Comparative studies of Interferon activity on different cell cultures using polio virus, semiliki forest virus and new castle disease virus

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Abstract

It was realized soon after chick interferon was discovered that all types of interferon has complex effect all these effects to inhibit or reduce the translation of mRNA to tRNA may be used like antibiotics for the prevention or possibly for the treatment of virus infection in man and animal, also used to treat some types of cancer, and this idea was supported by experiments showing that interferon would prevent or modify such diseases. In this work we used three different cell cultures HeLa cells (cell line), MRC5 (diploid cells and HAC (normal primary cells)) and three viruses (polio, SFV and NDV) belonging to three different families (enteroviridae, togaviridae and rhexoviridae). It was found that MRC5 and HAC are interferon sensitive while HeLa cells are interferon insensitive.

Introduction

Interferons (IFN) were originally characterized by their ability to protect cells from virus infection. Addition of IFN to the culture of mammalian cells prevents virus growth [1]. IFN is used as treatment for some types of cancer these include cancer of the kidney, malignant melanoma, multiple myloma, characinoid lumber

And some type of lymphoma and leukemia [2,3,4]. IFN is also used to treat diseases other than cancer such as chronic hepatitis C and B. Until the resent development of assays for the detection of HCV RNA by PCR no clear cut parameters were available to assess the response to IFN treatment in chronic HCV [5,6,7,8,9,10,11] IFN interact with cell and induces the antiviral state of the cell. Interferon vary in their antiviral activity in different cell systems making it difficult to define one type in relation to another [12] These difficulties led Stewart 1979 [13] to use the term interferon system in which divided his system into two parts, part I he related to the cell source, inducers and type of IFN produced Part II he related to the susceptible cells and susceptible viruses, Since IFN effect a diverse number of biochemical responses in cells, it is perhaps not surprising that there is no single mechanism of replication inhibition to which different viruses are equally susceptible, the degree of inhibition observed is dependent both on cell type and virus group [14,15].

In attempt to know how generally the antiviral activity induced by IFN *in vitro* a comparative studies on antiviral state of different cells choosing three different cell cultures, malignant cells (HeLa), normal cells treated with carcinogenic compound (MRC5) and normal cells (HAC); beside using three different viruses (polio, SFV and NDV).

Materials and methods

Viruses

1- Semiliki forest virus (SFV) was grown in HeLa cells monolayer maintained in BME (Gibco) supplemented with 2% foetal calf serum (FCS). Culturewere harvested after incubation for 48 hours at 37°C. Cells were frozen and thawed three times. And store at -70°C
2- Polio virus was grown in chick embryo fibroblast monolayer maintained in BME (Gibco) supplemented with 2% foetal calf serum (FCS). Culture were harvested after incubation for 48 hours at 37°C. Cells were frozen and thawed three times. And store at -70°C
3- Newcastle disease virus (NDV) was grown in chick embryo fibroblast monolayer maintained in BME (Gibco) supplemented with 2% foetal calf serum (FCS). Cultures were harvested after incubation for 48 hours at 37°C. Cells were frozen and thawed three times. And stored at -70°C and till used.

Cells

1-HeLa cells (Epithelial-like cells derived from human epitheloid cervical carcinoma) were grown in BME supplemented with 10% newborn calf serum (NCS) and antibiotics.

2-MRC5 (human diploid lung cells) were grown in BME supplemented with 10% newborn calf serum (NCS) and antibiotics.

3-Human amnion cells (HAC) (primary normal cells) were grown in BME supplemented with 10% newborn calf serum (NCS) and antibiotics.

Virus titration

Virus infectivity was assayed by titration in micro titer plates with confluent monolayer of HeLa cells. Using "half log" dilutions and 3 or 4 wells per dilution. Fifty percent end points were calculated by Karber's method. Certain titrations as indicated in the text were carried out after extracting the virus preparation with chloroform [6]. Antiviral activity was measured as the minimal concentration completely inhibiting cytopathic effect (CPE) induced by 100 TCID50 of virus. IFN was present throughout the assay.

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Human interferon α (INF) partially purified by selective precipitation was supplied by Dr. Cantell of public health laboratory (Finland).

Results and Discussion

Serial 2-fold dilutions of IFN were made and then added to the culture the day before adding the virus. On the second day IFN was removed from the monolayer, and the cells were then inoculated with viruses at concentrations of 100 TCID50. Results as shown in table (1) below. Concentrations of 2 unit/ml or more of IFN completely inhibited the production of CPE by polio virus in MRC5 and 1 unit/ml of IFN or more in HAC; and 4 unit/ml of IFN or more completely inhibited the production of CPE of SFV and NDV in MRC5 and HAC. Further more there was no antiviral activity of IFN against all viruses in HeLa cells using 4000 unit/ml of IFN. The toxicity of IFN was tested and it was found that there is no toxic effect up to 4000 unit/ml

Table (1): Effect of different concentrations of IFN on the activity of polio virus, SFV and NDV in different cell cultures

IFN Unit/m	CPE Produced by rubella virus ** Hu.IFN α		
	HeLa	MRC5	HAC
1000	++++	-	-
20	-+-+	-	-
10	—+-+	-	-
5	-+++	-	-
2.5	-—++	-	-
1.25	-+++	+	-
0.5	—++	+-++	-+++

IFN Unit/m	CPE Produced by rubella virus ** Hu.IFN α		
	HeLa	MRC5	HAC
1000	—++	-	-
20	-+++	-	-
10	-—++	-	-
5	—++	-	-
2.5	—++	+	+
1.25	-+++	+++	-
0.5	-—++	-+++	-+++

IFN Unit/m	CPE Produced by rubella virus ** Hu.IFN α		
	HeLa	MRC5	HAC
1000	-+++	-+++	-
20	-—++	+++	-
10	-—++	-+++	-
5	-+++	++++	-
2.5	+-++	----	+
1.25	+-++	+++	++
0.5	+-++	-+++	-++-

** - no CPE + 25% CPE +- 50 % CPE -+- 75% CPE +-+- 100%CPE

The evaluation of IFNs as well as all antiviral compounds can be quantified by using therapeutic index which is the ratio of the dose of drug or IFN just toxic (maximum tolerated dose) to the dose which is just effective (minimum effective dose) if this index is one or less, its not possible to use the drug under the conditions outlined without causing side effects; if the index is larger than the margin of safety is accordingly great [16]. Indeed it's important to beer in mind that the IFN toxicity was examined in this study using HeLa cells, MRC5 and HAC. No cytotoxicity was observed at concentration up to 4000 unit/ml.

The susceptibility of polio virus, SFV and NDV to IFN could be demonstrated by inhibition of CPE production by 100 TCID50 in tissue culture. However the apparent sensitivity of viruses varied between different cell culture, may be due to differences in type of receptors on the cell surface [14, 15], and the other reason may be partly because IFN treated cells undergoes several pathways to produce the antiviral state [17]. Thus HeLa cells were insensitive to IFN, while other cells *i.e.* MRC5 and HAC were sensitive to IFN. Its not surprising that some cells are sensitive to IFN and others are not sensitive since there's no single mechanism to give rise of same protean synthesis which are important enzymes for the activity of IFN, interferon's have complex effects but probably the main antiviral action is to degrade mRNA and/ or rRNA. It is surprising in some ways that different IFNs act synergistically, but this may partly because that different types of IFNs bind to specific receptors [18].

These results might give some explanation as to why HeLa cells are resistant to IFN, while MRC5 and HAC are sensitive. We found the antiviral action of IFN was greater in HAC than in deployed cells MRC5. These results with agreement with the result of Well *et al* [1] that (2-5) oligo (A) synthesize induced by IFN 50% more in trisomy-21vibroblas than deployed cells. This indication (2-5) oligo(A) synthesize is probably an important event after the initial interaction of IFN and receptor. However it was found that a total of seven of newly synthesized mRNA result in primary cell culture and deployed cell culture more than in cell lines, these mRNA coded for proteins of mol.wt.24000, 31000, 41000, 56000, 57000, 62000 and 68000 [19]. More studies need to know the mechanism of the reaction in each cell types.

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الخلاصة

لقد تحقق وبصورة بعد اكتشاف انترفيرون الدجاج وعلى ان جميع انواع الانترفيرون والذي له تأثيرات مختلفة تشترك بمنع تكاثر الفايروس بسبب منع او اختزال تحويل mRNA الى tRNA لذا فان الانترفيرون قد يلعب دوراً مهماً كما هو الحال في المضادات الحيوية لمنع وعلاج الامراض الفايروسية في الانسان والحيوان وكذلك لمنع وعلاج الامراض السرطانية وهذه الفكرة قد دعمت ببحوث ودراسات كثيرة بينت أن الانترفيرون يمنع او يعالج الامراض الفايروسية والسرطانية وقد بوشر باستخدامها طبيا لمعالجة هذه الامراض في الانسان .

في هذه الدراسة تم تقييم حساسية الانترفيرون لمنع نمو الفايروسات في انواع مختلفة من الزرع النسيجي فقد تم اختيار ثلاث انواع من الزرع النسيجي الاول من مصدر خلايا سرطانية (HeLa cells) والثاني من خلايا طبيعية عوملت بمواد مسرطنة (MRC5) والثالث من خلايا طبيعية (HAC) وقد استخدمت ثلاث فايروسات وهي NDV و SFV، polio ينتمي كلا منها الى عائلة مختلفة وهي [illegible] على التوالي . لوحظ أن هذه الفايروسات تنمو بصورة جيدة في هذه الخلايا وعند معاملة الخلايا بالانترفيرون بيوم سابق قبل حقنها بالفايروس وجد ان الخلايا من مصدر سرطاني (HeLa) غير حساسة للانترفيرون لكل الفايروسات المستخدمة بالتجربة أما النوعين الاخرين من الخلايا (MRC5,HAC) وجد انهما [illegible] حساسة للانترفيرون وبتراكيز قليلة (2 الى 4 وحدات)