ELSEVIER

Antiviral Research 36 (1997) 157–166

Antiviral Research

Evaluation of antioxidant healing formulations in topical therapy of experimental cutaneous and genital herpes simplex virus infections

J. Sheridan [a,*], E. Kern [b], A. Martin [c], A. Booth [c]

[a] *Ohio State University Health Sciences Center, Box 192 Postle Hall 305 West 12th Avenue, Columbus, Ohio 43210-1241, USA*
[b] *University of Alabama School of Medicine, Birmingham, Alabama, USA*
[c] *Warner-Lambert Worldwide Consumer Healthcare Research and Development, Morris Plains, New Jersey, USA*

Received 2 May 1997; accepted 2 September 1997

Abstract

A genital HSV-2 infection of guinea pigs and a cutaneous HSV-1 infection in mice were used to examine the ability of antioxidant components CRT[1] to reduce lesion development, duration, and severity. CRT is a patented antioxidant formulation developed by Warner-Lambert Worldwide Consumer Healthcare R. and D. CRT contains three components that work synergistically: vitamin E, sodium pyruvate and membrane stabilizing fatty acids. The MS strain of HSV-2 was utilized for intravaginal inoculation of guinea pigs with approximately 1.2×10^5 plaque forming units. The guinea pigs were treated on the external genital skin four times daily for 10 days beginning 48 h post viral inoculation. This study was designed to optimize the CRT formulations that would be used to quantify the synergistic effect of the CRT components. SKH-1 male hairless mice were inoculated with 1×10^7 HSV-1 (McIntyre strain) on the dorsal surface of the mouse and treated with CRT formulations starting on the afternoon of the day of infection, and treated for the following 14 days. In the guinea pig model, the CRT formula that contained all three CRT components, worked synergistically to reduce lesion development, duration and severity scores significantly compared to vehicle control or acyclovir. Acyclovir was the only compound that reduced viral titers, but in contrast to CRT, acyclovir did not reduce lesion development, duration or severity. The quantitative effect of the three CRT components was demonstrated in the mouse model. © 1997 Elsevier Science B.V.

Keywords: Antioxidant; Topical therapy; Herpes

1. Introduction

Primary and recurrent infections of the human oral cavity and the genital tract with herpes sim-

* Corresponding author. Tel.: +1 614 2922012; fax: +1 614 2926087; e-mail: sheridan.l@osu.edu

[1] CRT was known by the internal project name of Triad during these investigations.

PII S0166-3542(97)00049-1

plex virus type 1 (HSV-1) and type 2 (HSV-2) have reached epidemic proportions (Nahmias and Roizman, 1973; Embil et al., 1975; Johnson et al., 1989; Cowan et al., 1996). Primary infection of the genital tract is most often caused by HSV-2 and is generally more severe, resulting in multiple lesions on genitalia, accompanied by urinary tract involvement and neurologic complications (Nahmias and Roizman, 1973; Spruance et al., 1977; Hammer et al., 1980; Corey and Spear, 1986). HSV-1 is a common viral infection in humans which causes epidermal lesions in and around the oral cavity (Ship et al., 1967; Bastian et al., 1972; Embil et al., 1975; Young et al., 1976). The hallmark of an HSV infection is the ability of the virus to establish a latent infection in the nervous system, and to reactivate and cause recrudescent lesions (Bastian et al., 1972; Corey and Spear, 1986; Bonneau et al., 1991; Rodu et al., 1991). An estimated 98 million cases occur each year in the US (Embil et al., 1975; Cowan et al., 1996). Both HSV type 1 and 2 cause considerable discomfort to patients.

Several drugs are currently available for the treatment of HSV infections. Acyclovir is a prescription compound that interferes with viral DNA replication through its activation by viral thymidine kinase. Although extremely effective when given orally or intravenously for the treatment of primary or encephalitic HSV infections, acyclovir can be less effective topically.

During the inflammatory phase of the viral infection, there is a significant infiltration of white blood cells which release cytokines, proteases, and oxygen radicals, including hydroxyl radicals, hydrogen peroxide, and superoxide anions. Excess oxygen radicals can destroy or injure noninfected cells, thus prolonging the duration and severity of the HSV lesions. Excess oxygen radicals damage cell membranes, induce lipid peroxidation and can increase the intensity of the infection, leading to an increase in lesion duration and severity.

Antioxidants have been shown to enhance the healing of wounds (Slater and Block, 1991; Martin, 1996). They protect cells from oxygen radicals produced by exposure to UV light, injury, infections, or drugs (Martin, 1996). Each of the CRT components work differently to protect cells from oxygen radicals. Vitamin E, a lipid soluble antioxidant, protects cell membranes from oxygen radical damage. Sodium pyruvate is an intracellular antioxidant that protects DNA by degrading hydrogen peroxide, and enhances cellular growth and repair. Fatty acids are essential for membrane repair (Martin, 1996). The CRT components utilized in our studies (sodium pyruvate, vitamin E and membrane stabilizing fatty acids) have been shown to work synergistically to increase cellular proliferation (healing) and resuscitate injured cells (Martin et al., 1995; Martin, 1996). In pilot studies, CRT facilitated wound healing of bacterially-infected and noninfected wounds and reduced damage caused by over exposure to UV radiation (Martin, unpublished results). CRT was also used to reverse doxorubicin cytotoxicity in monocytes and reversed doxorubicin impaired wound healing in rats (Bauer et al., 1994).

The purpose of these studies was to determine the effect of topical CRT therapy on the development, duration and severity of primary lesions caused by HSV.

2. Materials and methods

2.1. Medications

The CRT preparations tested were provided by Warner-Lambert, Morris Plains, New Jersey. The 5% ACV-PEG was obtained from the University of Alabama Hospital Pharmacy. The experiment was placebo-controlled and the preparations were tested in a coded fashion (except for the ACV) (Spruance et al., 1977).

2.2. Genital HSV-2 infection of guinea pigs

2.2.1. Description of model

Intravaginal inoculation of weaning guinea pigs with HSV-2 results in a primary genital infection characterized by initial replication of virus in the vaginal tract followed by the development of external vesicular lesions. Virus titers peak on days 1–3 in the vaginal tract and gradually clear by days 7–10. The external genital lesions first appear on day 4, peak lesion severity occurs on days 6–8, and the lesions generally heal by days 15–18.

2.2.2. Virus and viral inoculation

The MS strain of HSV-2 was utilized for animal inoculation. Female Hartley guinea pigs (Charles River, Inc., Kingston, NY) weighing 300–350 g were inoculated intravaginally (i.vag.) with approximately 1.2×10^5 plaque forming units 1 h after being swabbed for removal of vaginal secretions. Viral inoculation was accomplished by inserting a swab soaked with virus into the vaginal tract and rotating about six times.

2.2.3. Treatment of guinea pigs

Groups of six guinea pigs were run in duplicate, consecutive experiments. The study was conducted as two identical experiments with six animals per group; a total of 12 animals were tested for each formulation. The guinea pigs were treated on the external genital skin with 0.1 ml each preparation, four times daily for 10 days beginning 48 h post-viral inoculation.

2.2.4. Sample collection and virus assays

To determine the effect of treatment on HSV-2 replication in lesions, swabs of lesions were obtained during primary infection on days 3, 4, 5, 6, 7 and 10 after HSV-2 inoculation. The swabs were taken prior to treatment each day from the external and internal vaginal areas. The swabs were placed in tubes containing 2.0 ml of media and frozen at -70°C until assayed for HSV. To identify the number of animals that became infected, vaginal swabs were obtained from all animals on day 5 and handled as above. When all samples were collected, they were thawed, vortexed, diluted serially and HSV-2 titers determined in rabbit kidney cells using a microtiter CPE assay.

2.2.5. Scoring of external lesions

To determine the effect of therapy on the development, spread and healing of external genital lesions, lesion severity was scored on 0–5+ scale through the primary infection.

2.2.6. Evaluation of efficacy

The data for each of the two experiments were combined and analyzed. Peak lesion scores, peak lesion virus titers, areas under lesion score-day and areas under the virus titer-day curves (area under the curve -AUC) between placebo- and drug-treated animals were compared using the Mann-Whitney U rank sum test. A P-value of 0.05 or less was considered significant.

2.3. Dorsal HSV infection of hairless mice (main study)

2.3.1. Description of model

Inoculation of hairless mice on abraded dorsal skin results in viral lesions that spread in a zosteriform pattern from the site of inoculation (midline on the back) to the abdominal area. The incubation period for this infection spans from day 0 to day 5 post infection (p.i.). Measurable herpetic lesions developed in all groups between 5 and 6 days p.i. The severity of the lesions continue to increase through day 7 p.i., and peak clinical signs occurred on day 8 p.i. The resolution phase of the infection occurs from day 9 to day 12 p.i. By day 10, lesions are crusted over and complete healing generally occurred by day 12 p.i.

2.3.2. Virus and viral inoculation

Male SKH-1 hairless mice (Charles River, Inc.), 6–8 weeks old were infected with HSV-1 McIntyre strain. Infection was achieved under general anesthesia (Ketamine, Xylazine) by the abrasion of a 1 cm square area (using a 25 gauge needle) centrally located on the dorsal surface of a mouse. Virus was then applied directly onto the abraded area (10 μl of a 1×10^9 PFU/ml virus stock). Following inoculation of 10^7 PFU of HSV-1 McIntyre strain by scarification of the epidermis, herpetic lesions developed by day 5 and persisted through day 12.

2.3.3. Scoring of external lesions

The viral lesions spread in a zosteriform pattern from the site of inoculation (midline on the back) to the abdominal area. By taking daily measurements of the lesions, a disease curve was constructed which consisted of three phases of the infection: incubation, log and resolution. The data, presented as the average lesion size (in mm^2) and lesion area (in mm^2), are shown in Table 3.

The incubation period for this infection spanned from day 0 to day 5 p.i. Measurable herpetic lesions developed in all groups between 5 and 6 days p.i. The severity of the lesions continued to increase in the log phase of the infection through day 7 p.i., and peak clinical signs occurred on day 8 p.i. The resolution phase of the infection occurred from day 9 to day 12 p.i. By day 10, lesions were crusted over and complete healing generally occurred by day 12 p.i. Data, including lesion scores, number of lesions, and lesion areas were recorded during the 07.00 h treatment session. Each animal was recorded as having one of five possible scores: 0, no signs; 1, redness and swelling; 2, single lesion; 3, multiple lesions; 4, sever spread of lesions in a zosterform pattern. In addition, the actual lesion area on the skin was measured using a micrometer (x and y axial values for each lesion were obtained in millimeters and then multiplied together to give the lesion area). For analysis, the individual lesion scores or areas within a treatment group were averaged on a daily basis.

2.3.4. Treatment of mice

Individual mice were treated with test compounds starting on the afternoon of the infection day and treatment was continued for 14 days. Treatments were administered at 07.00 and 16.00 h each day and involved the use of a sterile cotton-tipped applicator in such a manner that the affected area was evenly coated with the test compound. All groups contained a minimum of eight animals by the conclusion of the study. The test compounds consisted of either formulations that contained all combinations of two of the CRT components, or all three of the CRT components (vitamin E, sodium pyruvate and fatty acids). If no lesions were visible, only the site of infection was treated. Mice that showed no clinical signs for at least two consecutive days after inoculation of virus were considered to be uninfected and excluded from the study (the acyclovir control was an exception as this positive control was expected to prevent viral replication and reduce lesion formation).

2.3.5. Evaluation of efficacy

As stated above in Section 2.3.3, each animal was recorded as having one of five possible scores. For analysis, the individual lesion scores, or areas, within a treatment group were averaged on a daily basis. From the data gathered, mean lesion size, mean lesion peak area (severity), sum of lesion peaks, mean peak averages, and days to healing were determined and plotted.

3. Results

The purpose of these studies was to screen the activity of various CRT preparations administered topically in a primary genital HSV-2 infection of guinea pigs and to quantify the effect of the individual CRT components in an HSV-1 cutaneous infection of mice. Various preparations containing one, two or three CRT agents (vitamin E, sodium pyruvate and fatty acids) were evaluated to determine their ability to increase the rate of healing of lesions.

3.1. Effect of topical antioxidants on HSV-2 replication in genital lesions of guinea pigs

The commercial preparation of 5% acyclovir-PEG for cold sores was utilized as an internal control. The effect of topical antioxidants on virus titers is shown in Table 1. The only significant difference observed in lesion virus titer-day AUC's was with formulation 6 (acyclovir). All CRT formulations showed no significant reduction of viral titers, as evidenced by both virus titer AUC and mean peak virus titers. This was not unexpected since the CRT components should not alter viral replication. CRT was designed to reduce viral lesion severity, not as an antiviral agent.

3.2. Effect of topical antioxidants on lesion development in guinea pigs

A total of 20 different formulations of CRT with differing amounts of the three components were tested to determine the best ratio and bioavailibility of the components in the lotion

Table 1
Evaluation of topical antioxidants on lesion virus titers in a primary genital HSV-infection in guinea pigs

Formulation no.	Treatment[a]	No. vaginal virus/no. inoculated	Lesion virus titer-day area under curve	P value[b]	Mean peak lesion virus titer	*P* value[b]
1	Control—no CRT components	12/12	15.7	—	4.1	NS
2	Vitamin E and sodium pyruvate minus fatty acids	12/12	13.1	NS	3.5	NS
3	Vitamin E and fatty acids minus sodium pyruvate	12/12	14.0	NS	3.3	NS
4	Fatty acids and sodium pyruvate minus vitamin E	10/12	12.6	NS	3.3	NS
5 and 17	Vitamin E, fatty acids, sodium pyruvate (CRT)	12/12	13.2	NS	3.4	NS
6	5% ACV/PEG	12/12	6.1	<0.01	2.8	0.05

[a] Treatment was initiated at 48 h post-inoculation. Animals were treated four times per day for 10 days with 0.1 ml applied topically on external genitalia.
[b] All groups were compared with formulation no. 1 (vehicle only).
NS, not significant.

Table 2
Evaluation of topical antioxidants on lesion severity in a primary genital HSV-2 infection of guinea pigs

Formulation no.	Treatment[a]	Lesion score	*P* value[b]	Mean peak lesion score	*P* value
1	Control—no CRT components	27.4	—	3.1	NS[c]
17	Vitamin E, fatty acids and sodium pyruvate (CRT)	17.6	<0.01	2.4	0.06
6	5% ACV/PEG	27.8	NS	2.7	NS

[a] Treatment was initiated at 48 h post-inoculation. Animals were treated four times per day for 10 days with 0.1 ml applied topically on external genitalia.
[b] All groups were compared with formulation no. 1 (vehicle only).
NS, not significant.

base. The concentrations of the CRT components were either 0, 0.5, 4.75 or 9%. The results were analyzed and numerous three dimensional plots were made to show the multi-component effects. Since this was a pilot study and a means of screening for the optimized formulation, the range of formulations tested was very broad. The intent was to select a more refined set of formulation options to feed the main mouse study. The guinea pig model was chosen as the screening model because it produces a greater response to various formulations than the mouse model, thus allowing one to obtain an optimized formulation. The best formulations contained an equal ratio of the CRT components. The effect of the best CRT formulation on lesion development and severity are summarized in Table 2. The optimized three component CRT formulations that contained vitamin E, sodium pyruvate and fatty acids significantly reduced the lesion score compared with the vehicle control, acyclovir, and all other formulations. The three component CRT formulation also exhibited a significant reduction in peak lesion scores and time to healing. In contrast, all other formulations clearly resulted in significantly more severe disease than those that were treated with acyclovir. Formulations that contained all three components in approximately equal amounts performed best. Formulations that contained two of the CRT components produced poor results. Formulations that contained only one of the CRT components produced even worse results than the two component formulations. These results were used to select the formulations to be tested in the mouse model.

3.3. Effect of topical antioxidants on cutaneous HSV infection of mice

Results from the guinea pig experiment provided good evidence that for enhanced lesion healing all three CRT components were required. Whereas the guinea pig model was designed to measure the individual effects of each of the CRT components, the mouse study was meant to evaluate the synergistic effect of the three components. A 'One Minus' study design was selected. Three formulations, each lacking one of the three ingredients were prepared. These formulations and the full three component CRT formula were tested. The mouse model allowed us to quantitatively assess the contributions of each of the three CRT components. As shown in Table 3, the combination of two CRT components were less effective than all three components together. Lesion size, mean lesion peak area, sum of lesions, and mean peak averages were significantly reduced with formulations that contained all three of the CRT components. The data in Table 3 were analyzed using a linear component model. For each measurement variable (lesion size, mean lesion peak area, etc.) it was postulated that the difference from control (no CRT components) could be explained by a linear combination of a vitamin E effect, a sodium pyruvate effect, a fatty acid effect and a term called a synergy effect. For each measurement variable, four equations could be constructed and solved for the different effects. The results are shown in Table 4. For all measurement variables except 'days to healing' the syn-

Table 3
Synergistic analysis of CRT components, each at 4.75%[a], in SKH-1 hairless mice

Formulation no.	Treatment groups	Lesion size (mm^2)	Mean lesion peak area (severity) (mm^2)	Sum of lesion peaks (0–4 scale)	Mean peak averages (severity)	No. of days to healing
1	Control—no CRT components	130.8	51.1	17.5	3.38	12.0
2	Vitamin E and sodium pyruvate (CRT minus fatty acids)	110.4	40.9	18.5	3.14	12.0
3	Vitamin E and fatty acids (CRT minus sodium pyruvate)	100.2	36.9	16.8	3.43	11.8
4	Fatty acids and sodium pyruvate (CRT minus vitamin E)	128.7	48.3	18.1	3.24	12.0
5	Vitamin E, fatty acids and sodium pyruvate (CRT)	87.8	32.2	15.2	3.01	11.9

[a] Formulation no. 1: 100% petrolatum; formulation no. 5: 4.75% vitamin E, 4.75% sodium pyruvate, 4.75% fatty acids and 85.75% petrolatum.

ergy contribution was significant. This may be do to the fact that it takes mice approximately 5 days to develop lesions, 3 days to respond to the infection (inflammatory phase), leaving only 4 days to healing. The CRT components do not have antiviral activity, thus they can only enhance certain aspects of healing, lesion duration and severity. In order to express the three component synergy effect in more meaningful terms, the synergy effect was compiled as a percentage of the sum of the three individual components. For the five measurement variables showing synergistic effects the percentage synergy varied from 39 to 611%. These results suggest that the three components together (vitamin E, sodium pyruvate, and the membrane stabilizing fatty acids) have dramatically better effect than the sum of the individual component effect. CRT reduced lesion sizes 61% more than was predicted by the three component effect. CRT reduced mean lesion peak area (severity) 39% greater than the predicted three component effect. CRT reduced the sum of lesion peaks greater than 611% and reduced the mean peak averages 100% greater than the predicted three component effect. The days to healing showed very little variability. This, along with the analysis of the two component formulations, confirmed that the three CRT components were synergistic in their effect.

4. Discussion

The purpose of these studies was to determine the effect of topical CRT antioxidant therapy on lesion development, duration, severity and viral replication in the vaginal tract in guinea pigs and external skin lesions on mice. Acyclovir, a prescription compound that interferes with viral DNA replication, is effective when given orally or intravenously for the treatment of primary or encephalitic HSV infections. In this experiment, topical acyclovir reduced viral titers and also had some effectiveness reducing viral lesion development, duration, and severity (Whitley and Gnann, 1992). In contrast, antioxidants protect cells from oxygen radicals produced by exposure to UV light, injury, infection, or drugs and thus will enhance healing, but will not reduce viral replication (Martin, 1996).

Both the guinea pig and mouse models produced statistically and clinically significant results. The data from the studies showed that three CRT components reduced lesion development, duration and severity, but did not reduce viral titers. This was not unexpected, since CRT is not an antiviral agent. Acyclovir reduced viral titers, but its effect on viral lesion development, duration and severity was less effective than the three component CRT formulation (Tables 1–4). The mouse model was used to quantitatively measure the synergistic effect of the CRT components. In both models, the best formulas contained all three CRT components, and appeared to work synergistically to enhance healing. CRT components worked synergistically to reduce lesion development, duration, and severity. Excess oxygen radicals produced during the inflammatory phase of the viral infection may increase the ability of the viral infection to spread and grow, thus increase lesion size, duration and severity. Because CRT is not an antiviral agent, it can not reduce viral titers or viral infections, thus it may not be able to reduce the time to healing, which can only occur when viral replication is eliminated. Even though the time to healing did not change with the addition of CRT, the severity of the infection was decreased significantly. It would appear that the combination of components in CRT can protect cell membranes and cellular DNA from the damage produced by excess oxygen radicals, thus decrease inflammation that can increase the severity and duration of the lesions. The synergistic effect of the three components has already been demonstrated in other studies with keratinocytes, monocytes, and hepatocytes (Bauer et al., 1994; Martin, 1996). The CRT components utilized in these studies were also shown to increase cellular proliferation (healing) and resuscitate injured cells (Martin et al., 1995; Martin, 1996). In pilot studies, CRT facilitated wound healing of bacterially infected and noninfected wounds, and reduced damage caused by over exposure to UV radiation (Martin, unpublished results). CRT was also used to reverse doxorubicin cytotoxicity in monocytes and CRT also reversed doxorubicin-impaired

Table 4
Synergistic analysis of results of CRT components, each at 4.75%, in SKH-1 hairless mice

Row	Individual effects	Lesion size (mm^2)	Mean lesion peak area	Sum of lesion peaks (0–4 scale)	Mean peak averages (severity)	No. of days to healing
A	Vitamin E	−24.5	−10.8	−0.15	0.0	−0.1
B	Sodium pyruvate	+4.0	+0.6	+1.15	−0.2	+0.1
C	Fatty acids	−6.1	−3.4	−0.55	0.0	−0.1
D	Triad synergy	−16.4	−5.3	−2.75	−0.2	0.0
E	Total effect (CRT control)	−43.0	−18.9	−2.3	−0.4	0.1
F	Predicted 3 component effect	−26.6	−13.6	+0.45	−0.2	0.0
G	Synergy as a percentage of three component effect (row F)	61%	39%	611%	100%	0%

Row A–D, from model.
Row E, from Table 3; formulation no. 1–5 (also = sum of rows A+B+C+D).
Row F, predicted three component effect = sum of rows A+B+C.
Row G, row D as a percentage of row F.

wound healing in rats (Bauer et al., 1994). Elimination of any of the CRT components reduced the healing efficacy of the formulas in all the above studies. Thus, CRT can also act as a delivery system that protects drugs from destruction by oxygen radicals, producing a vehicle with sustained drug release capabilities. In subsequent studies, the combination of CRT and acyclovir reduced lesion development, duration, severity and viral titers in HSV infected animal models (Martin, unpublished data), indicating that CRT can enhance the activity of acyclovir and may also enhance the efficacy of other topical formulations for the treatment of HSV cutaneous and genital infections.

References

Bastian, F.O., Rabson, A.S., Yee, C.L., Tralka, T.S., 1972. Herpesvirus hominis: isolation from human trigeminal ganglion. Science 178, 306–307.

Bauer, G., O'Connel, S., Devereux, D., Martin, A., 1994. Reversal of doxorubicin-impaired wound healing using triad compound. Am. Surg. 60, 455–459.

Bonneau, R.H., Sheridan, J.F., Feng, N.G., Glaser, R., 1991. Stress-induction suppression of herpes simplex virus (HSV)-specific cytotoxic T-lymphocyte and natural killer cell activity and enhancement of acute pathogenesis following local HSV infection. Brain Behav. Immun. 5, 170–192.

Corey, L., Spear, P.G., 1986. Infection with herpes simplex viruses. New Engl. J. Med. 314 (1/2), 749–757.

Cowan, F.M., Johnson, A.M., Ashley, R., Corey, L., Mindel, A., 1996. Relationship between antibodies to herpes simplex virus (HSV) and symptoms of HSV infection. J. Infect. Dis. 174, 470–475.

Embil, J.A., Stephens, R.G., Manuel, F.R., 1975. Prevalence of recurrent herpes labialis and aphthous ulcers among young adults on six continents. Can. Med. Assoc. J. 113, 627–630.

Hammer, S.M., Buchman, T.G., D'Angelo, L.J., Karchmer, A.W., Roizman, B., 1980. Temporal cluster of herpes simplex encephalitis: investigation by restriction endonuclease cleavage of viral DNA. J. Infect. Dis. 141, 436–440.

Johnson, R.E., Nahmias, A.J., Madger, L.S., Lee, F.K., Brooks, C.A., Snowden, C.B., 1989. A seroepidemiologic survey of the prevalence of herpes simplex virus type 2 infection in the United States. New Engl. J. Med. 321, 7–12.

Martin, A., Kern, E.R., Sheridan, J., 1995. Evaluation of CRT healing components in two different topically treated cold sore animal models. Antiviral Res. A 26, 334.

Martin, A., 1996. The use of antioxidants in healing. J. Dermatol. Surg. 22, 156–160.

Nahmias, A.J., Roizman, B., 1973. Infection with herpes-simplex virus 1 and 2. New Engl. J. Med. 289, 781–789.

Rodu, B., Russell, C., Mattingly, G., 1991. Determining therapeutic efficacy in recurrent herpes labialis by lesion size analysis. Oral Surg. Oral Med. Oral Pathol. 72, 178–182.

Ship, I.I., Brightman, V.J., Laster, L.L., 1967. The patent with recurrent aphthous ulcers and the patient with recurrent herpes labialis: a study of two population samples. J. Am. Dental Assoc. 75, 645–654.

Slater, T., Block, G., 1991. Antioxidant vitamins and B-carotene in disease prevention. Am. J. Clin. Nutr. 53, 189–369.

Spruance, S.L., Overall, J.C. Jr., Kern, E.R., et al., 1977. The natural history of recurrent herpes simplex labialis: implications for antiviral therapy. New Engl. J. Med. 197, 69–75.

Whitley, R.J., Gnann, J.W., 1992. Acyclovir a decade later. New Engl. J. Med. 327, 782–789.

Young, S.K., Rowe, N.H., Buchanan, R.A., 1976. A clinical study for the control of facial mucocutaneous herpes virus infections. I. Characterization of natural history in a professional school population. Oral Surg. Oral Med. Oral Pathol. 41, 498–507.