Report on the study of interferonogenic activity of drug Flaraxin.

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Goal of experimental study

With aim to study the interferon inducing activity of Flaraxin in *in vitro* experiments on white outbred mice / males, body weight 18-20 g / were used.

Materials and methods

The drug was diluted with 5% glucose solution. Introduced in 0.2 ml intraperitoneally, at a concentration of 5.50g and 250g per mouse. As a control, the known interferon inducer Poly I:C /"Sigma"/, as well as 5% glucose solution or saline (placebo) were used.

After 6, 24, 48 and 72 hours, mise were decapitated, and plasma was obtained, in which the level of interferon was tested. In addition, tumour necrosis factor was determined.

The presence of interferon was determined by suppressing the cytopathogenic action of the test virus / vesicular stomatitis virus pcs. Indiana in dose of 100 Cytopathic Action of Viruses ($CPAV_{50}$)/ in the culture of transplantable cells L-929, or PTP grown in 96-well cultural panels /Linbro/. As an Interferon titter were taken the value reciprocal to the probe dilution, at which 50% of cells protection against cytopathogenic action of the test virus were observed.

Activity of plasma necrosis factor was determined by the cytolytic effect on L-929 target cells that were treated with actinomycin-D /Serva/ using a crystal violet spectrophotometric test. /Methodical recommendations, Kiev, 1994/.

Results of experimental study

The results of determining the levels of interferon are presented in table 1.

As follows from table 1, Flaraxin has interferogenic activity. So a dose of 5 g/mouse already possessed this ability. However, the most optimal dose is 50 g/mouse. When using this dose, the level of interferon in animals is highest and persists over a long period of time /for 72 hours/. At the same time, all known interferon inductors, including Poly I:C, have a short period of action when interferon is induced.

Table 1. The presence of interferon-similar activity in the plasma of mice after injection of the drug Flaraxin.

The titter of interferon in arbitrary units [units/ml]			
Time after drug administration			
6 hours	24 hours	48 hours	72 hours
Flaraxin in dose /5g/	40	40	40
Flaraxin in dose /50g/	320	320	160
Flaraxin in dose /250g/	80	80	40
Poly I:C in dose /50g/	320	40	<40
5% glucose solution	<4	<4	<4
Physiological solution	<4	<4	<4

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Table 2. The production of interferon by the splenocytes of mice that had previously been administered Flaraxin /*in vitro* experiments/.

The titter of interferon in arbitrary units [units/ml]				
Interferon inductor	Splenocytes of intact animals / 24h /	Splenocytes of mice after administration of Flaraxin / 24h /	Splenocytes of intact animals / 72h /	Splenocytes of mice after administration of Flaraxin / 72h /
NDV experiment 1	640	640	320	640
NDV experiment 2	320	640	320	640
PHG experiment 1	80	160	40	160
PHG experiment 2	80	160	80	320

Note: The following conventions are adopted here and in the following tables: NDV - Newcastle Disease Virus; PHG - phytohemagglutinin. Intact animals - are animals of the control group, which are observed in parallel with experimental animals.

Therefore, the property of Flaraxin for a long time to support the production of interferon makes it extremely interesting and promising for use in the treatment and prevention of pretumor, tumour processes, as well as viral infections of the central nervous system and the so-called slow viral infections which include AIDS, multiple sclerosis, etc.

In another series of experiments, 24 and 72 hours after administration of the optimal dose of the drug Flaraxin were (50g/mouse) to animals, the spleen were taken from the animals, a suspension of splenocytes were prepared and the functional capacity of immune cells for the production of interferon *in vitro* was studied, and the type of interferon induced was determined / IgA or IgG /.

For this purpose to the dredge of splenocytes were added inductors of interferon - Newcastle Disease Virus (NDV) and Phytohemagglutinin (PHG). Splenocytes of intact animals served as controls.

The results of these studies are presented in Table 2. The data obtained indicate a slight increase in the ability of the splenocytes of animals that were previously injected with the Flaraxin drug, to synthesize interferon *in vitro* under the influence of an interferonogen. As follows from Table 2, shows using the Newcastle Disease Virus (NDV) as an interferon inducer, i.e. during the induction of alpha-interferon, the synthesis of alpha-interferon increases by 2 times (if Flaraxin was administered 72 hours before taking the spleen from mice).

When Phytohemaglutinin PHG (alpha-interferon inducer) is used as the interferon inducer, the production of interferon is significantly increased - 4 times (if Flaraxin was administered 72 hours before taking the spleen from mice).

These data can be used to work out the optimal regimes of Flaraxin drug administration for treatment.

In addition, we studied the presence of Tumour Necrosis Factor (TNF) in the plasma of animals after administration of Flaraxin drug to mice. It was found that only the use of high doses of the drug (250 g/mouse) led to a significant increase in the level of Tumour Necrosis Factor (TNF) compared with the control.

As for the type of induced interferon, it was find out to be thermolabile (unstable to temperature changes), which is typical of Gamma-interferon, but for final approval it is necessary to conduct additional studies on acid-lability (instability to oxidative processes) and antigenic specificity (ability to cause antibody synthesis).

Conclusions

Thus, on the basis of the conducted studies, it was find out that the drug Flaraxin has interferonogenic activity and also induces the formation of tumour necrosis factor (TNF) in the body.

It is necessary to conduct additional studies to determine the optimal regimes of drug administration, taking into account the effects of hyporeactivity (weak resistance of the organism to diseases) on frequent administrations of the interferon inducer.

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http://www.logos.biz.ua/proj/vynahid/online/125.htm

https://www.ncbi.nlm.nih.gov/pubmed/?term=Spivak+M+Y

Notes

Cytopathic Action of Viruses (CPAV) is a destructive changes of individual cells and cell monolayer, resulting from productive viral infection of cells (see Viral infections) and cytotoxic action of virions. In the cellular monolayer, the CPAV manifests itself in the form of a solid or focal round or polymorphoncellular degeneration, the formation of multinucleated cells or cell symplasts, as well as in proliferative proliferation of cells. In virus-infected cells, CPD is manifested in pycnosis of the nucleus, margination and granularity of chromatin, the appearance of inclusions, bodies, crystals; vacuoles appear in the cytoplasm, it shrinks and degenerates. CPD is used to indicate the identification of viruses.

An important feature of Tumor Necrosis Factor (TNF) alpha is the influence on tumour cells due to apoptosis and on the generation of reactive oxygen and nitric oxide. TNF-alpha can eliminate not only the cells of the tumour, but also the cells affected by the virus. TNF-alpha participates in the development of the immune response, causing the proliferation of B- and T-lymphocytes and prevents the emergence of immunological tolerance. TNF-alpha also inhibits erythro-, myelo- and lymphopoiesis, but has a radioprotective effect.