

Efficiency of Izatizon against Experimental Hsv-1 Infection

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Abstract: *Izatizon possesses antiviral activity in the model of HSV-1 infection both in vitro and in vivo. The protective effect of Izatizon used in therapeutic regimen in the system of modeled herpesvirus meningoencephalitis in mice is comparable with that of reference antiviral drug Virolex. Izatizon may be considered as promising antiviral substance suitable for treating HSV infections. Besides, Izatizon possesses the immunotropic properties affecting the cells involved in antiviral immunity that is of importance for complex therapy of viral infections.*

Key words: *Izatizon, HSV-1 infection, modeled herpesvirus meningoencephalitis.*

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I. Introduction

Nowadays a dramatic increase of infectious disease incidence is evident in global scale with viral diseases being prevailed. Previously unknown viral infections are emerging and the diseases that were considered defeated return [1]. In the last decades, a number of pathogens have developed resistance to existing medicines obtained by chemical synthesis as well as to antibiotics. Moreover, the mixed infections in which the infectious agents interact one with another become more prevalent. Herpes virus infections are among the most prevalent infectious diseases all over the world. The characteristic feature of the epidemiology of such infections is the persistence of herpes viruses in more than 90% of the world population. Therefore, the problem of designing novel antiviral and antibacterial drugs of different spectrum is very important. A study of the mechanisms of their action in various models is a necessary step of their preclinical assessment. Izatizon is a drug of a new generation, which combines the antiviral activity and antitumor properties and also has immunotropic action [2].

Herpes virus infection is one of the most common infectious diseases. The treatment of chronic, often recurrent, HSV-1 infections to date presents some difficulties associated with the biological features of herpesviruses. A specific antiherpetic immune response is formed by contact of herpesvirus antigens with the immune system. The previous study of the antiviral activities of Izatizon in different models demonstrated low toxicity and immunotropic effects of this substance [3, 4]. In particular, Izatizon affects the proliferative activity of lymphocytes induced by mitogens *in vitro* and *in vivo*, the macrophage component of the immune system and NK cells [5]. Izatizon increases the resistance by helping to maintain homeostasis of the body [6]. There is every reason to believe that Izatizon can be an effective antiviral agent. The aim of the study was to assess the efficacy of Izatizon in the setting of the experimental herpetic infection.

II. Materials And Methods Of Research

Izatizon is a soluble form of the drug methisazone obtained by A.I. Potopalsky and co-authors in 1980 using a mixture of polyethylene glycol and dimethyl sulfoxide as the solvents. Izatizon was produced in the laboratory of IMBIG of NAS of Ukraine. As a reference drug, Acyclovir - acyclic nucleoside (Virolex) in the form of sodium salt ("KRKA", Slovenia) was taken.

Male outbred mice, 1 month old, weighing 20-22 g from the IMBIG vivarium of the NAS of Ukraine were used in the study.

Type 1 Herpes Virus - freeze-dried herpes simplex virus (HSV) type 1, strain VC was obtained from the Museum of Virus of D. Ivanovsky Institute of Virology, RAMS. The infectious titer by CPE in Vero cells was 6.0-6.5 lg TCD50 / 0.1 ml, in intracerebral injection of mice – 4.0-4.5 lg LD50 / 0.03 ml.

Vero cells (kidney cells of an African green monkey) were cultured performed in an atmosphere of 5% CO₂ in RPMI-1640 medium with 2 mM glutamine and inactivated 10% fetal calf serum (at 37 ° C)

Cytotoxic concentration (CC50) of drugs under study was assessed in cell culture plates. The cells were incubated in the presence of different dilution of the substances being assayed for 5 days. Experimental and control cultures were monitored on a daily basis to determine the presence or absence of cytopathogenic effect (CPE) assessed by the change in cell morphology (rounding, wrinkling of cells, detachment of degeneratively altered cells from the surface of the wells) and scored by 4+ plus system such as follows:

“-“ - complete absence of cell degeneration;

“+” - not more than 25% is affected;

“++” - not more than 50% of cell monolayer is affected; "+++" - not more than 75% of cell monolayer is affected; "++++" - complete degeneration of cell monolayer.

CC50 was defined as compound concentration (µg/mL) that reduces cell viability by 50%.

The effective dose EC50 was assessed as the concentration of a drug that gives half- maximal protection against the virus when cells are infected at a dose of 100 TCA50 / 0.1 ml. EC50 was calculated by the reduction of infectious titer of virus in cells treated with the substance under study. The conditions of cell culture were the same as above.

Finally, the index of selectivity was calculated as the ratio of CC50 to EC50.

Antiherpetic effect of Izatizon was studied in an experimental model of herpetic meningoencephalitis, which is convenient for assessing the severity of symptoms, is 100% reproducible and does not require the use of additional controls. For modeling herpetic meningoencephalitis in mice, the animals were infected by intracerebral administration of 10% brain suspension obtained from mice with HSV-induced meningoencephalitis (LD50, 0.03 ml per mouse). The depth of the needle insertion was controlled by the rubber stopper put on the needle. Before the administration of the virus, the mice were kept at 4°C to simulate their stress state and suppress the body defense. The needle insertion site was 1 mm above the midline extending from the outer corner of the eye to the middle of the base of the ear. The suspension was administered very slowly (in 3 steps without removing the needle), pausing after administration, preventing the suspension from leaving the syringe after removing the needle from the puncture site. The manipulation was considered technically successful by the survival of the animals within one hour after injection. The clinical signs of herpetic meningoencephalitis became evident in 4 days after inoculation with virus (deterioration of the general condition of the body, inadequate response to various sounds, vesicles in the oral cavity). In 13-14 days, 100% of mice died. Evaluation of the antiviral activity of the drug was performed by comparing the lethality of mice in the experimental and control groups. The effectiveness index was calculated as:

protection coefficient - 1

EI = ----- 100

protection coefficient

wherein protection coefficient equals the ratio of percent of dead animals inoculated with virus to percent of dead animals inoculated with virus and treated with the substance under study.

Detection of HSV DNA was provided by polymerase chain reaction (PCR) with Hybridization-Fluorescence

Detection Using the AmplitudeSensor^R HSVI, II-FL Reagent Kit. DNA was extracted in the presence of an internal control sample (ICS-FL). DNA fragments of HSV I and II types were amplified using the specific primers and the Tag polymerase enzyme.

Enzyme-linked immunosorbent assay was provided in the wells of microplates Maxisorp Nunc (Denmark) with adsorbed monoclonal antibodies to HSV.

For morphological studies, the internal organs of mice were fixed in 10% neutral formalin, processed by conventional histological method, paraffin sections were stained with hematoxylin- eosin. The changes in the organs of mice were evaluated by a semi-quantitative method with the allocation of four gradations - not found, 1+, 2+, 3+. In the spleen, the number of megakaryocytes was taken into account as an integral indicator of the proliferative activity of lymphoid tissue, as well as changes in the structure of the white pulp of the spleen. In the liver of mice the presence and nature of inflammatory infiltration, nuclear polymorphism, Kupffer cell response, the severity of dystrophic changes were evaluated. The size, cellularity, prevalence, and overall intensity of the inflammatory response (diffuse and focal) were evaluated in brain tissue.

III. Results And Discussion

Modeling of herpetic infection

Herpesvirus meningoencephalitis model was obtained by infection of white male outbred mice with virus-containing suspension of the brain of mice infected by HSV type 1. Infectious titer and viral load of HSV

1 and morphological changes in the brain, liver and spleen in the organs of experimental animals were determined.

The virological analysis of studies of viral load and viral titer in organs of mice infected with HSV type 1 are presented in table 1. Studies have shown that HSV was detected by both PCR (viral load) and the virological method (infectious titer of virus).

Table 1.

Tissues	Viral load g/eq.	Infectious titer in Ig ID50
Group 1 brain	4337.3	2.0
thymus	-	2.5
spleen	4133.1	4.0
lymph nodes	2733	2.5

Histological changes of the brain under herpetic infection

Morphologically herpes virus infection was manifested by an increase in the density of microglial cells, focal infiltration of macrophages / microglia. These changes were recorded mainly along the microvasculature and ependymes of the ventricles of the brain. Dystrophic changes of nerve cells were revealed around infiltrates, mainly due to apoptosis (Fig. 1).

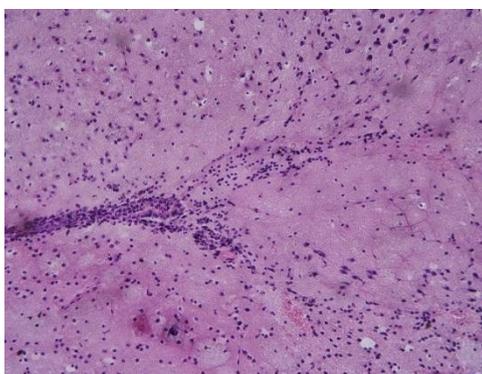


Fig. 1. Microglial / macrophagocytic infiltration of brain tissue around the microvessels and the lateral ventricular wall. H&E. x200

Morphological changes of the liver and spleen under herpetic infection

In HSV-infected mice we identified a number of morphological manifestations of pathological changes of liver. The attention is drawn to the appearance of foci of fibroblast organization, which is a manifestation of the development of cirrhosis. In such areas, dystrophic changes of hepatocytes were observed: cytoplasm swelling of cells, deformation of nuclei. A pronounced erythrocyte stasis of sinusoidal hemocapillaries, veins and arteries of the liver lobules was established. Diffuse cholestasis and focal diapedesis of erythrocytes were also evident (Fig. 2).

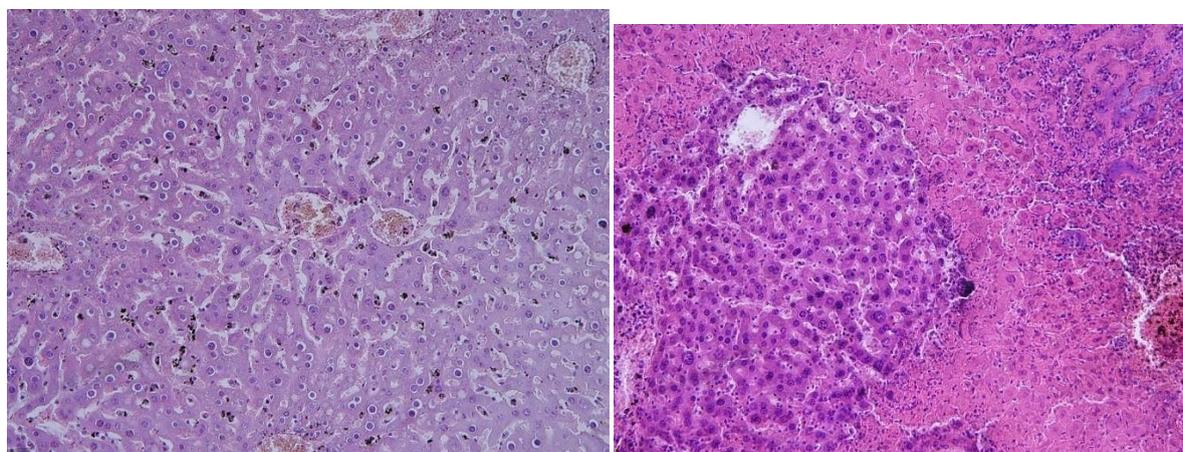


Fig. 2. A - sharp dilatation of hemocapillaries, stasis of the central veins, dystrophic changes of hepatocytes. B - cirrhosis - replacement of hepatic fibroblasts

Herpes virus infection in the spleen was manifested as a significant increase in the volume of white pulp of the organ, represented by the accumulation of leukocytes in the form of follicles. As a rule, the volume of white pulp prevailed over red, indicating a pronounced inflammatory reaction of the organ. The activated monocytes / macrophages were detected on the periphery of the lymphoid follicles. In red pulp, hemosiderin is detected indicating possible hemolysis (Fig. 3).

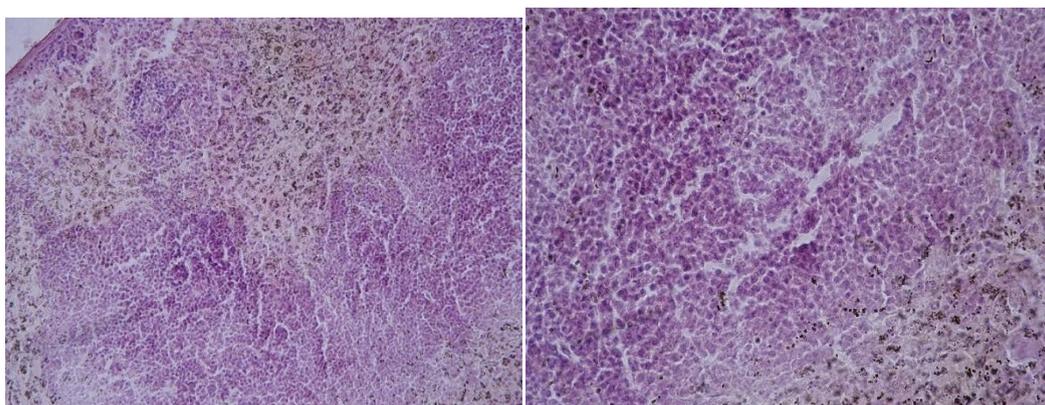


Fig. 3. Increase in white pulp volume, lymphoid follicles. H&E. x200 (a), x400 (b).

To study the antiherpetic effect of Izatizon, we assessed cytotoxic concentration (CC50), effective concentration (EC50) and selectivity index (IS) (Table 2).

Table 2 Cytotoxic Concentration (Cc50), Effective Concentration (Ec50) And Selectivity Index (Is) Of Izatizon In Vero Cells

Drug	CC50 (µg / ml)	EC50 (µg / ml)	IS
Izatizon	20	0.8	25.0
Virolex	> 100	0.39	384.6

Therefore, Izatizon is able to inhibit the reproduction of herpes virus in vitro system, which is shown in chart 1. Testing for the reproduction of herpes virus was performed by enzyme immunoassay using monoclonal antibodies to herpes virus.

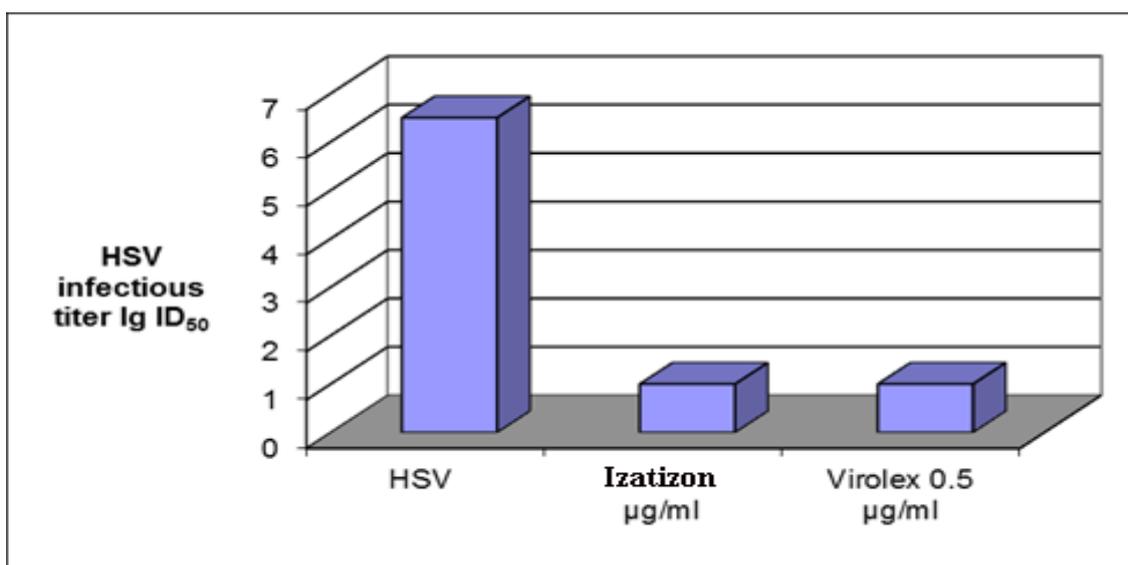


Chart 1. Reduction of HSV infectious titer in Vero cells treated with Izatizon

In in vivo experiment, we compared antiviral effect of Izatizon in prophylactic (24 h before inoculation

of virus) and therapeutic (24 h after inoculation of virus) regimens. Isatizon or Virolex as reference drug were administered intraperitoneally. The data presented in Table 3 demonstrate that in prophylactic regimen the protective effect of Isatizone was not observed.

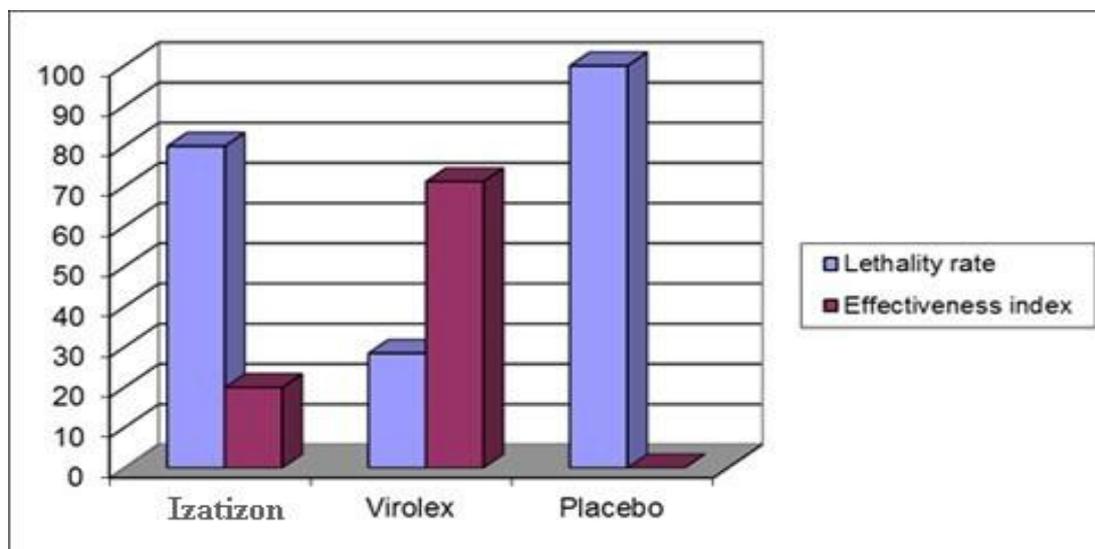


Chart 2. The effect of Izatizon in the prophylactic regimen of administration of mice infected with HSV-1

In therapeutic regimen (24 h after HSV infection), Izatizon was administered in doses 25 mg/kg and 10 mg/kg. The results represented in Chart 3 demonstrate significant protective effect when Izatizon was administered in 24 hours after infection.

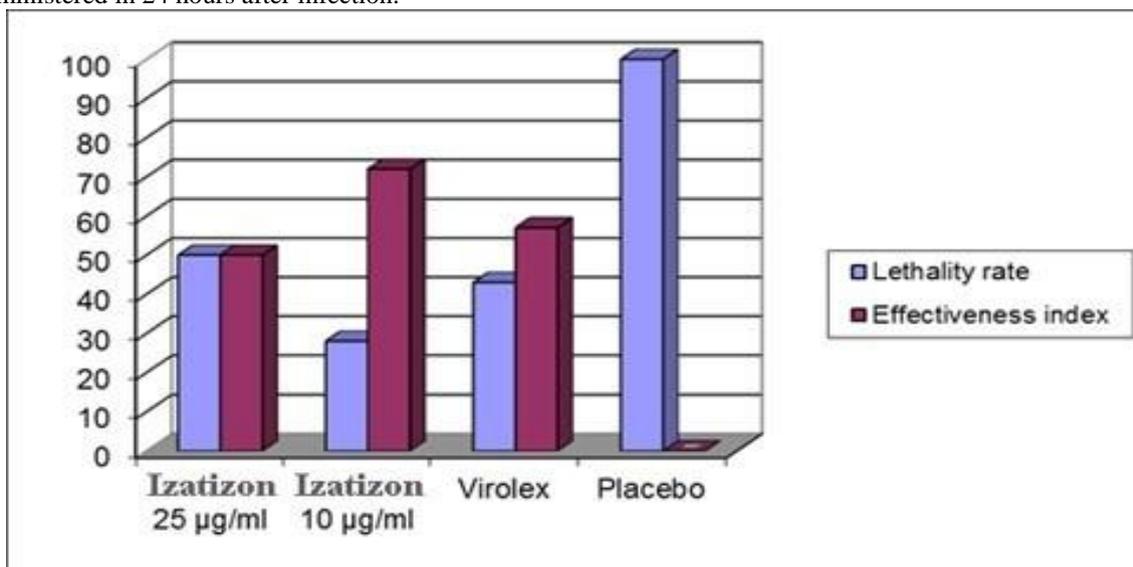


Chart 3. The effect of Izatizon in the prophylactic regimen of administration of mice infected with HSV-1

The protection coefficient of Izatizon in a dose of 25 mg/kg approached the value 2.3 for Virolex and in a dose of 10 mg/kg – exceeded the value for reference drug.

According to the criteria for evaluating the activity of an antiviral substance by index of effectiveness, the substance with IE exceeding 60% are considered as active [7]. The substances with IE of 70% and index of selectivity within 8-16 are considered as promising for the clinical trials and detailed toxicological and pharmacological studies [8]. Therefore, it can be concluded that Isatizone is a promising dosage form that can be successfully used in the treatment of herpes virus infection.

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