

Study of Protective Action of Plaferon and Phenowine on Some Indices of Experimental Influenza in White Mice

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ABSTRACT

The main purpose of our investigation was to study action of influenza virus on several immune indices, its mutagenic and embriotoxic effects in white mice, and evaluate a potential protective effect of plaferon-LB and phenowine. These preparations were highly successful in reversing immunosuppression, caused by influenza virus. Results of immunocorrective therapy proved, that heterologous plaferon demonstrated strong protective action in white mice. Plaferon/phenowin-therapy resulted in fewer aberrant metaphases of chromosomes (12,8% vs. 36,3% in the control of virus). In general, the protective effect of preparations was manifested as a decrease in the number of isolated aberrations, as well as a reduction in lysis of chromosomes. An antiembryotoxic effect of plaferon with improvement in indices of preimplantation mortality of fetus (from 13,1% to 8,8%) and total prenatal mortality of fetus (from 19,6% to 15,1%) were showed. These characteristics of plaferon and phenowine, observed in our clinical investigations and experimental study in white mice, serve to justify the use of these preparations in prophylaxis and treatment of influenza.

KEYWORDS: *influenza virus, plaferon, phenowine, immunocorrection, mutagenic/embriotoxic effects*

In our previous investigations moderate immunotrope action of phenowine in healthy volunteers was showed. Plaferon and phenowine possessed good medical effect in children during influenza infection - the period of treatment shortened, there were no complications [6]. Protecting humans from the influence of external biological agents, such as viruses, is a very important medical objective. There is sound experimental and clinical evidence, indicating an important role of influenza viruses in human pathology. Beside active reproduction of virus in upper respiratory tract and lungs, in addition to direct toxicity, influenza virus possesses mutagenic properties. A high degree of immunosuppression by viruses, even in minimal (non-toxic) doses, has been demonstrated [3]. In spite of this, many aspects of immunocorrection of influenza are largely unexplored.

Therefore, the main purpose of our investigation was to study action of influenza virus on several immune indices, its mutagenic and embriotoxic effects in white mice, and evaluate a potential protective effect of plaferon-LB and phenowine. In Georgia and other countries, plaferon has been used with a great success for treatment of different diseases. Plaferon is extracted from the 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 [2,4]. Phenowine is a phenol extract of red grapes peel and possess strong antioxidant action [1,5].

A model study of influenza infection in white mice was performed by nasal administration of influenza A0/PR8 virus. At different times, we examined antibody formation in the spleen, as well as phagocytosis and interferon in blood [8,9,15]. For immunocorrection we administered plaferon (1.0 mg protein per kg, i.m.) and phenowine (100mg per kg, orally), daily, over a 7day period of experiment, starting at the same day with virus.

Analysis of the data showed a gradual decrease in the measured parameters by the third day of experiment (Immunogramm-1, was compared with line 2 - control). Particularly sensitive were the interferon system (especially $IFN\gamma$) and the phagocytosis index (PhI). Moderate decrease of antibody formation (AbPC) and complete phagocytosis (CPh) were observed, along with an almost unchanged total percent of phagocytic neutrophils (PhN).

Plaferon and phenowine were highly successful in reversing immunosuppression. First, lethality of animals was decreased from 82,5% in the control group (without plaferon) to 17,5% in the plaferon/phenowine-treated group ($p < 0.001$). This effect of preparations was accompanied by a marked activation of immune homeostasis in the mice (Immunogramm-2). In particular, both types of interferon ($IFN\alpha, \gamma$) and the phagocytosis index (PhI) were notably stimulated.

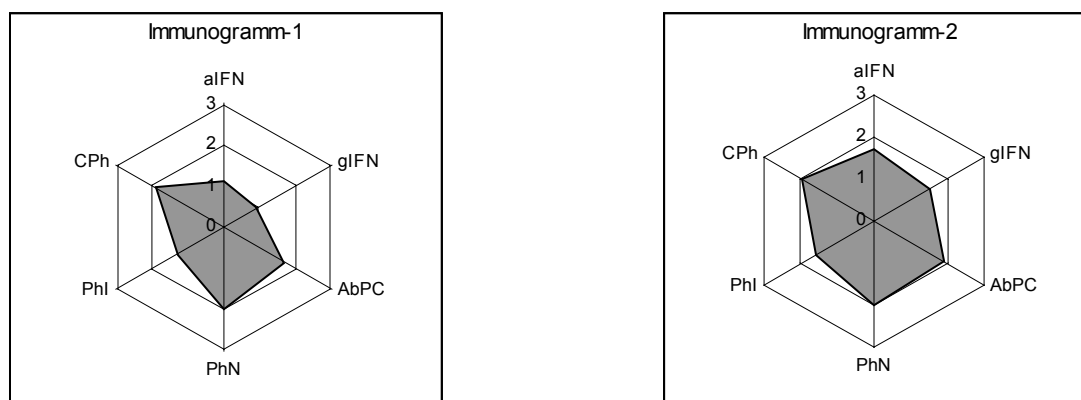


Fig.1 Action of plaferon and phenowine on immune status of white mice during influenz.

Group of mice	Isolated aberrations		Diffusive aberrations			Total aberrant metaphases
	Chromosomal	Chromatid	Despiralization	Fragmentation	Lysis	
Ctrl	0,3	1,1	0,7	0,1	0	2,2
1	3,2	8,4	5,3	2,3	17,1	36,3
2	0,6	2,3	2,7	0,8	6,4	12,8

1 – Influenza virus. 2 – Virus+plaferon/phenowine.

A – 1/Ctrl: all parameters $p < 0.001$. *B* – 2/Ctrl: all parameters are trustworthy. *C* – 2/1: all parameters are trustworthy. In each group, the number of metaphase analyses was 300.

Tab.1 Cytogenetic indices of influenza and plaferon/phenowine-therapy (expressed as percentage of aberrant chromosomes - 5th day of infection).

Group of mice	No of yellow bodies	Preimplantation mortality (I)	Postimplantation mortality (II)	Total prenatal mortality (III)
Ctrl	77	5 (6,5%)	4 (5,2%)	9 (11,7%)
1	76	10 (13,1%)	5 (6,5%)	15 (19,6%)
2	79	7 (8,8%)	5 (6,3%)	14 (15,1%)

1 – Influenza virus. 2 – Virus+plaferon/phenowine.

A – First group compared to Ctrl (1/Ctrl): **I, III** – $p < 0.001$; **II** – $p < 0.1$. *B* – 2/Ctrl: **I, II** – $p < 0.5$; **III** – $p < 0.05$. *C* – 2/1: **I** – $p < 0.02$; **II** – $p > 0.5$; **III** – $p < 0.05$.

In each group, the number of pregnant mice was 10.

Tab.2 Action of influenza virus and plaferon/phenowine-therapy on embryotoxicity in pregnant white mice (5th day of infection)

On the 5th day of the infection study in white mice, several parameters of chromosomal damage were examined in bone marrow cells, including isolated (chromosomal and chromatid) and diffusive (despiralization, fragmentation, and lysis) aberrations (Tab.1).

Cytogenetic analysis of marrow cells revealed marked damage to chromosomes, resulting from viral infections (36,3% aberrant metaphases vs. 2,2% in the control). Diffusive damage was detected more frequently (24,7%), than isolated damage (11,5%).

Isolated aberrations consisted mainly of paired acentric fragments, ruptures, and gaps.

Results of immunocorrective therapy proved, that heterologous plaferon demonstrated strong protective action in white mice. Plaferon/phenowine-therapy resulted in fewer aberrant metaphases (12,8%). In general, the protective effect of preparations was manifested as a decrease in the number of isolated aberrations, as well as a reduction in lysis of chromosomes.

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

Influenza A0 virus was administered nasally once, beginning on the first day of pregnancy (identified by discovery of a vaginal plug). Embryotoxic effects of virus were evaluated on the 19-20th days of pregnancy. Under a stereomicroscope, the number of yellow bodies (Corpus luteum) in both ovaries were counted (number of conceptions). 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.2*, influenza infection depressed normal embryo-genesis. Virus exerted a marked embryotoxic effect in pregnant mice, causing death of the embryo or fetus at various times during pregnancy, as well as postnatally. The maximum embryo-lethal effect of virus occurred before implantation into the uterus at a rate of 13,1%.

Because an immunocorrective effect of plaferon during infection in white mice was observed in our previous investigation, we examined the potential protective effect of plaferon in embryos. Plaferon and phenowine were

administered to pregnant mice, starting on the same day as virus administration). Data in *Tab.1* show an antiembryotoxic effect of plaferon with improvement in indices of preimplantation mortality of fetus (from 13,1% to 8,8%) and postnatal mortality of sucklings.

Our investigation demonstrated a pronounced protective effect of plaferon and phenowine during influenza infection in white mice. We suggest that the action of plaferon is due to its main properties - immunomodulating, and antimutagenic effects [2, 4,11] - which offset pathological factors present in the organism during infection.

Both our data, as well as data in the literature, also suggest that the protective action of plaferon is achieved by mechanisms related to DNA surveillance and repair systems. According to some authors [12,13], impaired DNA replication (induced by virus) is reversed during immuno-modulating therapy, ensuring viability of cells. At the same time, aberrant T-lymphocytes were decreased and immuno-competent cells were restored. With respect to interferon therapy, genes are activated, repair enzymes are synthesized, and damaged DNA is repaired. [12,10] 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 antimutagenic effect but does not eliminate damaged cells. [13,14] Both interferon and virus 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 [4], in addition to its antihypoxic and antimutagenic properties, and antioxidant action of phenowine, also. [7] These characteristics of plaferon and phenowine, observed in our clinical investigations [6] and experimental study in white mice, serve to justify the use of these preparations in prophylaxis and treatment of influenza. We can also suppose, that further investigations will allow us to reveal some other mechanisms of phenowine's action and to extend sphere of its application in clinics.

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Изучение протекторного действия плаферона и феновина на некоторые показатели гриппозной инфекции у белых мышей

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Р Е З Ю М Е

Цель исследования - изучение действия вируса гриппа на иммунологические показатели, его мутагенность и эмбриотоксичность у белых мышей, а также оценка потенциального протекторного эффекта плаферона и феновина. Эти препараты проявили выраженную нейтрализацию иммунодепрессии, вызванную гриппозной инфекцией. Результаты иммунокоррекции показали, что гетерологичный для белых мышей плаферон обладал защитным действием. Применение плаферона и феновина сопровождалось снижением количества aberrантных метафаз в хромосомах (12,8% против 36,3% в контроле). Протекторный эффект препаратов проявился в снижении числа изолированных aberrаций, а также лизиса хромосом. Установлен антиэмбриотоксический эффект плаферона: улучшение преимплантационной (с 13,1% до 8,8%), а также общей пренатальной гибели плодов (с 19,6% до 15,1%). Выявленные в наших клинических и экспериментальных исследованиях особенности действия плаферона и феновина обосновывают необходимость их применения для профилактики и лечения гриппозных инфекций.

КЛЮЧЕВЫЕ СЛОВА: вирус гриппа, плаферон, феновин, иммунокоррекция, мутагенный/эмбриотоксический эффекты