EFFECT OF NITROXYL RADICALS ON THE PATHOGENIC PROPERTIES OF HERPES VIRUS (A SPIN-PROBE STUDY OF VIRUS–MACROPHAGE INTERACTION)

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A leading role in the formation of tissue barriers to the spread of infection is ascribed to the mononuclear phagocyte system, which can inactivate viral antigen and inhibit reproduction of the virus in the host cell [5, 8]. On the other hand, the effect of a viral antigen may be accompanied by depression of phagocytic activity [4, 8] and may ultimately promote generalization of the infectious process. The development and use of new techniques to study the mechanism of virus–phagocyte interaction can help us to understand key aspects of the pathogenesis of acute virus infections and also to evaluate the efficacy of antiviral preparations and immunomodulators.

EXPERIMENTAL METHOD

Macrophages isolated from the spleen of CBA mice weighing 12-14 g [4] were used in the experiments. Highly virulent herpes simplex virus type 1 (HSV-1) (strain L-2) was propagated in a culture of transplantable Hep-2 cells. The virus was purified by ultracentrifugation in a sucrose density gradient [1]. The sucrose solutions were made up with Hanks' medium. To monitor the efficacy of virus–target cell interaction, a method based on analysis of the character of the change in the EPR spectra of nitroxyl radicals as a result of contact between spin-labeled HSV-1 virions with the macrophagal plasma membrane [6] was used. Spin-labeled HSV-1 was obtained by treating a suspension of the virus in Hanks' solution with the ethyl ester of 5-doxylstearic acid (M5DS, from "Sigma," USA), located in the lipid membrane of the virion, or with 4-maleimide-TEMPO (4MT, from "Sigma," USA), covalently binding with SH-groups of structural proteins. The conditions of treatment of the HSV-1 virus with the reagents 4MT and M5DS (15 min, 37°C) were chosen so that their effect on the pathogenic properties of the virus was minimal. For this purpose, alcoholic solutions of M5DS and 4MT were added to the suspension of virus made up in Hanks' solution (concentration $5 \times \log LD_{50}$) so that the final concentration of the reagents was 10^{-5}, $5 \cdot 10^{-5}$, 10^{-4}, and $5 \cdot 10^{-4}$ M. The ethanol concentration in the incubation medium of the control and experimental samples did not exceed 0.2%. The pathogenicity of the virus, treated with the reagents, was monitored in five parallel experiments on a test system of a 2-3-day cell culture of the transplantable Hep-2 line, and also by induction of experimental herpetic infection in CBA mice. The testing procedure on the cell culture was carried out as follows: growth medium was removed from the tubes containing the growing culture and replaced by Eagle's medium, containing HSV-1, diluted to $2 \times \log LD_{50}$ (intact or treated with solution of nitroxyl radicals). Reproduction of HSV-1 in the cell culture was judged from the cytopathic action exerted after 48 h. The titer of the virus was determined in 0.2 ml of virus-containing liquid. The animals were infected by intracerebral injection of 0.02 ml of the HSV-1 suspension with a concentration of between 1 and $5 \times \log LD_{50}$. The effect of the reagents was assessed from the number of surviving animals.

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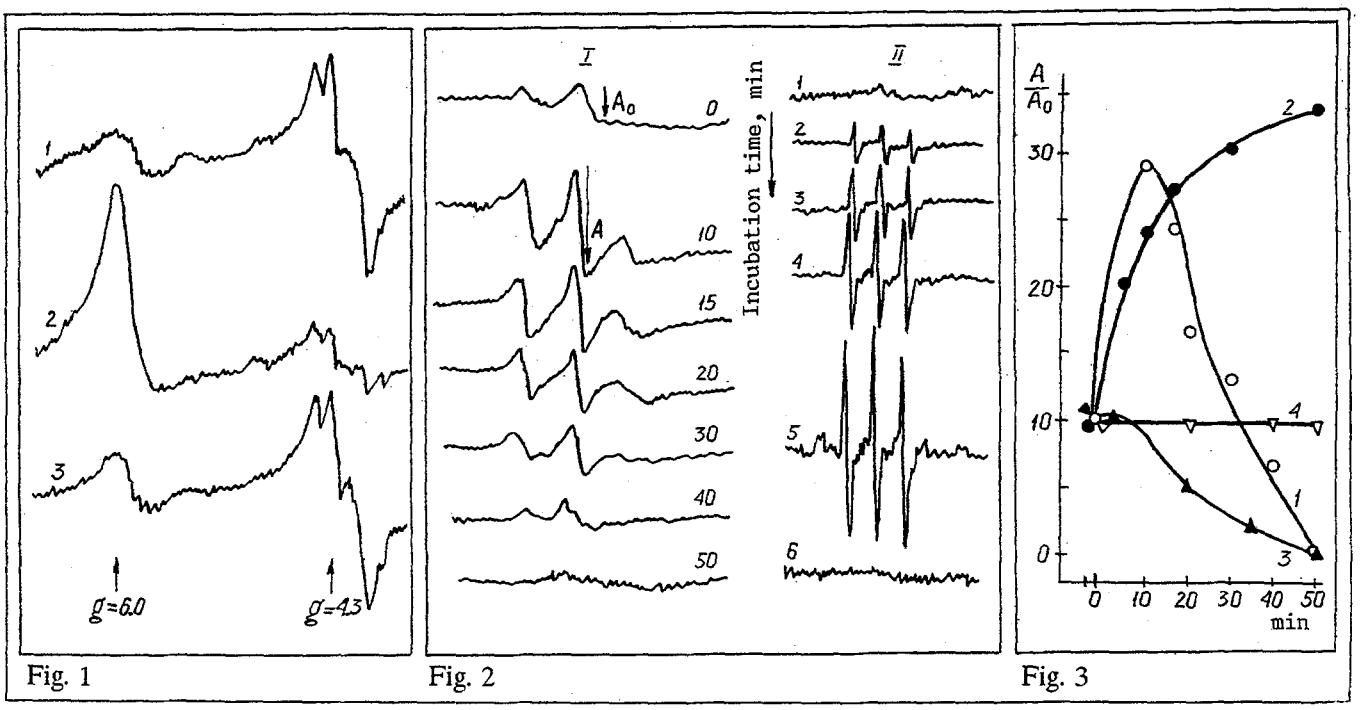

Fig. 1 Fig. 2 Fig. 3

Fig. 1. EPR spectra of blood samples from CBA mice: 1) blood of control animals, 2) blood of animals infected with HSV-1 (concentration of virus 3 × log LD_{50}), 3) blood of animals infected with spin-labeled HSV-1 (concentration of solution of 4-maleimide-TEMPO $5 \cdot 10^{-4}$ M); g = 4.3, transferrin signal; g = 6.0, methemoglobin signal.

Fig. 2. Nitroxyl radicals during estimation of dynamics of HSV-1 – macrophage interaction: I) character of change in EPR spectra of methyl ester of 5-doxylstearic acid, incorporated into virion membrane of HSV-1, depending on incubation time of virus in medium with macrophages; II) spectra of 5,5′-dimethylpyrroline-N-oxide (10^{-2} M): 1-5) reflecting dynamics of accumulation of free-radical products in incubation medium containing macrophages and HSV-1, inactivated by rabbit antiserum to HSV-1 (spectra recorded at interval of 10 min after addition of HSV-1 to medium), 6) absence of free-radical products in incubation medium after addition of intact HSV-1 (signal recorded 20 min after contact between virus and macrophage).

Fig. 3. Dynamics of relative change of intensity of midfield component (A in Fig. 2) of spectrum of nitroxyl radicals: methyl ester of 5-doxylstearic acid, incorporated into virion membrane of HSV-1, on incubation of HSV-1 with macrophages (1) and with liposomes (2); 4-maleimide-TEMPO, bound with HSV-1 proteins (3) or BSA (4), on incubation of HSV-1 with macrophages.

During development of an acute infectious process, the blood transferrin level of the animals was monitored. Blood samples were prepared in liquid nitrogen after decapitation of the animals (on the 4th-5th day after infection). The transferrin level in the samples was estimated from the intensity of the EPR signal g = 4.3 (at 77°K). The power of the microwave radiation was 20 mW.

Macrophages (about $4 \cdot 10^7$ cells in 1 ml Eagle's medium) were incubated in the presence of spin-labeled HSV-1 at 37.0 ± 0.5°C. The concentration of HSV-1 in the medium was 6 × log LD_{50}. Isolation of the plasma membranes of the macrophages, preparation of the lipid extracts, and determination of the lipid phosphorus content were carried out as in [2]. Liposomes were prepared from macrophagal lipids by injecting a 10% alcoholic solution of lipids into Eagle's medium, followed by dialysis of the suspension in Eagle's medium (5 h).

EPR spectroscopy was carried out on the small EPR spectrometer produced by the "Svetlana" Leningrad Optico-Electronic Factory.

EXPERIMENTAL RESULTS

Evaluation of the concentration effect of solution of M5DS and 4MT on the pathogenic properties of HSV-1 showed that the cytopathic properties of HSV-1 were depressed by 1 × log TCD_{50} on treatment of the virus with 10^{-5}, $5 \cdot 10^{-5}$ M solutions of M5DS or a 10^{-4} M solution of 4MT. The use of higher concentrations of the reagents gave a stronger effect. For instance, the decrease in cytopathic action of HSV-1 with a concentration of the reagents of $5 \cdot 10^{-4}$ M amounted to 5 and 4 × log TCD_{50} for M5DS and 4MT respectively. Similar concentration effects of nitroxyl radicals were observed in experiments on animals. HSV-1, treated with 10^{-5} M-$5 \cdot 10^{-5}$ M solutions of M5DS or a 10^{-4} M solution of 4MT caused encephalitis of varied severity in concentrations from 1 × log LD_{50} (i.e., with a dilution of 10^4 times). In this case reduction of the transferrin concentration and accumulation of methemoglobin (g = 6.0) were observed in the blood of the infected animals, a characteristic feature of an acute infectious process [3] (Fig. 1).

Treatment of HSV-1 with $5 \cdot 10^{-4}$ M solutions of nitroxyl radicals led to a marked decrease in pathogenicity of the virus; death of the animals was observed only after injection of high concentrations of spin-labeled HSV-1 (about 5 × log LD_{50}). On weakening of the pathogenicity of HSV-1 under the influence of M5DS and 4MT, the blood transferrin level of the infected animals agreed with the control level (Fig. 1).

To study interaction of spin-labeled HSV-1 with macrophages, virus treated with 4MT and M5DS solution in a concentration of $5 \cdot 10^{-5}$ was used. The efficacy of incorporation of spin-labeled HSV-1 was judged by the change in the midfield component of the spectrum (A_0) of the nitroxyl radical bound with the virion envelope. It follows (Fig. 2) from the dynamics of the time-dependent change in the intensity of the M5DS spectra on addition of spin-labeled virus to medium with macrophages, in the course of 10 min there was an increase in the intensity of the midfield component of the spectrum from the value of A_0 to the value of A (the increase A/A_0 correspondingly; curve 1 in Fig. 3). The cause of the observed effect was weakening of spin–spin exchange interaction between the paramagnetic spectra as a result of diffusion of M5DS molecules in macrophagal membranes on contact of the spin-labeled HSV-1 with macrophages. Next followed quenching of the signal of the probe (and, correspondingly, a decrease in A/A_0, see Fig. 3) under the influence of membrane-bound redox systems [9], as far as the complete disappearance of the component A. The effect of redox processes in macrophages was assessed by monitoring interaction of the spin-labeled HSV-1 with liposomes. In this case the ratio A/A_0 of the M5DS signal, having reached its maximal value, thereafter remained unchanged throughout the period of incubation (Fig. 3, curve 2).

The relative change in component A_0 of the 4MT spectrum on interaction of spin-labeled HSV-1 with macrophages differed in character. Virtually throughout the period of interaction of the spin-labeled HSV-1 with macrophages, the ratio A/A_0 fell slowly and ultimately the EPR signal disappeared completely (Fig. 3, curve 3), since the 4MT, covalently bound with the virion envelope, was restricted in its diffusion mobility in the lipid matrix of the macrophagal membranes. No such effect was observed when the EPR spectra of 4MT bound with BSA, which has low transmembrane permeability, were recorded: throughout the period of interaction of HSV-1 with macrophages there was no change in the A/A_0 ratio (Fig. 3). Absence of change in the intensity of the signal of spin-labeled BSA indicated that during interaction of HSV-1 with macrophages lysis of the cells or a change in their redox activity, accompanied by an oxidative burst, did not take place. Further evidence in support of this view was given by the results of monitoring the oxidative burst, using the free radical trap 5,5′-dimethylpyrroline-N-oxide (Fig. 2) [7].

Thus the effect of the nitroxyl radical derivatives M5DS and 4MT on the properties of HSV-1 is dose-dependent in character. With choice of the optimal concentration of nitroxyl radicals, to obtain spin-labeled virus, correct recording of interaction of highly virulent HSV-1 with the macrophagal plasma membrane can be carried out. Analysis of the kinetic parameters of interaction between virus and target cell can be used to assess the efficacy of antiviral preparations, capable of modulating the functional state of cell membranes.

LITERATURE CITED

1. A. S. Grishin and I. N. Titov, Purification, Concentration, and Fractionation of Animal Viruses [in Russian], Moscow (1971), pp. 18-25.
2. L. L. Prilipko, V. E. Kagant, V. A. Tyurin, et al., Dokl. Akad. Nauk SSSR, **269**, No. 5, 1260 (1983).
3. N. Gorbunov, A. Volgarev, N. Bykova, et al., Regulation of Free Radical Reactions, Varna (1989), p. 52.

4. K. N. Masihi, W. Lange, B. Rohde-Schulz, et al., Infect. Immun., **50**, 146 (1985).
5. S. C. Mogensen, Microbiol. Rev., **43**, No. 1, 1 (1979).
6. S. I. Ohnishi and T. Maeda, Structure, Dynamic and Biogenesis of Biomembranes, ed. by R. Sato and S.I. Ohnishi, London (1985), pp. 32-55.
7. L. H. Piette, L. H. Baxley, T. A. Grover, and P. J. Harwood, Oxygen Radicals in Chemistry and Biology, ed. by W. Bors, M. Saran, and D. Tait, New York (1984), pp. 134 145.
8. S. Plaeger-Marsall, L. A. Wilson, and J. W. Smith, Infect. Immun., **41**, 1376 (1983).
9. A. Stier and E. Sackmann, Biochim. Biophys. Acta, **311**, 400 (1973).

MODULATING ACTION OF ADENOSINE ON HUMAN PLATELET ACTIVATION

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The study of the functional state of human platelets is of great practical importance for the elucidation of the pattern of development of pathological processes taking place with the participation of these cells. These are, primarily, changes in the blood clotting system connected with hyper- and hyposensitivity of the platelets to the action of activating factors. The study of these cells in connection with their role in the formation of morphological and functional changes in the bronchopulmonary system also remains an urgent problem [7]. In addition, the use of platelets as a model with which to study the state of receptor systems can also help to shed light on the receptor-mediated processes that take place in other cell systems. In the light of these facts it is very interesting to study the action on the cell of physiological effector agents, with specific receptors on the platelet cell surface, such as platelet activating factor (PAF), adenosine diphosphate (ADP), and serotonin (5-HT) [2, 5, 8], and also the ability of adenosine to modulate the formation of the cell response [9]. The reason for the urgency of the study of the action of adenosine is the important role played by this endogenous compound in the regulation of the different systems of the body under both normal and pathological conditions.

The aim of this investigation was to compare changes in the cell response of platelets during modulation of the stimulating effect of PAF, ADP, and 5-HT by adenosine.

EXPERIMENTAL METHOD

Venous blood was obtained from healthy donors and mixed in the ratio 9:1 by volume with anticoagulant of the following composition (in %): sodium citrate 2.5, citric acid 1.37, glucose 20 (pH 6.5). The blood was centrifuged for 15 min at room temperature and at 200*g* to obtain platelet-enriched plasma (PEP). After removal the PEP was subjected to further centrifugation for 10 min at 2000*g* in order to obtain platelet-deprived plasma, which was used in order to adjust the cell count in PEP to $10^5/\mu l$. The aggregating power of the platelets under the influence of agonists was assessed with the aid of an "Elvi-840" two-channel aggregometer (Italy), with simultaneous recording of

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