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Immunotropic, Embryotoxic and Mutagenic Effects of Influenza Virus in Guinea-Pigs and Protective Role of Plaferon

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Abstract

Acute influenza infection in guinea-pigs was followed by a gradual decrease in the immunological parameters. Plaferon was highly successful in reversing immunosuppression (IFNa,g and the phagocytosis index were notably stimulated). Influenza virus exerted a marked embryotoxic effect in pregnant animals, causing death of the embryo or fetus at various times during pregnancy. An antiembryotoxic effect of plaferon with improvement in indices of preimplantation mortality of fetus was showed (from 13,6% to 8,8%). Cytogenetic analysis of marrow cells revealed marked damage to chromosomes, resulting from influenza (19,9% aberrant metaphases vs. 2.1% in the control). Results of plaferontherapy was manifested as a decrease in the number of aberrations (8,5%). These characteristics of plaferon serve to justify the use of plaferon in prophylaxis and treatment of influenza and his complications.

Keywords: *influenza virus, plaferon, immunocorrection, embriotoxic effect, mutagenic effect*

Protecting humans from the influence of external biological and chemical agents is a very important medical objective. There is sound experimental and clinical evidence, indicating an important role of viruses in human pathology. In addition to toxicity, viruses possess mutagenic, teratogenic, and carcinogenic properties. A high degree of immunosuppression by viruses, even in minimal doses, has been demonstrated [2]. In spite of this, many aspects of immunocorrection of influenza infection are largely unexplored.

Therefore, the main purpose of our investigation was to study of influenza virus influence on several immune indices in guinea-pigs and evaluate a potential protective effect of plaferon. In Georgia and other countries, plaferon has been used with great success for treatment of different diseases. Plaferon is extracted from amniotic membrane of the human placenta. The preparation contains several physiologically active substances (e.g., interferon, endorphins, enkephalins, cytokines), causing different pharmacological effects: antiviral, immunomodulating, antihypoxic, detoxifying [1]. Plaferon can be administered as an injection, an ointment, and as lingual drops.

A model study of acute influenza infection was performed by intranasal administration of influenza A1 virus (105 EID). At different times, we examined antibody formation in the spleen [10], as well as phagocytosis [4] and interferon [9] in blood. For immunocorrection, we administered plaferon-LB (1,0mg protein per kg, i.m., daily, starting at the same time with virus, during 10-th days).

Immunotropic Effect

Analysis of the data showed *(Fig.1)* a gradual decrease in the measured parameters. Particularly sensitive were the interferon system (especially IFNγ) and the

phagocytosis index (PhI). Moderate decreases of antibody formation (APC) and complete phagocytosis (CPh) were observed, along with an almost unchanged total percent of phagocytic neutrophils (PhN).

Plaferon was highly successful in reversing immunosuppression. First, lethality of animals was decreased from 22% in the control group (without plaferon) to 8% in the plaferon-treated group ($p<0.01$). This effect of plaferon was accompanied by marked activation of immune homeostasis in the guinea-pigs *(Fig.2)*. In particular, both types of interferon (IFNα,γ) and the phagocytosis index were notably stimulated.

This study demonstrates a protective action of plaferon during influenza infection in guinea-pigs. We suggest that the action of plaferon is due to its main properties - immunomodulating, antihypoxic, and antimutagenic effects [1], which offset pathological factors present in the organism during influenza.

Considering the cytogenetic influence of viruses [2], along with the genetic component of immunocompetence, it is instructive to study protective mechanisms of various interferon preparations relating to influenza infection in guinea-pigs. It is also well known that embryonic cells are generally sensitive to toxicant exposures. In this regard, embryotoxic effects of influenza virus in pregnant guinea-pigs were studied. Results of this research provided insight into mechanisms of influenza in relation to the immune system.

Embryotoxic Effect

Embryotoxic effects of influenza virus were evaluated on the 15-20th days of pregnancy. Under a stereomicroscope, the number of yellow bodies *(Corpus luteum)* in both ovaries (number of conceptions) were counted. We then considered number of implantations in the uterus (all viable, non-viable or immature fetuses, or only separate placentas, without "own" fetus). The difference between the number of yellow bodies and the number of implantations comprises the preimplantation mortality. The number of dead fetuses and empty placentas comprise the post-implantation mortality. Both pre and postimplantation mortalities comprise the total intrauterine (prenatal) mortality of fetuses. As shown in *Tab.1*, acute exposure to virus depressed normal embryogenesis. Influenza virus exerted a marked embryotoxic effect in pregnant animals, causing death of the embryo or fetus at various times during pregnancy. The maximum embryolethal effect of virus occurred before implantation into the uterus at a rate of 13,6%. Data in Table 1 show an antiembryotoxic effect of plaferon with improvement in indices of preimplantation mortality of fetus (from 13,6% to 8,8%).

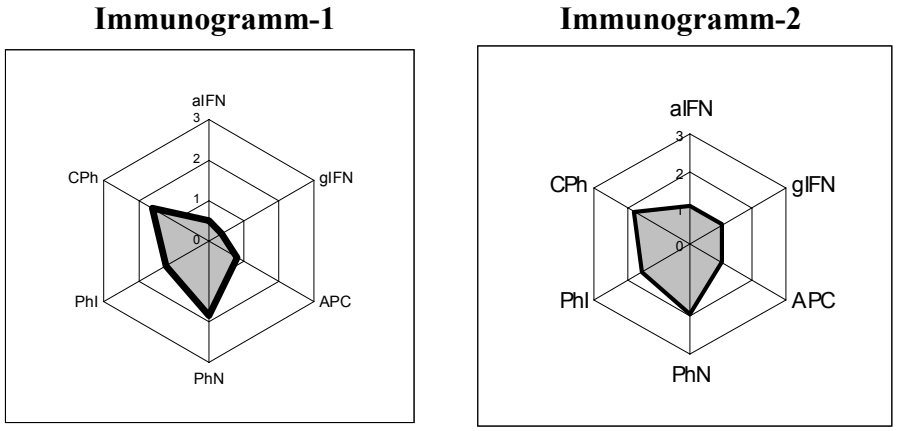

Fig.1 *Comparison with healthy control (Line-2).*

Group of pregnants	No. of yellow bodies	Preimplantation mortality	Postimplantation mortality	Total prenatal mortality
Ctrl	42	**2** (4.7%)	**2** (4.7%)	**4** (9.4%)
Virus	44	**6** (13.6%, $p<0.01$)	**3** (6.8%)	**9** (20.4%, $p<0.001$)
Virus+Plaferon	45	**4** (8.6%, $p<0.05$)	**2** (4.4%)	**6** (13.1%, $p<0.05$)

Tab.1 *Antiembryotoxic effect of plaferon with improvement in indices of preimplantation mortality of fetus.*

Group of animals	Isolated aberrations (%)		Diffusive aberrations (%)			Total aberrant metaphases (%)
	Chromoso-mal	Chromatid	Despiralization	Fragmentation	Lysis	
Ctrl	**0,2**	**0,9**	**0,8**	**0,1**	**0**	**2,1**
Virus	**1,8**(p<0.001)	**5,5**(p<0.001)	**2,3**(p<0.001)	**1,2**(p<0.001)	**9,1**(p<0.001)	**19,9**(p<0.001)
V+Plaf	**0,6**(p<0.01)	**1,3**(p<0.2)	**1,7**(p<0.01)	**0,6**(p<0.001)	**4,3**(p<0.001)	**8,5**(p<0.01)

Tab.2 *Antiembryotoxic effect of plaferon with improvement in indices of preimplantation mortality of fetus*.

Mutagenic Effect

On the 5th day of the influenza study in guinea pigs, several parameters of chromosomal damage were examined in bone marrow cells [3], including isolated (chromosomal and chromatid) and diffusive (despiralization, fragmentation, and lysis) aberrations *(Tab.2)*. Cyto- genetic analysis of marrow cells (in each group, the number of metaphase analyses was 300), revealed marked damage to chromosomes, resulting from influenza (19,9% aberrant metaphases vs. 2.1% in the control). Diffusive damage was detected more frequently (12,6%) than isolated damage (7,3%). Isolated aberrations consisted mainly of paired acentric fragments, ruptures, and gaps.

Results

Results of therapy proved somewhat unclear in that heterologous plaferon demonstrated protective action, which was manifested as a decrease in the number of isolated aberrations (1,9%), as well as a reduction in lysis of chromosomes (4,3%) and in total number of aberrant metaphases - 8,5%.

Conclusion

Our investigation demonstrated a pronounced protective effect of interferon preparations when simultaneously administered with influenza virus. Both our data, as well as data in the literature, suggest that the protective action of plaferon (interferon) is achieved by mechanisms related to DNA surveillance and repair systems.

According to some authors [6,7], impaired DNA replication (induced by viruses) is reversed during immunomodulating therapy, ensuring viability of cells. At the same time, aberrant T-lymphocytes were decreased and immunocompetent cells were restored. With respect to interferon therapy, genes are activated, repair enzymes are synthesized, and damaged DNA is repaired [5,6]. In contrast to well-known chemical antimutagens, interferons (plaferon) are extremely effective, because many chemical antimutagens which stimulate repair systems are often harmful to the organism (side effect). Additional investigations have reported that interferon elicits an antimuta-genic effect but does not eliminate damaged cells [7,8]. Both interferon and viruses interact with sites on DNA (e.g., purines).

We are uncertain, regarding the mechanism of the antiembryotoxic action of plaferon. The mechanism may involve membrane-stabilizing and hormone-like effects of plaferon [1], in addition to its antihypoxic and antimutagenic properties. These characteristics of plaferon, observed in our study on model influenza infection in guinea-pigs, serve to justify the use of plaferon in prophylaxis and treatment of influenza and his complications.

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Иммунотропные, эмбриотоксические и мутагенные эффекты вируса гриппа у морских свинок и протекторная роль плаферона

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Р Е З Ю М Е

При модельной гриппозной инфекции у морских свинок выявлено выраженное угнетение иммунокомпетентности организма животных, а также сильное эмбриотоксическое и мутагенное действие вируса гриппа. Плаферон проявил выраженное протекторное дейс-твие, способствуя нормализации иммунологических показателей (особенно интерферона и фагоцитарного индекса), снижению предимплантационной гибели плодов с 13,6% до 8,8%, ($p<0.05$). Цитогенетический анализ выявил достоверный антимутагенный эффект плаферона: снижение общего количества аберрантных метафаз с 19,9% (контроль вируса) до 8,5% (при 2,1% у интактных животных). Признается целесообразным профилактическое и лечебное применение плаферона при гриппозной инфекции, осложненной обструктивным бронхитом.

Ключевые слова: *плаферон, иммунокоррекция, ембриотоксический эффект, мутагенный эффект*