

Effectiveness of Levamisole on Herpetic Ganglionic Latency^{*1)}

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(Received March 25, 1983)

Key words: Herpes simplex virus, Levamisole, Recurrence

ABSTRACT

Levamisole therapy was tested for its effect on herpetic trigeminal ganglionic latency in mice. There was no significant difference between recovery rate from levamisole-treated mice and that from non-treated mice during 12 weeks treatment. These results show that levamisole does not suppress herpetic ganglionic latency.

INTRODUCTION

The stromal form of herpetic simplex virus (HSV) keratitis has a poor prognosis, and the incidence of the recurrence is high¹⁾. The use of topical or systemic steroids in combination with antivirals is the most effective in the treatment of severe herpetic corneal disease, but neither the steroids nor the antivirals will prevent a recurrence of the disease^{1,10)}.

Interest was, recently, aroused by studies on improvement in herpetic infection with levamisole as an immunopotentiating agent¹⁸⁾. Clinically, it has been recognized that levamisole reduces the occurrence of subsequent stromal disease, with remarkable improvement in chronic herpetic keratitis⁹⁾.

The purpose of this study is to evaluate the effect of levamisole on viral latency in murine trigeminal ganglion.

MATERIALS AND METHODS

Virus: The Moto strain of herpes simplex virus was used. It was isolated from the cornea of a patient with keratitis disciformis and dendritic ulcer, and passed sixteen times in SIRC cells in our laboratory. The final titer was $10^{6.5}$ TCID₅₀/ml.

Cell cultures: SIRC cells were grown and maintained in Eagle's minimum essential medium containing fetal calf serum.

Animals: Male mice weighing 12 to 25 g were used in all experiments.

Experimental infection: Herpetic infection was produced by lightly scratching the left corneal epithelium in a cross-hatch manner (five across and five down) utilizing a 27 gauge needle. A 0.05 ml volume of $10^{6.5}$ TCID₅₀/ml was instilled onto the cornea. And then, the lids were gently rubbed together for one minute. Levamisole 25 μ g/kg was given intramuscularly on 3 consecutive days every week until a sacrifice.

Mice with no treatment after inoculation of HSV were served as controls.

Co-cultivation of trigeminal ganglia with monolayer cells: Left-sided trigeminal ganglia were excised at one week, four weeks and twelve weeks after HSV inoculation, cut into small pieces, and co-cultivated with monolayer cells of SIRC for four weeks. Trigeminal latency was estimated to be positive when cytopathic effect (CPE) appeared in monolayer cells during co-cultivation.

Identification of isolates: 1) New monolayers of SIRC cells were grown on cover slips, inoc-

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ulated with the supernatant in cultures which had exhibited CPE during co-cultivation, and incubated at 37°C for 24 to 48 hours. And then, monolayers were stained by the direct immunoperoxidase technique which was described previously¹⁶⁾. 2) The virus-producing ganglionic tissues were fixed in glutaraldehyde, post-fixed in osmic acid. Specimens were dehydrated in graded alcohols and embedded in epoxy resin. After thin sectioning by a Porter-Blum ultramicrotome, they were stained with uranyl acetate and lead citrate and examined in an electron microscope (Nihon Denshi).

RESULTS

1. Recovery of HSV from trigeminal ganglion: The treatment of levamisole did not influence the rate of HSV recovery from trigeminal ganglion. The rate of recovery in levamisole-treated groups was not lower than that in the non-treated groups at all three time points; one week, four weeks and twelve weeks after HSV inoculation (Table 1, 2 and 3).

Table 1. Virus recovery rate from explanted trigeminal ganglia of levamisole-treated and non-treated mice after one week treatment

| | levamisole-treated | non-treated |
|---------------|--------------------|-----------------|
| recovery rate | 11/12 (91.7%) | 13/13 (100%) |

Table 2. Virus recovery rate from explanted trigeminal ganglia of levamisole-treated and non-treated mice after four weeks treatment

| | levamisole-treated | non-treated |
|---------------|--------------------|------------------|
| recovery rate | 10/12 (83.3%) | 10/12 (83.3%) |

Table 3. Virus recovery rate from explanted trigeminal ganglia of levamisole-treated and non-treated mice after twelve weeks treatment

| | levamisole-treated | non-treated |
|---------------|--------------------|------------------|
| recovery rate | 9/12 (75.0%) | 10/13 (76.9%) |

2. Immunoperoxidase technique: 1) Light microscopic examination revealed characteristic dark brown peroxidase reaction product in the

cytoplasm and the nuclei. No specific stain was observed in control samples prepared in parallel in each experiment using normal IgG. The control studies of normal SIRC cells, using anti-HSV were negative (Fig. 1). 2) Electron microscopic examination showed remarkable degeneration and loss of organelles of the neurons, and no viral particles in the cells. SIRC cells around the ganglion contained many

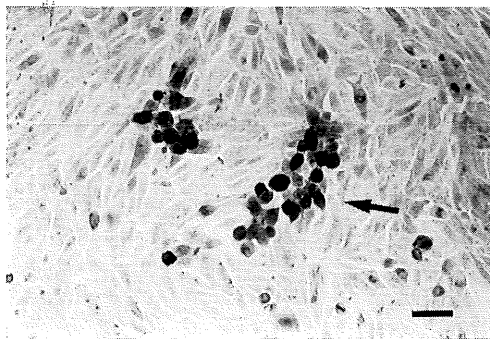


Fig. 1. Arrow indicates that HSV-infected cells are stained with peroxidase-labeled anti-HSV antibody. PLP fixative. $\times 200$. (Bar=0.5 mm)

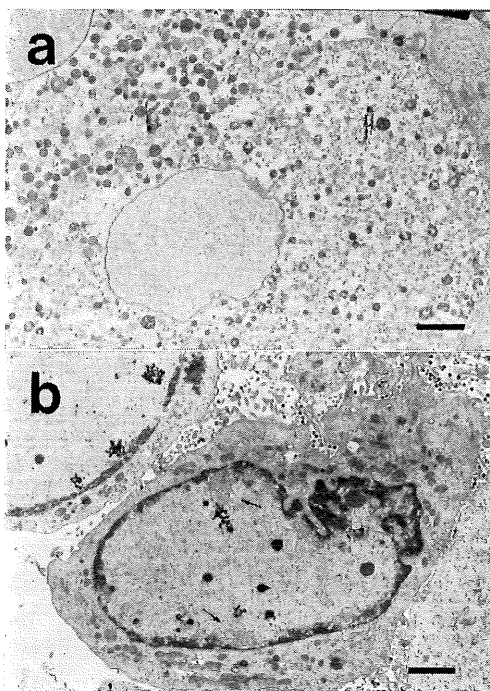


Fig. 2. Electron microscopic examination showed remarkable degeneration and loss of organelles of the neurons, and no viral particles in the cells (a). SIRC cells around the ganglion contained many viral particles (b). (Bar=0.5 μ)

viral particles (Fig. 2).

DISCUSSION

In 1970, Plummer and coworkers⁹⁾ demonstrated that HSV was recovered from local sensory ganglion and cord cells 6 to 11 months after rabbits had been inoculated intramuscularly with HSV. It had generally been assumed that HSV remains in genome within ganglion and cord cells, and is periodically reactivated to produce recurrent disease in peripheral organs.

Topical antimetabolite antiviral therapies^{8,10)} have been tried to suppress trigeminal ganglionic latency. However, these studies show that use of antivirals does not affect the recurrence or recovery rate.

As a radical therapy, an immunopotentiating agent is more hopeful than antivirals.

HSV antigens appears on the surface of the cells infected with HSV¹⁶⁾. Stevens and Cook¹⁴⁾, and Lehner and coworkers¹⁵⁾ suggested that antiviral antibody bound to the neuronal surface might be important in controlling latency in the ganglion. Hills and coworkers³⁾ described that virus recovery from the murine ganglion was increased after treatment with high dose of cyclophosphamide. Meanwhile, Openshaw and coworkers⁷⁾ reported that its ability to induce reactivation of HSV in the ganglion might be related to the ability of the drug to damage neuronal DNA rather than its immunosuppressive activity. But, the immunosuppressive mechanism is not always excluded, because Hill and coworkers⁷⁾ also described that treatment with antithymocyte serum induced recovery of HSV from the ganglia of mice. Ohashi and coworkers⁵⁾ presented that virus recovery rate was decreased in the mice treated with detergent-soluble extract of virus-infected cells. However, Scriba¹¹⁾ mentioned that inactivating vaccine of HSV was not effective on ganglionic latency of HSV in guinea pigs.

As described above, immunopotentiating therapy on ganglionic latency is not established, and controversial.

The recurrence rate for developing a subsequent lesion is very low in levamisole-treated patients with the stromal disease⁶⁾. But, the present study elucidated that levamisole dose not reduce ganglionic latency of HSV in mice. These observations led us to the "skin trigger theory" proposed by Hill and coworkers⁴⁾. It

was proposed that virus is frequently released from the ganglion but recurrent disease does not develop every time the virus invades the peripheral organs. Usually this virus is removed by host defence mechanisms and only when conditions in the peripheral organ particularly favour virus multiplication does a clinical lesion develop.

Levamisole enhances host defence mechanisms impaired, and particularly restores T cell²⁾ and macrophage function¹²⁾. Based on the clinical observations and our study, it is speculated that levamisole potentiates the defence mechanisms in the peripheral and local organs, and inhibits/reduces the development of a clinical lesion.

ACKNOWLEDGMENT

This work was supported in part by Grant-in-Aid for Encouragement of young scientist (No. 56770794) from the Ministry of Education, Japan.

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