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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-α 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

INTRODUCTION

MENINGIOMAS, tumours arising from the arachnoidial cell-layer surrounding the brain [1] are usually slowly growing, benign tumours. There are strong indications that the cause of meningiomas is the inactivation or loss of both copies of a putative tumour suppressor gene located on the long arm of chromosome 22 [2, 3]. This aetiology is probably shared with one of the inheritable forms of neurofibromatosis (bilateral acoustic neuro-fibromatosis or neurofibromatosis type 2) [3, 4] and possibly with some glial tumours [3].

The common treatment for meningiomas is surgical removal; however, the recurrence rate is considerable [5] and not all meningiomas are easily accessible to surgery. For these reasons other possibilities for treatment are investigated. The presence of receptors for progesterone in many meningioma tissues [6] has led to a number of research papers dealing with the possibilities of therapy using antiprogestins such as mifepristone [7–9].

Similarly, the discovery of the presence of high concentrations of specific receptors for somatostatin in meningioma membranes [10] has resulted in a number of reports about the effects of somatostatin (analogues) on the growth of meningiomas or cultured meningioma cells [11–13]. Contrary to one report in the literature [11], we have not been able to show inhibitory effects of somatostatin (analogues) on the growth of meningiomas (refs 12 and 13, and J.W.K. *et al.*).

Correspondence to J.W. Koper.
J.W. Koper and S.W.J. Lamberts are at the Department of Medicine, Room Bd 281; E.C. Zwarthoff is at the Department of Pathology; A. Hagemeijer is at the Department of Genetics and R. Braakman and C.J.J. Avezaat are at the Department of Neurosurgery, Erasmus University Rotterdam, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands; and M. Bergström is at the Department of Neurology, Uppsala University Hospital, Uppsala, Sweden.
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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 response to IFN-α of cells derived 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, suspended in fresh culture medium and seeded into 24-well tissue culture plates (Costar Europe, Badhoevedorp, The Netherlands) at 4×10^4 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 supernatants were collected into tubes and centrifuged. The mitotic cells were processed further according to standard cytogenetic procedures [18]. The remaining monolayer in the culture flasks was flooded with KCl–EGTA [19] for 20 min, scraped off with a rubber policeman, washed with 75 mmol/l KCl and processed as above. The chromosomes were identified using R-, Q- and G-banding techniques and analysed according to the International System for Chromosome Nomenclature [20].

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, 3.7×10^4 Bq of [methyl-^{3}H]thymidine was added to each well (2.2–3.3 $\times$ 10^{12} 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 NH₄OH, 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.

Cytogenic 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 meningioma/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

Table 1. Histological and cytogenetic analysis of the meningiomas

Case	M/F	Age	Histology	Karyotype
1	M	65	Syncitial and transitional	46, XY
2	M	42	Meningiotheliomatous	46, XY
3	F	74	Psammomatous	45, XX, −22
4	F	60	Psammomatous	45, XX, −22
5	M	70	Fibromatous	ND
6	F	75	Psammomatous and transitional	45, XX, −22
7	F	33	Meningioma/neurofibroma (type 2 neurofibromatosis)	44, X, −22

ND = not determined.

Table 2. Inhibition of EGF-stimulated [³H]thymidine incorporation in cultured meningioma cells by IFN-α

		EGF			
Case	Control	Alone	10 U/ml IFN-α	100 U/ml IFN-α	1000 U/ml IFN-α
1	100 (7)	321 (10)*	206 (7)*†	144 (4)*†	79 (10)†
2	100 (14)	232 (11)*	137 (4)*†	85 (5)†	53 (4)*†
3	100 (4)	106 (5)	107 (7)	100 (1)	94 (6)
5	100 (6)	150 (12)*	147 (5)*	114 (7)‖	93 (7)†
6	100 (3)	365 (3)*	255 (8)*†	163 (3)*†	82 (19)†
7	100 (21)	147 (7)*	92 (4)†	78 (2)•‖	69 (3)*†

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 reduction of thymidine incorporation to a level significantly below the level observed in unstimulated control cells (Table 3).

To assess whether IFN-α merely inhibited the stimulated

Table 3. Inhibition of FBS-stimulated [³H]thymidine incorporation in cultured meningioma cells by IFN-α

		FBS			
Case	Control	Alone	10 U/ml IFN-α	100 U/ml IFN-α	1000 U/ml IFN-α
3	100 (2)	199 (5)*	124 (4)*†	93 (4)†	68 (8)*†
4	100 (9)	503 (10)*	462 (4)*	379 (5)*†	200 (7)*†
5	100 (3)	271 (3)*	268 (13)*	262 (8)*	204 (5)*‖
6	100 (10)	653 (1)*	594 (3)*‖	489 (9)*†	298 (5)*†
7	100 (9)	207 (5)*	161 (15)*†	131 (5)•†	81 (1)†

Significantly different from untreated uncontrols: $*P<0.01$, $•P<0.05$; significantly different from stimulated controls: $†P<0.01$, $‖P<0.05$.
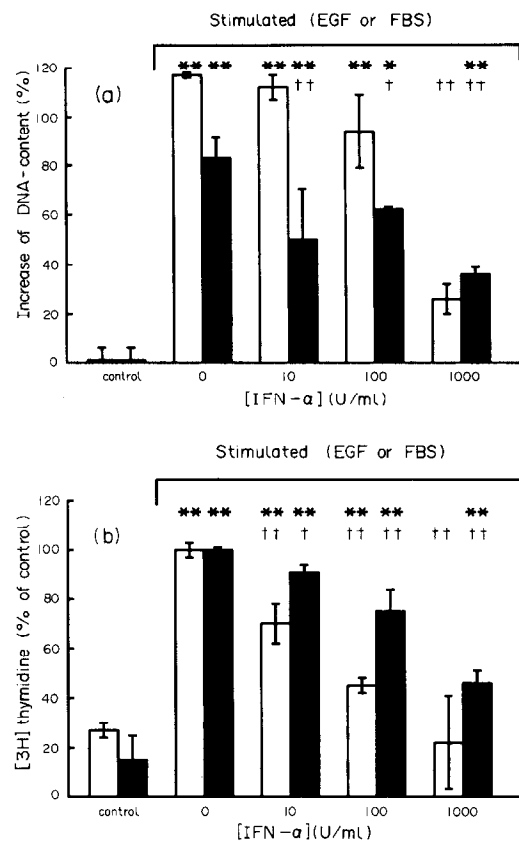

Fig. 1. **The effect of IFN-α on the increase of DNA-content (upper) and [³H]thymidine-incorporation (lower) in human meningioma cell-cultures. Cell cultures derived from meningioma 6 were stimulated with EGF (10 ng/ml, open bars) or FBS (10%, filled bars) in the presence of IFN-α as indicated. The increase of DNA-content is given as percentage increase (S.D.) ($n=3$) relative to the DNA content of unstimulated control cultures (these contained 1.58±0.08 μg DNA per well), thymidine incorporation as percentage (S.D.) relative to the stimulated controls. Incorporation by unstimulated cells is indicated by the bars on the left side. * and **: significantly different from untreated controls at $P<0.05$ and $P<0.01$, respectively; † and ††: significantly different from stimulated controls at $P<0.05$ and $P<0.01$, respectively.**

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 cytocidal, 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 [^{11}C]-L-methionine positron emission tomography [13]. In some of the patients IFN-α treatment resulted in a decreased growth-rate of their meningiomas.

We found that the growth of meningioma cells in culture was inhibited by IFN-α. The cells were derived from 6 different meningiomas of various histological types. In addition to the meningiomas, we used cultured cells derived from a falx-located tumour removed from a patient with neurofibromatosis type 2 [22]. The patient had multiple central and spinal meningiomas, bilateral acoustic neurinomas and bilateral optic tumours. This tumour had histological characteristics both of a meningioma and of a neurofibroma and is, therefore, referred to as "meningioma/neurofibroma". In all these tumour cells the growth, stimulated by specific (EGF) or non-specific (FBS) mitogens, was reduced or abolished by exposure to IFN-α. Interestingly, these inhibitory effects were observed at relatively low doses: significant reduction at 10 U/ml IFN-α in 3 of the 6 meningiomas tested and significant reduction at 100 U/ml IFN-α in all meningiomas. Moreover, in all meningiomas responsive to EGF, the stimulation of thymidine-incorporation by EGF could be abolished completely by 1000 U/ml IFN-α. This is especially interesting in view of the observed presence of EGF-receptors in a large proportion of meningiomas [12]. Presumably the meningioma that could not be stimulated by EGF was EGF-receptor negative; however, we did not have sufficient material to confirm this. Similarly, differences in response may represent differences in receptor density.

The effects of interferon-α were similar after stimulation with EGF and FBS, both with respect to thymidine incorporation and increase in DNA-content. Since meningioma cells—certainly in early passages—can be propagated quite easily in FBS containing medium, this indicates that interferon-α actually inhibited cell proliferation rather than specific parts of this process.

The cytogenetic analysis showed that loss of one copy of chromosome 22 had occurred in 3 out of 5 meningiomas tested and also in the meningioma/neurofibroma cells from a patient suffering from neurofibromatosis type 2. This gives additional support to the notion that these diseases have a common aetiology as suggested [2–4]. It also confirms that, certainly in the early stages of culture, the cultured meningioma cells used in this study were indeed meningioma cells and not stromal cells overgrowing the original tumour cells.

Cells derived from a different tumour (meningioma/neurofibroma) but with the same aetiology as meningioma [2–4] were also found to be responsive to the growth-inhibitory action of IFN-α. Patients suffering from neurofibromatosis type 2 usually have multiple meningiomas, in addition to the characteristic bilateral acoustic neurinomas; usually some of these meningiomas are inoperable. Possibly treatment with IFN-α is beneficial in particular to these patients and to patients with inoperable meningiomas in general.

The data presented in this paper suggest that IFN-α may be used successfully in the treatment of actively growing meningiomas that are difficult to reach by surgery or that cannot be removed completely. Dosages and routes of administration are matters to be investigated. However, the effects in this study, which were observed at low dosages, are promising. Since meningiomas are located outside the blood brain barrier, no problems are to be expected in getting the drug to the desired place.

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