Studies of human Interferon α, β and γ activities on different cell cultures against rubella virus

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Abstract

Human interferon (IFN) has complex effects but probably the main antiviral action is to reduce the translation of viral mRNA. IFNs induces the antiviral state of the cell when they bind to specific receptor. In our study, showed that at least two functional IFN receptors on human cells. IFN α and β bind to one type of receptor, whereas IFN γ bind to another.

Natural cell culture (HAC) which was used in the present study containing both receptors to IFN α, β and γ while MRC-5 which treated with some chemicals to be diploid cells lost the receptors IFN γ. HeLa cells on the other hand which is malignant cells lost both receptors on the other hand all types of interferon are non toxic at concentration up to 1000 unit/ml to all types of tissue culture involved in this study, while all types of interferon inhibit rubella virus growth at concentration of (2.5 unit/ml) and by use of therapeutic index (TI) which is the ratio of the dose of interferon which is just toxic to the dose which is just effective. If this index is one or les it is not possible to use in man, if this index is larger than the margin of study is great. The (TI) of interferon against rubella virus was more than 500, therefore interferon if used in such concentration (around 5 unit /ml) in human have no side effect.

Introduction

Since the discovery of interferon (IFN) it has been evident that IFN is in theory at least the ideal antiviral agent. It is naturally occurring, relatively non toxic and display a broad spectrum of activity against essentially all viruses. Clinical trials in man were generally disappointing until the purification IFN was developed in late 1970 [1]. After that time investigations about the antiviral effect of IFN have been numerous, but their results are difficult to compare because of lack of standardized dosages and titer. In addition, such research has until recently been hampered by the scarcity and expanse associated with obtaining natural IFN (1).

IFN is subdivided into three classes α, β and γ followed by the techniques of recombinant DNA to manufacture recombinant IFN [2,3,4,5,6,7,8] and various subtypes of IFN (α and β and also γ) are now available. However virtually all the studies that compare recombinant and natural IFN revealed that they are not identical and their effect is different [9,10,11.12,13,14].

IFN is administrated to treat many viral diseases such as viral hepatitis B, C and D [15,16,17], and it is generally assumed that IFN constitutes the first line of defense against viral infection in man and other animals. Although IFNs do not act directly on intact virus, they act on the cells and inducing antiviral state.

The development of antiviral activity requires metabolic activity on the part of the cells [22].Thus, the antiviral state induced by IFN is directed against a wide variety and maybe all viruses while antiviral antibodies which are

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produced by vaccination or infection are specific for one virus. Since that IFN can produce a complex effect in the cells, and it is not surprising that IFN act as anticancer drug, thus are many trials in man using IFN for treatment some types of cancer such as leukemia, lymphoma, multiple myeloma and cancer of the kidney [13,14,15,16]. IFN can be produced in most animal cells however nonucleated animal cells can not produced IFN [17]. IFN α, β and γ each type produce a different protein in the cell which intact with that type of IFN [18,19,20,21]. IFN have complex effect on the cells but main antiviral and anticancer action is to degreed mRNA and rRNA [22]. IFN α and β seem to share a common cell surface receptor which is distinct from that of IFN γ [22,23,24,25,26,27] .

In this study we evaluated the antiviral activity of interferon α, β and γ against rubella virus in tissue culture in addition to study the cell receptors to different types of interferon.

Materials and Methods

Viruses

Rubella virus were grown in chick embryo fibroblast monolayer and maintained in BME (Gibco) supplemented with 2% fetal 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

Cells

1-HeLa cells (epithelial-like cells derived from human epithelioid 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 microtiter 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.

Interferon

1- IFN α partially purified by selective precipitation was supplied by Dr. Cantell of public health laboratory (Finland).

2- IFN β was supplied by Dr. Parker (UK).

3- IFN γ was supplied by Adolf (Austria).

Results

Serial 2-fold dilution of IFN α, β and γ were made, and these were added to the culture the day before adding the virus on the second day. IFN α, β and γ was removed from the monolayer, and the cells were then inoculated with rubella virus at concentrations of 100 TCID50. As shown in the table below that concentrations of 5 unit/ml or more of IFN completely inhibited the production of CPE in MRC-5 and HAC when treated with IFN α or β while if cells treated with IFN γ only HAC induced antiviral activity while the MRC-5 did not. Furthermore there was no anti rubella activity of all types IFN (α, β and γ) against rubella virus in HeLa cells using 1000 unit/ml of IFNs.(α, β and γ)

The toxicity of IFN was tested and it was found that is no toxic effect up to 1000 unit/ml.

Table(1) inhibition of rubella virus by IFN α, β and γ in different cell cultures

IFN unit/m	CPE Produced by rubella virus **								
	IFN α			IFN β			IFN γ		
	HeLa	MRC5	HAC	HeLa	MRC	HAC	HeLa	MRC	HAC
1000	++++	-	-	++++	-	-	++++	++++	-
20	++++	-	-	++++	-	-	++++	++++	-
10	++++	-	-	++++	-	-	++++	++++	-
5	++++	-	-	++++	-	-	++++	-	-
2.5	++++	-	-	++++	-	-	++++	-	+
1.25	++++	++	-	++++	+++	-	++++	++++	++
0.5	++++	++++	++++	++++	++++	++++	++++	++++	++++

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

Discussion

Three different cell cultures were used; 1-human epitheliod cervical carcinoma (HeLa), 2-human diploid lung cells (MRC-5) and 3- primary human amnion cells (HAC) in monolayer at 37° C and treated with various concentration of IFN α, β and γ (0.5-1000 unit/ml). IFNs were incorporated into the tissue culture medium 24 hours before 100 TCID50 of rubella virus inoculation. After virus inoculation the medium was replaced by fresh medium containing the same IFN concentration for another 24 hours. For the next five days the media were changed every day without the addition of IFN, the harvested media were titrated to determine TCID50 in HeLa cells. It was found that concentration of 2.5unit/ml or less had no inhibitory effects on rubella virus replication in HAC and MRC-5 for IFN α and β while consternations of 5 units and more inhibit virus replication. IFN γ showed the same results when it was added to HAC *i.e.* 5 unit/ml or more inhibit rubella virus replication, while there is no effect on MRC-5 when treated with IFN γ up to 1000 unit/ml.

On the other hand all types of IFNs(α, β and γ) had no effect on HeLa cells up to 1000 unit/ml.

IFN vary in their antiviral activity in different cell system making it difficult to define one type of IFN in relation to another [30], since that IFN α and β sharing the same cell receptor therefore it may expected that some cell types are sensitive to IFN α and β but not for IFN γ, furthermore it has been shown that IFN γ induces the synthesis of unique set of cellular mRNA and proteins that are not induced by α or β IFNs [28,29,30,31,32].

On the other hand it was reported that some cells produced certain compounds while other cells are not when treated with the same types of IFN. We find that IFN γ had no inhibitory effect to rubella virus in MRC-5 while IFN α and β induce an inhibitory effect when applied at the same concentration. Our conclusion for these phenomena that MRC-5 lost some its properties during the preparation of such deployed cell including the lost of IFN γ cell receptor. On the other hand HeLa cells which is a malignant cells lost both IFN α β and IFN γ, while HAC cells which is normal cells containing both receptors and it should the same antiviral activity when applied to the same concentrations of IFN α, β and γ *i.e.* 5 unit/ml.

The final conclusion of this study that IFN must be tested in different cell cultures and organ cultures specially that organ or tissue infected with virus before use it in man.

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دراسة فعالية انواع الانترفيرون البشري الفا وبيتا وكاما لمنع نمو فايروس الحصبة الالمانية

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الخلاصة

الانترفيرون البشري كما هو الحال في جميع انواع الانترفيرون له تأثيرات مُعقده ولكنها جميعاً تشترك بتأثيرها على منع تكاثر الفايروسات وذلك بمنع او اختزال تحويل mRNA الى tRNA.

الانترفيرون البشري يُحدث حاله في الخليه تمنع نمو الفايروس عندما يتصل الانترفيرون بالمستقبل الخاص له عند جدار الخليه، في هذه الدراسه وجدنا ان المُستقبل للانترفيرون على نوعين نوع خاص باستقبال انترفيرون الفا وبيتا والنوع الثاني باستقبال انترفيرون كاما.

ولقد أثبتت هذه الدراسه على ان الخلايا الطبيعيه تفقد بعض خصائصه عند تعرضها لمواد كيماويه كما هو الحال في خلايا MRC-5 (خلايا رئويه سليمه) فقد فقدتت هذه الخلايا المُستقبل الخاص بأنترفيرون كاما. وكذلك فأن الخلايا تفقد من صفاتها عندما تتحول الى خلايا سرطانيه كما حدث لخلايا HeLa (خلايا بشريه من سرطان الرحم) حيث فقدت كلا المستقبلين لانترفيرون الفا وبيتا وكذلك لانترفيرون كاما في الجانب الاخر من هذه الدراسه حول فعاليه انواع الانترفيرون ضد فايروس الحصبه الالمانيه في انواع الزرع النسيجي وجد ان جميع انواع النترفيرون غير سامه للخلايا المعامله لاكثر من (١٠٠٠ وحده/ مل) وان جميع انواع الانترفيرون له تاثير واضح جدا على منع نمو فايروس الحصبه الالمانيه بتركيز (٢,٥ وحده/مل) فأكثر في الخلايا الحساسه للانترفيرون المذكوره اعلاه وبأستخدام الميزان العلاجي (TI) والذي هو نسبه اقل تركيز للانترفيرون مسببا تشحم الخلايا الى اقل تركيز للانترفيرون مسببا منع نمو الفايروس فاذا كان الميزان اقل من (١) فلا يسمح بأستخدامه وفي هذه العلاقة وجد ان الميزان العلاجي هو أكثر من ٥٠٠ لهذا فان هذه الماده في حاله استخدامها للعلاج البشري ليس لها اي تاثيرات سمية.