Antiviral Research, 17 (1992) 157–167

AVR 00527

Evaluation of 1-(2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)-5-ethyluracil in a rabbit model of herpetic keratitis

Melvin D. Trousdale[1], Judy L. Law[1], Frances A. Yarber[1], Kyoichi A. Watanabe[2] and Jack J. Fox[2]

[1]*Doheny Eye Institute and Department of Ophthalmology, University of Southern California School of Medicine, Los Angeles, California and*
[2]*Sloan – Kettering Institute for Cancer Research, New York, New York, U.S.A*

(Received 4 April 1991; revision accepted 16 August 1991)

Summary

The nucleoside analog 1-(2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)-5-ethyluracil (FEAU) was tested in a rabbit model of acute herpetic keratitis and its effectiveness compared with that of acyclovir (ACV). FEAU or ACV was applied topically 3 times daily, beginning 3 days post-HSV-1 inoculation and continued for a period of 7 days. FEAU at a concentration of 1% (w/v) or 3% ACV resulted in significant lessening of the severity of corneal lesions, conjunctivitis, iritis, and corneal clouding at 24 to 48 h after beginning chemotherapy. No toxic reaction was observed in any rabbit eyes treated with either FEAU or ACV. The duration of virus shedding into tear film and colonization of the trigeminal ganglia, however, were not reduced by either FEAU or ACV treatment begun 3 days post-inoculation. Fifty percent effective dose (ED_{50}) of FEAU determinations performed on isolates from tear film and on the virus inoculum in secondary rabbit kidney cultures yielded a range of 4.6–7 μM, with two in vitro resistant isolates having ED_{50}s of $\geqslant 1500$ μM of FEAU. Fifty percent cell growth inhibition for FEAU was 3000 μM at 72 h.

Antiviral; Ocular disease; Herpesvirus; FEAU

Correspondence to: Melvin D. Trousdale, Doheny Eye Institute, 1355 San Pablo Street, Los Angeles, CA 90033, U.S.A.

Introduction

Herpes simplex virus (HSV) infection of the eye is the most prevalent cause of corneal opacification and blindness and, to a lesser degree, iritis and secondary glaucoma in the Western world. Primary infections, usually in the oropharynx, occur in approximately 80% of children 6 months to 5 years old. Of the adult population who were infected as children, less than 1% had clinically apparent disease at the time of primary infection. Primary infection of the eye is rare and represents only 5–25% of all cases of herpetic eye disease. It is estimated that in the United States, 500 000 people each year suffer from episodes of ocular HSV that require medical attention. Approximately 90% of ocular herpes infections are caused by HSV-1, and the remainder by HSV-2 (Donaldson, 1980; Tullo, 1985; Weschler and Nesburn, 1988). Since HSV has the capacity to become latent and to subsequently reactivate, most successful treatments have been aimed at reducing the severity of recurrent clinical disease that is produced by active, replicating viruses.

A major breakthrough in antiviral therapy was made when it became possible to selectively inhibit virus activity while causing little or no host cell toxicity. This has been accomplished in part by using nucleoside analogs, such as acyclovir (ACV), which are believed to be preferentially monophosphorylated and thus activated by viral thymidine kinase (TK) (Lopez et al., 1980; Cheng et al., 1981; Hopkins and Furman, 1990). Subsequent phosphorylations are probably done by cellular kinases (Chen et al., 1984; McLauren et al, 1985; Hopkins and Furman, 1990). This phosphorylated product in turn inhibits viral DNA synthesis by selectively inhibiting viral DNA polymerase, possibly by involving competitive inhibition, chain termination and suicidal inactivation (Hopkins and Furman, 1990).

In addition to the well studied acyclovir (ACV), another family of compounds with this action are the 2′-fluoro-5-substituted arabinosyl pyrimidines (Fig. 1). Of these compounds, FEAU (Watanabe et al., 1984; Perlman et al., 1985; Chou et al., 1987; Mansuri et al., 1987) appears to show the most promise as a potential antiviral drug in humans because it: (i) is soluble and metabolically stable, (ii) is among the most selective inhibitors of HSV DNA synthesis (Kong et al., 1988), (iii) is exclusively incorporated into infected cells (Chou et al., 1987; Kong et al., 1988), and (iv) is relatively non-toxic both in vitro and in vivo (Chou et al., 1987). FEAU has also shown activity comparable to that of ACV in the mouse model and of systemic encephalitis, superior activity when compared with ACV in the cutaneous herpes infection model in guinea pigs (Mansuri et al., 1987). In addition to its effects on HSV, FEAU has been reported to inhibit hepatitis virus B-encoded DNA polymerase (Kong et al., 1988) and to be effective in treatment of simian varicella virus (Soike et al., 1990).

Herein we describe our studies that compared the efficacy of topical FEAU and ACV in minimizing the severity of herpetic keratitis in rabbits, and examined the antiviral activity of FEAU in vitro by plaque reduction assay in

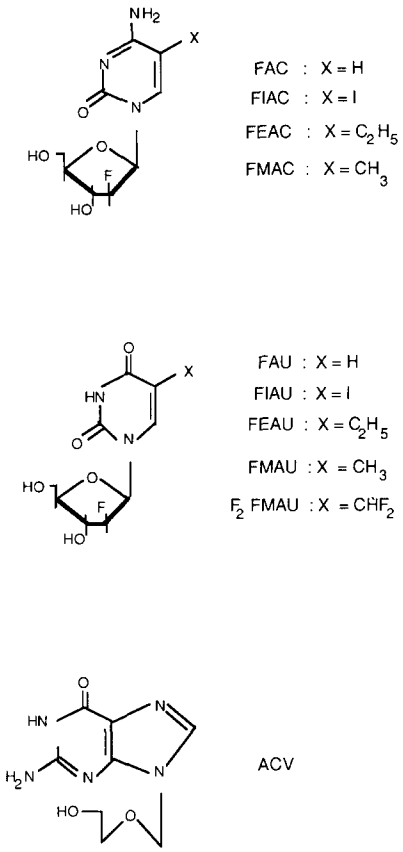

Fig. 1. Chemical structures of 2'-fluoro-β-D-arabinosyl pyrimidines and acyclovir (ACV).

SRK cultures. The selection of 3% ACV was based upon manufacturer's recommendation and previous studies (Trousdale et al., 1980,1981; Rotkins and Chandler, 1985). We also present data on the cell growth inhibition for FEAU in secondary rabbit kidney (SRK) cultures.

Materials and Methods

Virus strain and cell culture

The McKrae strain of HSV-1 was used in the ocular inoculations and in vitro analyses. Virus was propagated and quantitated on SRK cells. Cells were grown in minimum essential medium (Gibco Laboratories, Grand Island, NY) supplemented with 10% fetal calf serum (Irvine Scientific, Santa Ana, CA), 10% NCTC-135 (Gibco), 2 mM L-glutamine, 100 μg/ml gentamicin, 2 μg/ml of amphotericin B (Fungizone, E.R. Squibb and Sons, Princeton, NJ), 200 units

of penicillin and 200 μg/ml streptomycin. Cultures were maintained at 37°C in a 5% CO_2 environment.

Infection and treatment of rabbit eyes

Forty-one male New Zealand white rabbits weighing 2.5 to 3 kg were inoculated bilaterally with 50 μl of HSV-1 (McKrae) containing 3×10^5 plaque forming units (pfu). To facilitate transmission, the eyes were massaged with the lids closed for 15 sec prior to inoculation and then again for 15 sec post-inoculation. Inoculations were made directly onto the cornea, without scarification. Rabbits were examined daily by an uninformed examiner using slit lamp biomicroscopy; the degree of the keratitis, conjunctivitis, iritis and corneal clouding was assessed using a scoring system ranging from 0 to 4, as previously described (Trousdale et al., 1981). On day 3 postinoculation, animals were divided randomly into treatment groups (five animals, 3% ACV; seven animals, 1% FEAU; seven animals, 0.1% FEAU; six animals, 0.01% FEAU; and five animals, placebo). Treatment consisted of one of the following: placebo (phosphate buffered saline, PBS), 3% ACV (0.13 M) (Zovirax ophthalmic ointment in a petrolatum base from Burroughs Wellcome Co.), 0.01% FEAU, 0.1% FEAU or 1% FEAU (1% FEAU = 3.65×10^{-2} M). The FEAU was synthesized at the Sloan-Kettering Institute for Cancer Research. Appropriate concentrations of FEAU were prepared in PBS at the Doheny Eye Institute. Fifty microliters of the appropriate drug dilution was applied topically 3 times daily for 7 consecutive days, beginning 3 days post-inoculation. The coded treatments were delivered in a masked fashion after the daily examination had been performed.

Two-way analysis of variance was done to test for differences in the mean degree of severity across the treatment groups. Further comparisons between groups receiving drug versus placebo were performed using a multiple *t*-test with adjusted alpha level (Bonferroni-Dunn Test) (Zar, 1984). The overall significance level was set at 0.05.

Virus quantitation

On confluent SRK monolayers, 0.1-ml volumes of appropriately diluted HSV were inoculated into duplicate 35-mm dishes and absorbed for 1 h at room temperature. Overlay medium (2 ml) with 1.0% methylcellulose was added to each dish. Dishes were incubated at 37°C for 72 h, and then stained. Counts from replicate dishes were averaged and pfu/ml calculated.

Cytotoxicity and drug resistance

Three-fold serial dilutions of FEAU, up to 3000 μM, were added to replicate 35-mm wells containing 4×10^5 SRK cells. Cells were exposed continuously to the drug for 72 h, after which the toxic effect was assessed by a reduction in

viable cells in the treated wells relative to viable untreated control cells. To determine viability counts, cells were trypsinized and counted in a hemocytometer using the trypan blue exclusion method. Results were expressed as a percent of control cultures and were plotted against drug concentration. The theoretical concentration of drug that would give a 50% reduction in viable cells (ID_{50}) was then extrapolated.

Confluent SRK monolayers in 35-mm dishes were inoculated with 100 pfu of virus isolated from eye swabs of the 1% FEAU treatment group and from placebo treated animals taken at 2–3 weeks post-inoculation in vivo. Virus was allowed to adsorb for 1 h at room temperature on a rocker. Monolayers were then overlaid with 1% methylcellulose in culture medium containing three-fold serial dilutions of FEAU. After incubation for 72 h at 37°C in a humidified 5% CO_2 atmosphere, the cells were fixed with methanol-acetic acid (3:1) and stained with 1% crystal violet. Plaque number was determined, expressed as a percent of control and plotted against drug concentration. The theoretical concentration of drug that would give a 50% plaque reduction from control (ED_{50}) was then extrapolated.

Plaque purification of FEAU-resistant ocular isolates

Confluent SRK monolayers in 60-mm dishes were inoculated in duplicate with serially diluted 0.1-ml volumes of the resistant isolate. Virus was adsorbed for 1 h at room temperature on a rocker. Monolayers were overlaid with 1% methylcellulose medium containing 1000 μM FEAU and incubated for approximately 96 h. Harvested plaques were propagated on SRK monolayers until three-fourths of the cells showed cytopathic effect. The purification/propagation steps were repeated two additional times.

Isolation of HSV from tear film and trigeminal ganglion

Eye swabs of the ocular cul de sac were taken beginning on day 3 post-inoculation and for up to 19 days post-inoculation. Samples were inoculated onto SRK monolayers and incubated at 37°C for 10 days to observe typical HSV cytopathic effect. At the time of sacrifice, the trigeminal ganglia were removed, minced and cocultivated with SRK monolayers and examined for viral cytopathic effect for up to 28 days in order to detect latent HSV.

Results

Topical FEAU and ACV treatment of acute herpetic keratitis

Four parameters were used to evaluate the role of FEAU in lessening the severity of HSV ocular infection in rabbits (Fig. 2A–D). Overall, there was a significant reduction in severity of disease in the groups that received 1% or

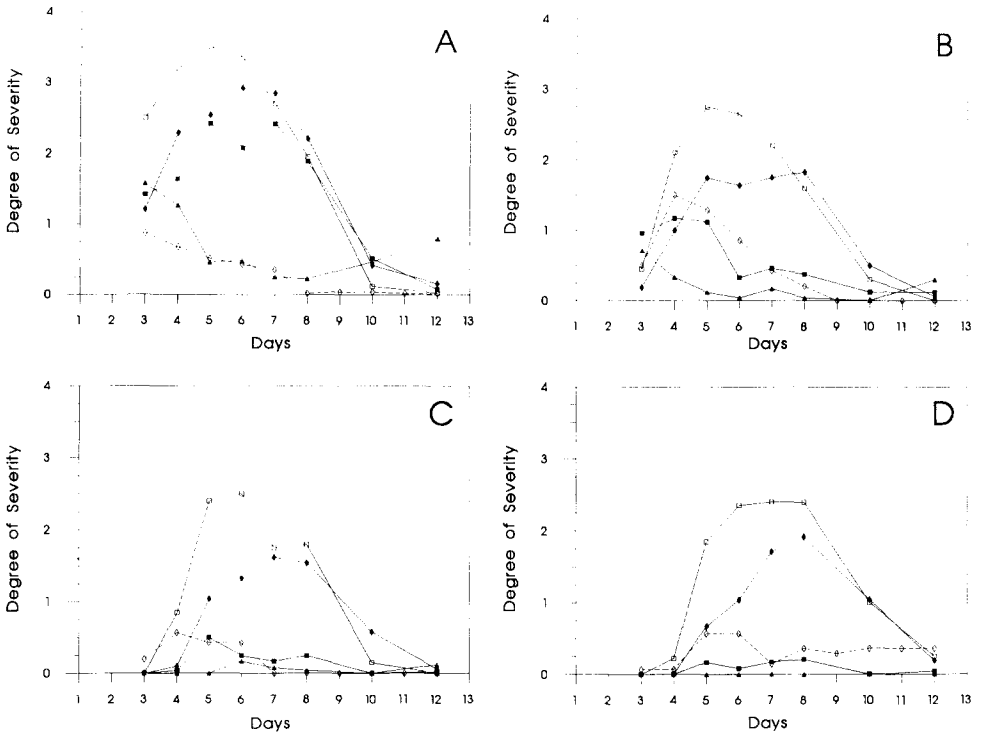

Fig. 2. Effect of topical FEAU (0.01%, 0.1% and 1%) and ACV (3%) treatment on corneal epithelial involvement (A), conjunctivitis (B), iritis (C) and corneal clouding (D) in rabbit eyes infected with HSV-1. Symbols: □, placebo; ▲, 1% FEAU; ■, 0.1% FEAU; ♦, 0.01% FEAU; and ◊, 3% ACV. The curve for 1% FEAU-treated eyes (▲) is barely discernible because corneal clouding was not observed.

0.1% FEAU or 3% ACV when compared with the placebo group. Results of treatment with 0.01% FEAU did not differ significantly from those of placebo. During the course of treatment, no noticeable adverse drug reactions were observed in any of the animals.

Prior to starting chemotherapy on day 3 post-inoculation, punctuate lesions involving the corneal epithelium were noted. Two days after the onset of treatment, there was significant reduction in severity of lesions in the group receiving either 1% FEAU or 3% ACV ($P=0.0001$); however, maximal corneal disease was present in animals treated with placebo or with more dilute concentrations of FEAU. At day 5 post-inoculation, severity score of corneal epithelial involvement ranged from 0.5 in the 1% FEAU and 3% ACV groups, to 3.5 in the placebo group. Marked reduction in epithelial keratitis occurred by day 10 post-inoculation in all treatment groups.

Conjunctivitis was significantly minimized (Fig. 2B) in groups receiving 1% FEAU, 0.1% FEAU or 3% ACV compared to 0.01% FEAU and placebo treated eyes ($P \leqslant 0.006$). Peak severity, as determined by the point of maximal conjunctival injection, occurred at 5 to 6 days post-inoculation, and eyes

TABLE 1

Isolation of HSV from tear film at peak corneal involvement and during clinical remission

Treatment[a]	Number of HSV positive ocular cultures/total eyes cultured after:		
	Day 5 p.i.	Day 7 p.i.	Day 14 p.i.
1% FEAU	12/12	9/12	5/10
0.1% FEAU	12/12	12/12	3/12
0.01% FEAU	12/12	12/12	2/8
Placebo	10/10	10/10	0/4

[a]ACV treated eyes were not cultured for HSV isolation.

appeared normal by day 12 in all treatment groups. Minimal iritis (Fig. 2C) was seen in 1% FEAU, 0.1% FEAU and 3% ACV ($P \leqslant 0.001$) groups, and this was much less severe than in 0.01% FEAU and placebo treated eyes. In the placebo group, corneal clouding was most severe between days 6 and 8 post-inoculation, but was nearly gone by day 12. Clouding of the cornea was absent in eyes receiving 1% FEAU and was significantly less in animals receiving 0.1% FEAU or 3% ACV ($P \leqslant 0.0003$) than in the placebo treated group (Fig. 2D).

Daily eye swabs were done for virus isolation, except for the ACV treated eyes. The number of eyes with positive virus cultures at peak infection and during clinical remission are shown in Table 1. Virus was isolated in 100% of the animals in all treatment groups at days 5 and 7 except the 1% FEAU group at day 7, in which only 75% (9/12) of the eyes were culture positive. At day 14, 50% (5/10) of the eyes in the 1% FEAU group were still positive, but none of the four placebo treated eyes were positive at this time. One animal from each treatment group died before day 14 except for the 3% ACV and 0.1% FEAU groups which had no deaths. The placebo group had two additional deaths on day 14. FEAU resistant isolates, as determined by in vitro plaque reduction, were isolated from two animals treated with 1% FEAU. There was significant difference in shedding between the 1% FEAU and the placebo groups during the period of days 3 to 18 ($P = 0.001$ by Chi square analyses and Cochran-Mantel-Haenszel statistics) (Zar, 1984). Culturing of trigeminal ganglia at the time of sacrifice did not reveal differences between the treatment groups of the ability of HSV to establish latency in neurons. HSV was isolated from the trigeminal ganglia of the treatment groups as follows: 3% ACV, 12 of 12; 1% FEAU, 11 of 12; 0.1% FEAU, 11 of 12; 0.01% FEAU, 9 of 10; and placebo, 8 of 8.

In vitro experiments with FEAU

The 50% effective dose of FEAU against HSV-1 McKrae strain ranged from 4.6 to 7 for the inoculum virus and for isolates from five different infected eyes. Two ocular isolates had an ED_{50} of equal to or greater than 1500 μM (data not shown), or more than a 200- to 300-fold increase. Cell growth inhibition studies for FEAU in rabbit kidney cells indicated toxicity at 3000 μM.

Discussion

Earlier work has suggested that a 2′-fluoro substitution in the 'up' (arabino) configuration in the sugar moiety and a 5-iodo, 5-methyl or 5-ethyl substitution in the pyrimidine ring has conferred more potent antiviral activity than was found in 2′-OH, hydrogen or other 2′-halogen-substituted nucleoside analogs (Watanabe et al., 1979, 1984; Fox et al., 1985; Perlman et al., 1985). Of the pyrimidine nucleoside analogs synthesized, 1-(2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)-5-iodocytosine [FIAC] and 1-(2′-deoxy-2′- fluoro-β-D-arabinofuranosyl)-5-methyluracil [FMAU] (Watanabe et al., 1979) were found to be among the most potent agents against HSV-1 and HSV-2 in vitro (Watanabe et al., 1979, 1984; Lopez et al., 1980; Fox et al., 1985; Kong et al., 1988). With FMAU, however, there is toxicity to the human central nervous system and bone marrow depression that limit its clinical use (Fanucchi et al., 1985). FIAC, although less toxic than FMAU, is rapidly deaminated intracellularly to FIAU, which shows cardiotoxicity at high doses and dose-limiting myelosuppression (McLauren et al., 1985).

Preliminary in vivo studies in mouse, dog and monkey, in which the drugs were administered via the interperitoneal or intravenous routes, showed that FEAU was tolerated at much higher doses than was FMAU and, unlike FMAU, caused no signs of encephalopathy (Chou et al., 1987). It was also reported that in normal rat bone marrow cells, the ED_{50} for FEAU was 415-fold higher than that for FMAU (Chou et al., 1987). FEAU is 14-fold (Mansuri et al., 1987) to 71-fold (Fox et al., 1985) less toxic to growth of Vero cells, and 134-fold less toxic to HL-60 cells (Chou et al., 1987) than is FMAU; however, FEAU is comparable to only 10-fold less potent against HSV (Fox et al., 1985; Mansuri, et al., 1987), thus giving FEAU a better therapeutic index. Depending on the study, FEAU is 9- to 12-fold less toxic than FIAC and ranges from being of similar potency to 46-fold less potent than FIAC against HSV (Fox et al., 1985; Watanabe et al., 1984). Our findings show low toxicity of FEAU in SRK cells, with a $TCID_{50}$ of 3000 μM. Comparing our current finding, that the ED_{50} of FEAU is 4.6 μM, with previous studies in our laboratory using HSV-1 (McKrae) on SRK monolayers, we reported that the ED_{50} for FMAU is 0.16 μM (Trousdale et al., 1983) and that for FIAC is 0.023 μM (Trousdale et al., 1981). It should be noted, however, that there is a great deal of variability in the ED_{50} determinations from study to study, depending on virus strains and culture systems used.

It is thought that the selectivity of the 2′-fluoro-5-substituted pyrimidines for antiherpetic activity is based on a higher affinity for viral TK than for host TK and a selective inhibition of viral DNA polymerase (Lopez et al., 1980; Cheng et al., 1981; Chou et al., 1987). The strength of this preference for viral enzyme varies among these antiviral agents. FMAU and metabolites of FIAC will incorporate into uninfected host cell DNA (Grant et al., 1982; Chou et al., 1987) while FEAU does not (Chou et al., 1987; Kong et al., 1988). FEAU has been shown to be a poor substrate for HL-60 host cell derived cytosolic TK

(Chou et al., 1987) or Vero cell TK (Mansuri et al., 1987), but to be an excellent substrate for HSV-1 and HSV-2 TK (Chou et al., 1987). This preference for viral TK not only accounts for some of the drug's specificity, but may also account for its low toxicity. Unlike FMAU, FEAU will preferentially incorporate into viral DNA, thus making FEAU a good inhibitor of virus replication, without harming the infected host cell (Kong et al., 1988).

Our current work with FEAU has demonstrated that it is as effective as ACV, as well as FMAU (Trousdale et al., 1983) and FIAC (Trousdale et al., 1981) in our rabbit model of acute herpetic keratitis. A 1% concentration of FEAU lessened clinical signs of herpetic keratitis, and the severity of ocular disease may have been further decreased if therapy had begun prior to the appearance of lesions and if therapy was continued until virus shedding stopped. Even though the 1% concentration lessens the severity of the disease, it does not stop virus shedding and, in fact, prolongs its duration relative to the placebo-treated group. It has been noted clinically that, when treating HSV strains sensitive to iododeoxyuridine (IDU), the virus is still recoverable from the eye at the end of a 7-day course of treatment (Rotkis and Chandler, 1985).

Virus isolation from trigeminal ganglia confirmed that HSV-1 could colonize the nervous system, regardless of treatment. We have found from previous work that HSV-1 can enter the trigeminal ganglia as early as 24 h after contact with corneal epithelium (unpublished data). To date, no available antiviral drug has been able to prevent neuronal latency or eradicate latent virus from the nervous system once established.

Two ocular isolates from animals treated with 1% FEAU were shown to be resistant to FEAU. Interestingly, the virus shedding pattern in the eyes from which the drug resistant virus was isolated resembled that seen in eyes of the placebo treated animals. The course of the clinical disease, however, followed a pattern similar to that seen in animals in the FEAU group (i.e., severity of ocular disease was reduced). One FEAU-resistant isolate was detected at day 9 post-inoculation; however, subsequent culturing of the eye did not detect virus. Drug resistant strains of HSV have been associated with ACV (Coen and Schaffer, 1980; Schnipper and Crumpacker, 1980; Furman et al., 1981) and FIAC (Trousdale et al., 1981). Evidence from complementation and recombination experiments on acyclovir-resistant HSV-1 isolates indicates that resistance may be conferred by mutations to either the TK or the DNA polymerase locus (Coen and Schaffer, 1980; Schnipper and Crumpacker, 1980; Furman et al., 1981). The second animal yielding the resistant isolate was sacrificed on day 10 post-inoculation because of encephalitis, even though the keratitis had responded to FEAU therapy.

Acknowledgements

This study was supported in part by EYO2957 and EYO3040. We thank Dr. Martha Lee for the statistical consultation, Ann Dawson for editorial assistance and Linda Montellano and Fay Sim for typing the manuscript.

References

Chen, M.S., Amico, L.A. and Speelman, D.J. (1984) Kinetics of the interaction of monophosphates of the antiviral nucleosides 2′-fluoro-1-β-D-arabinofuranosylpyrimidine and (E)-5-(2-bromovinyl)-2′-deoxyuridine with thymidylate kinases from Vero cells and herpes simplex virus types 1 and 2. Antimicrob. Agents Chemother. 26, 778–780.

Cheng, Y.C., Dutschman, G., Fox, J.J., Watanabe, K.A. and Machida, H. (1981) Differential activity of potential antiviral nucleoside analogs on herpes simplex virus-induced and human cellular thymidine kinases. Antimicrob. Agents Chemother. 20, 420–423.

Chou, T.C., Kong, X.B., Fanucchi, M.P., Cheng, Y.C., Takahashi, K., Watanabe, K.A. and Fox, J.J. (1987) Synthesis and biological effects of 2′-fluoro-5-ethyl-1-βD-arabinofuranosyluracil. Antimicrob. Agents Chemother. 31, 1355–1358.

Coen, D.M. and Schaffer, P.A. (1980) Two distinct loci confer resistance to acycloguanosine in herpes simplex virus type I. Proc. Natl Acad. Sci. U.S.A. 77, 2265–2269.

Dawson, C., Togni, B. and Moore, T.E. Jr. (1968) Structural changes in chronic herpetic keratitis studied by light and electron microscopy. Arch. Ophthalmol. 79, 740–747.

Donaldson, D.D. (1980) Atlas of external diseases of the eye, 2nd ed. Vol. III Cornea and Sclera. pp. 309–332, C.V. Mosby, St. Louis.

Dunkel, E.C., Green, M.T. and Rosborough, J.P. (1984) Suppression of HSV-1 infection in trigeminal ganglion cells: An in vitro model of latency. Invest. Ophthalmol. Vis. Sci. 25, 525–533.

Fanucchi, M.P., Leyland-Jones, B., Young, C.W., Burchenal, J.H., Watanabe, K.A. and Fox, J.J. (1985) Phase I trial of 1-(2′-deoxy-2′-fluoro-1-β-D-arabinofuranosyl)-5-methyluracil (FMAU). Cancer Treatment Reports 69, 55–59.

Fox, J.J., Watanabe, K.A., Schinazi, R.F. and Lopez, C. (1985) Antiviral activities of some newer 2′-fluoro-5-substituted arabinosylpyrimidine nucleosides. In: R. Kono and A. Nakajima (Eds) On herpes viruses and virus chemotherapy: Pharmacological and clinical approaches, pp. 53–56, Excerpta Medica, Amsterdam.

Furman, P.A., Coen, D.M., St. Clair, M.H. and Schaffer, P.A. (1981) Acylcovir-resistant mutants of herpes simplex virus type I express altered DNA polymerase or reduced acyclovir phosphorylating activities. J. Virol. 40, 936–941.

Grant, A.J., Feinberg, A., Chou, T.C., Watanabe, K.A., Fox, J.J. and Philips, F.S. (1982) Incorporation of metabolites of 2′-Fluoro-5-Iodo-1-β-D-arabinofuranosylcytosine into deoxyribonucleic acid of neoplastic and normal mammalian tissues. Biochem. Pharmacol. 31, 1103–1108.

Hopkins, S. and Furman, P. (1990) Molecular basis for the antiviral activity of acyclovir. In: D.A. Baker (Ed.), Acyclovir Therapy for Herpesvirus Infections, pp. 1–13. Marcel Dekker, New York.

Kaufman, H.E., Brown, D.C. and Ellison, E.D. (1968) Herpes virus in lacrimal gland, conjunctiva and cornea of man – a chronic infection. Am. J. Ophthalmol. 65, 32–35.

Kong, X.B., Scheck, A.C., Price, R.W., Vidal, P.M., Fanucchi, M.P., Watanabe, K.A., Fox, J.J. and Chou, T.C. (1988) Incorporation and metabolism of 2′-fluoro-5-substituted arabinosyl pyrimidines and their selective inhibition of viral DNA synthesis in herpes simplex virus type 1 (HSV-1)-infected and mock-infected Vero cells. Antiviral Res. 10, 153–166.

Lopez, C., Watanabe, K.A. and Fox, J.J. (1980) 2′-fluoro-5-iodo-aracytosine, a potent and selective anti-herpesvirus agent. Antimicrob. Agents Chemother. 17, 803–806.

Mansuri, M.M., Ghazzouli, I., Chen, M.S., Howell, H.G., Brodfuehrer, P.R., Benigni, D.A. and Martin, J.C. (1987) 1-(2′-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-ethyluracil. A highly selective antiherpes simplex agent. J. Med. Chem 30, 867–871.

McLauren, C., Chen, M.S., Barbhaiya, R.H., Buroker, R.A. and Oleson, F.B. (1985) Preclinical investigations of FIAU, an anti-herpes agent. In: R. Kono and A. Nakajima (Eds) Herpes Virus and Virus Chemotherapy: Pharmacological and clinical approaches, pp. 57–61, Exerpta Medica, Amsterdam.

O'Brien W.J. and Taylor J.L. (1989) The isolation of herpes simplex virus from rabbit corneas during latency. Invest. Ophthalmol. Vis. Sci. 30, 357–364.

O'Neill, F.J., Goldberg, R.J. and Rapp, F. (1972) Herpes simplex virus latency in cultured human cells following treatment with cytosine arabinoside. J. Gen. Virol. 14, 189–197.

Perlman, M.E., Watanabe, K.A., Schinazi, R.F. and Fox, J.J. (1985) Nucleosides. 133. Synthesis of 5-alkenyl-1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl) cytosine and related pyrimidine nucleosides as potential antiviral agents. J. Med. Chem. 28, 741–748.

Rotkis, W.M. and Chandler, J.W. (1985) Antiviral Agents. In: D.L. Easty and G. Smolin (Eds.), External Eye Disease, pp. 154–185. Butterworths, London.

Schnipper, L.E. and Crumpacker C.S. (1980) Resistance of herpes simplex virus to acycloguanosine: Role of viral thymidine kinase and DNA polymerase loci. Proc. Natl. Acad. Sci. U.S.A. 77, 2270–2273.

Shimeld, C., Tullo, A.B., Easty, D.L. and Thomsitt, J. (1982) Isolation of herpes simplex virus from the cornea in chronic stromal keratitis. Br. J. Ophthalmol. 66, 643–647.

Soike, K.F., Chou, T.C., Fox, J.J., Watanabe, K.A. and Gloff, C.A. (1990) Inhibition of simian varicella virus infection of monkeys by 1-(2-deoxy-2-fluoro-1-β-D-arabinofuranosyl)-5-ethyluracil (FEAU) and synergistic effects of combination with human recombinant interferon-β. Antiviral Res. 13, 165–174.

Trousdale, M.D., Nesburn, A.B., Su, T.L., Lopez, C., Watanabe, K.A. and Fox J.J. (1983) Activity of 1-(2′-fluoro-2′-deoxy-β-D-arabinofuranosyl)thymine against herpes simplex virus in cell culture and rabbit eyes. Antimicrob. Agents Chemother. 23, 808–813.

Trousdale, M.D., Nesburn, A.B., Watanabe, K.A. and Fox, J.J. (1981) Evaluation of the antiherpetic activity of 2′-fluoro-5-iodo-ara-C in rabbit eyes and cell cultures. Invest. Ophthalmol. Vis. Sci. 21, 826–832.

Tullo, A.B. (1985) Herpes simplex keratitis: latent and recurrent infection. In: D.L. Easty and G. Smolin (Eds) External eye disease, pp. 133–153, Butterworths, London.

Watanabe, K.A., Reichman, U., Hirota, K., Lopez, C. and Fox, J.J. (1979) Nucleosides. 110. Synthesis and antiherpes virus activity of some 2′-fluoro-2′-deoxyarabinofuranosylpyrimidine nucleosides. J. Med. Chem. 22, 21–24.

Watanabe, K.A., Su, T.L., Reichman, U., Greenberg, N., Lopez, C. and Fox, J.J. (1984) Nucleosides. 129. Synthesis of antiviral nucleosides: 5-alkenyl-1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)uracils. J. Med. Chem. 27, 91–94.

Weschler, S.L. and Nesburn, A.B. (1988) Recent advances in the molecular biology of herpes simplex latency: A short review. In: H.D. Cavanagh (Ed) The Cornea: Transactions of the World Congress on the Cornea III, pp. 511–516, Raven Press, New York.

Zar, J. (1984) Biostatistical Analysis, 2nd edn. Prentice-Hall, Inc., Englewood Cliffs, N.J.