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INTERACTIONS BETWEEN HUMAN AND PORCINE INTERFERONS

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Summary. - To investigate the possible interactions between human and porcine interferons (IFNs) *in vitro*, human transformed (FL) and nontransformed (HEF) cells were treated with either HuIFN alpha and/or gamma and porcine alpha and/or gamma. In both cases the antiproliferative activity was measured to determine the effects of different combinations between human and porcine IFNs on cell proliferation. Combinations of human and porcine IFNs acted mostly antagonistically with exception of IFN combination Hu-alpha/Po-gamma which showed a synergic cooperativity in therms of antiproliferative activity on human transformed cells.

Key words: human interferons alpha, gamma; porcine interferons alpha, gamma; antiproliferative activity; human transformed and nontransformed cells; interactions

Introduction

Interferons (IFNs) are a broad family of naturally occuring proteins regulating a number of different cellular functions and having a variety of antiviral, and antiproliferative as well as immunoregulatory effects (Gresser, 1978; Taylor *et al.*, 1984; Hubbell *et al.*, 1987). They can be divided into at least two classes (Type I - Alpha or Beta and Type II - Gamma) according to their induction (Type I - with viruses/pI: pC, Type II - with T cell mitogens).

Because of potential clinical usage, research on antiviral and antiproliferative/antitumour activity of IFNs is mainly focused on Human Alpha and Gamma IFNs. However, the antiproliferative/antitumour activity of Human Alpha IFN may increase in cooperation with nonhuman IFN. This phenomenon was first described in the mouse system (MuAlpha - MuGamma IFN) (Fleischmann *et. al.*, 1979) with respect to antiviral activity, and later, again in the mouse system, an enhancement of antiproliferative/antitumour activity was found (Fleischmann *et al.*, 1984). Different studies have shown that potentiation occurs in a similar manner in the mouse and in the human system.

Further, studies with recombinant DNA-derived mouse and human interferons have demonstrated that the molecules responsible for potentiation were interferons themselves (Czarniecki *et al.*, 1984). This is valid not only for Human and Murine IFN combination but also for combination with porcine IFN (Piasecki, 1988). Porcine IFN (PoIFN)-Alpha was found immunologically related to HuIFN-Alpha (Soloviev *et al.*, 1982; La Bonnardiere, 1986) acting as an acid labile „interferon-like inhibitor of foot-and-mouth disease virus" (Richmond, 1969). Additionally it was found, that PoIFN-Alpha successufully protects human cells against various viruses (Soloviev *et al.*, 1982). It makes sense to consider the clinical usage of PoIFN in man, despite of the risk of introducing an immunomodulating substance from one species into another.

We present the results of experiments performed to test the possible interactions between Human (Alpha and Gamma) and Porcine (Alpha and Gamma) IFNs *in vitro*.

Materials and Methods

Cells. Nontransformed human embryonal fibroblasts (HEF) and transformed human amniotic cells (FL) were used throughout. The cells were cultivated in Eagle's media supplemented with 10% of foethal calf serum (FCS) (Flow).

Interferons. HuAlpha-IFN (Institute of Immunology, Zagreb, Yugoslavia) with a specific activity of 10 000–100 000 units/mg of proteins was used; no further purification was performed. HuGamma IFN was prepared according to the method described by Gregoriades (1986) and Billiau *et al.* (1982). In brief: aseptically collected blood was separated on Ficoll-Hypaque gradient. After centrifugation, lymphocytes were collected and resuspended in Eagle's medium to a final concentration of 1 000 000 cells/ml. PHA (Phytohaemagglutinin) in concentration 20 μg/ml was used as inducer. After three days in culture, the suspension was centrifuged and the supernatant collected. IFN was partially purified using adsorption on silicic acid and elution with monoetyleneglycol. The preparation was analysed by SDS-PAGE; when used its specific activity was 100 000 – 1 000 000 units/mg protein.

PoAlpha IFN was obtained from the Institute of Microbiology, Medical Faculty of Szeged, Hungary; its specific activity ranged from 1 000 000 to 10 000 000 units/mg protein. PoGamma IFN (Mitogen stimulated) was prepared as described (Filipič *et al.*, 1986). In brief: citrate treated blood was collected aseptically, sedimented with 3.8 % Dextrane. The lymphocyte-leukocyte rich plasma was further purified by 6 % gelatine-10 % sucrose in ACD. After sedimentation (60 min) at room temperature, the erythrocytes were removed, the upper layer was collected and centrifuged at 2500 rev/min for 15 min. The leukocytes were resuspended in Eagle's medium containing 4 % porcine agamma plasma to obtain a suspension containing 10^7 cells/ml (counted in a haematocytometer) with a viability near 95 – 98 % (dye exclusion test with Trypan blue). PHA (50 μg/ml) was added, and the suspension was grown on a spinner for three days. Afterwards, the supernatant was collected by centrifugation at 2500 rev/min (30 min). The supernatant was handled by as used for preparation of HuGamma IFN (Billiau *et al.*, 1982), namely concentrated in an Amicon ultrafilter (cutoff 10 000), dialysed against PBS (Phosphate buffer saline, pH 7.4) and sterilised by membrane filtration. The preparation obtained had a specific activity of 100 000 – 1 000 000 units/mg protein.

Measurement of antiviral activity. Each sample of different IFNs (Human, Porcine) was tested by the 50% cytopathogenic inhibition assay against VSV as challenge virus (Forti *et al.*, 1986) in both FL and HEF cells. HuAlpha FIN (1000 units/ml) EGIS, Budapest, Hungary) was used as standard.

Measurement of antiproliferative activity. Cell growth inhibition assay was performed as described (Filipič *et al.*, 1985; Charley *et al.*, 1987; Dawson *et al.*, 1986). In brief: cells (HEF and FL) were cultured in microtitre plates (NUNC) with Eagle's medium supplemented with 10 % foetal calf serum. On the next day, the medium was replaced by a new medium containing 5 % of serum. The initial number of cells was determined separately. After 3 hours CO_2 incubation at 37 ^{0}C, the cells were fixed with glutaraldehyde and the plates were cooled to 4 ^{0}C. Different concentrations (in antiviral units) of IFNs (500, 100, 50, 10, 5, 1) were added, then cells were incubated for additional three days in CO_2 atmosphere at 37 ^{0}C. IFN treated and mock-treated control cells were fixed with a 0.25 % solution of glutaraldehdyde for 20 min, washed with PBS (Phosphate buffer saline, pH 7.4) and stained with Methylen blue (4 % solution) for 45 min at 37 ^{0}C. Finally, the plates were thoroughly washed with tap water, air dried, and extracted by adding 100 μl/well of 0.1 mol/HCl. The OD was measured in AUTOEIA (Labsystem) photometer at 570/650 nm. The growth index (GI) was calculated as follows:

$$GI = \frac{\text{OD after four days}}{\text{OD of initial number of cells.}}$$

Estimation of IFN interactions. The effect of the combined IFN tratment using a combination of drugs A and B was calculated by the isobole method (Berenbaum, 1981; Hubell *et al.*, 1987) from the equation:

$$D = \frac{Ac}{Ae} + \frac{Bc}{Be},$$

where Ac and Bc are the doses of the substance in combination and Ae and Be are the doses to be given separately to achieve the same magnitude of effect as in combination. If D is higher than 1, combination acts antagonistically, if D is equal to 1, effects are additive, and if D is lower than 1, the combination acts synergistically. The values for Ae and Be were derived from a line generated by dose-responses curve for each individual interferon.

Results

Antiproliferative activity of human and porcine IFNs on human cells

With HuAlpha and HuGamma IFN treatments the results obtained were more or less expected (Fig. 1-I, -II). Only in the case of HuAlpha small differences between nontransformed and transformed cells could be seen. When the cells (FL, HEF) were treated with the same amounts of porcine IFNs (Alpha and/or Gamma) the data were similar, though the antiproliferative activity of porcine Alpha IFN was much lower than that of human Alpha IFN. Concomitantly, it is interesting to note the relatively high antiproliferative activity of PoGamma on human transformed (FL) cells, in comparison to nontransformed cells (Fig. 1-III, -IV).

Comparison of the four IFNs (HuAlpha, HuGamma, PoAlpha, and PoGamma) shows differences in terms of the antiviral units/ml (Table 1) required to obtain 50 % reduction of GI (GI/C %) in nontransformed (HEF) and transformed (FL) cells. Proximately ten times more (in terms of antiviral units) PoIFN was required for the same antiproliferative effect.

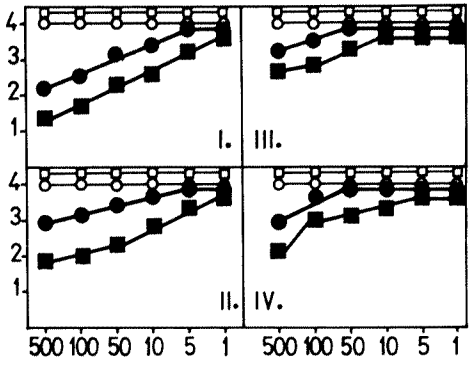

Fig. 1
Antiproliferative activity of human alpha (I), gamma (II) and porcine alpha (III), gamma (IV) IFNs
HEF (●) and FL (■) cells were treated with different concentrations (500, 100, 50, 10, 5, 1) of human porcine IFNs for four days, and the growth index was determined (see Materials and Methods).
Abscissae: antiviral units of IFN/ml; ordinates: growth index values.

Interactions between human and porcine interferons

In the same experimental system, the possible interactions between human and porcine interferons were studied using the following combinations: HuAlpha: HuGamma, HuAlpha: PoGamma, HuAlpha: PoAlpha, and HuGamma: PoGamma using the ratio (in antiviral units/ml) 500:500, 500:100, and 100:500. The data (Figs. 2-I, -II) show that the highest increase of the antiproliferative activity was obtained, when HuAlpha was combined with HuGamma or PoGamma in the ratio of 500:100 units/ml. In both cases the combination used was much more effective on FL (transformed) than on HEF (nontrans-

Table 1. Efficacy of Hu and Po interferons as antiproliferative agents

Type of interferon	GI/C % (50%) (IAU/ml required)	
	HEF	FL
HuAlpha	500	100
HuGamma	368	50
PoAlpha	2870	1000
PoGamma	800	500

LAU/ml – International antiviral units
GI – Growth index
C – Control

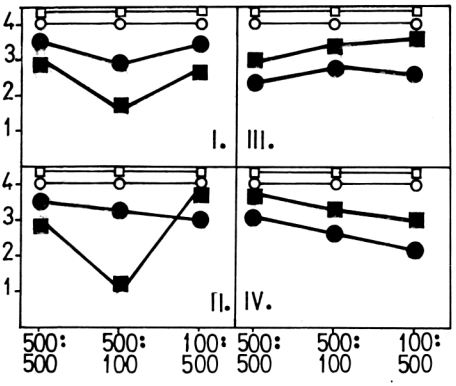

Fig. 2
Interactions between human and porcine IFNs
Human alpha and human gamma IFNs (I) were mixed in different ratios of antiviral units and the growth index was measured (see Materials and Methods) on HEF (●) and FL (■) cells. Human alpha and porcine gamma IFNs (II) were mixed in different ratios of antiviral units and the growth index was measured. Human alpha and porcine alpha IFNs (III) were mixed in different ratios of antiviral units and the growth index was measured. Human gamma and porcine gamma IFNs (IV) were mixed in different ratios of antiviral units and the growth index was measured.
Abscissae: ratios of antiviral units of IFN/ml; ordinates: growth index values.

formed) cells. Completely different data were observed (Figs. 2-III, -IV) when combinations of HuAlpha and PoAlpha and of HuGamma and PoGamma were used. In both cases effects were practically the same as in the single dosage experiments. Calculations (Tables 2 and 3) showed that most of the combinations acted antagonistically with exception of combination HuAlpha: PoGamma (500:100) in FL cells and of combination HuGamma: PoGamma (100:500) in HEF cells, which acted synergistically.

Discussion

Based on abovementioned data it can be emphasized that the effects of Hu and Po IFNs in human cells are comparable since the human cell lines used (FL and HEF) aer sensitive for porcine IFNs (Alpha/Gamma). It is more complicated to explain the interactions between Hu and Po IFNs. In the case of HuAlpha and HuGamma and of HuAlpha and PoGamma, the same tendency was observed. When the optimal ratio of AV units was obtained, this resulted in enhancement of antiproliferative activity in terms of selectivity for transformed cells. On the other hand, the combination of HuAlpha with PoAlpha inversed the selectivity for nontransformed cells. Similar data were obtained in the case of HuGamma and PoGamma.

When tests were designed to investigate the possible enhancement of antiviral activity (Tóth and Filipič, 1991) the obtained data were similar, i. e. the highest increase was obtained with the HuAlpha: PoGamma mixture. The question remains open as to the nature of interactions concerning their antiviral and antiproliferative activities. Antigenic similarity between Hu and Po

Table 2. Interactions between human and porcine IFNs, tested in FL cells

IFN (Ac)		IFN (Bc)		GI/C %	IFN (Ae)	IFN (Be)	D
HuAlpha	500	HuGamma	500	60.046	50	10	60
	500		100	32.333	100	500	5.2
	100		500	55.427	10	10	60
Hu Alpha	500	PoGamma	500	64.665	50	100	15
	500		100	16.166	1000	1000	0.6
	100		500	85.450	5	5	120
HuAlpha	500	PoAlpha	500	60.046	50	100	15
	500		100	73.903	5	50	102
	100		500	83.141	5	50	30
HuGamma	500	PoGamma	500	87.760	5	10	150
	500		100	73.903	5	10	110
	100		500	64.665	5	10	70

IFN (Ac): IFN (Bc) values are expressed in IAU (International antiviral units)/ml.
IFN (Ae) value was estimated from a standard curve to obtain the same effect as in combination. The value is given in IAU/ml.
IFN (Be) value was estimated from a standard curve to obtain the same effect as in combination. The value is given in IAU/ml.
D = Combination index calculated as given in Materials and Methods.
GI = growth index
C = control

Table 3. Interactions between human and porcine IFNs, tested in HEF

IFN (Ac)		IFN (Bc)		GI/C %	IFN (Ae)	IFN (Be)	D
HuAlpha	500	HuGamma	500	80.831	10	50	55
	500		100	64.665	100	500	5.2
	100		500	73.903	50	100	7.0
Hu Alpha	500	PoGamma	500	80.831	10	500	51
	500		100	73.903	50	500	10.2
	100		500	83.141	10	500	11.0
HuAlpha	500	PoAlpha	500	55.427	100	1000	5.5
	500		100	64.665	50	500	10.2
	100		500	62.356	100	1000	1.5
HuGamma	500	PoGamma	500	73.903	100	500	6.0
	500		100	62.356	100	500	5.2
	100		500	50.808	1000	1000	0.6

For legend see Table 2.

IFNs should be taken into account. In the case of Alpha IFNs the homology is of about 78.5 % (at nucleotide level) (Lefevre and La Bonnardiere, 1986). The homology between Gamma (Hu and Po) IFNs remains open because of the nature of PoGamma (mitogen stimulated) IFN. The degree of the sequence homologies between rPoIFN Gamma/rHuIFN Gamma was estimated at 59 % (Charley *et al.*, 1987). Opinions on PoIFN Gamma continue to differ, especially in view of its physicochemical properties (stability at pH 2 and 56 ^{0}C, antigenic similarity in terms of antiserum interactions). Differences also exist between PoGamma prepared by mitogen stimulation and by the recombinant technology.

The presented data point at possible clinical exploitations of PoIFNs (Alpha /Gamma), even though the importance of possible antigenic differences must be taken into account.

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