Photodynamic Inhibition of Infection Caused by Drug-Resistant Variants of Herpes Simplex Virus Type I

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Membranotropic amphiphilic chromophore merocyanine 540 sensitized photodynamic inhibition of drug-resistant and sensitive variants of type I herpes simplex virus in cultured Vero cell. Optimal conditions of photodamage to virus particles and infected cells were determined (merocyanine 540 concentration 1 μM, illumination dose 32.5-65.0 kJ/m^2, exposure at early stages of infection). Infected cells actively bind the photosensitizer, which explains their selective photodamage.

Key Words: *herpes simplex virus; Vero cell culture; photodynamic exposure; merocyanine 540*

Herpetic infection in humans is often associated with life-long latent carriership of herpes simplex virus type I (HSV-1); activation of the virus is associated with specific clinical symptoms and development of immunodeficiency. Virus variants with defective genome resistant to antiviral inhibitors, such as acyclovir (AC_{res} variant) and phosphonacetic acid (PA_{res} variant), were detected using highly active antiherpetic officinal preparations [11]. Therefore, the development of alternative methods of virus inhibition acquires special importance.

Photosensitizing reactions in biological systems attracted special attention of scientists in recent years. These reactions provided the basis for new methods of photodynamic therapy of various diseases [6,8]. Photosensitizers generate free radicals after absorption of visible light, for example, they generate active oxygen forms, which easily oxidize proteins, lipids, and other biomolecules. Merocyanines (MC) binding to virus envelope [7] are perspective agents for suppressing viral infections. The efficiency of membranotropic sensitizers in photodynamic destruction and elimination of the virus and infected cells is explained by differences in membrane structure and functions in infected and intact cells [2].

Here we investigated the possibility and determined the optimal conditions for photodynamic inhibition of viral infection caused by drug-resistant and sensitive HSV-1 variants in a cell culture using MC 540 photosensitizer.

MATERIALS AND METHODS

The study was carried out on cultured green monkey kidney cells (Vero). The cells were cultured on slides in flasks with Eagle's medium (Sigma) supplemented with 10% FCS and gentamicin (100 μg/ml). HSV-1 strain 1C (virus collection of Institute of Microbiology and Epidemiology, Minsk) and its PA_{res} and AC_{res} variants detected as described previously [3] were used.

Virus reproduction was evaluated by the specific cytopathogenic effect. Quantitative accumulation of virus particles in the studied cells was evaluated by infectious titer and expressed as logarithm of the titer of cytotoxic dose (lg TCD_{50}/ml) [1]. Herpetic infection was modeled in monolayer cultures (day 3 after subculturing). Multiplicity of infection was 0.1 and 1.0 TCD_{50}/ml. The cultures were monitored for 1 week; the degree of cell monolayer destruction was determined.

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Antiviral effect of photodynamic exposure in the presence of MC 540 (Sigma) was evaluated by the decrease in infectious titers of HSV-1 variants (at least 75% intact monolayer) in cultures exposed to illumination at different stages of virus reproduction: adsorption and penetration into the cell (0.5-2.0 h), uncoating and synthesis of products of intracellular virus development (4-7 h), and accumulation of mature particles (24 h). Cell morphology and mitotic activity were studied by microscopic examination of preparations fixed in ethanol and stained with hematoxylin-eosin.

Binding of MC 540 (0.1-15.0 μM) to cytoplasmic membranes was evaluated after cell transfer into phosphate-buffered saline. Spectral studies were carried out on a Jobin Ivon spectrofluorometer. The fluorescence intensity was recorded at λ=600 nm (λ_{ex}=575 nm). Dye toxicity was evaluated by the cytomorphological test. Photodynamic exposure of intact and infected cells was carried out after replacement of the growth medium in the flasks with Hanks' solution containing MC 540. The samples were exposed to light using Peleng 500 K slide projector (150 W KGM lamp) in doses of 19.5-98.0 kJ/m^2 at light flow intensity of 108.5 W/m^2.

The results were processed by routine statistical methods.

RESULTS

Variants of herpes virus caused similar cytopathic changes in Vero cultures typical of herpes simplex virus: swelling of the nucleoli, appearance of multinuclear cells, simplasts, and nuclear and cytoplasmic inclusions [2]. The first virus-induced morphofunctional changes in cultured cells were seen as early as 4-6 h postinfection and manifested in swelling of nucleoli and stimulation of cell proliferation. The maximum cytoproliferative effect of HSV-1 was observed at the lower dose of the virus (0.1 TCD_{50}/ml). The mitotic index in cultures infected with 0.1 TCD_{50}/ml was 120.0-98.0‰ *vs.* 58.0 in the control (p<0.01). At later

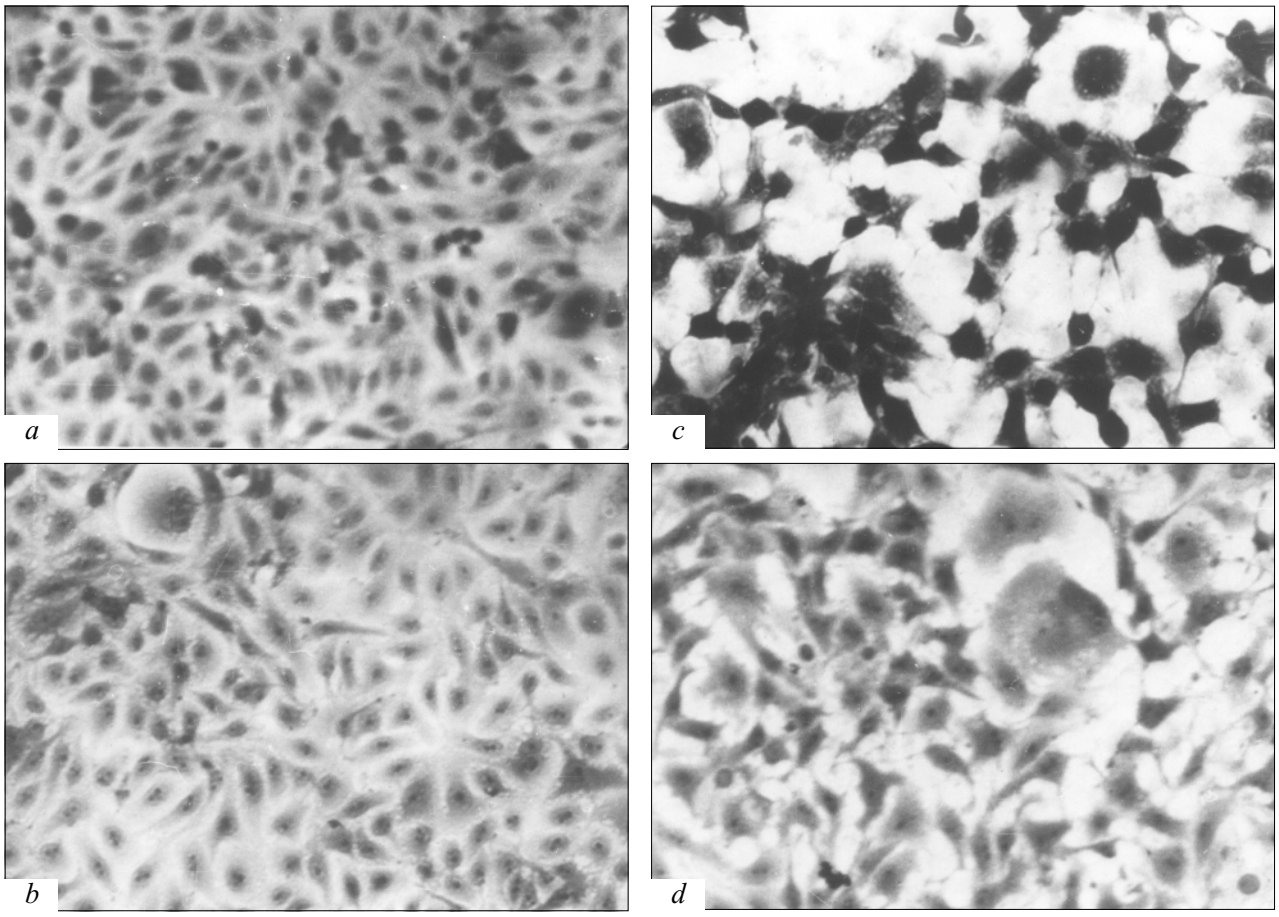

Fig. 1. Morphology of Vero cell culture exposed to different factors. Hematoxylin and eosin staining, ×200. *a*) intact monolayer; *b*) cell culture 48 h after merocyanine 540 (1 μM) treatment and photoexposure (65.0 kJ/m^2). Monolayer of viable cells, solitary cells with changed morphology are seen; *c*) cytodestructive changes in Vero culture caused by type 1 herpes simplex virus (1C), 48 h postinfection; *d*) preserved monolayer of infected cells after photodynamic exposure sensitized with merocyanine 540 (1 μM) at early stage of infection (4 h). Protective (virus-inhibitory) effect, 48 h after exposure.

stages (48-56 h) appearance of ball-shaped cells and simplasts, detachment of the monolayer, and total cell death were noted. The cycle of virus reproduction varied from 24 to 78 h depending on the multiplicity of infection. Twelve hours postinfection virus accumulation in cultures was 2.5-3.0 lg TCD_{50}/ml, while after 48-52 h its level was 6.0-6.5 lg TCD_{50}/ml for the parental HSV-1, 1C, and AC_{res} variant and 5.2-7.2 lg TCD_{50}/ml for PA_{res} variant.

Photosensitizer binding to cells is an obligatory condition of photodynamic damage of the cells. Interactions between MC 540 and cells were evaluated by the fluorescent parameters of the probe: dye binding to the plasma membranes sharply increased fluorescence intensity and shifts the maximum in the stimulation and fluorescence spectra toward longer waves [5]. Under our experimental conditions (2×10^5 cells/ml, 1 μM MC 540) about 10^9 MC 540 molecules bound to one intact Vero cell. Addition of 5% blood serum to the culture medium (serum albumins exhibit high affinity to MC 540 [4]) 3.5-fold decreased MC binding, which indicates the presence of high affinity binding centers for the dye on membranes of cultured Vero cells. The presence of MC 540 binding centers on the plasma membrane is characteristic of dedifferentiated and actively proliferating cells. We showed that the total amount of MC 540 bound by the cell did not depend on the stage of culture growth. On the other hand, MC 540 binding in high affinity centers increased by 25-30% at the peak of cell proliferative activity. MC 540 binding to infected cells in the presence of the serum depended on the stage of virus reproduction: it increased by 22% during the adsorption stage, remained at this level during early stages of the virus multiplication, and decreased during synthesis of mature virus particles. Since proliferative activity of cells increased at the early stages of infectious process, the nature of MC 540 binding centers was presumably similar to that in blast and transformed cells. It is also possible that increased binding of MC 540 was due to interactions of the dye with virus particles adsorbed on the cell surface. Control experiments showed that addition of MC 540 (to a concentration of 1 μM) to intact monolayer and subsequent 60-min incubation in the dark did not impair the monolayer integrity and caused no appreciable changes in cell morphology.

The capacity of infected cells to high accumulation of MC 540 helped us to determine the conditions of their selective photodamage. Photodynamic expo-

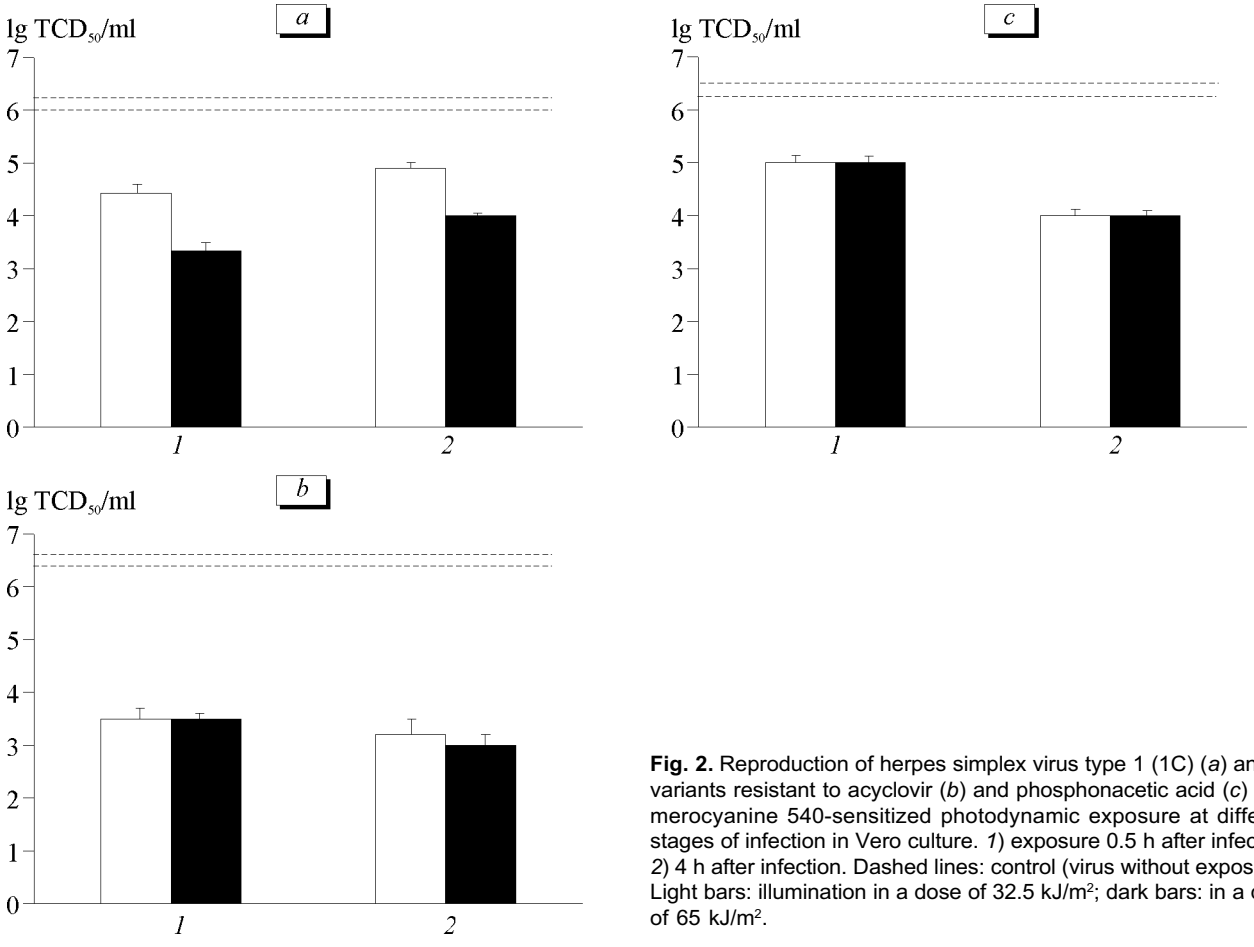

Fig. 2. Reproduction of herpes simplex virus type 1 (1C) (*a*) and its variants resistant to acyclovir (*b*) and phosphonacetic acid (*c*) after merocyanine 540-sensitized photodynamic exposure at different stages of infection in Vero culture. *1*) exposure 0.5 h after infection; *2*) 4 h after infection. Dashed lines: control (virus without exposure). Light bars: illumination in a dose of 32.5 kJ/m^2; dark bars: in a dose of 65 kJ/m^2.

sure of intact and infected cells with MC 540 was studied. Photoexposure of intact Vero cells in doses of up to 65.0 kJ/m^2 in the presence of MC 540 (1 μM) did not impair cell monolayer over 5 days of observation (Fig. 1, *a*, *b*). Increasing MC 540 concentration (15 μM) and illumination dose (98.0 kJ/m^2) led to the appearance of pronounced phototoxicity: the cells were fixed to the flask surface, their morphology changed, pyknosis of the nuclei was observed.

Virus-inhibitory effect of MC 540-sensitized exposure on infected cultures (4 h after infection) was observed at a dye dose of 0.2 μM and illumination dose of 32.5 kJ/m^2. However, the optimal conditions of photodynamic inhibition of the infection was 1 μM MC 540 and illumination dose of 65.0 kJ/m^2 (Fig. 1, *c*, *d*; Fig. 2). The cells infected with drug-resistant HSV-1 variants showed high sensitivity to photodynamic exposure. The infective titer of HSV-1 strain 1C and its variants PA_{res} and AC_{res} after exposure of the cell monolayer in the presence of MC 540 decreased by 1.5, 2.4, and 3.5 lg TCD_{50}/ml, respectively. Photodynamic treatment of the monolayer at later stages of infection (15-24 h), when the majority of cells were involved in the infectious process, produced a pronounced cytotoxic effect. Inhibition of virus infection in the culture can be due to photosensitized destruction of not only infected cells, but also viral particles. In order to evaluate the virucidal effect of photodynamic exposure, chromophore MC 540 was added to virus-containing medium and after illumination infectious activity of the samples was studied. Illumination in a dose of 32.5 kJ/m^2 in the presence of 1 μM MC 540 decreased infectious titer of HSV-1, 1C by 2.0 lg TCD_{50}/ml and of PA_{res} and AC_{res} variants by 3.6 lg TCD_{50}/ml. It is noteworthy that 1-h exposure of all HSV-1 variants with MC 540 in the dark did not modify infectious titer of the virus.

Close interrelations between macromolecular virus-specific syntheses and biosynthetic processes in cells determines modification of the structure and antigenic specificity of cytoplasmic membranes and promotes selective binding of MC 540 to infected cells making them sensitive to photodamage.

Our findings open good prospects of photodynamic therapy by inhibition of herpesvirus infection as a method alternative to drug therapy. Clinical use of this method in patients with drug-resistant HSV-1 variants and in combined therapy for preventing drug resistance of viruses is perspective.

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