Activity of izatizon concerning adenoviral infection in vitro.

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Methyl-isatihn β-tiosemicarbazon or metisazon is a compound that has a wide antiviral spectrum of action and is approved as a preparation for smallpox prevention. But it has a high toxicity. 2% solution of 1-metilizatin-β-tiosemicarbazon in universal solvent was named as izatizon. This preparation is nontoxic, and has a wide antiviral spectrum and is used with success in veterinary for curing and prevention of respiratory viral infections and Marek's disease of birds.

The goal of the research is to study molecular-biological mechanisms of izatizon antiviral action.

Materials and methods of the research:

During the work human adenoviruses of 1 and 2 serotypes were used. Cytotoxicity of the preparations was determined on adenovirus infected cells with the help of the method of fluorescence microscopy including fluorochromic evaluation of acridine orange fixed cells. From this we can predetermine about the morphology of cell and simultaneously reveal in it DNA and RNA.

For testing of viral hexone synthesis (main albumen of virus capsule) immune-enzyme assay was used with MCAb to hexone of the 1 type of human adenovirus, rabbit antiserum to adenovirus of 6 type and conjugated with peroxidase of antibody against rabbit immunoglobulin. For cloning of the gene VAI RNA HindIII-fragment (6231 -11555 np according to Ad2 map) was cut out and cloned in consisting of plasmid pUC19 (pAdH5.3). On the third stage with the help of cutting out the X-bal-fragment we received plasmid pVA224 that contains the whole copy of VAI RNA gene.

Results:

Obtained data testify that there are no essential distinctions between izatizon and metisazon in vitro.

We have checked out antiviral activity of izatizon on different cell lines: Hela, Vero and HEp-2. We found that izatison stops the viral reproduction (in 50% of infected cells and hexone synthesis level) only on HEp-2cell line. Recombinant human interferon β-2 (r-

IFN) had no antiviral activity in concentration 2000 units/ml, but stimulated antiviral activity of methisazone and izatizon. It displayed in strong decrease of cytocidal activity of virus and in two-fold decrease synthesis of capsule albumen. The decrease of synthesis of the viral hexone we observed in Ad1- infected cells of HEp-2 line treated with izatizon. It is necessary to mention that antiviral effect was observed only when izatizon, methisazon and interferon were applied on the early stage of viral infection – up to 8 hours after infection and there was no antiviral effect when the preparations were used later. In that way, antiviral action of izatizon and methisazone needs the presence of interferon in a medium from the one hand and from the other hand - an early activation of viral genes.

Izatizon influences the induction of interferon synthesis only in Ad1 infected HEp-2 line cells. After infection, synthesis of interferon was low till 8 hours. Than this synthesis was rising and then lowering after 12-14 hours. Cytocidal effect appeared only after 96 hours. Both methisazon and izatizon don't stimulate synthesis of interferon neither in vitro nor in vivo and haven't straight influence on the transmission. This shows that the preparation can influence the transcription of early viral genes and as a result, it modifies an expression of interferon in HEp2 cells.

It was detected that the ability of adenovirus to induct β-interferon synthesis in the infected cells in vitro determined an antiviral activity of izatizon. An insertion of exogenous interferon didn't influence infected adenovirus cells, but provoked an antiviral effect only in the presence of izatizon.

Study of the molecular mechanism of viral stability transgression to interferon has shown that izatizon blocks an activation of transcription of early adenovirus genes that delaying the beginning of replication and synthesis VAI RNA (the last one determines stability of adenovirus to interferon activity). The resistance of virus to interferon sharply decreases and this leads to antivirus effect.

A toxicity of izatizon on HEp-2 cell line doesn't differ from that one indicated in previous researches. It is not excluded that used Hela cell line genetically insensitive to the preparations of this type. Previously printed information points on such a possibility.

Cytomorfological analysis of fixed cell samples show that the action of izatizon connected with the delay of the promotion of viral infection, cessation of the reproduction of virus on early stages of the process.

It has been detected that methisazone and izatizon effects concerned with the interferon presence in the medium or with its induction by virus in infected cells. Yet adenoviruses are persistent to the interferon activity because they have, like many other viruses, specific protection system. Mentioned viral persistence possibly caused by

synthesis of big quantity of VAI RNA. This low-molecular RNA is formed by the transcription of corresponding gene with cell RNA polimerase III on the late stage of adenoviral reproduction and it can block protein kinase activity of P1/elF2 dependent protein kinase that activates by interferon and little quantity of dcRNA. Protein kinase phosphorylates the factor of initiation elF2, blocking the translation and provoking polysome disintegration in infected cell. With the help of VAI RNA virus blocks protein kinase, and the translation of viral albumen goes with equal speed both with presence of interferon and without it.

Izatizon in combination with interferon blocks the synthesis of adenoviral hexone. Izatizon doesn't influenced the translation by itself, so we supposed that izatizon somehow inhibited the viral protection against interferon. As the viral replication detains in the presence of izatizon and VAI RNA transcribes mainly after the beginning of virus replication, exactly here may be a trouble with VAI RNA synthesis. Indeed, in the infected cells, treated with antiviral concentrations of izatizon synthesis of VAI RNA begins with couple hour delay. As a result, its level in 4 - 5 times lower in the experiment than in control. Nozern hybridization data also confirm the results received with the help of dot analysis.

Conclusion:

Izatizon antiadenoviral activity can depend on genetic characters of test-cell culture.

Mechanism of izatizon action consists in delay of expression of early viral genes, replications and synthesis of VAI RNA, this conduced to quick weakening of viral protection regarding to interferon. Thus, the viral gene is a target for izatizon.