Effect of L-Lysine-A-Oxidase on the Development of Genital Herpes Infection in Guinea Pigs

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Positive effect of local treatment with L-lysine-a-oxidase on the course of herpes genital infection was demonstrated on guinea pigs even after the appearance of infection symptoms. In animals treated with L-lysine-a-oxidase, the severity of the disease, virus reproduction and virus-induced changes in cells were significantly less pronounced than in untreated animals. The preparations exerted no toxic effects.

Key Words: *herpes simplex virus type II; L-lysine-a-oxidase*

L-lysine-a-oxidase (LO) is a promising antitumor enzyme. The growth-inhibiting effect of LO was first discovered by Japanese scientists and then confirmed by Russian researchers using a *Trichoderma harzianum Rifai* strain [1-[3,6].

Herpetic infection often serves as an ethiological factor for cancer development. Our previous studies showed that LO exerts a powerful antiherpetic effect *in vitro*. The present study investigated the antiherpetic activity of LO *in vivo*. This article describes the effects of LO on genital herpes infection in guinea pigs.

MATERIALS AND METHODS

Herpes simplex virus type II (HSV-2), a MS strain (National Collection of Viruses of the USA) was used. It was maintained in sensitive Vero cells cultured in MEM medium containing 2% fetal calf serum and 500 U/ml benzylpenicillin.

Infectious activity of viruses isolated from herpetic lesions was determined by a standard titer technique in Vero cell culture. The results were expressed in tissue cytopathic doses (TCD_{50}). Specific cytopathic effects of HSV-2 were verified by an immunofluorescent method using antiherpetic rabbit antibodies labeled with fluorescein isothiocyanate [4,5].

Male guinea pigs weighing 300 g were intramuscularly anesthetized with ketalar (300 mg/ml) and infected with herpes by application penis of 0.1 ml virus suspension in a dose of 10^5 plague-forming units/ml corresponding 100% lethal dose on preliminary scarified. Clinical symptoms of acute genital herpes infection were assessed using a 5-point scale [7].

Three groups were formed (15-20 animals each): group 1 (control) received no treatment; group 2 animals were daily treated with 0.5 ml LO gel (70 μg/ml) applied on herpetic lesion areas and given intramuscular injection of 0.25 ml LO (100 μg/ml); group 3 animals received only gel applications (0.5 ml, 70 μg/ml) on penis mucosa lesions.

The treatment was started 1 or 48 h postinfection. The severity of viral infection was scored according to D. Mayo *et al.*, [7] (0.5-1 points: erythema or 1-2 clusters of blisters; 2 points: 3-10 clusters of herpetic blisters; 3: 11-20 clusters; 4: more than 21 clusters, fused blisters) and the mean score was calculated.

RESULTS

In the control group, typical symptoms of the disease appeared 24-48 h postinfection (Fig. 1, *a*). First, single vesicles appeared on the penis mucosa, then their

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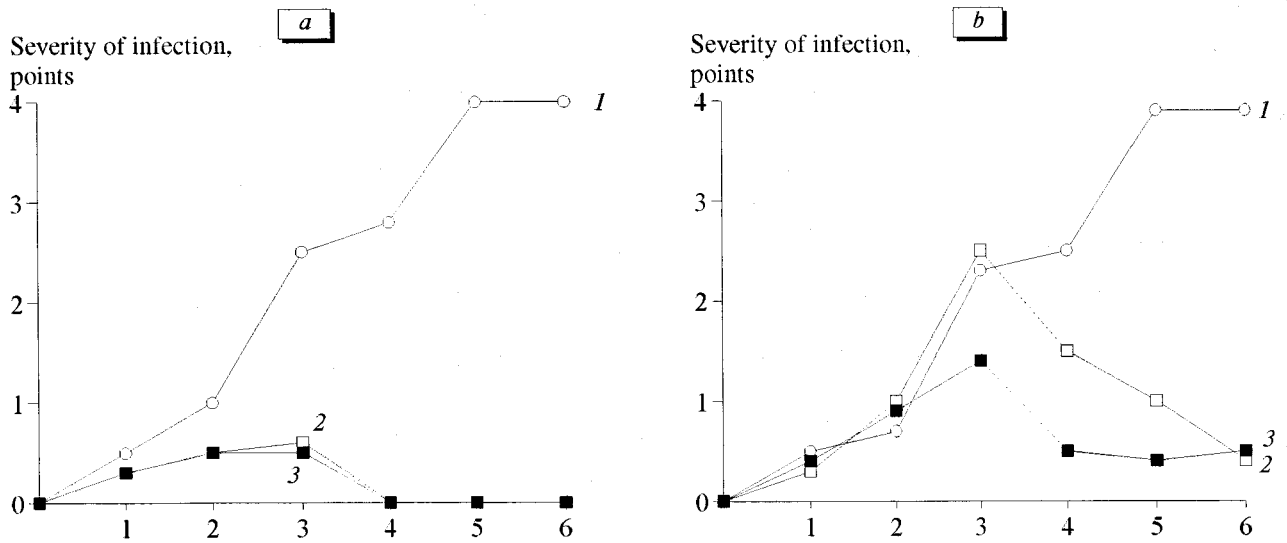

Fig. 1. Effect of treatment with L-lysine-a-oxidase started 1 (*a*) and 48 h (*b*) postinfection on the course of genital herpes in guinea pigs. *1*) control (without treatment), *2*) local gel application, *3*) combined treatment.

number increased with the appearance of fused blisters with hemorrhagic content. The symptoms of generalized herpes infection (fever, flaccidity, paresis of pelvic organs, and hind leg paralysis) appeared on days 4-5. The animals died on days 7-8 from increasing symptoms of meningoencephalitis.

Both local and combined treatment exerted considerable positive effects on the course of the disease (Fig. 1). The best results were observed when the treatment was started 1 h postinfection. In these animals local symptoms were limited to erythema which appeared 24 h postinfection and completely disappeared on day 4 of treatment.

Despite more pronounced lesions after later onset of treatment (48 h postinfection, Fig. 1, *b*), the therapeutic effect of LO was considerable and the severity of local lesions in the control and experimental groups differed significantly. The mean score in the control group was 2.8, herpetic blisters tended to fuse and form large exudates with inflammatory changes. In animals treated with gel or gel in combination with LO

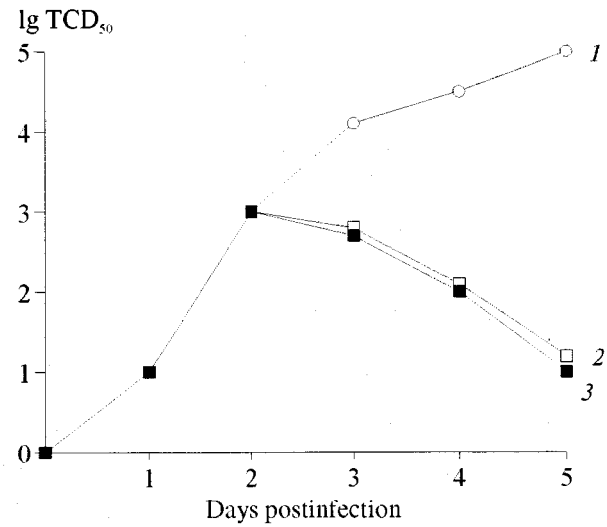

Fig. 2. Antiherpetic effect of L-lysine-a-oxidase (LO) measured by suppression of reproduction of herpes simplex virus type II isolated from herpetic vesicles, when the treatment was started 48 h postinfection. *1*) control (without treatment), *2*) treatment with gel, *3*) combined treatment.

TABLE 1. Effect of Treatment with Lysine-A-Oxidase on the Course of Genital Herpes in Guinea Pigs

Treatment	Start of treatment			
	1 h postinfection		48 h postinfection	
	incidence of pareses, %	mortality, %	incidence of pareses, %	mortality, %
Control (without treatment)	100/40	0/60	50/20	0/80
Local gel application	0/0	0/0	40/60	0/40
Combined treatment	0/0	0/0	20/60	0/20

Note. The indices on day 5 are presented in numerator, on day 7 — in denominator.

injection, the mean score was 2.2 and 1.8, respectively. The disease showed no significant progress, lesion foci did not fuse, the phase of exudation was absent.

The most pronounced antiherpetic effect was observed on day 5 postinfection in animals receiving combined treatment. Herpetic blisters ruptured followed by rapid healed and epitelization of erosions. The positive local effect of the enzyme prevented infection aftereffects and reduced the incidence of paralyses, pareses and lethal outcomes (Table 1). In the control group, mortality on day 7 was 4 and 2 times higher, than in groups 2 and 3, respectively.

The therapeutic effect of LO was confirmed by the analysis of biological activity of HSV-2 isolated from herpetic blisters (Fig. 2). In the control group, the infectious activity of the viruses in sensitive Vero cell culture increased, while treatment started 48 h postinfection reduced their activity by 3-4 lg TCD_{50}.

Thus, LO efficiently suppressed genital herpes infection in guinea pigs showing the best results after combined treatment.

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