EFFECT OF DOUBLE-STRANDED RNA AND TYPE I INTERFERONS ON CYTOMEGALOVIRUS REPRODUCTION IN HUMAN FIBROBLASTS

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Studies of the biological activity of various preparations of double-stranded DNA (dsRNA) conducted in the USSR and elsewhere have indicated that they are comparable with interferons in their antiviral and antiproliferative activity [2]. In recent years a number of new preparations of dsRNA and recombinant interferons have been created in the USSR and have undergone preclinical and clinical trials in viral and neoplastic diseases [3, 4]. Cytomegalovirus infection is a particular hazard for debilitated and immunosuppressive patients with disseminated malignancy, including for patients with AIDS, patients with transplanted organs, and pregnant women [6]. Previous investigations with natural and recombinant type I and II interferons have demonstrated their protective prophylactic action against human cytomegalovirus (CMV) [8, 13]. Encouraging results have been obtained in patients after renal transplantation [12].

The action of two clinically promising preparations of dsRNA (larifan and ridostin) and of recombinant α- and β-interferons, produced in the USSR, in a culture of human fibroblasts infected with CMV, was investigated for the first time in the study described below. The preparation larifan, whose toxic and pharmacologic properties have been studied, possesses marked antiviral activity against herpes virus in cell culture and in animals [1]. Comparison of the effect of preparations and combinations of them, when given prophylactically and therapeutically, is of particular scientific interest.

EXPERIMENTAL METHOD

Human CMV of strain AD 169 was obtained from the Virus Museum of the D. I. Ivanovskii Institute of Virology, Academy of Medical Sciences of the USSR. The virus was subcultured with multiplicity of infection of 0.1 CPD_{50} per cell in a continuous culture of human fibroblasts (line M19) for 48-96 h at 37°C in Eagle's medium with 5% calf serum. The yield of virus was determined by titration by a micromethod based on the cytopathic action of human fibroblasts. The titer of cultural virus was 5-6 log. units. The dsRNA preparations studied were the larifan-replicative form of RNA of phage F_2 (A. Kirchenstein Institute of Microbiology, Riga) and ridostin — plasmid dsRNA of the yeast *Saccharomyces cerevisiae* (Research Institute of Tissue Culture and Biologically Active Substances, Novosibirsk) and also preparations of natural leukocytic human interferon ("Egis," Hungary), and human recombinant interferons α_2 (Glavmikrobioprom, Moscow) and β_1 ("Diagnostikum" Research and Production Combine) were used. Experiments were carried out in 96-well microplates on a monolayer culture of human fibroblasts, each version being duplicated in 4-6 wells. Dependence of the action of the preparations on the multiplicity of viral infection, on the dose of the preparations and the duration of treatment was studied. In the prophylactic mode the cells were treated with the preparations first, whereas in the therapeutic mode, the cells were first infected, after which the virus was adsorbed (2 h, 37°C), the monolayer washed off, and the preparations of dsRNA and interferons added. Analysis of the results was based on the development of the cytopathic action, under the light microscope, and titration of 1:10 dilutions of the cultural virus.

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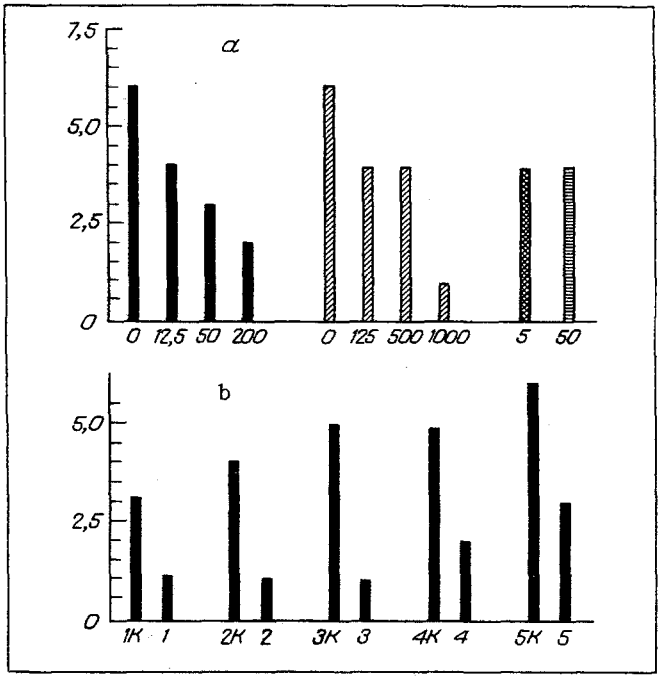

Fig. 1. Inhibition of CMV reproduction by dsRNA: a) dependence on concentration of dsRNA; b) dependence on multiplicity of infection. Treatment with dsRNA for 16 h at 37°C before infection (prophylactic mode). K) Virus control (b). Black columns denote larifan, obliquely shaded columns denote ridostin, horizontally shaded columns ridostin 50 μg/ml + DEAE-dextran 25 μg/ml, cross-hatched columns denote larifan 5 μg/ml + DEAE-dextran 12.5 μg/ml. Abscissa: for a) concentration of dsRNA (in μg/ml); for b) multiplicity of infection (in log. CPD_{50}/ml). Ordinate, titer of cultural virus (in log. CPD_{50} ml).

EXPERIMENTAL RESULTS

Dependence of the antiviral action of dsRNA (larifan and ridostin) on the concentration of the preparations and multiplicity of CMV infection is illustrated in Fig. 1. Both dsRNA preparations gave a marked protective effect which increased with an increase in dose of the preparations. The antiviral action of larifan was manifested at lower concentrations (12.5 μg/ml) than that of ridostin, due to the higher content of dsRNA in the preparation (about 90%). Depression of the yield of the virus reached 4 log. units at concentrations of larifan of 200 μg/ml, and 5 log. units with ridostin in 8 dose of 1000 μg/ml (corresponding roughly to a dose of dsRNA of 100 μg/ml). The antiviral effect of ridostin was therefore actually higher than that of larifan. In maximal concentrations, the dsRNA preparations had no visible toxic action on human fibroblasts throughout the period of testing (96 h). Addition of the polycation DEAE-dextran to the dsRNA reduced the effective dose of the preparation tenfold in order to obtain a comparable protective action, evidently as a result of their more rapid penetration into the cell. We found a similar result previously with vesicular stomatitis virus and alphavirus [10]. The magnitude of the inhibitory action of dsRNA depended on the multiplicity of infection. The antiviral effect was maximal with low multiplicity (1 log. CPD_{50}/ml) and decreased with higher multiplicity However, it still remained at the 3 log. level when the infecting dose of CMV was 5 log CPD_{50}/ml (1 CPD_{50} cell), and the larifan concentration was 200 μg/ml.

The same dependence of antiviral action of preparations of natural α-interferon and recombinant α_2- and β_1-interferons illustrated in Fig. 2. Dependence of the effect on the duration of interferon treatment was studied at the same time. Of the interferons tested, natural leukocytic interferon had the strongest protective effect, and it was about 10 times more active than the recombinant preparations. With a high infecting dose (1 CPD_{50} cell) natural α-interferon in a concentration of 1000 units/ml had an antiviral action comparable with that of the dsRNA preparation larifan in a dose of 200 μg/ml,

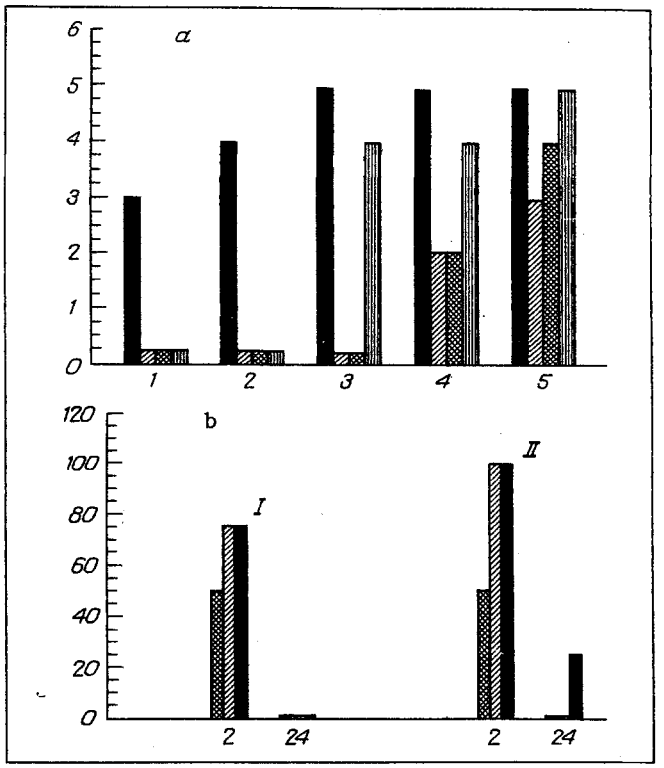

Fig. 2. Inhibition of CMV reproduction by interferons: a) dependence on multiplicity of infection; b) dependence of multiplicity of infection on duration of treatment by interferons. Abscissa: for a) multiplicity of infection (in log. CPD_{50}/ml), for b) time, in h. Ordinate: for a) titer of cultural virus (in log. CPD_{50}/ml); for b) value of CPD_{50} (in %). For a: black columns — virus control, obliquely shaded columns — natural α-interferon (1000 units/ml), cross-hatched — recombinant β-interferon in a dose of 10,000 units/ml, vertical shading — recombinant β-interferon in a dose of 1000 units/ml. For b: black columns — multiplicity of infection 5 log. CPD_{50}/ml, obliquely shaded — 4 log. CPD_{50} ml, cross-hatched — 3 log CPD_{50}/ml; I) natural α-interferon in a dose of 10,000 units/ml; II) recombinant α-interferon in a dose of 10,000 units/ml. Treatment with interferons for 24 h at 37°C (a).

lowering the yield of the virus by 2 log. units. To exhibit the maximal antiviral effect, the cells had to be treated for 24 h; however, with low multiplicity of infection a protective action of the interferon preparations was observed after 2 h.

Having discovered the marked antiviral action of the dsRNA preparations and interferons on the prophylactic mode (addition of the preparations before infection with the virus), we studied their therapeutic action in cell culture (treatment after CMV infection), The results are given in Fig. 3. Clearly, with a high multiplicity of infection (1 CPD_{50} cell) the preparation had a stimulating action in the early stages of infection on reproduction of CMV virus, increasing the yield of the virus by 1-3 log. units. The antiviral action of the preparations was exhibited at low multiplicity of infection (0.01 CPD_{50} cell) only in a combination of natural α-interferon with the dsRNA preparation larifan and with complexes of dsRNA with DEAEdextran. The stimulating effect of dsRNA and interferon after infection with CMV is not unexpected and can be explained by similarity of the mechanisms regulating transcription of viral and interferon genes, which has been demonstrated recently by a number of investigations [7, 9, 11]. Similar DNA sequences activated by nuclear transcription factor NK-KB have been found in promotors of CMV genes and interferon regulated genes. For that reason, the stimulating effect which we found confirms the possibility of additive stimulation of transcription of the early viral genes of CMV by dsRNA and interferon at the cellular level. At the same time, this suggests that dsRNA and interferon can prevent the

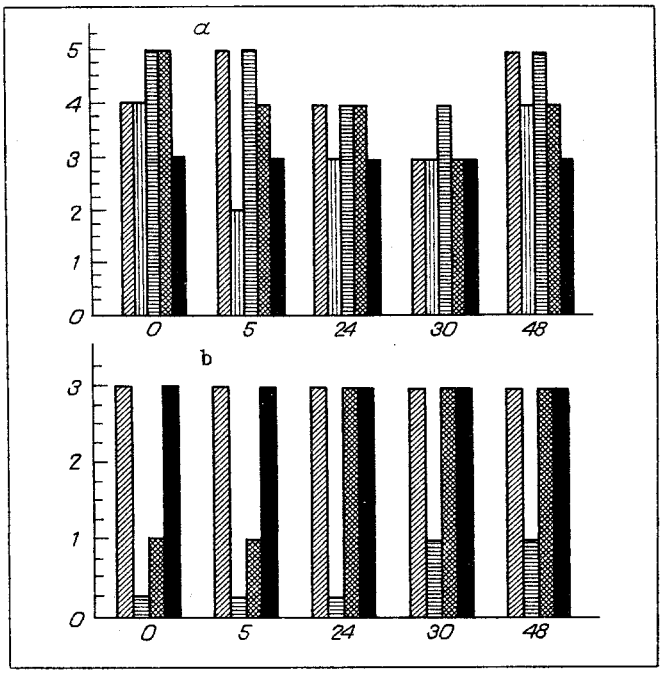

Fig. 3. Effect of dsRNA and interferons at different times after CMV infection: a) high multiplicity of infection (1 CPD_{50}/cell), b) low multiplicity (0.01 CPD_{50}/cell). Black columns — virus control, obliquely shaded — larifan 200 μg/ml, vertical shading — natural α-interferon in a dose of 100,000 units/ml, horizontal — combination of larifan (200 μg/ml) with α-interferon (10,000 units/ml), double-hatched — complex of larifan (100 μg/ml) with DEAE-dextran 25 μg/ml. Abscissa, time after infection with virus (in h) when preparation was added; ordinate, titers of cultural virus (in log. CPD_{50}/ml).

onset of chronic infection and a further search for effective combinations with chemotherapeutic agents inhibiting synthesis of viral DNAs and proteins is essential.

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