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Study of the Mechanism of the Inhibitory Effect of Yeast RNA-Tiloron Molecular Complexes on HIV-I Reproduction *In Vitro*

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Time characteristics of anti-HIV activity of the yeast RNA-tiloron molecular complex are studied *in vitro* and the antiviral effect is compared with the effectiveness of low temperature of incubation and the effects of azidothymidine, larifan, and poly(I)-poly(C). The effectiveness of cell protection against the cytopathogenic activity of HIV-1 and the levels of p24 antigen in culture medium and cell lysates were studied in MT-4 cells infected with HIV-I/IIIB. The molecular complex had the highest inhibitory effect at the stage of virus penetration in the cell. The probable mechanism of its anti-HIV activity is discussed.

Key Words: *HIV-I; polyribonucleotides; interferon inductors; tiloron; MT-4 cells*

α-Interferon [12] and its polyribonucleotide inducters [4,6,7,9] are prospective in anti-HIV therapy. Previously we detected an interferon-inducing [2,3] and anti-HIV effects of a molecular complex forming in reaction of yeast RNA with tiloron [1].

In this study we investigated the parameters of the reproduction of HIV inhibition by this complex *in vitro* in order to elucidate the probable mechanisms of its anti-HIV action.

MATERIALS AND METHODS

The RNA-tiloron molecular complex yeast (MC) was prepared as described previously [2] from commercial yeast RNA (Biokhimreaktiv) and tiloron hydrochloride (Sigma). The reference agents were azidothymidine (Wellcome), poly(I)-poly(C) (Calbiochem), and larifan (bacteriophage f2 dsRNA dosage form, A. Kirchenstein Institute of Microbiology).

Experiments were carried out on human lymphoblastoid cell culture MT-4 infected with HIV-1/IIIB laboratory strain (virus-containing culture fluid). Cells were grown in RPMI-1640 with 10% fetal calf serum (inactivated at 56°C for 30 min), 300 μg/ml L-glutamine, and 100 μg/ml gentamycin at 37°C and 5% CO_2. Multiplicity of infection was 0.5-1.0 lg TCD_{50}/cell. Suppression of virus replication by the drugs was estimated on days 1-12 of experiment, the degree of cell protection was evaluated [4], and the level of HIV-I p24 antigen was measured by enzyme immunoassay using an Abbott kit in accordance with the manufacturer's recommendations.

RESULTS

For studies of the time characteristics of anti-HIV activity, the drug in a concentration of 10 μg/ml

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was added into cell culture at different intervals before, during, and after infection.

A short-term anti-HIV effect of a single MC dose was observed 1 h after its infection (Fig. 1, Table 1). Starting from day 4, the content of p24 viral protein in culture medium increased. A similar decrease in anti-HIV effect under analogous conditions was observed with anti-HIV agents azidothymidine and double-stranded synthetic polyribonucleotides.

This effect, as well as that of poly(A)-poly(U) [7], is probably due to partial degradation of the nucleic component of MC during incubation of infected cells at 37°C and the loss of its anti-HIV activity. Re-addition of MC to infected cells on day 5 of culturing markedly prolonged the protective effect (Fig. 1), which confirms this hypothesis.

MC was added to the culture at various intervals before and after cell infection; its highest anti-HIV activity was observed after the addition 1 h before and simultaneously with infection with HIV-I. The activity was manifested in a better cell protection and a lowered content of p24 antigen. With prolongation of the period between the addition of MC and cell infection, the effect of the agent decreased. This may indicate that MC directly inhibits the early stages of HIV infection. Therefore, we compared the effects of MC and other polyribonucleotides with anti-HIV activity of azidothymidine at the stage of HIV-I entry into the cell. As expected, incubation of cells infected with HIV-I during this period at a low temperature effectively decreased the content of p24 antigen in cell lysates (Fig. 2). It is explained as follows: at 4°C HIV binds to CD4 receptors but does not enter the cell [11]. Reproduction of this effect in our experiments demonstrated the control level of probable suppression

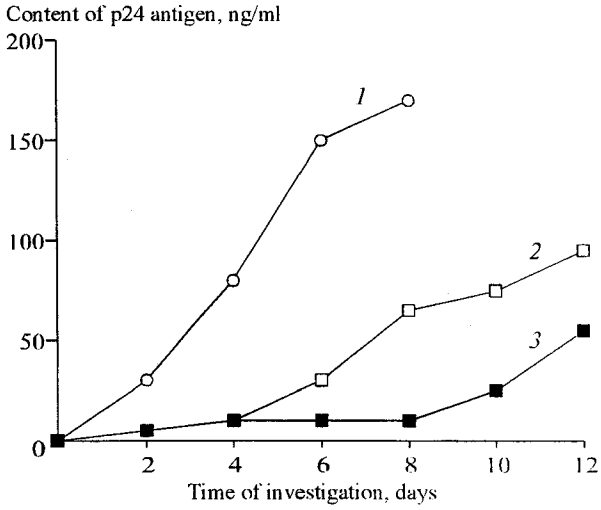

Fig. 1. Prolongation of HIV-1 inhibitory effect of the yeast RNA-tiloron molecular complex by repeated addition of the complex. *1*) control; *2*) single addition of molecular complex 1 h after cell infection; 3) repeated addition of molecular complex 5 days after cell infection.

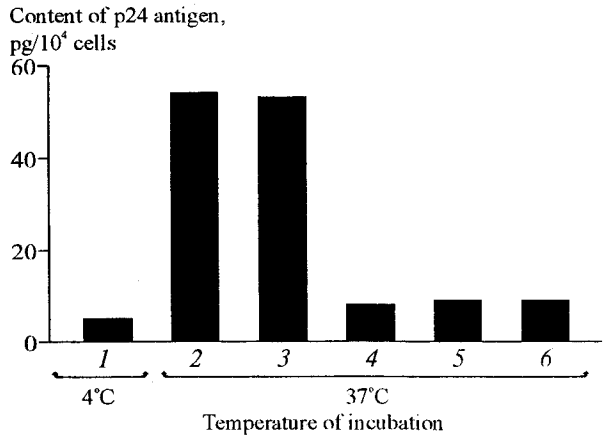

Fig. 2. Effect of the yeast RNA-tiloron molecular complex and other anti-HIV agents on the stage of HIV-I/IIIB entry into MT-4 cells. *1,2*) cell infection without treatment; *3-6*) addition of 5 μg/ml azidothymidine; molecular complex, larifan, and poly(I)-poly(C) in doses of 10 μg/ml.

TABLE 1. The Relationship between Anti-HIV Activity of MC and the Time of the Drug Addition (Day 8, *M±m*)

Time of MC adding (before, during, or after infection)	Degree of cell protection, %	Content of p24 antigen, ng/ml
3 days before	5.7±1.2	125
2 days before	15.4±3.2	110
1 day before	30.5±7.1	84
1 h before	62.5±6.2	32
Simultaneously with HIV-I	60.3±3.8	45
1 h after	45.1±7.3	68
4 h after	40.3±5.6	95
2 days after	10.4±3.3	124
5 days after	3.2±0.8	145
No treatment (control)	—	168

of HIV-I entry into cells. The suppression during treatment with MC and polynucleotides was comparable to this level, while the values for azidothymidine, which does not influence this stage of infection [6], were comparable to the control (Fig. 2).

As polyribonucleotides and other polyanions [6] MC is active mainly during the stage of HIV-I entry into the cell. It may directly react with positively charged residues of CD4 receptors [8] and with gp120 domain of HIV [10], which may be the mechanism underlying the direct anti-HIV effect of MC. The interferonogenic effect of MC [2,3] and the antiviral effect of the monomolecular component of MC tiloron [5] may have an additional impact on the inhibition of HIV reproduction in an organism during treatment with this drug. This represents MC as a promising agent for AIDS therapy.

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