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Influence of Synthetic Oestrogens on the Synthesis of Interferon-γ in Human Leukocytes

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Abstract

Immune interferon (interferon-γ) is induced in leukocytes as a result of the influence of specific bacterial or viral antigens. The addition of oestrogen preparations to interferon biosynthesis growth media is considered to be a very important factor for the intensification of interferon synthesis. In order to study the influence of synthetic oestrogens on the synthesis of interferon-γ (IFN-γ) in human blood leukocytes, diethylstilbestrol (in the form of the soluble sodium salt of a diphosphoric acid ether, cf. Honvan) was used. There was a twofold increase of interferon titre in comparison with control tests.

Keywords: immune interferon; leukocytes; synthetic oestrogens; T-lymphocytes; interferon biosynthesis.

Introduction

The main characteristic of interferon (IFN) is its ability to suppress the reproduction of viruses and thereby hinder the process of viral infection. Interferon also acts on other intercellular parasites (Chlamydiales and the protozoöns) and on the products of bacteria (toxins).

As opposed to cultured cells that grow up outside an organism (e.g., laboratory-reared cells), leukocytes appear to have the ability to produce interferon with non-viral inducing agents such as phytohaemagglutinin (PHA) and other mitogens, bacterial and viral antigens, endotoxins and other substances of microbial origin [1, 2].

Because the interferon induced in mitogen-stimulated leukocyte cultures differs with respect to some properties from classical interferon (type I), and hence is called type II interferon [3]. Interferon of type II is induced only with immune-competent cells; the name immune interferon (interferon-γ) was introduced by Falcoff [4]. The properties of virus-induced leukocyte interferon as distinct from those of immune interferon are: instability in strong acids (pH ≤ 2.0) or alkalis (pH ≥ 10.0); and thermolability (complete inactivation by keeping for one hour at 56 °C) [5].

Immune interferon is distinguished by its special activity as one of the mediators of cell immunity. Immune interferon possesses much more anticellular and immune modulation activity than interferons α and β (Type I), and may be much more effective as an anticancer treatment than

other interferons [6, 7]. Interferon-γ has a direct cytological action on some tumour cells, and intensifies the actions of interferons α and β when used jointly [8, 9].

Usually immune interferon production is induced in proliferating lymphocytes in leukocyte cultures that are activated by mitogens of organisms possessing an immune system responding to particular viral or bacterial antigens [10]. Which type of interferon (I or II) will be produced by lymphocytes in response to a given inducer depends on the method of preparing the cells for cultivation [11], the immune status of the blood donors [12] and on the nature of those cells that help lymphocytes. Although interferon is synthesized by lymphocytes and not macrophages, macrophages are necessary for the stimulation of interferon production, the amount of type II interferon (interferon-γ) produced in the system of lymphocytes plus macrophages is three to five times more than its production by lymphocytes alone [13, 14].

It was asserted by Epstein that T-lymphocytes are the main producer of interferon, mainly because of the observation that anti-T-cellular serum containing complement helping to exclude T-lymphocytes restrained the synthesis of type II interferon [15]. However, Stewart (1979) considers that B-lymphocytes produce immune interferon as well as T-lymphocytes and the production efficiency of both depends on the existence of macrophages. The interferon produced in a mouse by T- and B-cells is only differentiated by the amount [16].

The addition of oestrogen preparations to interferon biosynthesis growth media is considered to be a very important factor for the intensification of interferon synthesis [17]. The purpose of this work was to further investigate this.

Material and methods

Immune interferon was prepared from human blood lymphocytes, leukocytes and leucomass. The material was received from a haemotransfusion station.

The Newcastle disease virus was used as interferon inducer (interferonogen). The titre infected by this virus equalled 10^{10}–10^{11} CPA_{50} [The cytopathogenic action (CPA_{50}) is the virus dose that engenders cytopathogenic changes in 50% of the tissue culture tubes] on hen embryos. The indicator virus was vesicular stomatitis with the 10^5 CPA_{50} titre on the cell culture [18, 19].

Culture growth media in the experiments were: medium 199, Eagle's medium, lactalbumin hydrolysate added to Hanks solution, 0.25 % trypsin solution, sugar broth, 1% agar, thioglycol medium, pH buffer and stabilizing solutions.

Blood lymphocytes were isolated from blood newly taken from the donor with a ficoll-verografin density gradient. After washing out the ficoll, cells were diluted to 2×10^6 or 4×10^6 cells/mL. Interferon-γ was induced by adding clean phytohaemoglutinin (PHA, Difco) at 10–100 g/mL. During induction, cells were placed in a thermostat at 37°C for 72–96 h. The collected liquid containing interferon was obtained after centrifuging for 5–10 min at 1500–2000 rpm. Our test criterion for interferon-γ is its stability in acid solution (pH 2.0 for 24 h) and while warming to 56 °C (for 30 min) [20].

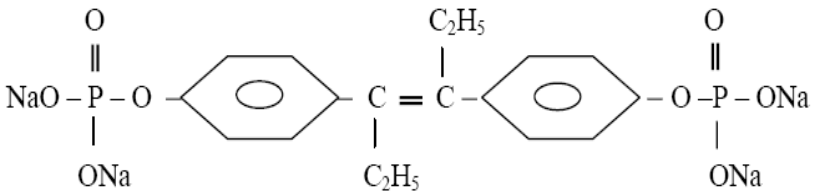
Induction of immune interferon took place in the total fraction of leucocytes that was isolated with a 0.83 % aqueous solution of ammonium chloride and then further fractionated with haemolysis.

To define the interferon titre 1 mL of the preparation was added to a series of tubes containing cell culture medium. At the same time we made the titration of vesicular stomatitis virus (the indicator). The next day 100 CPA_{50} vesicular stomatitis virus (0.1 mL) was placed in each tube. Simultaneously, a dose titration of the indicator virus was made. After cultivation in a thermostat for 24–48 h the tubes were examined under the microscope. For the interferon titre, the final dilution ensured 50 % cells cultural protection from the stomatitis 100 CPA_{50} virus [20].

Results and conclusion

It is known that female sex hormones (notably the oestrogens: oestrodial, oestrone and oestriol) effect changes of carbohydrate, protein and purine substances. They act as activators to enzyme deoxyribonuclease through the Krebs cycle; i.e., they promote oxidation of organic substances to the final product (carbon dioxide and water). This is especially important, because anaerobic glycolysis appears to be the basic energy resource for leucocytes. Because of inefficiencies of the early stages of the fermenting system associated with the Krebs cycle, the

oxidation of glucose in the cells ends in the lactate stage and only a small amount (less than 5 %) is subjected to further fission [21, 22]. That is why the addition of oestrogen preparations to interferon biosynthesis growth media is considered to be a very important factor for the intensification of interferon synthesis. However, the extremely low aqueous solubilities of female sex hormones necessitates that production preparations use oil solutions, which complicates use of the preparations. We chose a synthetic preparation of diethylstilbestrol soluble in water as the diphosphoric ether, tetrasodium salt (Honvan) [23] (Scheme).



Scheme

We carried out experiments adding 1 mg Honvan to 10^7 and 2×10^7 leucocytes in 10 mL. The concentration of the preparation in the culture medium was 0.1 mg/mL. We also carried out experiments to receive human immune interferon, also by adding the glucose and Honvan to the culture medium. The concentration of glucose (the purpose of which was to provide a carbon source for sell growth) in the culture medium was 1%. The results of titration of interferon-γ tests are shown in Table.

Table. The influence of diethylstilbestrol diphosphoric ether tetrasodium salt (Honvan) on interferon-γ production by blood leucocytes

Stimulator	Concentration of stimulator in preparation /10 mg/mL	Interferon-γ titre in 1 mL	
		Leucocyte concentration = 10^7 cells/mL	Leucocyte concentration = 2×10^7 cells/mL
Honvan	0.1	80	100
Honvan + 1% glucose	0.1	40–80	160
Control	0.0	40	40–80

In all variants of the experiment compared with the control test, double growth of the interferon titre is observed.

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Влияние синтетических эстрогенов на продукцию γ-интерферона человеческими лейкоцитами

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Аннотация. Иммунный интерферон (γ-интерферон) индуцируется в лейкоцитах иммунного организма при воздействии специфических бактериальных или вирусных антигенов. Для изучения влияния синтетических эстрогенов на продукцию γ-интерферона человеческими лейкоцитами крови мы использовали тетранатриевую соль дифосфорного эфира – диэтилстильбэстроль (Hov). Добавка эстрогенных препаратов в питательную среду для биосинтеза интерферона расматривается нами как весьма важный фактор интенсификации интерфероногенеза. Во всех вариантах опытов в сравнении с контрольными пробами наблюдалось двухкратное увеличение титров интерферона.

Ключевые слова: Иммунный интерферон; лейкоциты; синтетические эстрогены; Т-лимфоциты; биосинтез интерферона.