ELSEVIER

Antiviral Research 33 (1996) 65–72

Antiviral Research

Effect of 9-(4-hydroxybutyl)-N^2-phenylguanine (HBPG), a thymidine kinase inhibitor, on clinical recurrences of ocular herpetic keratitis in squirrel monkeys

Herbert E. Kaufman[a], Emily D. Varnell[a,*], George E. Wright[b], Hongyan Xu[b], Bryan M. Gebhardt[a], Hilary W. Thompson[a]

[a]*Lions Eye Research Laboratories, LSU Eye Center, Louisiana State University Medical Center School of Medicine, 2020 Gravier Street, Suite B, New Orleans, LA 70112, USA*
[b]*Department of Pharmacology, University of Massachusetts Medical School, Worcester, MA 01655 USA*

Received 27 March 1996; accepted 29 July 1996

Abstract

9-(4-Hydroxybutyl)-N^2-phenylguanine (HBPG) is a new viral thymidine kinase inhibitor that we tested for the ability to prevent recurrences of herpetic keratitis. Eighteen squirrel monkeys (*Saimiri scuireus*) were infected in both corneas with the Rodanus strain of herpes simplex virus type 1 (HSV-1). All corneas showed typical dendritic keratitis 3 days after infection, followed by spontaneous healing. On day 21, the monkeys were randomized into two coded groups and ocular examinations were begun. One group received intraperitoneal (i.p.) injections of HBPG, 150 mg/kg, in a corn oil suspension every 8 h, and the other group received i.p. injections of the corn oil vehicle only. On day 22, recurrences were induced by reducing the temperature of the room in the late afternoon so that a low of 18°C was achieved during the night. After the morning treatment, room temperature was raised to the normal ambient temperature (24–27°C), and treatment was discontinued. Treatment was reinstituted on day 27, the room temperature was lowered again on day 28, and treatment was again discontinued as before. Third and fourth cycles of treatment and cold stress were begun on days 34 and 69. Ocular examinations were continued until day 73, at which point the code was broken. We found that the HBPG treatment significantly reduced the number of corneas with recurrences during the treatment periods, compared with recurrences in untreated, cold-stressed animals ($P = 0.01$).

Keywords: Antiviral; Herpes simplex virus (HSV); Recurrent infections; *Saimiri scuireus*; Thymidine kinase

* Corresponding author. Tel.: +1 504 5686700, ext. 336; fax: +1 504 5684210; e-mail: evarne@lsumc.edu

0166-3542/96/$15.00 © 1996 Elsevier Science B.V. All rights reserved
PII S0166-3542(96)00997-7

1. Introduction

The herpes viruses are common pathogens in humans and a variety of animal species (Straus et al., 1985; Mertz, 1990). Herpes simplex virus type 1 (HSV-1) and herpes simplex virus type 2 (HSV-2) infect epithelial and mucosal surfaces, causing painful lesions and considerable emotional stress (Whitley, 1990). It is well known that, coincident with acute herpes infection, the virus enters the nervous system and that viral latency and the potential for reactivation and recurrent disease are a lifelong threat (Mertz, 1990; Roizman and Kaplan, 1992).

Many years ago, we developed the first effective antiviral for the topical treatment of ocular HSV-1 infection (Kaufman, 1962; Kaufman and Maloney, 1962; Kaufman et al., 1962). Since then, numerous anti-herpetic drugs have been designed and tested; the current armamentarium includes thymidine analogs, arabinonucleosides, and various acyclonucleosides (Elion, 1985; Beyer et al., 1989; Mansuri and Martin, 1991; Lapucci et al., 1993). Virtually all of the drugs that are presently available shorten the duration of acute herpes infection of end organs such as the ocular surface and the genital tract.

A major goal in antiviral drug development is the design of chemotherapeutic agents that specifically inhibit viral reactivation from latency. Because HSV-1 achieves latency in the nervous system and because reactivation from latency depends on certain viral enzymes, many investigators have sought to target virally encoded enzymes with drugs that inactivate or compete with viral synthetic mechanisms (Elion, 1985; Leib et al., 1990; Nsiah et al., 1990; Kaufman et al., 1991; Bourne et al., 1992; Lapucci et al., 1993; Taylor et al., 1994). Viral thymidine kinase has been widely studied in this regard (Nutter et al., 1987; Martin et al., 1989a,b; Spadari and Wright, 1989; Klein and Czelusniak, 1990; Kim et al., 1993), and the status of inhibitors of this enzyme has been reviewed (Wright, 1994). Inhibitors of HSV thymidine kinase were first proven to be effective in preventing viral reactivation in an in vitro explant-cocultivation model of latently infected murine trigeminal ganglia (Leib et al., 1990).

Some of the currently available chemotherapeutic agents have been shown to be effective in reducing HSV-1 and HSV-2 recurrences in very specific circumstances (Park et al., 1979; Saral et al., 1981; Straus et al., 1984; Douglas et al., 1984; Meyrick Thomas et al., 1985, Spruance et al., 1988; Rooney et al., 1993). For example, acyclovir is effective in reducing the viral recurrence rate in patients with urogenital HSV-2 infection (Corey et al., 1982; Douglas et al., 1984; Mindel et al., 1984, 1988; Straus et al., 1984; Gold and Corey, 1987). Similarly, acyclovir is effective in reducing the frequency of recurrences of oral–facial HSV-1 (Spruance et al., 1988; Rooney et al., 1993). Unfortunately, neither systemic nor topical acyclovir has been proven effective in preventing ocular recurrences of HSV-1 infection (Sanitato et al., 1984; Collum et al., 1986). Barney and Foster (1994) found that acyclovir reduced the recurrence rate of ocular herpes after keratoplasty, but steroids were used after the surgery and antivirals are known to reduce recurrence rates after topical corticosteroid administration. Legmann and Langston (1995) presented a non-randomized, retrospective study of a small group of patients who seemed to have fewer recurrences when given oral acyclovir, and there is an NIH-sponsored study (Herpetic Eye Disease Study) in progress to determine if oral acyclovir is useful in preventing herpetic recurrences. To date, however, there are no conclusive data that establish the value of acyclovir in preventing ocular herpetic recurrences in patients who are not treated with steroids.

Recently, we have been successful in synthesizing a compound that potently and specifically inhibits herpetic viral thymidine kinases (Xu et al., 1995). This compound, 9-(4-hydroxybutyl)-N^2-phenylguanine (HBPG), has physicochemical properties that make it suitable for testing in vivo. HBPG has been found to be active in preventing HSV-1 reactivation in a mouse model of heat stress-induced reactivation (Gebhardt et al., 1996). Using our recently described primate model of hypothermic induction of HSV-1 reactivation (Varnell et al., 1995), we tested HBPG for the ability to reduce ocular recurrences of herpetic keratitis in monkeys.

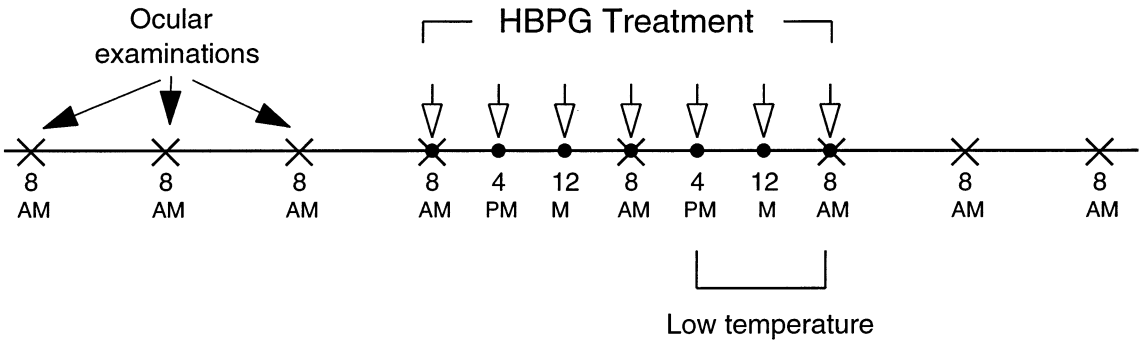

Fig. 1. Diagram of experimental design. Monkeys were treated every 8 h with i.p. injection of 150 mg/kg HBPG or corn oil. At the time of the fifth injection, the ambient temperature was reduced to 16–18°C for a period of 16 h, during which time two additional treatments were given. Eyes were examined every morning at 08:00 (X) for several days before, during, and several days after the period of hypothermic stress (see Fig. 3).

2. Materials and methods

2.1. Animals

Eighteen male or female young adult squirrel monkeys (*Saimiri scuireus*) weighing 0.46–1.08 kg were used. All animals were handled in accordance with the NIH guidelines on the care and use of animals in research, the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research, and the approval of the Institutional Animal Care and Use Committee of the LSU Medical Center in New Orleans. The monkeys had previously been used in breeding research but had not been involved in any infectious disease research.

2.2. Pharmacokinetic studies

Four monkeys were given single i.p. injections of 150 mg/kg HBPG in corn oil suspension (10 mg/ml) and bled 2, 5, 8 and 10 h later. Two monkeys were given single intravenous (i.v.) injections of 40 mg/kg HBPG dissolved in 90% dimethylsulfoxide (10 mg/ml), and bled 0.5, 1, 2 and 3 h later. The primates were anesthetized with 10–40 mg/kg intramuscular ketamine prior to injection of the drug, and were reanesthetized as necessary for phlebotomy from the femoral vein. No more than 0.2 ml of blood was removed at each time point. Plasma was separated, frozen, and subjected to analysis for HBPG content. Diluted plasma samples were analyzed by high-performance liquid chromatography (HPLC) on a C8 reverse-phase column, and concentrations of HBPG were calculated from the absorbance of the compound at 275 nm. Details of the conditions have been published (Xu et al., 1995).

2.3. Efficacy trial

The corneas of the 18 squirrel monkeys were anesthetized with a drop of proparacaine hydrochloride (Alcaine, Alcon, Humacao, PR). The superficial corneal epithelium was minimally traumatized with a 27-gauge needle, after which 25 μl of Rodanus strain HSV-1 was dropped into the conjunctival cul-de-sac, and the eyelids were gently rubbed over the cornea for 10 s. The corneas were stained with fluorescein (Fluor-I-Strip, Wyeth-Ayerst, Philadelphia, PA) 3 days after infection and the presence of herpetic keratitis was verified in all corneas by slit-lamp biomicroscopic examination. Based on the pharmacokinetic data, we decided to use 150 mg/kg HBPG, i.p. every 8 h, and to inject drug or vehicle a total of five times before lowering the room temperature.

Fig. 1 illustrates the experimental design. To induce recurrences with hypothermic stress, the room temperature was lowered at 16:00. Drug treatment was continued two additional times

(midnight and 08:00), and the desired low temperature was noted at 06:00. Corneas were examined at 08:00, and the room temperature was raised to the normal level. Animals were exposed to four cycles of temperature stress, with treatments starting on days 21, 27, 34 and 69. A cross-over study was not undertaken in order to avoid any drug carryover from a possible depot of drug that might slowly dissolve. Each animal was followed serially because it is known that the numbers of recurrences decrease with time after infection. The corneal evaluations were done in a masked manner so that the observer had no knowledge of the treatments.

2.4. Statistical methods

The outcome was expressed as a binary variable with the value 1 if an eye had a typical herpetic lesion, 0 if it did not. The dependent variable was the treatment, either HBPG or the vehicle. The analyses were conducted separately for the time during treatment and the time of no treatment. The analysis was a Wilcoxon rank sums test using a correction for a normal approximation with a continuity correction for the two sample case (i.e. the two levels of the treatment, HBPG and the vehicle (Siegel, 1956)).

3. Results

Plasma from HBPG-treated animals was analyzed by HPLC to measure the time course of drug residence in the circulation. The results of i.v. dosing (Fig. 2(A)) showed an elimination half-life of 1.05 h and a volume of distribution, 0.83 l/kg, that indicated uniform distribution of the drug throughout body tissues. By contrast, after i.p. injection, HBPG had an apparent plasma half-life of 12.5 h (Fig. 2(B)), indicating that elimination of the drug was dependent on the absorption rate from the peritoneal cavity. The prolonged plasma half-life suggested that i.p. injection at convenient dosing intervals would provide adequate and consistent concentrations of drug at the target site, the trigeminal ganglion. Intravenous dosing would have been impossible, given the short half-life of the administered drug and the volume of fluids required for i.v. administration in such small animals. Indeed, the dosing frequency by the i.p. route was chosen in order to achieve higher peak drug concentrations at steady state than was possible with a single dose.

The dosing frequency was chosen to cause a degree of cumulation of drug in the plasma at

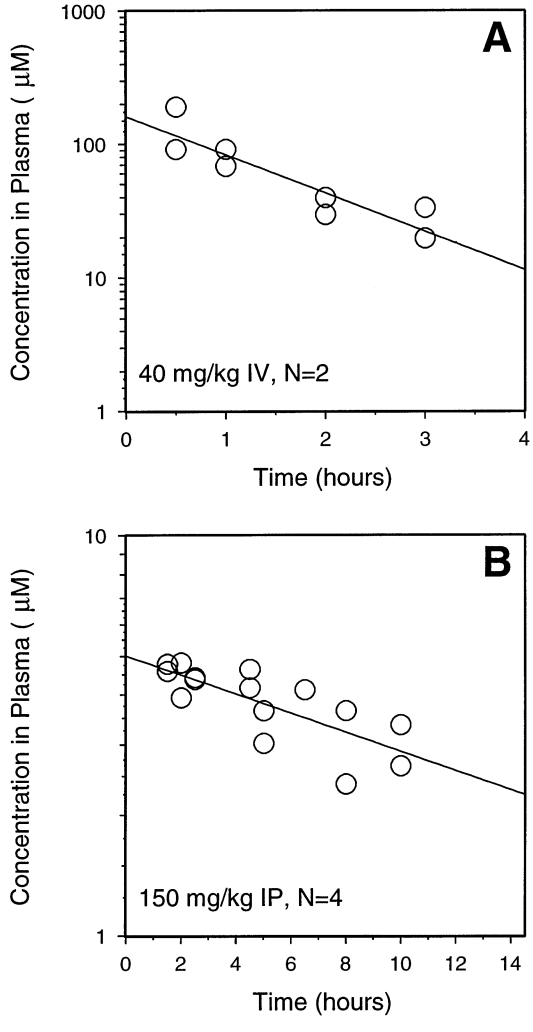

Fig. 2. (A) Plasma concentration of HBPG in two squirrel monkeys that had been given a single i.v. injection of 40 mg/kg HBPG dissolved in 90% dimethylsulfoxide shows an elimination half-life of 1.05 h and a volume of distribution of 0.83 l/kg. (B) Plasma concentration of HBPG in four squirrel monkeys that had been given a single i.p. injection of 150 mg/kg HBPG in corn oil shows an apparent half-life of 12.5 h.

Table 1
Recurrent epithelial herpetic keratitis in squirrel monkeys infected with HSV-1 strain Rodanus

Treatment[a]	No. of observations[b]	Eyes		Eye lesion days
		Recurrent lesions	No recurrent lesions	
Corn oil	432	9/18	9/18	47
HBPG	372	10/18	8/18	19

[a] HBPG 150 mg/kg i.p. every 8 h on PI days 21, 22, 27, 28, 34, 35, 69, 70, 71 and 72, and at 08:00 on PI days 23, 29, 36 and 73; corn oil given i.p. on same schedule.
[b] Total eyes examined over all days of study (PI days 16–73).

steady state and to reduce the peak-to-trough variation during the reactivation protocol. By beginning drug treatments before lowering the temperature, the plasma concentration of drug would be near steady state (about 4 half-lives). Assuming that the plasma concentration just before the next dose, a, is given by $a = b\exp(-0.693/t^{1/2})t$, where b is the peak concentration and t is the dosing interval, the extent of cumulation with a dosing frequency of 8 h is 2.8, i.e. $b/(b-a)$. Thus, the peak plasma concentration at steady state would be 14 μM (2.8×5 μM (see Fig. 2(B)) and the trough would be 9 μM.

All nine monkeys randomized to the HBPG-treated group and six of the nine monkeys randomized to the vehicle-treated group had at least one incident of recurrent herpetic keratitis during the observation period (Table 1 and Fig. 3). During the treatment periods, the frequency of corneas showing specific recurrent herpetic keratitis was significantly lower in the HBPG-treated group than in the untreated control group ($P = 0.01$, Wilcoxon rank sums test). In the periods before and after treatment, there were no significant differences in the frequency of herpetic keratitis between the two treatment groups (before: $P = 0.6536$; after: $P = 0.1106$).

Three monkeys died during the study, one on the night of day 28 when the temperature was lowered to 16°C, one on the night of day 34, and one during the night of day 69. Although all three events were associated with treatment periods (6, 3, and 3 consecutive doses, respectively), the veterinarian who performed the post mortem examinations did not find any gross or histologic findings suggesting HBPG toxicity. He found non-specific changes suggesting hypoglycemia/shock/possible stroke that could have been related to the low temperature. Further studies of drug toxicity will be required in the future.

4. Discussion

It is essential to demonstrate the safety and efficacy of drugs that target viral enzyme systems in primate species before testing these drugs in the clinical setting. In the present study, we used a model of hypothermic stress in primates (Varnell et al., 1995). In this model, latently infected squirrel monkeys subjected to brief intervals of lowered ambient temperatures (to approximately 18°C) exhibited a significantly greater number of corneal herpetic lesions compared with latently infected animals maintained in an ambient temperature range of 24–27°C. The results of the present study provide evidence that a new drug, HBPG, which inhibits viral thymidine kinase, is effective in preventing viral reactivation and recrudescence of ocular viral disease in hypothermically stressed primates. These results support and broaden our previous investigations with this and related compounds (Xu et al., 1995; Gebhardt et al., 1996).

Xu et al. (1995) demonstrated that HBPG is an effective inhibitor of HSV-1 thymidine kinase in vitro and provided convincing evidence that this compound achieves effective systemic distribution and plasma levels, making it an ideal candidate for further study as an antiviral. Subsequently, Gebhardt et al. (1996) showed that HBPG is effective in reducing viral reactivation in a murine model; they demonstrated a reduced frequency of

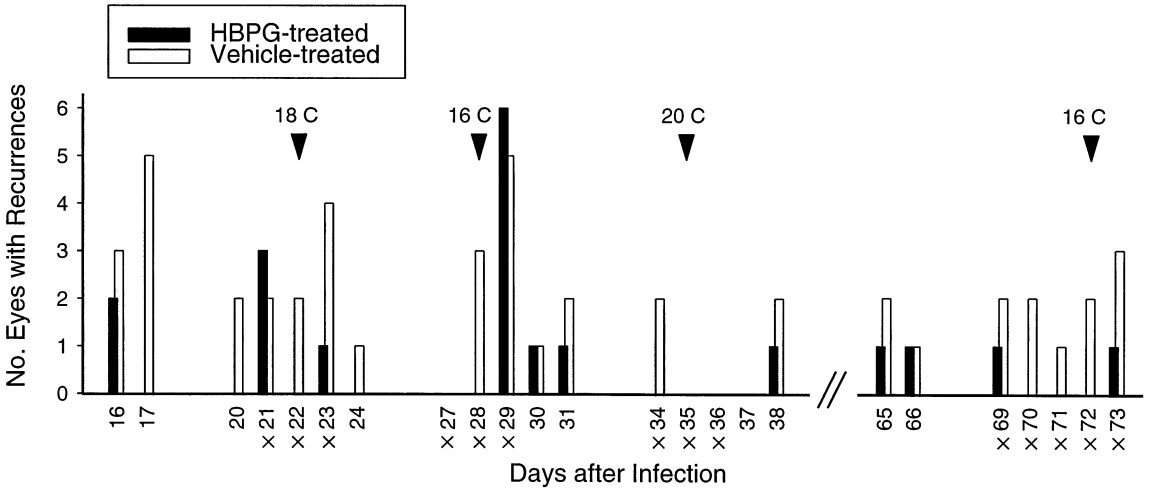

Fig. 3. The animals were examined only on the numbered days after infection; they were not examined on weekends or during the period of post-infection (PI) day 39 through PI day 64. X denotes days of treatment. Vehicle-treated group = nine monkeys (18 eyes). HBPG-treated group = nine monkeys (18 eyes) through PI day 28, eight monkeys (16 eyes) through PI day 34, seven monkeys (14 eyes) through PI day 69, and six monkeys (12 eyes) to end of study. On PI day 35, the room temperature could not be lowered to the desired range of 16–18°C; a low of 20°C was attained. On PI days 35, 36 and 37, no recurrent keratitis was seen in either group.

ocular shedding of infectious virus, as well as reduced synthesis of viral DNA in the trigeminal ganglion, the site of viral latency, in HBPG-treated animals compared with untreated controls. In the present study, however, corneas were not cultured for the presence of herpesvirus because swabbing the eyes can damage the cornea and cause other trauma which may mimic viral lesions or perhaps even induce viral shedding.

Research into antiviral drugs has been focused on compounds that shorten the duration of acute infection and the destructive effects of the virus in the infected organs (Elion, 1985; Beyer et al., 1989; Mansuri and Martin, 1991; Lapucci et al., 1993). For HSV-1 and HSV-2, a major thrust of drug development is aimed at preventing viral reactivation. An especially desirable goal is to develop a safe, nontoxic, effective antiviral that can be self-administered and that can prevent viral reactivation in carriers of latent herpesviruses. For many years, we have focused our attention on the design and testing of antiviral compounds directed against metabolic pathways that are involved in viral synthesis and assembly during reactivation from latency (Kaufman, 1993), and we believe that viral thymidine kinase inhibitors that do not affect host enzymes have great potential for both safety and efficacy. For example, we tested the thymidine kinase inhibitor 5′-ethynylthymidine and found that this drug suppressed ocular recurrences of HSV-1 (Kaufman et al., 1991). Unfortunately, in order to achieve the desired effect, the drug had to be administered i.p. every 4 h for many days. Our present study with HBPG in primates provides a stimulus for further investigation into the use of such compounds in preventing viral reactivation in the clinical setting. Ultimately, it should be possible to provide patients with an oral medication that can be taken routinely in circumstances where viral reactivation is a likely event.

Acknowledgements

This work was supported in part by US Public Health Service grants EY02672, EY02377 and EY08701 from the National Eye Institute, National Institutes of Health, Bethesda, MD, Department of the Army, Cooperative Agreement

DAMD17-93-V-3013 (this does not necessarily reflect the position or the policy of the government, and no official endorsement should be inferred), as well as an unrestricted departmental grant (LSU Eye Center) from Research to Prevent Blindness, New York, NY. This study was presented in part at the 8th International Conference on Antiviral Research, Santa Fe, NM, April 24, 1995.

References

Barney, N.P. and Foster, C.S. (1994) A prospective randomized trial of oral acyclovir after penetrating keratoplasty for herpes simplex keratitis. Cornea 13, 232–236.

Beyer, C.F., Hill, J.M. and Kaufman, H.E. (1989) Antivirals and interferons. Ophthalmol. Clin. North Am. 2, 51–63.

Bourne, N., Bravo, F.J., Ashton, W.T., Meurer, L.C., Tolman, R.L., Karkas, J.D. and Stanberry, L.R. (1992) Assessment of a selective inhibitor of herpes simplex virus thymidine kinase (L-653,180) as therapy for experimental recurrent genital herpes. Antimicrob. Agents Chemother. 36, 2020–2024.

Collum, L.M.T., McGettrick, P., Akhtar, J., Lavin, J. and Rees, P.J. (1986) Oral acyclovir (Zovirax) in herpes simplex dendritic corneal ulceration. Br. J. Ophthalmol. 70, 435–438.

Corey, L., Nahmias, A.J., Guinan, M.E., Benedetti, J.K., Critchlow, C.W. and Holmes, K.K. (1982) A trial of topical acyclovir in genital herpes simplex virus infections. N. Engl. J. Med. 306, 1313–1319.

Douglas, J.M., Critchlow, C., Benedetti, J., Mertz, G.J., Connor, J.D., Hintz, M.A., Fahnlander, A., Remington, M., Winter, C. and Corey, L. (1984) A double-blind study of oral acyclovir for suppression of recurrences of genital herpes simplex virus infection. N. Engl. J. Med. 310, 1551–1556.

Elion, G.B. (1985) Selectivity — key to chemotherapy: presidential address. Cancer Res. 45, 2943–2950.

Gebhardt, B.M., Wright, G.E., Xu, H., Focher, F., Spadari, S. and Kaufman, H.E. (1996) 9-(4-hydroxybutyl)-N^2-phenylguanine, HBPG, a thymidine kinase inhibitor, suppresses herpes virus reactivation in vivo. Antiviral Res. 30, 87–94.

Gold, D. and Corey, L. (1987) Acyclovir prophylaxis for herpes simplex virus infection. Antimicrob. Agents Chemother. 31, 361–367.

Kaufman, H.E. (1962) Clinical cure of herpes simplex keratitis by 5-iodo-2′-deoxyuridine. Proc. Soc. Exp. Biol. Med. 109, 251–252.

Kaufman, H.E. (1993) Introduction: the first effective antiviral. In: J. Adams and V.J. Merluzzi (Eds), The Search for Antiviral Drugs, pp. 1–21. Birkhäuser, Boston.

Kaufman, H.E. and Maloney, E.D. (1962) IDU and hydrocortisone in experimental herpes simplex keratitis. Arch. Ophthalmol. 68, 396–398.

Kaufman, H.E., Nesburn, A.B. and Maloney, E.D. (1962) IDU therapy of herpes simplex. Arch. Ophthalmol. 67, 583–591.

Kaufman, H.E., Varnell, E.D., Cheng, Y.C., Bobek, M., Thompson, H.W. and Dutschman, G.E. (1991) Suppression of ocular herpes recurrences by a thymidine kinase inhibitor in squirrel monkeys. Antiviral Res. 16, 227–232.

Kim, C.U., Misco, P.F., Luh, B.Y., Terry, B., Bisacchi, G. and Mansuri, M.M. (1993) (2*R*,4*S*,5*S*)-1-(tetrahydro-4-hydroxy-5-methoxy-2-furanyl)thymine: a potent selective inhibitor of herpes simplex thymidine kinase. Bioorg. Med. Chem. 3, 1571–1576.

Klein, R.J. and Czelusniak, S.M. (1990) Effect of a thymidine kinase inhibitor (L-653,180) on antiviral treatment of experimental herpes simplex virus infection in mice. Antiviral Res. 14, 207–214.

Lapucci, A., Macchia, M. and Parkin, A. (1993) Antiherpes virus agents: a review. Farmaco 48, 871–895.

Legmann, A. and Langston, D.P. (1995) Long-term oral acyclovir therapy: effect on recurrent infectious herpes simplex keratitis. Ophthalmology 102 (Suppl. 9A), 121.

Leib, D.A., Ruffner, K.L., Hildebrand, C., Schaffer, P.A., Wright, G.E. and Coen, D.M. (1990) Specific inhibitors of herpes simplex virus thymidine kinase diminish reactivation of latent virus from explanted murine ganglia. Antimicrob. Agents Chemother. 34, 1285–1286.

Mansuri, M.M. and Martin, J.C. (1991) Antiviral agents. Annu. Rep. Med. Chem. 26, 133–140.

Martin, J.A., Duncan, I.B. and Thomas, G.J. (1989a) Design of inhibitors of herpes simplex virus thymidine kinase. In: J.C. Martin (Ed), Nucleotide Analogues as Antiviral Agents. pp. 103–115. American Chemical Society, Washington, D.C.

Martin, J.A., Duncan, I.B., Hall, M.J., Wong-Kai-In, P., Lambert, R.W. and Thomas, G.J. (1989b) New potent and selective inhibitors of herpes simplex virus thymidine kinase. Nucleosides Nucleotides 8, 753–764.

Mertz, G.J. (1990) Herpes simplex virus. In: G.J. Galasso, R.J. Whitley and T.C. Merigan (Eds), Antiviral Agents and Viral Diseases of Man, 3rd Edn., pp. 265–300. Raven Press, New York.

Meyrick Thomas, R.H., Dodd, H.J., Yeo, J.M. and Kirby, J.D.T. (1985) Oral acyclovir in the suppression of recurrent non-genital herpes simplex virus infection. Br. J. Dermatol. 113, 731–735.

Mindel, A., Weller, I.V.D., Faherty, A., Sutherland, S., Hindley, D., Fiddian, A.P. and Adler, M.W. (1984) Prophylactic oral acyclovir in recurrent genital herpes. Lancet ii, 57–59.

Mindel, A., Faherty, A., Carney, O., Patou, G., Freris, M. and Williams, P. (1988) Dosage and safety of long-term suppressive acyclovir therapy for recurrent genital herpes. Lancet i, 926–928.

Nsiah, Y.A., Tolman, R.L., Karkas, J.D. and Rapp, F. (1990) Suppression of herpes simplex virus type 1 reactivation from latency by (±)-9-{[(*Z*)-2-(hydroxymethyl)-cyclohexyl]methyl}guanine (L-653,180) in vitro. Antimicrob. Agents Chemother. 34, 1551–1555.

Nutter, L.M., Grill, S.P., Dutschman, G.E., Sharma, R.A., Bobek, M. and Cheng, Y.-C. (1987) Demonstration of viral thymidine kinase inhibitor and its effect on deoxynucleotide metabolism in cells infected with herpes simplex virus. Antimicrob. Agents Chemother. 31, 368–374.

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

Roizman, B. and Kaplan, L.J. (1992) Herpes simplex viruses, central nervous system, and encephalitis. A two-body problem, with one outcome and too many questions. In: R.P. Roos (Ed), Molecular Neurovirology, pp. 3–21. Humana Press, Totowa, NJ.

Rooney, J.F., Straus, S.E., Mannix, M.L., Wohlenberg, C.R., Alling, D.W., Dumois, J.A. and Notkins, A.L. (1993) Oral acyclovir to suppress frequently recurrent herpes labialis. A double-blind, placebo-controlled trial. Ann. Intern. Med. 118, 268–272.

Sanitato, J.J., Asbell, P.A., Varnell, E.D., Kissling, G.E. and Kaufman, H.E. (1984) Acyclovir in the treatment of herpetic stromal disease. Am. J. Ophthalmol. 98, 537–547.

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

Siegel, S. (1956) Nonparametric Statistics for the Behavioral Sciences, 312 pp. Ch. 5. The case of two related samples. The Wilcoxon matched-pairs signed ranks test, pp. 75–78. McGraw-Hill, New York.

Spadari, S. and Wright, G. (1989) Antivirals based on inhibition of herpesvirus thymidine kinases. Drug News Perspectives 2, 333–336.

Spruance, S.L., Hamill, M.L., Hoge, W.S., Davis, L.G. and Mills, J. (1988) Acyclovir prevents reactivation of herpes simplex labialis in skiers. J. Am. Med. Assoc. 260, 1597–1599.

Straus, S.E., Takiff, H.E., Seidlin, M., Bachrach, S., Lininger, L., DiGiovanna, J.J., Western, K.A., Smith, H.A., Lehrman, S.N., Creagh-Kirk, T. and Alling, D.W. (1984) Suppression of frequently recurring genital herpes: a placebo-controlled double-blind trial of oral acyclovir. N. Engl. J. Med. 310, 1545–1550.

Straus, S.E. (moderator), Rooney, J.F., Sever, J.L., Seidlin, M., Nusinoff-Lehrman, S. and Cremer, K. (discussants) (1985) Herpes simplex virus infection: biology, treatment, and prevention. NIH Conference. Ann. Intern. Med. 103, 404–419.

Taylor, J.L., Tom, P., Guy, J., Selvarajan, R.M. and O'Brien, W.J. (1994) Regulation of herpes simplex virus thymidine kinase in cells treated with a synergistic antiviral combination of alpha interferon and acyclovir. Antimicrob. Agents Chemother. 38, 853–856.

Varnell, E.D., Kaufman, H.E., Hill, J.M. and Thompson, H.W. (1995) Cold stress-induced recurrences of herpetic keratitis in the squirrel monkey. Invest. Ophthalmol. Vis. Sci. 36, 1181–1183.

Whitley, R.J. (1990) Herpes simplex viruses. In: B.N. Fields, D.M. Knipe, R.M. Chanock, M.S. Hirsch, J.L. Melnick, T.P. Monath and B. Roizman (Eds), Virology, 2nd Edn., pp. 1843–1887. Raven Press, New York.

Wright, G.E. (1994) Herpesvirus thymidine kinase inhibitors. Int. Antiviral News 2, 84–86.

Xu, H., Maga, G., Focher, F., Smith, E.R., Spadari, S., Gambino, J. and Wright, G.E. (1995) Synthesis, properties, and pharmacokinetic studies of N^2-phenylguanine derivatives as inhibitors of herpes simplex virus thymidine kinases. J. Med. Chem. 38, 49–57.