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Antiproliferative effect of interferons on human prostate carcinoma cell lines*

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Summary. The effect of purified human fibroblast beta-interferon (B-IFN) and recombinant alpha-2b-interferon (A-IFN) on cell proliferation was investigated in two human prostate carcinoma cell lines, named PC-3 and DU-145. Both cell lines respond to the antiproliferative action of interferon, B-IFN being more effective than A-IFN. PC-3 is more sensitive than DU-145 cell line, showing 95% inhibition of cell proliferation at the highest concentration of B-IFN. As interferons, besides reducing cell growth, are able to modify steroid receptor content in different hormone-sensitive human tumours, our results may be of some relevance as these drugs might be used to regulate both cell proliferation and hormone-sensitivity in prostate cancer.

Key words: Human natural beta-interferon – Human recombinant alpha-2b-interferon – Growth inhibition in cell culture – Prostatic cancer

Introduction

It has been reported that interferons have antiproliferative effects in different experimental models. However, this information was from studies carried out on cells grown in tissue culture. A wide range of cell types have been used, including normal cells [13], and relative tissue affinities for different interferons have been postulated [3, 7]. As far as transformed tumour cells are concerned, cell growth inhibitory action of interferons on cell lines derived from lymphomas [11], myelomas [8], breast carcinomas [1, 14, 16], osteosarcoma and soft tissue sarcomas [18], ovarian carcinomas [9], melanomas [4], and bladder carcinomas [2] has been established. Moreover, a number of studies dealing with inhibition of the synthesis of macromolecules such as DNA and protein [10], effects on cell cycle [4], variation of cyclic nucleotides [19] have been attempted to clarify the mechanism by which the antiproliferative action of interferons is mediated. To our knowledge, insufficient data are available regarding the action of interferons on prostatic carcinoma cell lines. The aim of the present study was to investigate the effect of B-IFN and A-IFN on the proliferation of two human prostatic carcinoma cell lines (PC-3 and DU-145), which have been reported to be hormone-insensitive [12, 17].

Materials and methods

Interferons

Natural beta-interferon (FRONE), induced in human fibroblasts by poly I·poly C was kindly supplied by SERONO, Rome, Italy.

Human recombinant alpha-2b-interferon (INTRON A) was obtained from ESSEX ITALIA, Milan, Italy. Both interferons were dissolved in culture medium at the appropriate concentrations just before use.

Cell lines

PC-3 cell line was derived from a human prostatic adenocarcinoma which metastasised to bone [12]. DU-145 cell line has been obtained from a human prostatic adenocarcinoma brain metastasis [17].

PC-3 cell line was kindly donated by Prof. F. Labrie, Laval University, Quebec, Canada. DU-145 cell line was a gift from Prof. K. Griffiths, Tenovous Institute for Cancer Research, Cardiff, UK.

The cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM, Flow Laboratories, Irvine, UK), supplemented with

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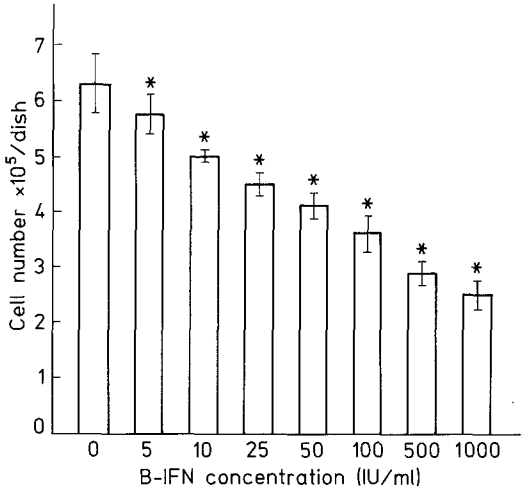

Fig. 1. Effect of various concentrations of B-IFN on PC-3 cell proliferation after a 3-day treatment. Values represent the mean of two different experiments. Bars represent standard deviation. * $P<0.01$, Dunnett's test [6]

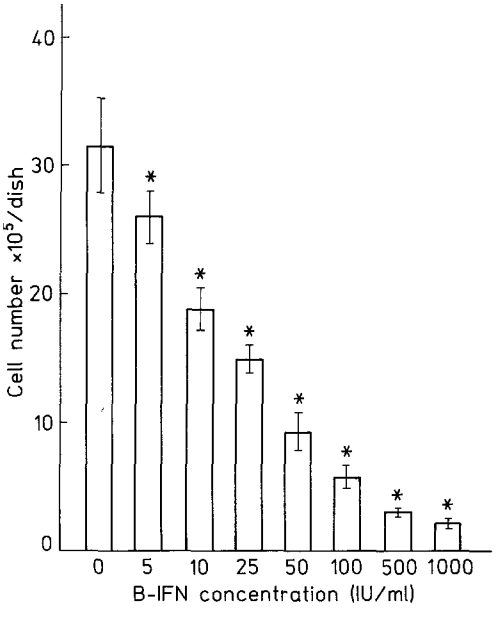

Fig. 2. Effect of various concentrations of B-IFN on PC-3 cell proliferation after a 6-day treatment. Values represent the mean of two different experiments. Bars represent standard deviation. * $P<0.01$, Dunnett's test [6]

10 mM Hepes buffer (N-2-hydroxyethylpiperazine-N'2-ethansulfonic acid, Sigma, St. Louis, Mo), 10% foetal calf serum (FCS, Flow Lab.), 100 IU/ml penicillin, 100 µg/ml streptomycin (Flow Lab.), 2.5 µg/ml amphotericin B (Flow Lab.), under a humidified atmosphere in a 5% CO_2 incubator.

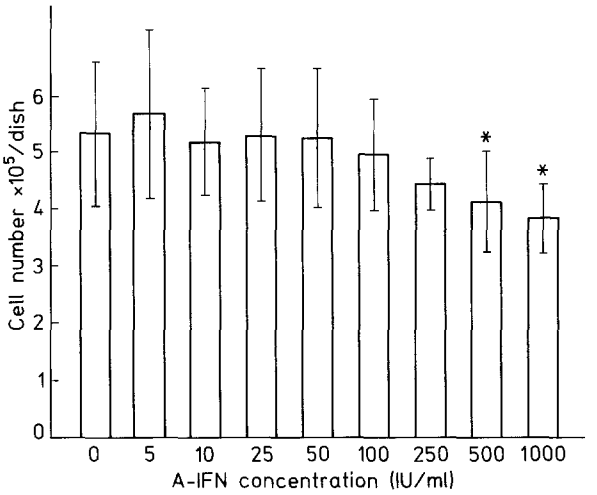

Fig. 3. Effect of various concentrations of A-IFN on PC-3 cell proliferation after a 3-day treatment. Values represent the mean of two different experiments. Bars represent standard deviation. * $P<0.05$, Dunnett's test [6]

Antiproliferative assays

Cells at logarithmic growth phase were trypsinized and plated out at the density of 25,000 cells/ml, into 60 mm plastic Petri dishes in DMEM supplemented as described above.

Twenty-four hours after plating, by which time the cells became attached to the surface of the dish, the medium was renewed with fresh medium containing B or A-IFN at concentrations ranging from 5 to 1,000 IU/ml. Triplicate cultures were set up for each IFN concentration. Control dishes were run in parallel. Cell counts were performed with a hemocytometer after 3 days of exposure to the drug. Medium containing IFN was replaced in the remaining dishes, which were counted again after 3 days. Cell viability, as assessed by trypan blue exclusion technique, was observed to be at least 90% for each experiment. Statistical analysis was performed using one-sided Dunnett's test [6].

Results

Figures 1 and 2 show the effect of various concentrations of B-IFN on Pl-3C cell proliferation after 3 and 6 days of treatment. On the third day, B-IFN produced an inhibition of cell proliferation which was statistically significant at all the concentrations tested ($P<0.01$), reaching 60% with respect to control at 1,000 IU/ml of B-IFN.

On the sixth day, even at low doses of IFN, inhibition was relevant (about 40% with respect to control at 10 IU/ml of IFN) and became pronounced at highest doses (from 80 to 95% at concentrations ranging from 100 to 1,000 IU/ml). The difference between treated cells and controls was statistically significant ($P<0.01$).

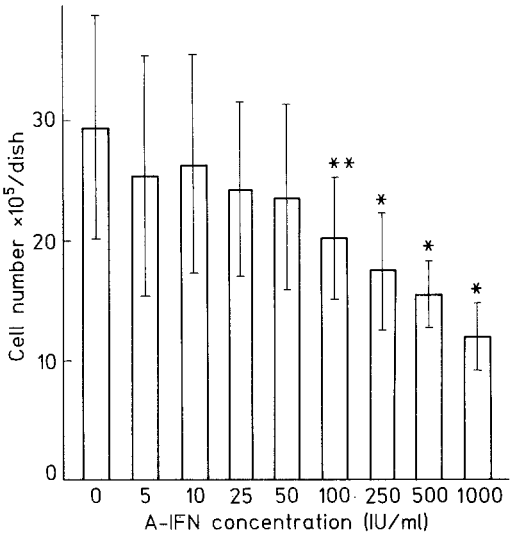

Fig. 4. Effect of various concentrations of A-IFN on PC-3 cell proliferation after a 6-day treatment. Values represent the mean of two different experiments. Bars represent standard deviation. * $P<0.01$; ** $P<0.05$, Dunnett's test [6]

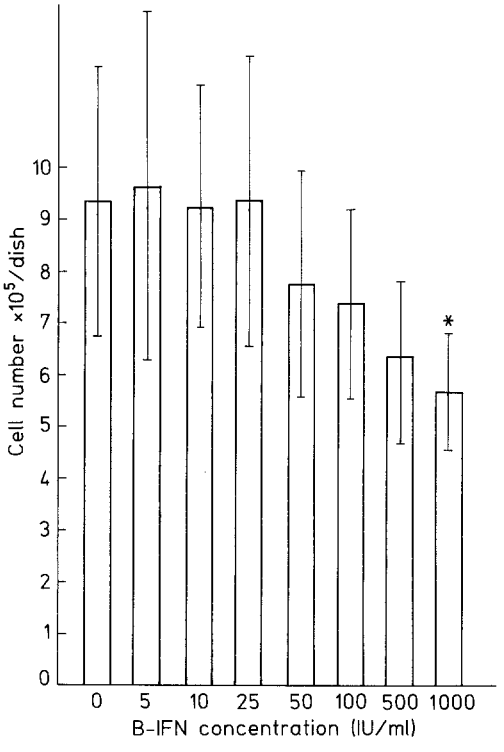

Fig. 5. Effect of various concentrations of B-IFN on DU-145 cell line proliferation after a 3-day treatment. Values represent the mean of two different experiments. Bars represent standard deviation. * $P<0.05$, Dunnett's test [6]

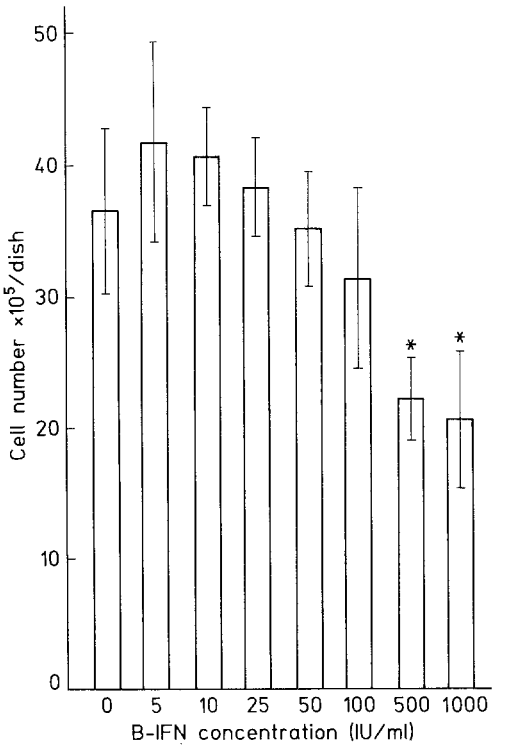

Fig. 6. Effect of various concentrations of B-IFN on DU-145 cell line proliferation after a 6-day treatment. Values represent the mean of two different experiments. Bars represent standard deviation. * $P<0.01$, Dunnett's test [6]

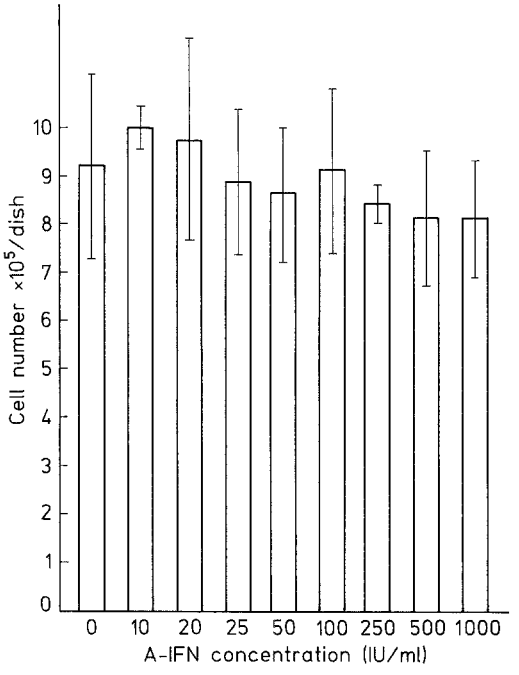

Fig. 7. Effect of various concentraions of A-IFN on DU-145 cell line proliferation after a 3-day treatment. Values represent the mean of two different experiments. Bars represent standard deviation. Difference between treated and control cells was not statistically significant at all the IFN concentrations tested (Dunnett's test [6])

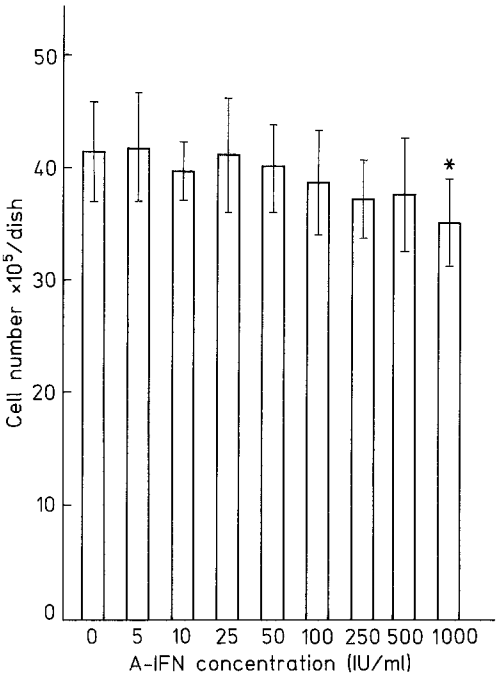

Fig. 8. Effect of various concentrations of A-IFN on DU-145 cell line proliferation after a 6-day treatment. Values represent the mean of two different experiments. Bars represent standard deviation. *$P<0.05$, Dunnett's test [6]

Figures 3 and 4 show the effect of various concentrations of A-IFN on PC-3 cell proliferation. After a 3-day treatment a significant inhibition of cell growth was seen only at IFN concentrations ranging from 500 to 1,000 IU/ml ($P<0.05$). On the sixth day, the difference between treated and control cells became significant at concentrations ranging from 100 to 1,000 IU/ml ($P<0.05$ at 100 IU/ml; $P<0.01$ from 250 to 1,000 IU/ml). The inhibition was about 30% at 100 IU/ml of IFN and reached 60% at 1,000 IU/ml. The effect of various concentrations of B-IFN on DU-145 cell proliferation is depicted on Figs. 5 and 6. Inhibition of approximately 40% was reached after a 3-day treatment at the concentration of 1,000 IU/ml of medium ($P<0.05$). After 6 days of treatment, a significant inhibition of cell proliferation was seen at concentrations ranging from 500 to 1,000 IU/ml ($P<0.01$). The maximum inhibition (42%) was obtained at the highest IFN concentration used.

Figures 7 and 8 show the antiproliferative effect of various concentrations of A-IFN on DU-145 cell line.

After a 3-day treatment no effect on cell growth was seen at all the concentrations tested. On the sixth day a slight inhibition of cell proliferation was observed only at the highest concentration of A-IFN used (about 16% with respect to control $P<0.05$).

Discussion

Our data indicated that both cell lines were sensitive to the antiproliferative action of interferons, but that they differed in the degree of their sensitivity under the experimental conditions employed. Naturally produced B-IFN was more active than recombinant A-IFN in inhibiting cell growth both in PC-3 and DU-145 cell lines. The decrease in cell growth, induced in PC-3 cell line by B-IFN was noteworthy, considering that it is produced by very low B-IFN concentrations (10 IU/ml) both after 3 and 6 days of treatment in standard culture conditions, i.e. presence in the culture medium of 10% FCS, which contains factors that can antagonize interferon action. Higher doses of B-IFN from 25 to 1,000 IU/ml caused a massive reduction of cell number. PC-3 and DU-145 cell lines were not stimulated by testosterone in our own experience and in that of other authors [12, 17]. Nevertheless, preliminary results obtained in our laboratory indicate that, unlike reports by Kaighn et al. [12], these cells contain androgen receptors as evaluated by a whole cell assay (unpublished data).

As recently demonstrated, interferon not only affected cell proliferation, but also modified oestrogen and progesterone receptor levels in breast cancer cells [15, 16] and in neoplastic endometrium [5]. Work is in progress in prostatic carcinoma cell lines to assess whether the inhibitory action of IFN is associated with the modulation of androgen receptor content. If such an effect could be confirmed, it would be of clinical relevance especially if interferon could affect not only the growth but also the hormone sensitivity of prostatic cancer cells.

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