VIROSTATIC ACTION OF RIMANTIDIN STUDIED ON A PHAGE – BACTERIUM MODEL

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The action of rimantidin on reproduction of phages T3, T4, and λ was investigated. The maximal inhibitory action of the compound was observed between the first and sixth minute of infection with phage T3, but no such effect was observed on replication of phages T4 and λ. It is postulated on the basis of the results of incorporation of labeled precursors into phage DNA, RNA, and protein and the character of manifestation of the action of the compound on the phage yield that the inhibitory effect of rimantidin is evidently determined by its effect on bacterial and phage RNA polymerases, mainly on the latter.

KEY WORDS: rimantidin; phage and bacterial RNA polymerase; DNA polymerase.

The chemotherapy of virus infections deservedly attracts the attention of virologists, chemists, and clinicians. Despite some progress in this field, many problems concerning the synthesis, selection, and study of the mechanisms of action of chemotherapeutic preparations still remain unsolved. To shed light on them it is evidently desirable to carry out investigations at all levels of biological organization, using convenient model systems. In this connection the phage–bacterium system, which has now been studied much more thoroughly than the virus–cell system, is particularly interesting and, although it does not fully reproduce the principles of interaction of vertebrate viruses with the cell, it can be used to study the mechanism of action of antivirus preparations and their selection [3].

The object of this investigation was to study the action of the known antiinfluenzal preparation rimantidin (α-methyl-1-adamantan-methylamine hydrochloride) on reproduction of phages T3, T4, and λ. There is information in the literature on the effect of this compound on replication of several viruses [2], although the action of rimantidin on a phage model has not been investigated.

EXPERIMENTAL METHOD

Experiments were carried out with the following strains of Escherichia coli: E. coli B, E. Coli BB, E. coli K-12 (λ), E. coli K-12 CSH-2 [R-4 pro–met–(λ)], E. coli K-12 SF-14 (try^-, S^r), and phages T3, T4, and λ.

The phage was concentrated as follows. After clarification at 6000 rpm, the phage lysate was centrifuged at 30,000 rpm for 1.5-2 h in the fixed-angle rotor of the MSE Superspeed-65 centrifuge. The residue was resuspended in physiological saline and homogenized. The concentrated phage was kept in a refrigerator at 4°C. The action of rimantidin on phage replication was studied by the usual methods [1]. The phage yield in the control was taken as 100%. The results given are the mean values of six to eight experiments. They were highly reproducible.

Experiments with radioactive precursors were carried out on phage T3 and E. coli BB as the model with a multiplicity of infection of 2.5. Labeled H^3-uridine, H^3-thymidine, and C^{14}-amino acids were added to the nutrient medium in a concentration of 5-20 μCi simultaneously with the infecting virus. At different times after infection, samples measuring 0.1 ml were taken and placed on disks of Whatman No. 4 chromatographic

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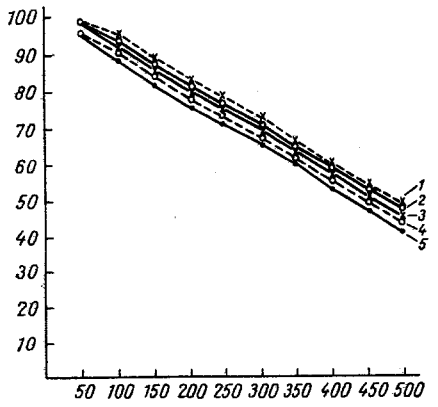

Fig. 1

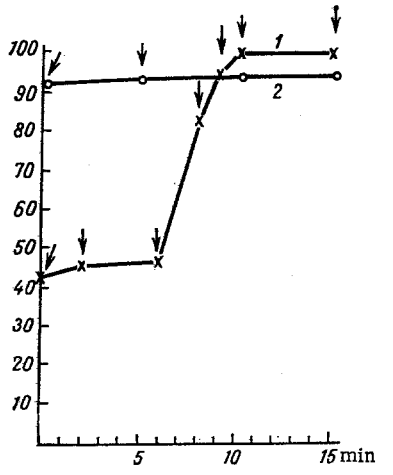

Fig. 2

Fig. 1. Survival of different strains of E. coli as a function of rimantidin concentration. Exposure 1 h. 1) E. coli K-12 CSH-2 (λ); 2) E. coli BB; 3) E. coli B; 4) E. coli K-12 SF-14; 5) E. coli K-12 (λ). Abscissa, concentration; ordinate, survival rate (in %).

Fig. 2. Effectiveness of action of rimantidin on phages as a function of time of addition of the compound. Arrows indicate time of addition of compound: 1) phage T3; 2) phage T4. Ordinate, yield of phage (in % of control).

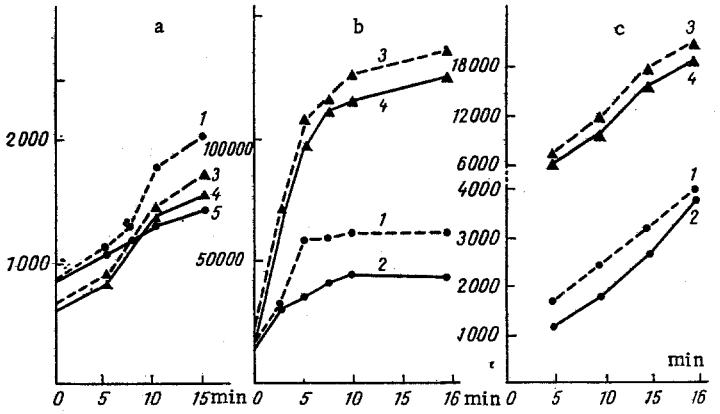

Fig. 3. Effect of rimantidin on synthesis of phage and bacterial macromolecules: a) DNA; b) RNA; c) proteins. Rimantidin added simultaneously with infection. 1) Synthesis of phage macromolecules without rimantidin; 2) synthesis of phage macromolecules in the presence of rimantidin; 3) synthesis of bacterial macromolecules without rimantidin; 4) synthesis of bacterial macromolecules in presence of rimantidin. Ordinate, intensity of synthesis (in counts/min).

filter paper. After two or three washings with 5-10% TCA solution, alcohol, and ether the disks were dried and placed in flasks containing toluene scintillator. The radioactivity was determined in a Packard (USA) counter.

EXPERIMENTAL RESULTS

The doses of rimantidin close to the maximal tolerated values were determined in preliminary experiments. A reduction in the phage yield of 50% compared with the control was taken as the index of activity of the compound.

As Fig. 1 shows, rimantidin in concentrations of 50 and 100 μg/ml had no appreciable inhibitory action on E. coli during incubation for 1 h. Similar results were obtained when the action of the compound was studied for 24 h. Rimantidin likewise had no action on intact phages. During incubation for the period specified rimantidin had no significant effect either on cell metabolism, as shown by the incorporation of labeled precursors into bacterial macromolecules.

In the next series of experiments the effect of rimantidin was studied on the reproduction of phages when the compound was added at different stages of infection. The results (Fig. 2) indicate that the inhibitory action of rimantidin on the replication of phage T3 was strongest until the sixth minute. The action of rimantidin was specific in character, for it had no appreciable effect, in the concentrations used, on the yield of phages T4 and λ.

The action of rimantidin was next studied on the synthesis of DNA, RNA, and proteins of phage T3, as reflected in incorporation of the labeled precursors. As Fig. 3 shows, the compound reduced the synthesis of phage macromolecules. It inhibited RNA synthesis at the fifth minute by 40% and protein synthesis by 30%. The action of rimantidin on the synthesis of phage DNA was manifested later and amounted to 28%. It will be noted that rimantidin depressed synthesis of bacterial DNA, RNA, and proteins to some degree, which could explain its very slight effect on the reproduction of phages T4 and λ, which are known to utilize bacterial enzymes and, in particular, RNA polymerase during replication [4, 7]. According to data in the literature, phage T3, which utilizes bacterial RNA polymerase in the early stages of infection, induces its own specific enzyme, which appears at the fifth minute of infection, and from that time it transcribes the "late" region of the phage genes which carries information for the synthesis of DNA polymerase, deoxyribonuclease, and other proteins [5]. It is logical to suggest that the different action of rimantidin on replication of phages T4 and λ, on the one hand, and T3, on the other hand, is connected with its effect on the formation and function of phage RNA polymerase. Inhibition of the synthesis of phage DNA can also be explained by inhibition of phage RNA polymerase, which is essential not only for the formation of DNA polymerase, but also to initiate the synthesis of phage DNA [6].

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