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Selective Mechanisms of Antiviral Effect of Triazole Derivatives in a Transplantable Virus-Producing Cell Culture of Hamadryas Baboon.

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ABSTRACT

Lymphoblastoid cell lines of monkeys represent an alternative object in the study of various antineoplastic preparations, because they are usually latent or productively infected by the lymphotropic herpesviruses of baboons (LHB). One of the characteristics of LHB is its ability to induce expression in the infected cells of the new DNA-dependent DNA polymerase. This enzyme differs from cellular DNA polymerases by matrix specificity, sensitivity to a high salt concentrations and different chemical inhibitors that enables to evaluate selectively the virus inhibiting properties of the studied preparations. The article presents the results of the molecular biological studies of the preparation lozeval and its active component triazole derivative (morpholinium) in a transplantable virus-producing cell culture of hamadryas baboon.

Keywords: triazole derivatives, DNA polymerases, indirect immunofluorescence, transplantable cell cultures, viral infections, medical therapy.

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INTRODUCTION

As a result of constant evolution of the various widely circulating in the nature pathogens and the emergence of their new forms, involvement in the pathological process of the opportunistic microorganisms that are present as commensals in a normal microflora of animals and humans, various endogenous and exogenous influences, etiological structure of infectious diseases in recent years has significantly changed.

New infectious diseases that are subjected to pathomorphism, both natural and induced manifested in changing the general panorama of infectious diseases, are constantly emerging. A spectrum of infectious agents has dramatically changed in favor of the viruses; the incidence of infections caused by so-called opportunistic infections has also significantly increased [1].

On this background the role of the new infections with a complex etiology, caused by an increase in the virulence of opportunistic microorganisms and viruses, in the occurrence of diseases is becoming increasingly important. Diseases acquire the character of mixed and associated bacterial and viral infections, which differ from the classical forms of manifestation of a particular disease with a complicated course. At the same time possessing a pronounced biological and ecological plasticity such mixed infections are capable to widespread in the environment and long-term persistence in a living organism.

All this requires a search and development of the new chemotherapeutic means with different mechanism of the antimicrobial action. World experience of the struggle with the infectious diseases of animals has shown that the main role is given to medical therapy and prophylaxis that can significantly reduce the economic damage they cause [2]. A variety of pharmacological means is used for this, the most widespread of them are antibiotics, fluoroquinolones, sulfonamides, nitrofurans. However, their efficiency has decreased significantly in the recent years due to the changes in the biological properties of microorganisms, display of the multiple resistance after repeated passaging and strengthening of their virulent and pathogenic properties. Resistant forms of infectious diseases pathogens as a new biological population circulate in the nature and infect animals and poultry that significantly hamper their prevention and therapy [3].

The aim of the work was to study the mechanisms of antiviral preparation lozeval, which is representative of a group of chemotherapeutic preparations – triazole derivatives. The active component of the antiviral preparation is morpholinium-3-methyl-1,2,4-triazolyl-5-thioacetate [4].

Antiviral activity of lozeval is caused primarily by its immunomodulatory properties and by the distinct membrane stabilizing action, wherein there is a stabilization of membranes of basophils, mast cells and eosinophils, limiting the release of histamine, serotonin and other biogenic amines. The anti-inflammatory effect is manifested by a rapid leveling of the symptoms of inflammation and return to a normal level of leukocytes in a blood, C-reactive protein and sialic acid. While the immunomodulatory means of the preparation are reduced to increase the phagocytic activity of leukocytes, normalization of T-lymphocytes and reduction of the circulating immune complexes in a blood.

Preliminary studies of the virus inhibitory activity of the compound on a cell of chicken fibroblasts and in developing chicken embryos infected with influenza virus of type A and A2 have shown that when the infectious activity of the virus A in 6.0 Lg TCD50 for chicken fibroblast cells and virus A2 6.0 -7,0 Lg EID50 (embryo infectious dose) for chicken embryo cells, the preparation lozeval has showed a virus inhibitory action against the influenza virus A (WSN) to 3,3 Lg, and the reproduction of the virus strain A2 (England) decreased to 4,0 Lg. Similar results were obtained in studies of the vaccineia and antiherpetic action of the preparation.

MATERIALS AND METHODS

To investigate the molecular mechanisms of the antiviral effect of the preparation lozeval and its active component morpholinium we conducted experiments in vitro on a model of baboons virus producing culture 594-S / F9 [5]. For this purpose we determined their selective effect on the activity of DNA-dependent DNA polymerase – viral and cellular, as well as the expression of viral antigens, that are typical for cell-associated with this culture herpes virus of baboons (HVB).

At the first stage of the research a synchronization of the cell culture was carried out for the simultaneous introduction of the maximum number of those into mitosis. For this purpose the day after subpassaging to a culture that had a density of 1×10^6 cell/ml was added 0,2 mg/ml of a solution of 12-o-tetradecanoylphorbol-1,3-acetate (TPA) and cyclohexamide (CH) in a dimethylsulfoxide to a concentration of 20,0 ng /ml (solutions of the company «Siqma»). Cells were incubated in this environment for 24-96 hours. By the end of the 3-day synchronization cells were twice washed from unbound TPA and CH and were placed into a fresh environment with the studying preparations that are known as blockers of the cellular DNA polymerases - TPA and 5-iodinedeoxyuridin (IDU) and with the studying by us lozeval and morpholinium. The concentration of morpholinium and lozeval corresponded 1:10 LD_{50}.

RESULTS

The studies found out that during the first 24 hours the speed of cellular DNA polymerase was blocked more intense in the presence of IDU (9,1%) and TPA (34,8%) as expected. In these conditions morpholinium blocked cellular polymerase on 39,7% and lozeval – on 40,2% at 100% of the activity of this enzyme in a control group. After 72 hours the block of the synthesis of cellular polymerases became much weaker in the presence of the IDU and TPA and gradually returned to a normal in the environment of lozeval and morpholinium.

A viral DNA polymerase has shown itself quite differently under these conditions. During the first 24 hours after removal from the culture environment the inhibitory cell division TPA+CH there came an intensive synthesis of viral DNA in the samples with TPA+CH (control comparisons) - 706,0 ± 016,6 impulses/min on 1×10^3 cells and in the environment of inducers of the viral polymerase synthesis, that accounting for TPA 445,8 ± 9,8 and for IDU 598,0 ± 10,0 impulses/min on 1×10^3 cells respectively. On the other hand, the speed of synthesis of the enzyme in the presence of morpholinium decreased to 1,4 ± 0,09 and in the presence of lozeval to 1,2 ± 0,05 impulses/min on 1×10^3 cells. By the third day (72 hours) of culturing cells in the presence of compared agents an activity of viral polymerase decreased by half to the control (in the environment with the comparing preparations TPA and IDU), while in the presence of morpholinium and lozeval the synthesis of virus DNA polymerase was almost blocked and was only 0,45±0,002 for morpholinium and 0,5±0,004 impulses/min on 1×10^3 in the environment with lozeval respectively (Table 1).

Table 1. Activity of the viral DNA polymerase in a cell culture

Inductor	impulses/min on 1×10^3 cells (24 hours)	impulses/min on 1×10^3 cells (72 hours)
Intact control	56,4±0,6	53,0±5,2
Control after TPA+ CH	706,0±16,6 <0,001	24,6±2,5 <0,02
IDU	598,0±10,0 <0,001	7,0±0,6 <0,001
TPA	445,8±9,8 <0,001	32,0±3,3 <0,05
Morpholinium	1,4±0,09 <0,001	0,45±0,002 <0,001
Lozeval	1,2±0,05 <0,001	0,5±0,004 <0,001

The obtained results are consistent with the data of indirect immunofluorescence under fluorescent microscopy studies of investigated cell culture 594 S/F 9. We determined that in comparison to the intact control, where the percentage of luminous cells with viral antigens in a continuous culture did not exceed 8,1-

10,1%, the joint introduction of TPA+CH resulted in a significant increase in the number of those cells to 12,8-13,3% that persisted after the removal of the protection of these agents.

It should be noted that the effect of the studied preparations on the cells, in which the synthesis of viral DNA polymerase has been additionally induced, was different. Thus, after the first 24 hours of incubation there was a slight increase in the percentage of the luminous cells, primarily in the experiment with the IDU (12,8 ± 0,5 vs. 8,10 ± 0,9 in the control), where the speed of the synthesis of viral polymerase increased almost 10 times. After 72 hours the amount of the luminous cells increased more than 2 times, being kept in this range within 96 hours.

Similar results were obtained in the experiment with another inducer of the polymerase synthesis – TPA (Table 2). In experiments with morpholinium and preparation lozeval was the opposite situation. By 24 hours in their presence a percentage of luminous cells in comparison with the control group was identical, but by 72 hours the number of cells with viral antigens decreased nearly 3 times in both experiments and it was maintained even after 96 hours.

Table 2. Percentage of virus-containing cells in the indirect immunofluorescence

Inductor	24 hours	72 hours	96 hours
Intact control	8,1±0,9	8,7±0,5	10,1±0,5
Control after TPA+ CH	12,8±0,5 <0,02	13,3±0,9 <0,02	12,7±0,7 <0,05
IDU	14,9±1,2 <0,02	17,6±0,6 <0,01	20,1±0,8 <0,01
TPA	13,9±1,1 <0,02	13,2±0,3 <0,02	17,5±1,1 <0,01
Lozeval	7,9±0,8 <0,5	3,4±1,5 <0,05	4,1±0,4 <0,05
Morpholinium	7,2±1,0 >0,5	2,9±0,4 <0,05	3,4±1,3 >0,05

DISCUSSION

A wide spread of the leukemia viruses in the nature, which cause leucosis and lymphomas both of primates and other animals, stimulate the use of molecular biology methods for searching new effective antineoplastic preparations. A development of the optimal methods for identifying metabolic and virus-producing activity in a virus-associated cell cultures in vitro is of a particular importance [6]. Thereby we used the classic inductors for stimulating the proliferative activity of a cell culture. Previously it has been found that in latently infected cells it is rarely possible to induce the virus production [7]. The use of TPA for these purposes in combination with CH allowed to enhance harshly the metabolic processes of the cells, that led to an additional synthesis of viral DNA. In this case both the number of cells, bearing the viral antigens, and the content of these antigens have increased in the infected cells (Table 2). Adding in this medium active blockers of DNA polymerase has shown that this enzyme is more sensitive to the IDU than to the preparations lozeval and morpholinium. In contrast, the viral DNA polymerase activity sharply decreased in the presence of the studied preparations within the first day and completely blocked by 72 hours of incubation.

Apparently an expression of the viral DNA polymerase is an early viral function implemented by the cellular enzymes, and thus the appearance of virally encoded DNA polymerase occurs earlier then the phase of viral DNA synthesis, which occurs in the synchronized cells in the end of the first day in the early S-phase of the cell cycle [8]. In the presence of the studied preparations at the end of the mass synthesis of the viral DNA the activity of the virus-induced enzyme reduced dramatically due to the damage of the cellular control mechanisms of nucleic acid synthesis.

CONCLUSION

Therefore, studies have shown that the preparations morpholinium and lozeval selectively inhibit the activity of viral DNA polymerase, wherein reversibly blocking the activity of cellular DNA polymerases. This may

explain the fact that in experiments in vitro and in vivo morpholinium and lozeval have a pronounced selective antiviral effect. On this basis it is possible to use the virus-producing lymphoblastoid lines of baboons as a test system for studying simultaneously antimetabolic and virus-inhibiting action of antineoplastic compounds, what is especially important in detecting the signal parameters of their activity and for correcting existing treatment regimens of lymphoproliferative diseases both of humans and animals.

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