Effect of a Cytostatic on the Course and Outcomes of Experimental Herpetic Infection in Rabbits

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Herpetic infection (HI) caused by the herpes simplex virus (HSV) is characterized by the prolonged persistence of the pathogen in the organism in a latent form of infection which is reactivated periodically, the disease relapsing in the form of acute local or regional lesions or in a generalized form of infection. The mechanisms of latency and the factors promoting HSV reactivation are not fully studied. However, numerous clinical observations attesting to frequent relapses of the disease or of the development of generalized infection in patients with different forms of immunodeficiency confirm the important role of the immune system in these processes [1,2,4].

It is well known that herpetic infection is a serious threat to the life of oncological patients and individuals with transplanted organs and tissues, because diverse immunodepressants and chemical preparations having a cytostatic effect, including an influence on the cells of the immune system, are used to treat such patients [2,4].

The aim of the present study was to perform an experimental investigation of the cytostatic effect of holoxan on the course and outcomes of acute herpetic infection in rabbits.

MATERIALS AND METHODS

The experiments were carried out on 24 chinchilla rabbits of both sexes weighing 1.0-1.5 kg. Local herpetic lesions in the form of keratoconjuctivitis were induced by rubbing a 10% brain suspension containing HSV of type I (strain CI, titer 3.8 log LD_{50}) into scarified cornea preliminarily treated epibulbarly with a 0.5% dicain solution. The degree of expression of the clinical signs of herpetic keratoconjuctivitis was assessed one or two times a week according to a four-point scale [10]. The method of fluorescing antibodies [3,4] was used for determination of HSV antigens. HSV in blood clots was titered by the routine method using a cell culture of chick embryo fibroblasts. A single injection of holoxan (ASTA Pharma AG, Germany) was introduced intraperitoneally (150 mg/kg) into six animals during the period of remission, after an acute primary herpetic infection in the form of keratoconjuctivitis. Rabbits with induced herpetic keratoconjuctivitis which did not receive an immunodepressant served as the control. The results were subjected to statistical analysis after Moroz [5].

RESULTS

The dynamics of development of primary herpetic keratoconjuctivitis (HKC), of the first relapse induced by the cytostatic holoxan, and of the ensuing spontaneous relapses manifested as chronic herpetic infection in the rabbits, is shown in Fig. 1. The mean index of the clinical manifestations of acute ophthalmoherpes attained the maximum on days 6-10 of infection in the

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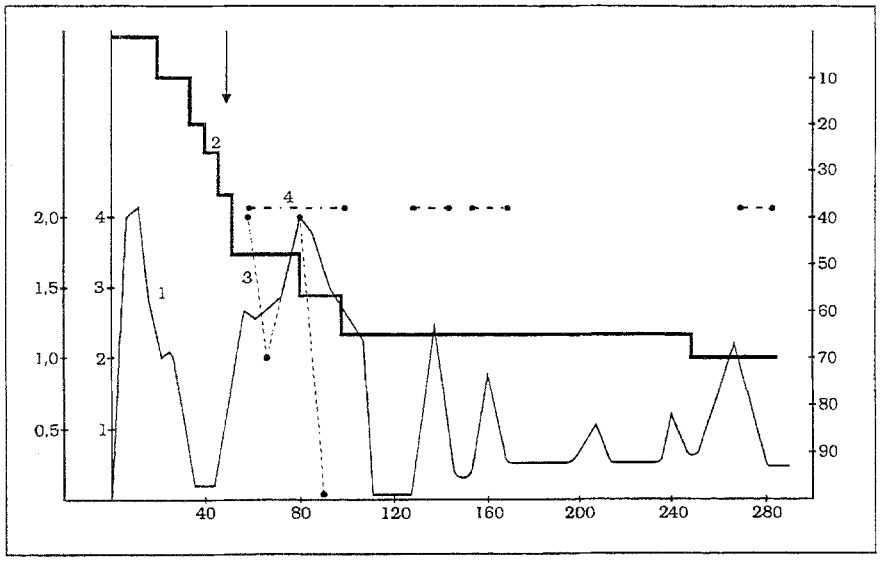

Fig. 1. Dynamics of development and of the course of herpetic infection (acute and chronically relapsing) in animals. 1) development of ophthalmoherpes manifestations; 2) mortality; 3) virus titer in blood clots; 4) HSV antigens determined in blood cells; the time of holoxan injection being marked by an arrow. Ordinate: mean index of clinical manifestations of HKC, percentage mortality, virus titer in blood clots (log LD_{50}). Abscissa: days of observation.

12 rabbits included in the experiment. Conjuctivitis was found in practically all animals; the vesicular form of keratitis, characterized mainly by lesions of the surface layers of the stroma, developed in 70% of the animals. During the following 25 days, the intensity of the clinical signs of keratoconjuctivitis diminished gradually, with a slight delay on days 20-26. The disease ended in a spontaneous healing of the herpetic eye lesions in the animals still alive on days 30-34 of the experiment. Of the 12 rabbits with induced HKC, three animals (25%) exhibiting clinical signs of encephalitis died on days 16, 30, and 33.

The dynamics of development of eye lesions and the outcomes of disease previously noted in rabbits of the same age (2-3 months) studied in an experimental HKC model [2,3] were similar to those of the control group. Viremia was observed during days 8-15, and the level of lethal outcomes of herpetic encephalitis (confirmed by morphological and virological investigations) varied in a range of 20-30%. However, in these series of experiments no further observations of surviving animals were performed.

On days 40-42, after spontaneous healing of the eye lesions had occurred in the period of remission, three more rabbits demonstrating signs of encephalitis died.

In order to induce immunosuppression, holoxan injection was performed on days 12-14 in the surviving six rabbits (intraperitoneally, 150 mg/kg). One day after the substance was introduced, signs of keratitis development were found in 4 out of the 6 animals (66.7%). Two days later, all the animals exhibited such signs, their maximum clinical manifestation being reached in the next 4-5 days, after which these signs were preserved with virtually no changes during the following 10 days. The vesicular corneal lesions discovered during biomicroscopic observations were complicated by the development of dull spots on the stroma. During the following 20 days the severity of keratitis increased gradually to the level noted in the rabbits in the acute period after primary infection. At the same time, against the background of arborescent and geographic forms of corneal lesions, uveitis development without any signs of conjunctivitis was observed in the majority of animals. Discoid keratitis was observed in one rabbit. For 5 to 7 days the course of ophthalmoherpes was severe, but during the following 15-18 days a gradual regression of the disease manifestations was noted.

During the development of the first herpetic infection relapse induced by holoxan injection, two rabbits out of six had died (33.3%). One rabbit died on day 35 of the relapse, its death occuring during the period of the maximum clinical manifestations of ophthalmoherpes and of the secondary peak of viremia. One more rabbit died on day 50, when the clinical signs of eye lesions, as well as the HSV titer in the blood, were reduced. Morphological and immunological studies of HSV antigens in the blood confirmed the development of a generalized herpetic infection, affecting not only the visceral organs (liver, lungs, kidneys) but also the brain.

A comparison of a number of parameters of the herpetic infection development, of the course of primary HKC, and of the relapse induced by holoxan injection showed a number of specific manifestations both of local lesions and of systemic infection developing in the organism against the background of immunosuppression. In the animals with induced immunosuppression, a more prolonged course of local lesions was observed, assuming the character of a lingering, subacute process. At the same time the lesions of the eye structures were more profound and severe, not only the inner layers of the corneal stroma but also the retina and blood vessels being involved. Viremia was noted during a more prolonged period this promoting a hematogenous dissemination and generalization of the herpetic infection against the background of immunosuppression.

The subsequent observation of the surviving animals over 6 months showed that a chronically relapsing form of ophthalmoherpes developed. The relapses appeared spontaneously each month with equal intervals of remission. At the end of one of the relapses, the death of one rabbit (out of four) demonstrating signs of encephalitis was observed on day 245. We should like to mention that all these relapses of ophthalmoherpes differ from the acute signs of primary infection and from the first relapse of the infection induced by holoxan; the severity of the corneal lesions was 2-3 times lower and the duration was 3-4 times lower.

Reactivation of latent HSV in the form of the development of herpetic keratitis relapse in rabbits injected with the cytostatic holoxan is in agreement with the results of other investigations [6,9,11-13], when ultraviolet radiation, cyclophosphamide or dexamethasone have been used for the induction of immunosuppression. The model described here is appreciably different. First of all, clinical manifestations of a relapse are noted in all the rabbits which received holoxan, while in the rabbits with an induced relapse of ophthalmoherpes, symptom-free virus secretion is observed as a rule [7,8]; in mice receiving cyclophosphamide, dexamethasone, and ultraviolet radiation clinical manifestations of a relapse were noted in just 22.2% of the animals (in mice treated with one of the drugs or ultraviolet radiation alone, there were no clinical signs of a relapse, and a symptom-free HSV secretion was all that was only observed) [11]; in guinea pigs with a latent infection UV radiation was shown to induce a relapse of genital herpes in 60% of animals [13].

A second difference is that the dose of holoxan injected into the rabbits is two times lower than the maximum single dose usually used for immunosuppression and does not produce any toxic effect, whereas during a combined application of cyclophosphamide and dexamethasone in separate series of experiments, the mortality of animals suffering from systemic toxicity has been shown to reach 44% [11].

A third difference is that the first signs of the development of a relapse induced by holoxan are observed earlier, only 24-48 hours after injection, while for application of the other chemical and physical immunodepressants, they have been shown to appear after 36-96 hours [11,13]. Besides, analysis of our own results and of data in the literature provides evidence that the reduced capabilities for self-restriction of virus reproduction and of infection inhibition do not depend upon the clinical form of primary infection in the animals during the latent HSV infection developing against the background of immunosuppression caused by cytostatic injection. As a result, not only do the duration and the severity of the local manifestations of the relapse increase but so does mortality. The latter is due to a higher degree of viremia and HSV dissemination in the organism, inducing lesions in almost all of the visceral organs, not just the brain. This occurs, as a rule, due to intraneural spreading of the virus in immunocompetent animals exhibiting one or another local or regional form of the clinical manifestations of herpetic pathology.

Thus, the results attest to the possibility of using holoxan for reactivating latent HSV and for inducting relapses of herpetic infection experimentally. The model described above has a number of advantages in comparison with models known previously, and can be used for an investigation of the molecular mechanisms of HSV latency and reactivation, as well as for analysis of the virological, immunological, and biochemical aspects of the development of the generalized form of herpetic infection.

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