Antiviral Research, 5 (1985) 83–91
Elsevier

AVR 00159

Effects of combined use of acyclovir and antibody in athymic nude mice inoculated intracutaneously with herpes simplex virus*

Masahiro Yamamoto, Yoshinobu Hayashi, Lie-Liu Tang and Ryoichi Mori**

Department of Virology, School of Medicine, Kyushu University, Fukuoka 812, Japan

(Received 26 June 1984; accepted 30 August 1984)

Summary

Antiviral effects of acyclovir (ACV) and antibody were studied in athymic nude mice inoculated intracutaneously in the midflank with herpes simplex virus type 1. Three hours after virus inoculation, treatment was initiated. In ACV-treated mice, the development of skin lesions was inhibited and the mean survival time was prolonged as compared with controls. Treatment with ACV markedly reduced the viral titers both at the inoculation site and in the neural tissues (dorsal root ganglia, spinal cord and brain). Transfer of antibody was also effective in controlling infection as described previously. The use of ACV plus antibody was more effective than the use of ACV alone and in 5 of 17 mice the disease was completely inhibited. Furthermore, in the mice that survived, latent infections in the ganglia were also prevented. These results indicate that treatment with ACV plus antibody is highly effective against herpes simplex virus infection in the immunocompromised host.

acyclovir; herpes simplex virus; nude mouse; immunocompromised host; antibody

Introduction

Acyclovir [ACV, 9-(2-hydroxyethoxymethyl)guanine, or acycloguanosine] has

* Supported by Grant-in-Aid for Scientific Research from Ministry of Education, Science and Culture of Japan.

** To whom all correspondence should be addressed.

been introduced as a potent and selective antiviral agent against herpes simplex virus (HSV) infection [4]. Since HSV can cause severe infections in immunocompromised humans [18], the potential of ACV for therapy of HSV infections in such patients has been investigated [8,15,16,23,25,27,28]. In animal studies, the effects of ACV on infection with HSV have been investigated in nonimmunosuppressed mice [1,5–7,11,13,19,20], guinea pigs [21,24,26], and rabbits [22], as well as in cyclophosphamide-treated mice [14].

We have reported dissemination of HSV in nude mice after intracutaneous inoculation at the midflank [10,12,17]. Because of the complete deletion of a mature T cell population, infection with HSV in athymic nude mice provides a useful model of HSV infection in immunocompromised hosts. Furthermore, this system has the advantage that skin lesions are readily visible and to a certain extent, can be assessed quantitatively, although it has only rarely been used to assess the efficacy of antiherpes drugs [3].

In the present study, we have evaluated the protective effects of ACV against HSV infection in the nude mouse system. ACV was used as such or concomitantly with antibody.

Materials and Methods

Virus

HSV type 1 (HSV-1), strain Hayashida, an isolate from a vesicular skin lesion of herpes labialis, was passaged 5 times in Vero cells and used for inoculation. Vero cells grown in Eagle's minimal essential medium (MEM) supplemented with 5% calf serum were used for virus propagation and titration.

Inoculation of mice

6-week-old female nude (nu/nu) mice with BALB/c genetic background were purchased from the Central Laboratory of Experimental Animals, Co., Ltd., Osaka, Japan. The mice were inoculated intracutaneously in the midflank with 1×10^5 plaque-forming units (p.f.u.) of HSV-1 per 0.05 ml as previously described [17]. The skin lesions were scored every day. Scores of skin lesions are as follows: 0, no lesion; 2, local lesion (vesicle or erosion); 6, mild zosteriform lesion; 8, moderate zosteriform lesion; 10, severe zosteriform lesion; and death.

ACV

ACV (kindly provided by Nippon Wellcome Co., Ltd., Osaka, Japan) was dissolved in phosphate-buffered saline (PBS), pH 7.2, at a concentration of 5 mg/ml.

Human gamma-globulin

Human gamma-globulin (kindly provided by the Chemo-Sero-Therapeutic Research Institute, Kumamoto, Japan) is a preparation from pooled human plasma and had a neutralizing titer of 1 : 64 against HSV-1. The neutralizing activity of human gamma-globulin was measured using the microplate method described previously [9], where the neutralizing antibody titer was expressed as the highest dilution which showed 80% plaque reduction.

Determination of virus titers in mouse tissues

At various times after virus inoculation, mice were sacrificed for determination of virus titers in their tissues. Tissue samples included the skin (the minimum area encompassing the inoculated site and the whole lesion), dorsal root ganglia from L (lumbar)-1 to L-5, spinal cord in the lumbosacral area, brain, spleen, liver and kidney. Dorsal root ganglia were homogenized with a mortar and pestle in 1 ml MEM supplemented with 2% calf serum, and 10-fold serial dilutions were made from the homogenates. The other organs and tissues were frozen at −70°C until they were assayed. The organ samples were homogenized with a Teflon homogenizer, and the skin was separately homogenized with quartz sand and a mortar and pestle in 5 ml of medium. After centrifugation at 2000 rev/min for 10 min, serial 10-fold dilutions were made from the supernatant fluids. Each dilution was inoculated onto Vero cell monolayers in 24-well tissue culture plates and allowed to adsorb for 1 h before the cells were overlaid with MEM containing 2% calf serum and 2% methyl cellulose.

After incubation for 3 days, the cells were stained with 0.5% crystal violet, and the plaques were counted. The titers of virus recovered from the various homogenates were expressed as p.f.u. per organ or tissue.

Cocultivation of dorsal root ganglia with Vero cells

Mice that survived 60 days after inoculation were sacrificed. The dorsal root ganglia from L-1 to L-5 were removed and were put onto monolayers of Vero cells. The cultures were examined daily for four weeks for the appearance of viral cytopathic effect.

Results

Protective effects of ACV on the course of HSV infection

In the first experiment, treatment with ACV was initiated 3 h after intracutaneous inoculation with HSV at the midflank in nude mice. ACV was administered intraperitoneally (25 mg/kg of body weight) every 12 h for a total of 28 injections (700 mg/kg of body weight). As shown in Fig. 1, local skin lesions (vesicle or erosion) appeared by day 4 after infection in the PBS-treated controls. The lesion began to spread after day 6 and developed into a zosteriform skin lesion, and all mice died within 20 days of infection. The development of HSV skin lesions in control nude mice was similar to the pattern described in our previous papers [10,12,17].

In the ACV-treated mice, the skin lesion developed more slowly. In this group, the local lesions appeared after day 9, and development of skin lesions at a later time was suppressed as long as the ACV treatment was continued. The lesion remained at the site of inoculation for up to 15 days and developed into zosteriform lesions after day 20. Five of 6 ACV-treated mice eventually died between day 21 and day 30. The mean survival time was significantly prolonged in ACV-treated mice (24.0 days) as compared with controls (12.3 days, $P<0.001$).

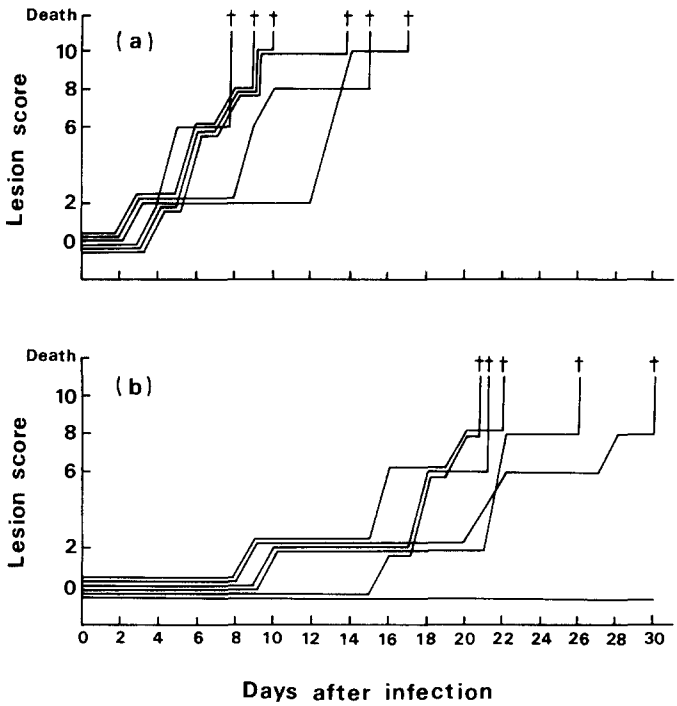

Fig. 1. Effect of ACV on the development of skin lesions after intracutaneous inoculation with HSV-1 in nude mice. (a) PBS-treated control mice. (b) ACV-treated mice. ACV was administered intraperitoneally (25 mg/kg of body weight) every 12 h beginning 3 h after inoculation and continuing for 14 consecutive days. Nude mice were inoculated with 1×10^5 p.f.u. HSV-1 intracutaneously in the midflank. Each line represents the development of skin lesions of one mouse. The symbol (+) indicates the death of the mouse.

Effects of ACV on viral growth in mouse tissues

Fig. 2 presents the virus titers of tissue homogenates from control and ACV-treated mice at various intervals after infection. The organs from which the virus could be recovered were the skin, dorsal root ganglia, spinal cord and brain. No virus could be recovered from the liver, spleen and kidney throughout the experiment.

In the control mice, the virus titer in the skin exceeded 10^4 p.f.u. per tissue from the first day after inoculation, and rose as the zosteriform lesions began to develop. The virus could be recovered from the homogenates of dorsal root ganglia as early as day 4 after infection, and from the spinal cord by day 7. The titers of dorsal root ganglia and spinal cord rose gradually until death of the mice. The virus could be recovered from the brain after day 10 mostly in moribund mice. The highest titer in each organ was recorded just before death.

Tissue samples from the ACV-treated mice showed a striking reduction in the viral titers. In mice treated with ACV, the virus titer in the skin was about 10-fold less than that in control mice on day 1 after infection, and declined slowly until day 13. A 5000-fold reduction of virus titer in the skin was observed on day 13. From day 16, the

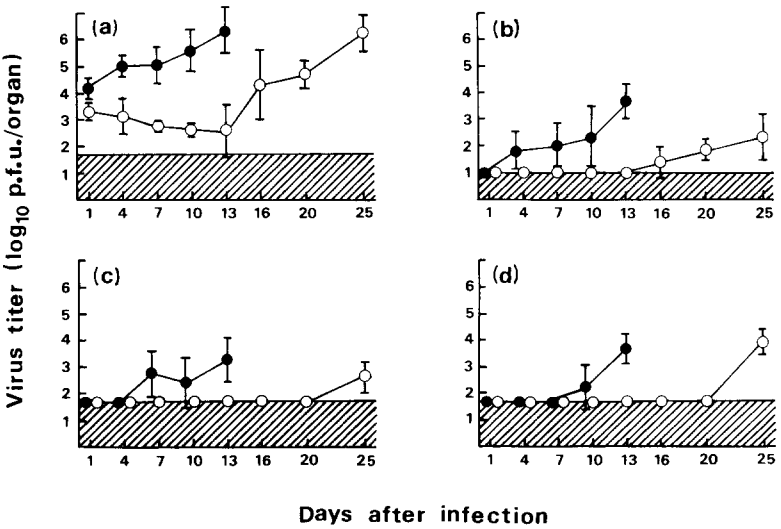

Fig. 2. Effect of ACV on viral growth in mouse tissues after intracutaneous inoculation with HSV-1. (a) Skin; (b) dorsal root ganglia; (c) spinal cord; (d) brain. Nude mice were inoculated with 1×10^5 p.f.u. of HSV-1 intracutaneously in the midflank and given PBS (●) or 25 mg of ACV per kg of body weight (○) every 12 h intraperitoneally. Treatment was initiated 3 h after inoculation and continued for the times indicated until day 14. Each point represents the geometric mean titer of virus from 3 mice ± the standard deviation. The hatched portion of each graph indicates the threshold of virus detection.

titer rose along with the development of the zosteriform skin lesions. This is likely due to cessation of ACV treatment. For 13 days after infection, no virus was recovered from the dorsal root ganglia as long as the skin lesions were limited to the inoculated area. Virus could be recovered from the spinal cord and brain only from moribund mice.

Effects of combined use of ACV and human gamma-globulin

Since cessation of administration of ACV caused multiplication of HSV and finally death of nude mice, the effects of combined use of ACV and human gamma-globulin were tested. ACV was administered intraperitoneally (25 mg/kg of body weight) beginning 3 h after the virus inoculation and was given every 12 h for a total of 22 injections (550 mg/kg of body weight). One milliliter of human gamma-globulin was injected intraperitoneally only once 3 h after the virus inoculation. Mice which received only ACV, gamma-globulin or PBS served as controls.

The development of skin lesions in each group is shown in Fig. 3 and the results are summarized in Table 1. When human gamma-globulin was given to lethally HSV-infected nude mice, the development of zosteriform skin lesions was delayed and survival time was also prolonged as described previously [10,12]. Effects of ACV were almost the same as those indicated in the experiment shown in Fig. 1.

The use of ACV plus human gamma-globulin was more effective. The development of skin lesions was suppressed and the mean survival time was significantly prolonged as compared with the use of ACV alone. Furthermore, 5 out of 17 nude mice that were

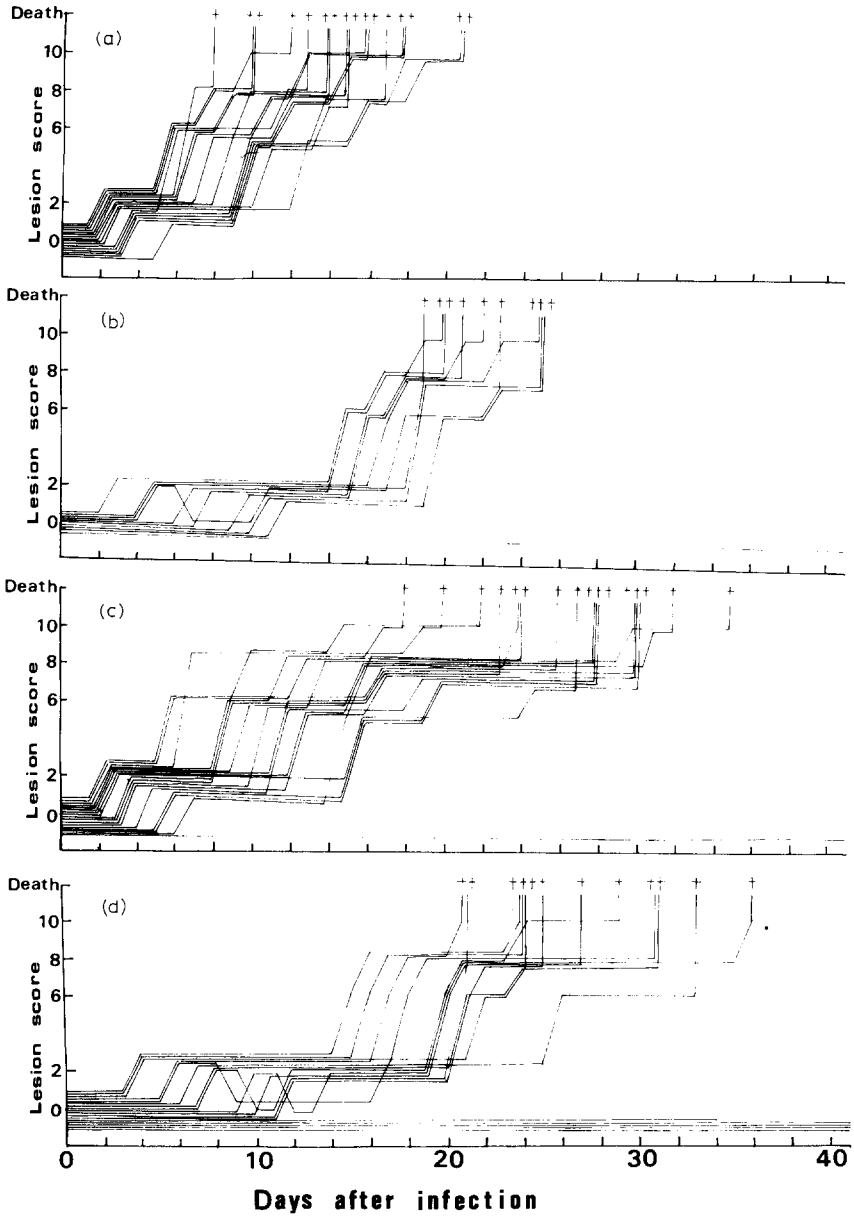

Fig. 3. Effect of ACV and human gamma-globulin on the development of skin lesions after intracutaneous inoculation with HSV-1 in nude mice. (a) PBS-treated control mice. (b) ACV-treated mice. ACV was administered intraperitoneally (25 mg/kg of body weight every 12 h) beginning 3 h after inoculation and continuing for 11 consecutive days. (c) Human gamma-globulin-treated mice. One milliliter of human gamma-globulin with a titer of 1 : 64 was administered intraperitoneally 3 h after inoculation. (d) ACV plus human gamma-globulin-treated mice. ACV was administered intraperitoneally in 25 mg/kg doses beginning 3 h after inoculation and was given every 12 h for 11 consecutive days. One milliliter of human gamma-globulin with a titer of 1 : 64 was administered intraperitoneally only once 3 h after virus inoculation.

TABLE 1

Summary of results of the experiments shown in the Fig. 3

Group*	Mean survival days** ± S.D.	Number of mice without skin lesions
(a) Control	14.9 ± 3.7 (n = 16)	0 (0%)
(b) ACV	22.3 ± 2.5 (n = 9)	1 (10%)
(c) HGG***	26.6 ± 4.5 (n = 16)	1 (6%)
(d) ACV + HGG	27.7 ± 5.0 (n = 12)	5 (30%)

*For (a), (b), (c) and (d), see legend of Fig. 3.
**For calculation, mice surviving longer than 60 days were excluded.
***Human gamma-globulin.

treated by ACV and human gamma-globulin did not develop diseases. Those mice which failed to develop any skin lesions were killed 60 days after challenge, but no virus was recovered from the dorsal root ganglia even after cocultivation with Vero cells.

Discussion

Our findings demonstrate that ACV treatment is effective against intracutaneous HSV infection in athymic nude mice. The use of ACV plus antibody completely protected some mice from latent infection of the regional nerve ganglia, as evidenced by the failure of detection of HSV after cocultivation with Vero cells. Using euthymic animals, significant therapeutic efficacy of ACV against cutaneous HSV infections has been established in mice [1,5–7,11,13,19] and in guinea pigs [21,26].

It is well known that HSV can cause severe localized and disseminated infections in immunocompromised humans [18]. HSV infection in athymic nude mice serves as an adequate model for the study of HSV infection in immunocompromised hosts [10,12,17]. Although the potential of ACV for therapy of HSV infection in immunocompromised patients has been investigated [8,15,16,23,25,27,28], more basic data on experimental studies are needed. The present study was designed to evaluate the protective effects of ACV and antibody against HSV infection in the nude mouse system.

In ACV-treated nude mice, the development of skin lesions and virus replication in the skin were strongly inhibited, and no virus could be recovered from the dorsal root ganglia, spinal cord and brain, as long as ACV treatment was continued. ACV appeared to suppress acute ganglionic infection and subsequent retrogressive and ascending infection in nude mice. However, after cessation of treatment with ACV, skin lesions developed and viral titers of dorsal root ganglia rose gradually. Eventually, the mice died.

We have previously reported [10,12] that in nude mice given antibody, the development of skin lesions is inhibited and the mean survival time is prolonged significantly.

The inhibitory effect of antibody on the course of infection originates from the suppression of virus growth, not only at the inoculated skin site, but also in the dorsal root ganglia and subsequent retrogressive and ascending infection. However, HSV reaches the dorsal root ganglia as early as 48 h after infection from the midflank, and the mice die eventually with the clearance of administered antibody [12].

The prominent effect of concomitant use of ACV and antibody is that complete suppression of infection is achieved in at least a certain proportion of the mice, although each treatment alone is not sufficient to bring about complete suppression of the infection. The same potentiating effect has been reported by Cho et al. by using adenine arabinoside and human immune globulin in a euthymic mouse system [2]. In their experiments, synergistic antiviral effects of adenine arabinoside and human antibody were obtained only in normal mice and not in the thymus deficient nude mice, thus emphasizing the necessity of intact host defense mechanisms for the enhanced protection. However, our results indicate that the concomitant use of ACV and antibody is effective in nude mice. The reason for this discrepancy is not clear.

The concomitant use of ACV and antibody may be effective in the prophylaxis of HSV infections in immunocompromised patients and should be the subject of further studies.

Acknowledgement

We thank Dr. Cynthia C. Smith for reviewing the manuscript.

References

1 Blyth, W.A., Harbour, D.A. and Hill, T.J. (1980) Effect of acyclovir on recurrence of herpes simplex skin lesions in mice. J. Gen. Virol. 48, 417–419.

2 Cho, C.T., Feng, K.K. and Brahmacupta, N. (1976) Synergistic antiviral effects of adenine arabinoside and humoral antibodies in experimental encephalitis due to herpes virus hominis. J. Infect. Dis. 133, 157–167.

3 De Clercq, E., Zhen-Xi, Z., Descamps, J. and Huygen, K. (1981) E-5-(2-Bromovinyl)-2′-deoxyuridine vs. interferon in the systemic treatment of infection with herpes simplex virus of athymic nude mice. J. Infect. Dis. 143, 846–852.

4 Elion, G.B., Furman, P.A., Fyfe, J.A., de Miranda, P., Beauchamp, L. and Schaeffer, H.J. (1977) Selectivity of action of an antiherpetic agent, 9-(2-hydroxyethoxymethyl)guanine. Proc. Natl. Acad. Sci. U.S.A. 74, 5716–5720.

5 Field, H.J., Bell, S.E., Elion, G.B., Nash, A.A. and Wildy, P. (1979) Effect of acycloguanosine treatment on acute and latent herpes simplex infections in mice. Antimicrob. Agents Chemother. 15, 554–561.

6 Field, H.J. and De Clercq, E. (1981) Effect of oral treatment with acyclovir and bromovinyldeoxyuridine on the establishment and maintenance of latent herpes simplex virus infection in mice. J. Gen. Virol. 56, 259–265.

7 Field, H.J. and Neden, J. (1982) Isolation of bromovinyldeoxyuridine-resistant strain of herpes simplex virus and successful chemotherapy of mice infected with one such strain by using acyclovir. Antiviral Res. 2, 243–254.

8 Gluckman, E., Latsberg, J., Devergie, A., Zhao, X.M., Melo, R., Gomez-Morales, M., Mazeron, M.C. and Perol, Y. (1983) Oral acyclovir prophylactic treatment of herpes simplex infection after bone marrow transplantation. J. Antimicrob. Chemother. 12, Suppl. B, 161–167.

9 Hayashi, Y., Nagata, M., Yoshizumi, H., Mori, R., Navarro, C.V. and Yang, H.D. (1984) Difference in age distribution of antibody to herpes simplex virus in Fukuoka, Manila and Busan. Jpn. J. Med. Sci. Biol. 37, 35–39.

10 Hayashida, I., Nagafuchi, S., Hayashi, Y., Kino, Y., Mori, R., Oda, H., Ohtomo, N. and Tashiro, A. (1982) Mechanism of antibody-mediated protection against herpes simplex virus infection in athymic nude mice: requirement of Fc portion of antibody. Microbiol. Immunol. 26, 497–509.

11 Hill, T.J., Blyth, W.A. and Harbour, D.A. (1982) Recurrent herpes simplex in mice; topical treatment with acyclovir cream. Antiviral Res. 2, 135–146.

12 Kino, Y., Hayashi, Y., Hayashida, I. and Mori, R. (1982) Dissemination of herpes simplex virus in nude mice after intracutaneous inoculation and effect of antibody on the course of infection. J. Gen. Virol. 63, 475–479.

13 Klein, R.J., Friedman-Kien, A.E. and DeStefano, E. (1979) Latent herpes simplex virus infections in sensory ganglia of hairless mice prevented by acycloguanosine. Antimicrob. Agents Chemother. 15, 723–729.

14 Mayo, D.R., Richards, J.C. and Rapp, F. (1979) Acycloguanosine treatment of herpesvirus infections in footpads and nervous tissue of normal and immunosuppressed mice. Intervirology 12, 345–348.

15 Meyers, J.D., Wade, J.C., Mitchell, C.D., Saral, R., Lietman, P.S., Durack, D.T., Levin, M.J., Segreti, A.C. and Balfour, H.H., Jr. (1982) Multicenter collaborative trial of intravenous acyclovir for treatment of mucocutaneous herpes simplex virus infection in the immunocompromised host. Am. J. Med. 73 (1A), 229–235.

16 Mitchell, C.D., Bean, B., Gentry, S.R., Groth, K.E., Boen, J.R. and Balfour, H.H., Jr. (1981) Acyclovir therapy for mucocutaneous herpes simplex infection in immunocompromised patients. Lancet 1, 1389–1392.

17 Nagafuchi, S., Oda, H., Mori, R. and Taniguchi, T. (1979) Mechanism of acquired resistance to herpes simplex virus infection as studied in nude mice. J. Gen. Virol. 44, 715–723.

18 Nahmias, A.J. and Roizman, B. (1973) Infection with herpes-simplex viruses 1 and 2. N. Engl. J. Med. 289, 781–789.

19 Park, N.H., Pavan-Langston, D. and McLean, S.L. (1979) Acyclovir in oral and ganglionic herpes simplex virus infection. J. Infect. Dis. 140, 802–806.

20 Park, N.H., Pavan-Langston, D., McLean, S.L. and Albert, D.M. (1979) Therapy of experimental herpes simplex encephalitis with acyclovir in mice. Antimicrob. Agents Chemother. 15, 775–779.

21 Park, N.H., Pavan-Langston, D., McLean, S.L. and Lass, J.J. (1980) Acyclovir topical therapy of cutaneous herpes simplex virus infection in guinea pigs. Arch. Dermatol. 116, 672–675.

22 Pavan-Langston, D., Campbell, R. and Lass, J. (1978) Acyclic antimetabolite therapy of experimental herpes simplex keratitis. Am. J. Ophthalmol. 86, 618–623.

23 Prentice, H.G. (1983) Use of acyclovir for prophylaxis of herpes infections in severely immunocompromised patients. J. Antimicrob. Chemother. 12, Suppl. B, 153–159.

24 Pronovost, A.D., Lucia, H.L., Dann, P.R. and Hsiung, G.D. (1982) Effect of acyclovir on genital herpes in guinea pigs. J. Infect. Dis. 145, 904–908.

25 Saral, R., Burns, W.H., Laskin, O.L., Santos, G.W. and Lietman, P.S. (1981) Acyclovir prophylaxis of herpes virus infections: a randomized, double-blind, controlled trial in bone-marrow transplant recipients. N. Engl. J. Med. 305, 63–67.

26 Schaeffer, H.J., Beauchamp, L., de Miranda, P., Elion, G.B., Bauer, D.J. and Collins, P. (1978) 9-(2-Hydroxyethoxymethyl)guanine activity against viruses of the herpes group. Nature (London) 272, 583–585.

27 Straus, S.E., Smith, H.A., Brickman, C., de Miranda, P., McLaren, C. and Keeney, R.E. (1982) Acyclovir for chronic mucocutaneous herpes simplex virus infection in immuno-suppressed patients. Ann. Intern. Med. 96, 270–276.

28 Wade, J.C., Newton, B., McLaren, C., Flournoy, N., Keeney, R.E. and Meyers, J.D. (1982) Intravenous acyclovir to treat mucocutaneous herpes simplex virus infection after marrow transplantation – a double blind trial. Ann. Intern. Med. 96, 265–269.