cytogenetic analysis (case 5).

We tested the EGF-response of 5 of these meningiomas (cases 1, 2, 3, 5 and 6) and of the meningioma/neurofibroma (case 7) and examined the effect of IFN-α on this stimulation in a thymidine incorporation assay. The results of these experiments are summarised in Table 2.
Table 2. Inhibition of EGF-stimulated [3H]thymidine incorporation in cultured meningioma cells by IFN-α

<table>
<thead>
<tr>
<th>Case</th>
<th>Control</th>
<th>Alone</th>
<th>IFN-α 10 U/ml</th>
<th>100 U/ml</th>
<th>1000 U/ml</th>
<th>IFN-α</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>100</td>
<td>321 (10)*</td>
<td>206 (7)**</td>
<td>144 (4)**</td>
<td>79 (10)**</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>100 (14)</td>
<td>232 (11)*</td>
<td>137 (4)**</td>
<td>85 (5)</td>
<td>53 (4)**</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>100 (4)</td>
<td>106 (5)</td>
<td>107 (7)</td>
<td>100 (1)</td>
<td>94 (6)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>100 (6)</td>
<td>150 (12)*</td>
<td>147 (5)*</td>
<td>114 (7)</td>
<td>93 (7)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>100 (3)</td>
<td>365 (3)*</td>
<td>255 (8)*</td>
<td>163 (3)**</td>
<td>82 (19)**</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>100 (21)</td>
<td>147 (7)*</td>
<td>92 (4)**</td>
<td>78 (2)</td>
<td>69 (3)**</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>100 (2)</td>
<td>199 (5)*</td>
<td>124 (4)**</td>
<td>93 (4)**</td>
<td>68 (8)**</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>100 (9)</td>
<td>503 (10)*</td>
<td>462 (4)*</td>
<td>379 (2)**</td>
<td>200 (7)**</td>
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<tr>
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<td>271 (3)*</td>
<td>268 (13)*</td>
<td>262 (8)*</td>
<td>204 (5)**</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>100 (10)</td>
<td>653 (1)*</td>
<td>594 (3)*</td>
<td>489 (5)**</td>
<td>298 (5)**</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>100 (9)</td>
<td>207 (5)*</td>
<td>161 (15)**</td>
<td>131 (5)**</td>
<td>81 (14)</td>
<td></td>
</tr>
</tbody>
</table>

Means (S.D.) expressed as percentage of the unstimulated controls; n = 3.

Significantly different from untreated controls: *P < 0.01, **P < 0.05; significantly different from stimulated controls: †P < 0.01, ‡P < 0.05.

(Table 2) show that 4 (cases 1, 2, 5 and 6) out of the 5 meningiomas tested and also the meningioma/neurofibroma (case 7) responded to EGF by an increased incorporation of tritiated thymidine. The response to EGF was rather variable and ranged from 50% to 265% increase in the incorporation of [3H]thymidine. In these 4 meningiomas and in the meningioma/neurofibroma IFN-α significantly inhibited the effect of EGF. In one meningioma (case 2) and in the meningioma/neurofibroma (case 7) IFN-α even reduced the thymidine incorporation to a level significantly below that observed in unstimulated control cells.

In view of the observed lack of response of one meningioma (case 3) to EGF we carried out a similar thymidine incorporation assay with this meningioma and three other meningiomas (cases 4, 5 and 6) and with the meningioma/neurofibroma (case 7), now using a less specific stimulant: FBS. Table 3 shows that all 4 meningiomas tested in this way and the meningioma/neurofibroma responded to FBS by an increased incorporation of [3H]thymidine. The response to FBS was rather more vigorous than that to EGF (cf. cases 5, 6 and 7). In all 5 cases this stimulation was significantly reduced by IFN-α. In the meningioma (case 3) shown in Table 2 not to respond to EGF, IFN-α at 1000 U/ml, after FBS stimulation, resulted in a reduced incorporation of thymidine to a level significantly below the level observed in unstimulated control cells (Table 3).

To assess whether IFN-α merely inhibited the stimulated incorporation of tritiated thymidine or really slowed the proliferation of cultured meningioma cells, we stimulated meningioma cultures (case 6) with either EGF or FBS and measured their DNA content after a week of treatment with IFN-α. The results are presented in Fig. 1, together with the data on thymidine incorporation for the same meningioma. IFN-α significantly inhibited growth stimulation by EGF and FBS in this meningioma. The inhibition of FBS-stimulated growth by IFN-α was less than the inhibition of EGF-stimulated growth. As can be seen these results approximately paralleled those obtained in the thymidine-incorporation assay (Fig. 1).

DISCUSSION

The IFNs are a family of proteins first discovered (and named) by their interference with viral infections. Only during the last 20 years it has become clear that they can also influence growth and differentiation of (tumour) cells [15, 16]. Work in vitro has shown that IFNs inhibit the growth of a remarkable variety of cells [15]. Even though the action of IFNs may be cytostatic rather than cytotoxic or cytoidal, this growth-inhibitory action makes them interesting subjects in research about the treatment...
of tumours. There are now a number of tumours that can be treated with good to reasonable success with IFNs [17].

We have observed an inhibitory effect of IFN-α on the metabolism of meningiomas in patients, using [13C]-L-methionine positron emission tomography of tumours. There are now a number of tumours that can be problems are to be expected in getting the drug to the desired place.


Acknowledgement—This research was supported by the Dutch Cancer Society.