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Enhanced efficacy of nucleoside analogs and recombinant alpha interferon in weanling mice lethally infected with herpes simplex virus type 2

Lawrence R. Crane and James C. Sunstrum

Department of Medicine, Division of Infectious Diseases, Wayne State University, Detroit, Michigan, U.S.A.; Division of Infectious Diseases, Veterans Administration Medical Center, Allen Park, Michigan, U.S.A.

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Summary

Weanling ICR Swiss mice were inoculated intranasally with a lethal dose of herpes simplex virus type 2, and treated with either vidarabine, vidarabine-5′-monophosphate, acyclovir or a hybrid recombinant human alpha interferon which is active in murine tissues. Treatment with antiviral drugs was initiated 2, 24, 48, 72 or 96 h following virus inoculation. Single drug treatment showed little effect on mortality, with only acyclovir showing some slight reduction. Four dual drug combinations (vidarabine/acyclovir; vidarabine/interferon; vidarabine 5′-monophosphate/acyclovir and vidarabine 5′-monophosphate/interferon) were all associated with marked reductions of mortality when treatment was begun at 2 h, and this beneficial effect increased further when therapy was delayed until 24 or 48 h following virus inoculation. However, the combination of acyclovir/interferon was consistently toxic to the mice, unless a reduction in dosages was employed. These results suggest that certain antiviral combinations might be useful for serious human infections caused by herpes simplex virus.

Herpes simplex virus; Combination antivirals; Interferon

Correspondence to: James Sunstrum, Division of Infectious Diseases, Veterans Administration Medical Center, Allen Park, MI 48101, U.S.A.

Introduction

Acyclovir has emerged as the current drug of choice for herpes simplex virus (HSV) infections, but morbidity following treatment of serious diseases such as herpes encephalitis and disseminated herpes infections in neonates remains a therapeutic problem (Nahmias et al., 1983; Skoldenberg et al., 1984). We have previously reported the enhanced efficacy of certain antiviral combinations both in vitro and in mice inoculated intravaginally with HSV type 2 (HSV-2) (Crane et al., 1984). While all combinations tested in vitro appeared to show enhanced or synergistic effects, this did not always predict the in vivo response as we occasionally observed serious toxicity with certain combinations. We found the combination of full doses of vidarabine plus acyclovir to be the most effective combination, and this also reduced the proportion of latency among surviving animals. However, vidarabine (ara-A) plus an interferon inducer was highly toxic, a result not predicted by the in vitro assays (Crane et al., 1984). Similarly, several groups have also explored the potential utility of combinations of antiviral compounds using animal models in a search for enhanced therapeutic efficiency (Cho and Feng, 1980; Connell et al., 1985; Fraser-Smith et al., 1984a, 1984b; Karim et al., 1985; Park et al., 1984; Schinazi et al., 1982, 1983, 1986). We recently have reported in vitro observations of combinations employing a recombinant interferon and various antiviral nucleosides (Crane et al., 1985). In this study, we have extended the study of those various combinations using a weanling mouse model of HSV-2 infection, and report the efficacy of several antiviral combinations. This model has recently been reported to resemble the pathogenesis of disseminated neonatal HSV infection in human neonates (Kern et al., 1986). The potential for HSV to develop resistance to acyclovir is also a concern, and we addressed this issue in mice treated with acyclovir.

Materials and Methods

Virus, cells and media

HSV-2, strain MS, was kindly provided by Dr. Andre Nahmias, Emory University, Atlanta, GA. Virus pools were grown in continuous cell lines of human foreskin fibroblasts (FS) and generally titered 1.0×10^8 PFU/ml on Vero cells. Cells were routinely cultured in Eagle's minimal essential media (MEM) containing L-glutamine. Ten percent fetal calf serum (FCS) was added for cell growth or freezing of stock virus (−70°C). Two percent FCS was used for cell maintenance and virus titrations. Cell lines and virus stocks were mycoplasma-free.

Drugs and interferon

Vidarabine (ara-A), and vidarabine 5′-monophosphate (ara-AMP) were obtained from Dr. Edwin L. Marcus, Warner-Lambert/Parke-Davis (Ann Arbor, MI). Recombinant interferon (rHuIFN-A/D) was a gift from Dr. Patrick H. Trowne, Hoffmann-La Roche Laboratories (Nutley, NJ). Interferon activity was estab-

lished by calibration with NIH standard mouse IFN (G-002-904-511) in mouse L-929 cells using vesicular stomatitis virus (VSV) as the challenge. Activity was expressed as international units (IU) per ml (WHO Expert Committee on Biological Standardization, 1984). Acyclovir (ACV) was obtained from the manufacturer (Burroughs-Wellcome, Research Triangle Park, NC).

Mouse model

The weanling mouse model of herpes infection as described by Kern and coworkers was used (Kern et al., 1982). Three-week-old male ICR-Swiss mice (Harlan-Sprague-Dawley, Inc., Indianapolis, IN), weighing 12–14 g each, were caged in groups of 10 or 15 mice. Mean weight mice per cage did not vary by more than one gram. After anesthetizing with intraperitoneal (i.p.) sodium phenobarbital, mice were allowed to inhale 0.03 ml HSV-2 suspension delivered in drops from a 26 gauge needle. Each animal received about 3×10^6 PFU of HSV-2 which resulted in 100% mortality. Mice were observed daily for 21 days and day of death recorded for each.

Drug therapy

Antiviral therapy began 2, 24, 48, 72, or 96 h after inoculation with virus and continued for five days. Drugs were prepared immediately before use and given intraperitoneally in 0.1 ml volumes. Injections of drug or diluent were given in rapid sequence in combination experiments. Vidarabine or ara-AMP was suspended in sterile 0.4% carboxymethylcellulose or phosphate buffered saline (PBS) respectively and given once daily. The interferon rHuIFN-A/D was diluted in sterile PBS and given in single daily doses. Acyclovir was dissolved in an appropriate volume of PBS, to which an equimolar amount of sodium hydroxide was added, and given in two divided doses at 9 a.m. and 5 p.m.

Dose-response curves were established for each antiviral and compared with infected, untreated control mice which were given i.p. injections of appropriate diluent. Full-dose, single-drug regimens were defined as that highest dose regimen in mg/kg/day (ara-A, ara-AMP, or ACV) or IU/mouse/day (rHuIFN-A/D) that did not result in toxicity or death up to 3 days post-treatment. The definition of toxicity was an average weight loss of three or more grams for uninfected, treated control mice. Fifteen mice were tested for toxicity for each antiviral regimen.

Acyclovir sensitivity assay

Virus recovered from brain and lung homogenates of ACV treated mice were compared with the HSV-2 MS stock inoculum for any change in ACV sensitivity. Virus sensitivities were determined using the 50% plaque reduction method. 60 mm dishes with confluent monolayers of Vero cells were gently washed once with sterile PBS. Approximately 200 plaque forming units of HSV-2 in 0.2 ml PBS were adsorbed on duplicate dishes at 37°C in a CO_2 incubator for one hour. The cells were then washed once with PBS, and a 5 ml overlay of 0.7% methylcellulose in medium with 2% FCS and ACV was added. Two-fold dilutions of ACV (1–32 μM) were prepared for incorporation in the overlay. Cells were incubated for 3–5 days

at 37°C in a 5% CO_2 incubator. The overlay was then removed and the monolayer fixed and stained with one percent crystal violet in 50% ethanol for 5 min. Plaques were counted with a dissecting microscope and a graph was generated to determine the concentration of drug which inhibited 50% of plaque development.

Statistical analysis

Median day to death (MDD) was computed for animals that did not survive. Meier-Kaplan survival curves were generated for treated and untreated mice. The Lee-Desu statistic was used for pairwise comparisons. Animals surviving past day 21 were included as censored data (Hull and Nie, 1981). Synergy was defined as a significant difference between survival curves of the combination regimen as compared with each corresponding monotherapy. Antagonistic interactions displayed significant decreases in these parameters, otherwise the interaction was termed additive or indifferent (Crane et al., 1984). Proportions were compared using Fisher's exact test. Confidence levels of 95% defined significance.

Results

We selected four antiviral agents which are established (i.e., ACV and ara-A) or potential (i.e., ara-AMP and rHuIFN-A/D) therapeutic agents for use in human HSV infections, and studied them in the weanling mouse model.

TABLE 1

Mortality of 3 week ICR-Swiss mice inoculated intranasally with herpes simplex virus type 2, and treated without antivirals, and with vidarabine (ara-A), its monophosphate (ara-AMP), acyclovir (ACV) or recombinant interferon (rIFN). Treatment groups had therapy initiated 2, 24 or 48 h post-inoculation

Agent and dosage[a]				Mortality at a window of:								
mg/kg/day			IU/day	rIFN ($\times 10^3$)	2 h			24 h				
ara-A	ara-AMP	ACV			No. dead/inoc.	% dead	MDD[b]	No. dead/inoc.	% dead	MDD		
	48 h											
	No. dead/inoc.	% dead	MDD									
				30/30	100	(6.6)	15/15	100	(6.4)	14/14	100	(6.3)
250				14/15	93	(9.0)[c]	14/15	93	(8.0)[c]	13/15	87	(8.3)
	250			13/15	87	(9.5)[c]	14/15	93	(7.4)[c]	13/15	87	(6.3)
		60		11/15	73	(11.3)[c]	12/15	80	(8.8)[c]	13/15	87	(8.5)
		15		15/15	100	(7.9)	14/14	100	(6.9)			
			100	14/15	93	(10.0)[c]	15/15	100	(6.7)	15/15	100	(6.6)
			50	13/15	87	(6.7)	14/14	100	(6.0)	14/14	100	(6.4)

[a] ACV given in 2 divided doses; ara-A, ara-AMP, and rIFN given once daily. All drugs given intraperitoneally for 5 days.

[b] MDD, median day to death. Calculated on Day 21 of infection.

[c] Probability that observed value compared to control owing to chance: $P < 0.05$ (Lee-Desu).

Antiviral treatment, single-drug therapy

None of the full-dose single-drug regimens provided adequate protection against mortality, regardless of the time from inhalation of the viral inoculum to first drug dose (Table 1). Administration of higher levels of antivirals to the mice resulted in unacceptable toxicity as determined by excess weight loss, and was observed for each of the four drugs (data not shown). Full-dose ACV (60 mg/kg per day) was the only monotherapy that reduced mortality (11/15 or 78% dead), and this effect was evident only at the 2-h treatment window. Although the MDD was significantly extended with full doses of ara-A (250 mg/kg), ara-AMP (250 mg/kg), or rHuIFN-A/D (1×10^5 IU/kg) monotherapies, mortality remained at or near 100% (Table 1).

We tested for any shift in sensitivity to ACV in virus recovered from brains and lungs of ACV-treated mice. No differences were observed for the in vitro 50% plaque reduction results of virus recovered from infected tissues as compared to the original HSV-2 MS inoculum (data not shown), indicating that in vivo development of resistance to ACV was not occurring in this model.

Antiviral treatment, combination therapy

Table 2 presents the results of simultaneous treatments with combination antivirals versus their respective monotherapies on mortality and MDD for the 2, 24 and 48 h windows. Delay of treatment to 72 or 96 h did not alter mortality and thus the data is not presented. When treatment was begun 2 h following viral inoculation, four combinations demonstrated an increase in survival of the animals: (i) ara-A + rHuIFN-A/D; (ii) ara-AMP + rHuIFN-A/D; (iii) ara-A + ACV; and (iv) ara-AMP + ACV. However, the combination of full doses of ACV + rHuIFN-

TABLE 2

Mortality of 3 week ICR-Swiss mice inoculated intranasally with herpes simplex virus type 2, and treated with dual combinations of the four antivirals in Table 1, at 2, 24 or 48 h post-inoculation

Agent and dosage[a]				Mortality at a window of:								
mg/kg/day			IU/day	2 h			24 h			48 h		
ara-A	ara-AMP	ACV	rIFN ($\times 10^3$)	No. dead/ inoc.[b]	% dead	MDD	No. dead/ inoc.	% dead	MDD	No. dead/ inoc.	% dead	MDD
250			100	$9/15_A$	60	(10.5)	$3/15_S$	20	(10.5)	$2/15_S$	13	(13.0)
	250		100	$7/15_S$	47	(11.3)	$7/15_S$	47	(8.8)	$3/15_S$	20	(11.3)
250		60		$7/15_A$	47	(12.5)	$2/15_S$	13	(14.0)	$1/15_S$	7	(15.5)
	250	60		$5/15_A$	33	(10.5)	$1/15_S$	7	(7.5)	$5/15_S$	33	(11.8)
		60	100	$7/15_T$	47	(14.3)	$6/15_T$	40	(9.5)	$10/15_T$	66	(9.3)
		15	50	$12/15_S$	80	(6.7)	14/14	100	(8.3)			

[a] ACV given in 2 divided doses; ara-A, ara-AMP, and rIFN given once daily. All drugs given intraperitoneally for 5 days.

[b] Probability that observed value compared to control owing to chance: $P < 0.05$ (Lee-Desu). Inferior suffix: S = synergy; A = addition; T = toxicity.

A/D was toxic to the animals, resulting in diarrhea and weight loss greater than 10% of initial body weight. Accordingly, we tested a reduced dose ACV + rHuIFN-A/D combination (ACV 15 mg/kg plus IFN 50 IU/kg) on infected mice, and found some beneficial effect on overall mortality and survival, although this was not nearly as effective as the other full-dose combination regimens.

We then delayed the administration of the combination antiviral regimens to 24 or 48 h following viral inoculation of the weanling mice (Table 2). This allowed further viral replication to occur in vivo, and thus would more likely resemble the therapeutic situation faced with human neonates, where antiviral therapy is initiated well after active viral replication has begun. This delay of antiviral administration to 24 or 48 h after viral inoculation was associated with even greater reductions of mortality for the four effective combinations when compared to the 2 h treatment window, with mortality reduced to as low as 1/15 animals (7%). None of these four effective combinations resulted in toxicity for the animals (data not shown).

Discussion

The rationale for combining two antiviral drugs for HSV infections is based on three possible advantages: (i) enhanced antiviral activity; (ii) decreased host toxicity if lower doses of potentially toxic drugs are given; and (iii) reduced emergence of drug-resistant strains. Previous studies in our laboratory demonstrated that dual combinations of these four antivirals showed enhanced activity against HSV-2 in mouse embryo fibroblasts (Crane and Milne, 1985). However, in vitro studies of antiviral combinations against HSV may not reliably predict in vivo responses or toxicity, as we have reported (Crane et al., 1984). The model of HSV-2 infection in weanling mice has been used by other groups for studies of antiviral efficacy (Kern et al., 1982), and a recent report by Kern et al. (1986) has described similarities in the pathogenesis of this model to human neonatal HSV infection. The recent availability of a human hybrid IFN which also has activity in murine tissue allowed the study of human interferon in a mouse model (Fish et al., 1983, 1986).

The HSV-2 strain MS used was uniformly 100% lethal for the mice, thus presenting a challenge for testing efficacy of the antiviral agents. None of the four full-dose monotherapy regimens in this study produced an adequate response in terms of survival of infected mice, prompting us to pursue combination regimens. Dual drug combinations of ara-A + rHuIFN-A/D; ara-AMP + rHuIFN-A/D; ara-A + ACV; and ara-AMP + ACV were all associated with decreased mortality when they were administered 2 h following viral inoculation; ara-AMP + ACV was the most effective combination at this window. We observed marked toxicity among mice treated with full-doses of ACV + rHuIFN-A/D. A reduced dose combination resulted in elimination of toxicity, and some enhanced survival when compared to the reduced monotherapy doses. However this reduced dose ACV + rHuIFN-A/D combination was not nearly as effective as the other full-dose combinations.

Surprisingly, delaying administration of the four effective combinations to 24 h or 48 h following inoculation was associated with even greater reductions of mortality when compared to the 2 h window; as low as 7% mortality was observed among animals receiving either ara-A or ara-Amp + ACV (Table 1). The reason for this increased survival of animals is unknown since considerable viral replication is occurring 24 h after viral inoculation (Kern et al., 1986). The trauma of four consecutive procedures (anesthesia, inhalation of virus, two intraperitoneal injections) with 2 h may have played a role. On the other hand, administration of antiviral combinations when active viral replication is occurring (i.e. at 24 or 48 h) may offer greater enhancement of host defenses (i.e. endogenous interferons; macrophages, etc.) which are now activated.

Other groups studying antiviral combinations against HSV have used varying animal models, dosing intervals, routes of inoculation of virus, and statistical calculations, thus making comparisons difficult. Connell et al. (1985) reported enhanced efficacy of ACV plus rHuIFN-A/D in weanling mice infected intraperitoneally with HSV-1. Toxicity for mice receiving greater than 100 mg/kg ACV together with interferon (2.5×10^4 IU) was noted, a result similar to our findings of toxicity with this combination. Fraser-Smith et al. (1984a, b) have reported positive results of combining gancyclovir (DHPG; 9-(1,3-dihydroxy-2-propoxymethyl)guanine) and either alpha or beta IFN in adult mice inoculated intraperitoneally with HSV-2.

Schinazi et al. (1982) reported enhanced reductions of mortality in mice receiving both ara-A and ACV. This same group has recently reported on the effects of a delayed treatment regimen (72 h) with dual combinations of ACV, ara-A, 2′-fluoro-5-iodoaracytosine (FIAC), and 2′-fluoro-5-methylarauracil (FMAU) on mice inoculated intracerebrally with this HSV-2 (Schinazi et al., 1983). A new statistical analysis, the median effect method, was used to demonstrate synergy with combinations combining FMAU plus ara-A or ACV. The median effect method uses a dose-response analysis of drug effect, from which may be calculated the dose at which a defined proportion of animals will survive (i.e. 50%; 90%) (Hartshorn et al., 1986; Schinazi et al., 1986). However, if a single drug has only marginal effect on producing a beneficial effect, the median effective dose will be calculated to be far greater than therapeutically useful doses. For example, Schinazi et al. (1986) calculated the 50% effective dose of ACV to be 7587 μM/kg per day (1708 mg/kg per day), which exceeds the reported 50% lethal dose for ACV in mice (4400 μM; 1000 mg/kg) (Schaeffer et al., 1978). The lack of efficacy of single drug regimens in our study precluded meaningful calculation of a median dose effect. Nevertheless, the high mortality among mice receiving one drug alone is in contrast to the nearly complete elimination of mortality with several dual drug combinations we tested.

One of the potential advantages of combination therapy for HSV infections is to reduce or eliminate the emergence of resistant virions. We tested the sensitivity of virus recovered from ACV treated mice, but could not prove any shift in 50% ACV plaque reduction levels.

We conclude that certain combinations of antiviral drugs produce significantly

enhanced survival in this mouse model of HSV-2 infection. The two drugs currently approved for human use, ACV and ara-A, proved to be the most synergistic pair, in agreement with earlier results from our laboratory (Crane et al., 1984) and others (Karim et al., 1985; Park et al., 1984; Schinazi et al., 1982). A combination of these two antivirals may prove the first useful pair to study in clinical trials for serious HSV infections.

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References

Cho, C.T. and Feng, K.K. (1980) Combined effects of acycloguanosine and humoral antibodies in experimental encephalitis due to Herpes virus hominis. J. Infect. Dis. 142, 451.

Connell, E.V., Cerruti, R.L. and Trowne, P.W. (1985) Synergistic activity of combinations of recombinant human alpha interferon and acyclovir, administered concomitantly and in sequence, against a lethal herpes simplex virus type 1 infection in mice. Antimicrob. Agents Chemother. 28, 1–4.

Crane, L.R. and Milne, D.A. (1985) Comparative activities of combinations of acyclovir, vidarabine or its 5'-monophosphate, and cloned human interferons against herpes simplex virus type 2 in human and mouse fibroblast cultures. Antiviral Res. 5, 235–238.

Crane, L.R., Milne, D.A., Sunstrum, J.C. and Lerner, A.M. (1984) Comparative activities of selected combinations of acyclovir, vidarabine, arabinosyl hypoxanthine, interferon, and polyriboinosinic acid-polyribocytidylic acid complex against herpes simplex virus type 2 in tissue culture and intravaginally inoculated mice. Antimicrob. Agents Chemother. 26, 557–562.

Fish, E.N., Banerjee, K. and Stebbing, N. (1983) Antiviral activities of cloned human leukocyte interferons against herpes simplex virus type 2 infections of mice. J. Gen. Virol. 64, 2317–2321.

Fish, E.N., Banerjee, K., Levine, H.L. and Stebbing, N. (1986) Antiherpetic effects of a human alpha interferon analog, IFN- Conl, in hamsters. Antimicrob. Agents Chemother. 30, 52–56.

Fraser-Smith, E.B., Eppstein, D.A., Vivienne Marsh, Y. and Matthews, T.R. (1984a) Enhanced Efficacy of the Acyclic Nucleoside 9-(1,3-Dihydroxy-2-Pro (propoxymethyl) Guanine in Combination with Alpha-Interferon Against Herpes Simplex Virus Type 2 in Mice. Antimicrob. Agents Chemother. 26, 937–938.

Fraser-Smith, E.B., Eppstein, D.A., Vivienne Marsh, Y. and Matthews, T.R. (1984b) Enhanced Efficacy of the Acyclic Nucleoside 9-(1,3-Dihydroxy-2-Propoxymethyl) Guanine in combination with Beta-Interferon Against Herpes Simplex virus Type 2 in Mice. Antimicrob. Agents Chemother. 25, 563–565.

Hartshorn, K.L., Sandstrom, E.G., Neumeyer, D., Paradis, T. J., Chou, T., Schooley, R.T. and Hirsch, M.S. (1986) Synergistic inhibition of human T-cell lymphotropic virus type III replication in vitro by phosphonoformate and recombinant alpha interferon. Antimicrob. Agents Chemother. 30, 189–191.

Hull, C.H. and Nie, N.H. (ed.) (1981) Survival: life table analysis. SPSS update 7–9. New procedures

and facilities for releases 7–9, 205–219. McGraw-Hill Book Co., New York.

Karim, M.R., Marks, M.I., Benton, D.C. and Rollerson, W. (1985) Synergistic antiviral effects of acyclovir and vidarabine on herpes simplex infection in mice. Chemotherapy 31, 310–317.

Kern, E.R., Richards, J.T., Glassgow, L.A., Overall, J.C., Jr. and De Miranda, P. (1982) Optimal treatment of herpes simplex virus encephalitis in mice with oral acyclovir. Am. J. Med. 73, (Suppl) 125–131.

Kern, E.R., Richards, J.T. and Overall, J.C., Jr. (1986) Acyclovir treatment of disseminated herpes simplex virus type 2 infection in weanling mice: Alteration of mortality and pathogenesis. Antiviral Res. 6, 189–195.

Nahmias, A.J., Keyserling, H.L. and Kerrick, B.N. (1983) In: Infectious Diseases of the Fetus and Newborn Infant, J.S. Remington and J.O. Klein (Eds), W.B. Saunders, Co., Philadelphia.

Park, N., Callahan, J.G. and Pavan-Langston, D. (1984) Effect of combined acyclovir and vidarabine on infection with herpes simplex virus in vitro and in vivo. J. Infect. Dis. 149(5), 757–762.

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

Schinazi, R.F., Peters, J., Williams, C.C., Chance, D. and Nahmias, A.J. (1982) Effect of combinations of acyclovir with vidarabine or its 5′- mono-phosphate on herpes simplex viruses in cell culture and in mice. Antimicrob. Agents Chemother. 22, 499–507.

Schinazi, R.F., Peters, J., Sokol, M.K. and Nahmias, A.J. (1983) Therapeutic Activities of 1-(2-fluoro-2-deoxy-B-D-arabinofuranosyl)-5-iodocytosine and -Thymine alone and in combination with acyclovir and vidarabine in mice infected intracerebrally with herpes simplex virus. Antimicrob. Agents Chemother 24, 95–103.

Schinazi, R.F., Chou, T., Scott, R.T., Yao, X. and Nahmias, A.J. (1986) Delayed treatment with combinations of antiviral drugs in mice infected with herpes simplex virus and application of the median effect method of analysis. Antimicrob. Agents Chemother 30, 491–498.

Skoldenberg, B., Alestig, K., Burman, L., Forkman, A., Lovgren, K., Norrby, R., Stiernstedt, G., Forsgren, M., Bergstrom, T., Dahlquist, E., Fryden, A., Norlin, K., Olding-Stenkvist, E., Uhnoo, I. and de Vahl, K. (1984) Acyclovir versus vidarabine in herpes simplex encephalitis. Lancet ii, 707–711.

WHO Expert Committee on Biological Standardization (1984) Standardization of interferons. Report of a WHO informal consultation. Antiviral Res. 4, 76–98.