INHIBITING EFFECT OF 6-AZAURIDINE UPON REPRODUCTION OF HERPES SIMPLEX VIRUS

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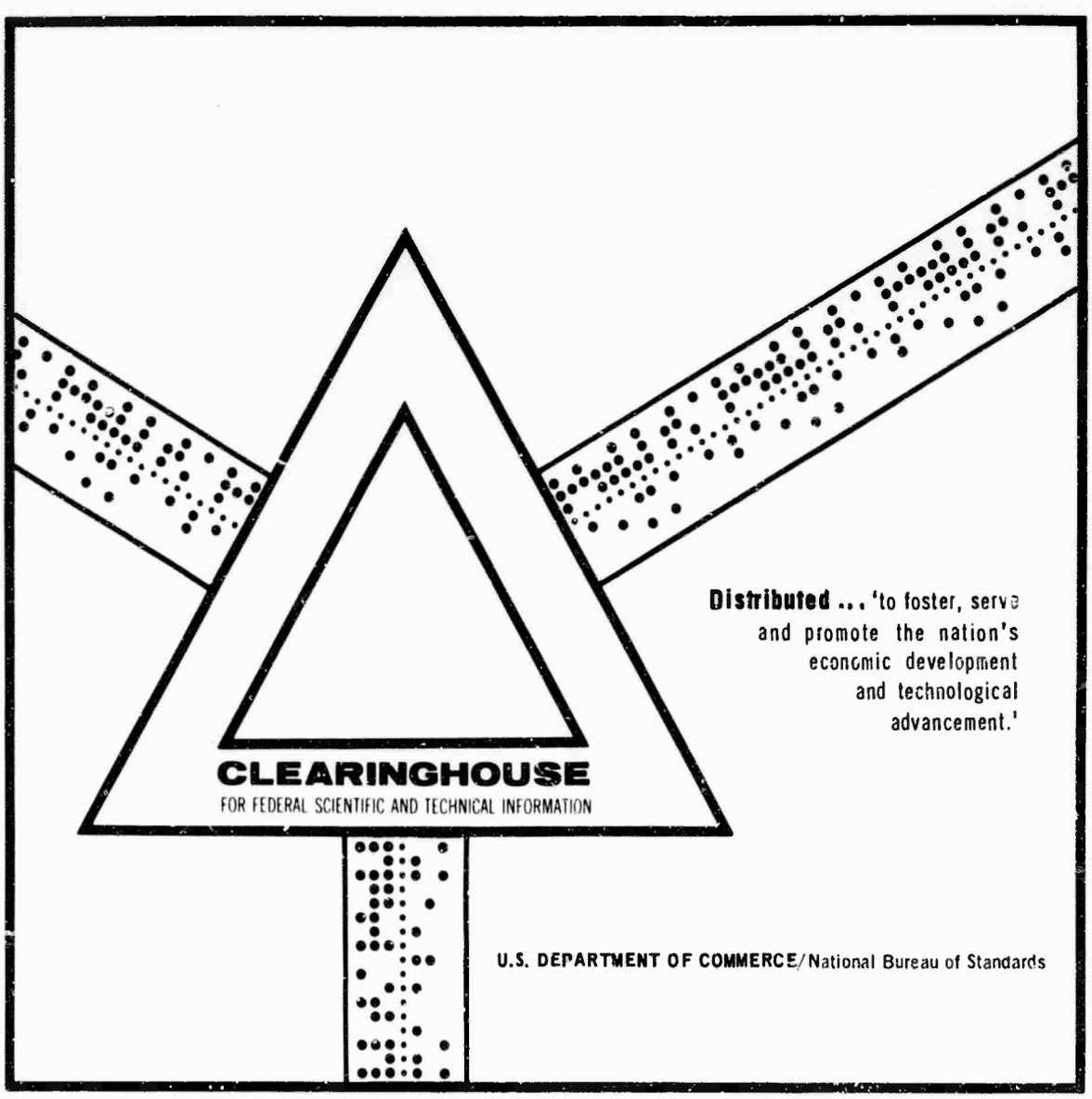

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INHIBITING EFFECT OF 6-AZAURIDINE UPON REPRODUCTION OF HERPES SIMPLEX VIRUS

[Following is the translation of an article by G. A. Galegov, R. M. Bikbulatova, K. A. Vanag, and R. N. Shen, Institute of Virology imeni D. I. Ivanovskiy, AMN USSR, Moscow, published in the Russian-language periodical Voprosy Virusologii (Problems of Virology) No 1, 1968, pages 18-21. It was submitted on 14 Feb 1967.]

The antimetabolic properties of azapyrimidines, which were discovered by Sorm and others, are of tremendous interest not only for biochemistry and microbiology, but also for the chemotherapy of malignant tumors and viral infections. It has been demonstrated that 6-azauracil in the form of its nucleoside 6-azauridine (Azur) suppresses the development of a number of malignant tumors [3, 4] and also the reproduction of vaccinia virus [5, 6], adenoviruses [7], and polyoma virus [8]. It is very important that the antiviral effect of Azur was demonstrated in vivo, when it was possible to suppress to a considerable degree the cutaneous lesions caused by the vaccinia virus in rabbits [9].

The present report deals with the study of the inhibiting effect of Azur on the reproduction of herpes simplex virus (HSV) in a culture of human embryo fibroblasts.

Materials and Methods

An initially trypsinized culture of human embryo fibroblasts was infected with the L-2 strain of the herpes simplex virus with a calculation of $10LD_{50}$ for white mice following infection in the brain per cell. Cultivation of cells was carried out in test tubes by the conventional method using medium No 199 with 10% bovine serum; sera were not added to the supporting medium and the infected cultures were incubated at 34°. Azur was used in a concentration of 2.85×10^{-3}M.

Three series of tests were set up. In series I the cells were preincubated with Azur for one hour, then the antimetabolite was washed off, after which the cells were infected with virus (0.2 ml). After contact for one hour medium No 199 containing Azur was added. In series II the cells were infected with virus and medium containing Azur was added. The control series of human embryo fibroblasts were infected with virus of herpes simplex and medium No 199 without Azur was added.

In 6, 12, 24, 48, 72, and 96 hours three test tubes were taken from each series and a determination was made of the infectious activity of the virus by means of intracerebral titration on white mice with a subsequent calculation of the LD_{50} (0.03 ml) according to the method of Reed and Mench. At the same time in the same period of investigation in all 3 series a study was made of the dynamics of development of the cytopathic effect of the virus on a cell culture. The results were evaluated by the four-star system. [2]

Results

As it follows from Figure 1, Azur possessed an obvious capacity to partially inhibit the development of a cytopathic effect under the influence of virus infection. The nature of development of a cytopathic effect in both test series was practically the same, and the differences between the control and the test series was very apparent in 24 hours from the moment of infection. In subsequent periods this difference nagnified considerably (up to 96 hours after infection of a culture of the herpes simplex virus; see Fig. 1).

Influence of 6-azauridine on yield of infectious virus (Results expressed in $\log_{10} LD_{50}/0.03$ ml)

(a) Время после заражения клеток (в часах)	(b) Серии[1] I	II	III
6	<1,00	<1,00	1,00
12	1,00	2,00	1,25
24	2,05	2,00	2,50
48	3,25	2,00	1,50
72	5,50	3,00	2,00

[1]Обозначения, см. рис. 1.

Key: (a) Time after infection of cells (in hours); (b) Series, [1] for explanation see Figure 1.

In the concentrations used Azur did not cause any apparent changes in the monolayer of cells which would have made it difficult to evaluate the results.

It follows from data in the Table that Azur possessed a doubtless capacity to suppress the reproduction of herpes simplex virus in a tissue culture. A lag in the growth of infectious titers in the control and test series was noted by 24 hours, and later this difference increased, being most expressed in series III.

In this series after 24 hours, apparently, the infectious titer of the herpes simplex virus did not increase, while in series I it increased progressively. In series II the infectious titer was increased to a considerably lesser degree.

Fig. 1. Suppression by 6-azauridine of the cytopathic effect in a cell culture of human embryo fibroblasts infected with the herpes simplex virus.
a - series I (control); b - series II (Azur introduced immediately after infection of cells with virus); c - series III (Azur introduced one hour prior to infection of cells and present after their infection).
Key: (a) Hours after infection.

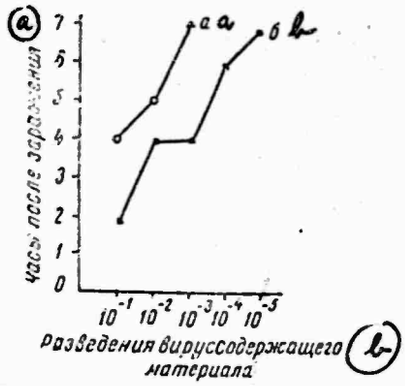

Fig. 2. Dynamics of death of mice (infected with material taken 72 hours after infection of cells). Legend the same as for figure 1.
Key: (a) Hours after infection; (b) Dilution of virus-containing material.

The logical consequence of this were the results of a calculation of death of mice following intracerebral infection with virus-containing material from series I, II, and III (Fig. 2). In series I animals died which were infected with virus in dilutions from 10^{-1} to 10^{-5} inclusively. In series II the death of animals was noted following administration of virus-containing material in dilutions from 10^{-1} to 10^{-3}. In series III animals died which were infected with virus in dilutions of 10^{-1} and 10^{-2}. Mice, infected with the virus of herpes simplex in a dilution of 10^{-1}, in series II and III died 2 days later than mice infected with material from series I. A lag in the periods of death of mice became more expressed in time in a comparison of series I and II under conditions of infection of animals with virus in a dilution of 10^{-3}.

Discussion

At present it has become apparent that Azur primarily inhibits the synthesis of RNA. The mechanism of its action is that this antimetabolite, being subjected to phosphorylation, i.e., transformation into azauridine-5[1]-monophosphate, may inhibit the biosynthesis of uridine-5[1]-monophosphate, forming as a result of decarboxylation of orotidylic acid [10]. This is the main path for the synthesis of uridylic acid and an intermediate for cytidylic and thyminic acids in objects of animal origin. Under such conditions uridylic acid becomes completely inadequate for its intermediate use in the synthesis of RNA. However, in order for Azur to convert into an inhibitor of orotidylate-decarboxylase its phosphorylation is necessary, and its intensity should be quite high in order to ensure an inhibiting concentration of azauridylic acid in the cell. Phosphorylation of Azur is realized with uridine kinase. In tissues of malignant tumors and also in tissues infected with the vaccinia virus the process of Azur phosphorylation is more intensive than in normal tissues [11]. This is connected with the induction of uridine kinase activity. The possibility is not excluded that in a cell, infected with the herpes simplex virus, there is also an intensification of uridine kinase activity, thus ensuring in the cell an inhibiting concentration of azauridylic acid. This atypical nucleotide may stop the reproduction of HSV and, apparently [1, 6], also prevent the synthesis of RNA of this virus at the same that 5-iodo-2 desoxyuridine (IDUR) suppresses the synthesis of DNA of this virus (see sketch).

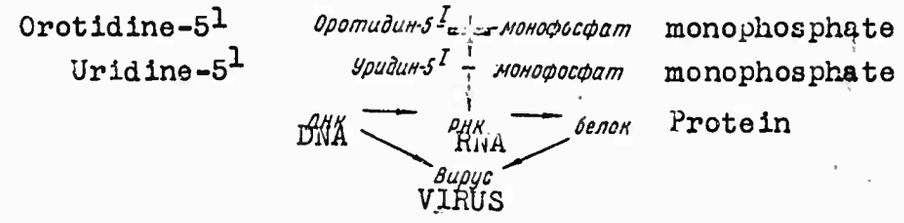

Figure 3.

In our experiments with inhibitor a sharp lowering of uridylic acid could have taken place which would entail the lowering of the infectious titer of the virus. The experiment with preincubation turned out to be more effective, since in this case the lowering of the concentration of uridylic acid preceded the infection of the cells. It is interesting to note that Azur did not suppress the reproduction of influenza virus and the virus of Newcastle disease [5, 12].

This could indicate that phosphorylation of Azur takes place inadequately to disrupt the utilization of uridylic acid for the synthesis of RNA of these viruses.

Conclusions

It was revealed that 6-azauridine suppresses the reproduction of herpes simplex virus and partially inhibits the development of a cytopathogenic effect under the influence of virus infection. Possible mechanisms of the antivirus action of 6-azauridine are discussed.

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