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# Biological activity of rat fibroblast interferon beta

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*The biological activity of rat fibroblast beta interferon was studied. Rat embryonal fibroblasts (Wistar strain-WiREF) were used as a source of interferon. Cells were grown in suspension and on the microcarriers (Cytodex 3). In the exponential phase of cell growth the induction was performed. The biological activity of the obtained interferon was tested on the homologous cells. Interferon's influence on cell growth, morphology and intracellular level of some hydrolases was measured.*

*Key words:* fibroblasts-drug effects; interferon- beta; rats

## Introduction

Generally, interferons (IFNs) are very small glycoproteins/proteins produced by eukaryotic cells in response to induction by certain chemicals or infection with various viruses.[1] They are divided into two groups: Type I with three distinct families: alpha, beta and omega, and Type II with only one family: gamma.[2, 3]

Beta IFN can be obtained from various types of mammalian fibroblasts, such as human, bovine and murine[4, 5] by viral or pI:C induction. An interferon molecule has a molecular weight of around 22.000, although some smaller components with molecular weights between 17.000 and 18.000 can be found. It is less stable than alpha interferon. Schiff's staining revealed that beta interferon is a glycoprotein. Aminosugar and aminoacid analyses confirmed its hydrophobic characteristics.

Among other biological activities of beta IFNs, the antiproliferative activity was described.[6] This activity became important because of possible use of IFN as an antineoplastic agent. Mostly, the attention has been focused on the human interferons.

In our previous experiments[7, 8] we have found that spontaneously transformed rat embryonal fibroblasts of Wistar strain (WiREF) can produce relatively large amounts of beta interferon. In search of the possible mechanisms of the anticellular activity of IFN the growth inhibition was found to be parallel to the intracellular enzymatic changes in various model systems.[9, 10] Different hypotheses were postulated on the anticellular effects of IFN, though little is known about the distinct changes of the normal or transformed cells after a short-term treatment. It is generally accepted that at least 18-hour treatment is needed to produce an inhibition of cell growth in vitro.

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The experiments presented are aimed to show the distinct changes (morphological, growth characteristics, nuclear blebs, alkaline phosphatase) after the treatment of nontransformed and transformed WiREF cells with IFN for a short time (15, 30, 45, 60, 90 and 180 minutes).

## Materials and methods

### *Cells*

In these experiments we used rat embryonal fibroblasts (WiREF) in their nontransformed (Phase A) and transformed (Phase E) form.[7] Cells were grown in Eagle's medium supplemented with 10% of FCS (foetal calf serum).

### *Interferon*

To obtain rat fibroblast (beta) interferon, the transformed WiREF cells were grown in up to two liters of suspension ($10^7$-$10^8$ cells/ml). IFN was induced using pI:C (polyionosinic: polycitidilic acid) at a concentration of 25 μg/ml. After 8 hours Actinomycin (10 μg/ml) was added. The cells were then sedimented (1200 RPM/20 minutes) and resuspended in fresh medium. The cultivation was carried on for the next 24 hours. Thereafter, the cells were pelleted (1200 RPM/ 20 minutes) and the supernatant was tested for the IFN content (antiviral units/ml). The IFN was further purified using the selective precipitation method.[8] The IFN used in the experiments has a specific activity of $10^8$ antiviral unit/mg of proteins.

### *Analysis of cell morphology*

In order to establish the differences in cell morphology, the cells were cultivated by two methods: (i) on small glass slides for four days, and (ii) on small glass slides in test tubes for 15, 30, 45, 60, 90 and 180 minutes after adsorbtion (control) and treatment with 1000 units of IFN/ml or mock IFN for 15, 30, 45, 60, 90 and 180 minutes. To analyse morphological changes, the cells were fixed with 4% paraformaldehyde, washed with PBS (phosphate buffer saline pH 7.2) and stained with Giemsa. In the parallel experiments, the cells were fixed and washed as in the case of staining with Giemsa, but afterwards stained with acetoorcein for two minutes. The following parameters were analysed: the diameter of the nucleus (>1/2 of the cell), presence or absence of phylopodia, presence or absence of binuclear cells.

### *Analysis of nuclear blebs*

Throughout the experiments the number of nuclear blebs was determined using the method described by Fraccaro et al.[11] In summary, cells were adsorbed for 45 minutes and treated with 1000 units/ml of IFN (mock in control) for 15, 30, 45, 60, 90 and 180 minutes. The cells were then detached using trypsin (0.25%) and resuspended in 5 ml of 0.83 mM of Ammonium chloride for 45 minutes. The nuclei were sedimented by centrifugation for 10 minutes at 1200 RPM, and resuspended in PBS and put onto glass slides, air dried and fixed with methanol. Thereafter, they were stained with Giemsa and analysed by dark field microscopy.

### *Growth characteristics*

The following growth characteristics were analysed: growth index (GI), cumulative population density (CPD) and calcium dependence growth assay.[7]

Growth index (GI): Cells were seeded into 5 cm Petri dishes for 45 minuts to allow the adsorbtion. After the treatment with 1000 units of IFN/ml (mock in control) for 15, 30, 45, 60, 90 and 180 minutes, the medium was replaced with medium containing 1% and/or 10% of FCS. The incubation was continued for four days. Cells were detached using trypsin (0.25%) and counted by means of a hematocytometer.

Cumulative population density (CPD): The values were obtained from the data (number of cells/ml) of growth experiments.

Calcium dependence growth assay: To obtain the values for calcium dependence growth in

vitro, the cells were seeded into 5 cm Petri dishes for 45 minutes and treated with 1000 units of IFN/ml (mock in control) for 15, 30, 45, 60, 90 and 180 minutes. Afterwards, the medium was replaced with medium containing 10% FCS, and incubated for 18 hours. On the next day, the medium was replaced with another one containing 0.01 mM Ca or 1.00 mM Ca with 10% of dialysed FCS. The number of cells was determined after four days of incubation by means of a hematocytometer.

*Alkaline phosphatase*

The enzyme level was measured according to the method described by Chou.[12] In summary, 1 ml of distilled water was added to 50 µl of substrate (p-nitrophenyl phosphate in 0.05 M Tris-HCl, pH 8.6). After 5 minutes of preincubation, cell supernatant (100 µl) was added. Thereafter, the incubation was continued for 20 minutes, in the presence of PBS pH 9.0. The extinction was measured at 550 nm using a spectrophotometer.

*Protein content*

In each sample the protein content was determined by modified Lowry method.[13]

## Results

*Cell morphology*

Even though a significant differences can be seen between nontransformed and transformed WiREF cells, we were interested to find out whether a short time IFN treatment could change the following parameters: diameter of the nuclei, number of binuclear cells and the number of cells without philopodia. The results obtained (Figure 1) show a relatively fast increase in the number of cells with nuclei bigger than 1/2 of the cell. In contrast to this, in transformed cells these changes were much slower.

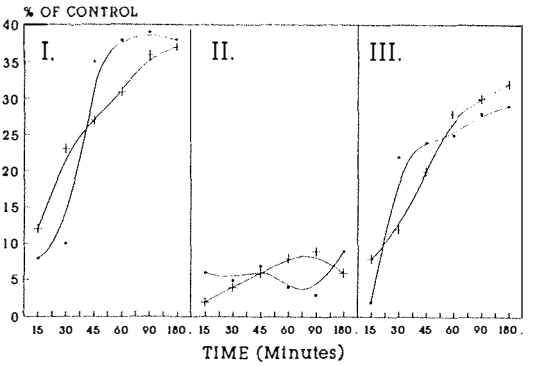


**Figure 1.** Morphological changes after interferon treatment of nontransformed (•) and transformed (+) WiREF cells. I. % of big nuclei, II. % of fused nuclei, III. % of cells without phylopodia.

The analysis of binuclear cells showed no differences between nontransformed and transformed cells at a higher rate, but it can be seen, that the percentage of binuclear cells begins to increase at a higher rate in nontransformed than in transformed cells.

When the phylopodia were analysed, the following results were obtained: in nontransformed cells the increase of their number began after 30 minutes, whereas in transformed cells this began after 15 minutes of treatment.

*Growth characteristics*

When the effect of IFN on the growth characteristics (Table 1, Figure 2) was analysed, in general the expected data were observed, i.e. a higher sensitivity of transformed versus nontransformed cells. A completly reverse situation, however, was observed in the case of CPD. The reduction rate was higher in nontransformed cells.

*Alkaline phosphatase*

In the case of alkaline phosphatase (Figure 3), the kinetics of enzyme levels seemd similar in both nontreated and IFN- trated transformed cells, but quantitative differences could be seen. In nontransformed cells, the enzyme level decreased in untreated cells, whereas in IFN-trea-

**Table 1.** Growth characteristics of nontransformed and transformed rat embryonal fibroblasts (Wistar strain) (WiREF).

| Cells | Growth index[1] | | Cumulative[2] population doublings: | | Ca Dependence[3] |
|---|---|---|---|---|---|
| | (1%)[x] | (10%)[x] | (1%)[x] | (10%)[x] | |
| Nontransformed (Phase A) | 2.19 | 3.19 | 1.29 | 1.67 | 0.42 |
| Transformed (Phase E) | 3.11 | 4.87 | 2.09 | 2.29 | 0.96 |

1) Values obtained by equation: $\frac{\text{No. of cells after 4 days}}{\text{No. of cells on day 0}}$

2) Values obtained by equation: $\frac{(\text{Log N1} - \text{Log N0})}{\text{Log 2}}$

N0 = Number of cells on day 0
N1 = Number of cells after four days

1) Values obtained by equation: $\frac{\text{No. of cells at. 0.01 mM Ca}}{\text{No. of cells at 1.00 mM Ca}}$

x) Denotes the presence of 1% or 10% of FCS in Eagle's medium

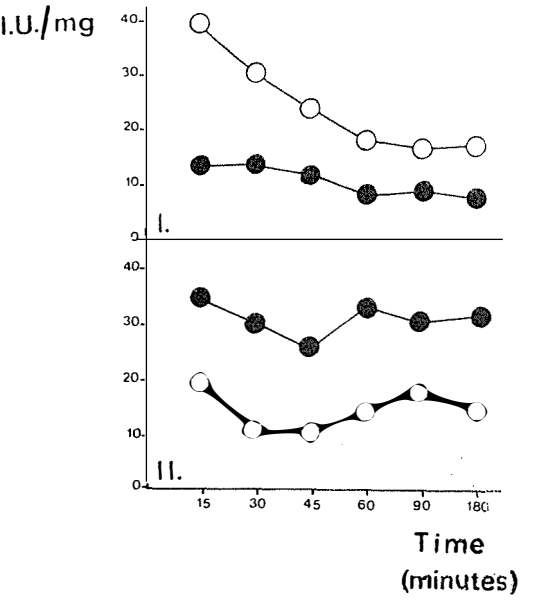


**Figure 3.** Effect of a short term rat Beta IFN on the intracellular level of alkaline phosphatase (specific activity). I. Nontransformed WiREF, II. Transformed WiREF. (o) Nontreated cells, (•) Treated cells.

ted ones a time independent decrease was seen throughout.

## Discussion

The experiments were conducted on spontaneously transformed rat embryonal fibroblasts (WiREF) cell line and on its nontransformed counterpart. We have been interested to find out whether homologous rat (beta) interferon exerts an effect on cell morphology, growth characteristics and enzyme level. The experiments showed differences in the sensitivity of nontransformed cells. As to the morphology, in general there was no difference, but the effects on transformed cells were observed earlier than on nontransformed cells. The first morphological change in the diameter of cell nucleus can be found after a 15 minute treatment. It is interesting to note that immunochemical methods have shown that interferon can be detected around the cell nucleus even after 30 minutes.[14] These results can be correlated with the changes in the number of nuclear blebs and calcium dependent cell growth *in vitro*. In contrast to this, in our study of IFN effect on the actin organisation,[15] the nontransformed cells were found to be more sensitive than transformed ones. Having all this in mind, we attempted to find out whether the effects of IFN listed above (morphology, growth characteristics, nuclear blebs, actin organisation) were

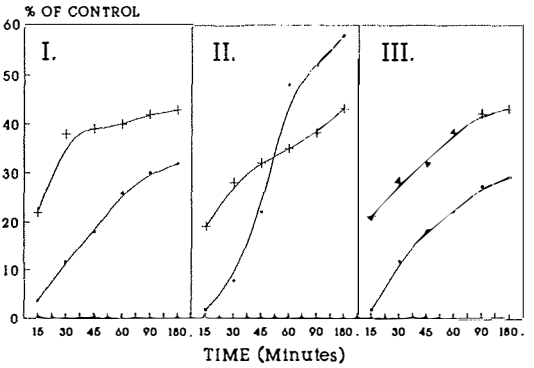


**Figure 2.** Growth characteristics of nontransformed (•) and transformed (+) WiREF cells after treatment with 1000 units of IFN/ml for 15, 30, 45, 60, 90 and 180 minutes. The values are expressed as the percentages of the control (Table 1). I. Growth index, II. Cumulative population doubling, III. Ca dependence growth *in vitro*.

parallelled by changes in enzyme levels (alkaline phosphatase). In notransformed cells the decrease of alkaline phosphatase was time independent, in contrast to transformed cells, where time-dependent changes were observed (alkaline phosphatase reached a maximum after 180 minutes).

It can be concluded that after the binding to cell surface, interferon acts relatively fast, and that the intracellular changes (enzyme level, morphology) are probably different depending on the phase of the cell cycle as well as on the phase of transformation. But it seems that the majority of the changes connected with the anticellular activity are triggered during the first 60–180 minutes. Concomitantly, the transformed cells show a trend toward a time dependent sensitivity of IFN action.[16, 17] The mechanism of a relatively greater sensitivity of transformed cells to IFN remains unknown, even though it seems possible that the regulatorly role of different intracellular hydrolases and consequently also the role of proteases and their inhibitors should be taken into account.

## Acknowledgment

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