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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

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Summary

The incorporation and metabolism of 2′-fluoro-5-substituted arabinosyl pyrimidine analogs, and their selective inhibition of viral DNA synthesis in herpes simplex virus type 1 (HSV-1)-infected and mock-infected Vero cells were studied by HPLC and CsCl isopycnic density gradient analysis of isolated DNAs. The amounts of radiolabeled analogs incorporated as parent compound following 10 μM exposure for 4 h were 10-fold higher in HSV-1-infected vs mock-infected cells for 2′-fluoro-5-difluoromethyl-Ara-U (F_2FMAU); 4.3-fold higher for 5-ethyl deoxyuridine (EdU); 2.6-fold higher for 2′-fluoro-5-methyl-Ara-U (FMAU) and 1.7-fold higher for dThd. For 2′-fluoro-5-ethyl-Ara-U (FEAU), 3.0 pmole of unchanged moiety was incorporated per 10^6 HSV-1-infected cells but no incorporation was detected in mock-infected cells. HPLC profiles showed that the percentages of radiolabeled analogs incorporated as parent compound in the DNA extracted from HSV-1-infected cells were 31.0% for F_2FMAU, 99.6% for EdU, 83.5% for FEAU and 98.3% for FMAU; from mock-infected cells, they were 63.6% for F_2FMAU, 96.7% for EdU, 97.3% for FMAU and no incorporation into DNA for FEAU was detected. CsCl density gradient analyses of isolated DNA showed that viral DNA synthesis was inhibited 98% by 10 μM FEAU, 92% by 10 μM F_2FMAU, 90% by

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2 μM FMAU and 80% by 50 μM EdU, whereas cellular DNA synthesis was inhibited by 53, 44, 61, 66 and 54%, respectively. We conclude that: (a) FEAU incorporation into host-cell DNA was not detectable but FEAU was selectively incorporated into HSV-infected cells; (b) FMAU and FEAU were metabolically stable; however, F_2FMAU was extensively metabolized; (c) FEAU and F_2FMAU were among the most selective inhibitors of HSV-1 DNA synthesis while allowing cellular DNA synthesis to continue.

2′-Fluoro-5-substituted-ara-pyrimidine; Herpes simplex virus; Incorporation; Metabolism; Selective inhibition; DNA synthesis

Introduction

Our early studies of structure-activity relationships have suggested that a 2′-fluoro substituent in the 'up' (arabino) configuration conferred more potent antiviral activity than did a 2′-OH, hydrogen, or other 2′-halogens (Fox et al., 1981a,b) (Fig. 1). 2′-fluoro nucleosides were resistant to catabolic cleavage by nucleoside phosphorylase, presumably as a result of the increased metabolic stability of N-glycosyl linkage imposed by this electronegative 2′-substituent. Of several 2′-fluoro-5-substituted-arabinosyl pyrimidine nucleosides synthesized in this Institute, FIAC and FMAU were found to be potent agents against herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) in vitro (Fox et al., 1981 a,b; Fox et al., 1988; Lopez et al., 1980; Watanabe 1979) (Table 1). These agents were selective substrates of viral thymidine kinase (TK) and the nucleoside 5′-triphosphates are selective inhibitors

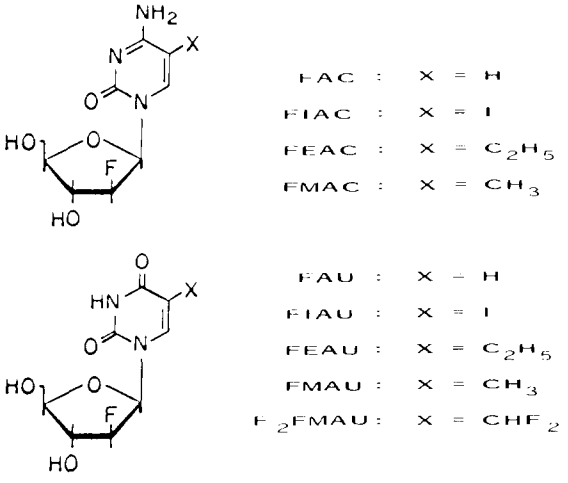


Fig. 1. Chemical structures of 2′-fluoro-arabinosylpyrimidines.

TABLE 1

Antiherpetic activity and cytotoxicity of 5-substituted 2′-fluoro-ara-pyrimidines

	Antiherpetic activity ED_{50} (μM)[1]		Cytotoxicity ID_{50} (μM)[2]	ID_{50}/ED_{50}	
5-substituted cytosine analogs (X)[3]	HSV-1 (F)	HSV-2 (G)		HSV-1	HSV-2
Iodo (FIAC)	0.023	0.03	21.7	940	720
CH_3 (FMAC)	0.006	0.026	1.5	250	57
CH_2CH_3 (FEAC)	0.035	0.32	143	4085	476
H (FAC)	–	–	0.11[4]	–	–
5-substituted uracil analogs (X)[3]					
CH_3 (FMAU)	0.010	0.23	2.8	280	120
CH_2CH_3 (FEAU)	0.024	0.24	>200	>8330	>833
CH_2F_2 (F_2FMAU)	0.34	1.04	97	285	93
H (FAU)	0.8	6.0	>100	–	–
CH_2CH_3 (EdU)[4]	3.9	–	–	–	–

[1] Antiherpetic activity was determined by plaque reduction assay in Vero cells (Fox et al., 1981, 1985).
[2] Cytotoxic activity was determined by inhibition of Vero cell growth (Fox et al., 1981, 1985).
[3] For chemical structure, see Fig. 1.
[4] Data of 5-ethyl 2′-deoxyuridine (EdU) are from De Clercq and Shugar (1975).

of viral DNA polymerase (Cheng et al., 1981; Allaudeen et al., 1982). Recently [2-^{14}C]FIAC and [2-^{14}C]FMAU were found to be incorporated into viral as well as mammalian cellular DNA (Chou et al., 1987a,b). FMAU was incorporated unchanged, whereas FIAC was extensively metabolized (Chou et al., 1987a,b). Although FIAC and FMAU are among the most potent anti-herpes virus compounds in animal models, toxicity toward the human central nervous system, and bone marrow depression at high doses, limit their clinical utility (Fanucchi et al., 1985).

Recent studies have shown that FEAU is 71-fold less toxic than FMAU toward host cells, but is only 4 to 10-fold less potent than FMAU against HSV-1 (Chou et al., 1987a,b); a similar specificity for virus-infected cells has been suggested for F_2FMAU (Matulic-Adamic et al., 1986; Matulic-Adamic et al., 1988). In addition, a preliminary in vivo study has shown that FEAU selectively inhibits the production of the virus-encoded DNA polymerase of woodchuck hepatitis virus in chronically infected woodchucks (an animal model for evaluation of potential antihepatitis B virus agents in humans) (Fourel et al., 1987). The inhibitory effect of FEAU is immediate, nontoxic and long-lasting when administered intravenously or orally (Fourel et al., 1987). FEAU is also highly effective in the treatment of simian varicella virus when given by intravenous or oral routes (Fox et al., 1987). In the present report, the metabolism and selectivity of these 2′-fluoro-5-substituted-arabinosyl nucleosides in HSV-1-infected and mock-infected Vero cells are studied. We found that FEAU was not detectable in host cell DNA, and that

FEAU and F_2FMAU are among the most selective inhibitors of HSV-1 DNA synthesis that we have investigated.

Materials and Methods

Compounds and radiolabeled chemicals

FMAU, FEAU, FIAC, F_2FMAU, and their radiolabeled compounds; [2-^{14}C]FMAU (9.18 mCi/mmole), [2-^{14}C], F_2FMAU (10.93 mCi/mmole), and [2-^{14}C]FIAC (13 mCi/mmole) were synthesized in this institute (Fox et al., 1981a,b, Fox et al., 1985; Lopez et al., 1980; Watanabe et al., 1979). [Ethyl-^{3}H]FEAU (6.22 Ci/mmole) was custom synthesized by Amersham Inc. (Arlington Heights, IL). EdU and [^{14}C]EdU (48.1 μCi/mmole) were generous gifts from Leonard I. Wieb (University of Alberta, Edmonton, Canada). Acyclovir [ACV, 9-(2-hydroxy-ethoxy-methyl guanine)] was from Burroughs Wellcome Co., Research Triangle Park, NC. [^{3}H-methyl]dThd (2.0 Ci/mmole) was purchased from ICN Radiochemicals, Irvine, CA. The purities for all the labeled compounds were found to be over 98% by HPLC. All other chemicals, unless otherwise indicated, were purchased from Sigma.

Cells and virus

African green monkey kidney (Vero) cells, were propagated in Eagle's modified minimal essential medium containing 10% fetal calf serum (FCS), 2 mM glutamine and antibiotics (penicillin and streptomycin). Cells were infected with one plaque-forming unit per cell of HSV-1 (strain F) in the same media containing 2% FCS. Virus- or mock-infected cells were treated with the indicated antiviral compound at the time of infection and incubated at 37°C for 12 h followed by the addition of [^{3}H]dThd for 2 h prior to DNA isolation. Alternatively, ^{14}C-radiolabeled FIAC, FMAU, F_2FMAU, EdU, [^{3}H]FEAU or [^{3}H]dThd were added to the cells 12 h postinfection; 4 h later the cells were washed in phosphate-buffered saline and DNA was isolated for further analysis.

Incorporation of labeled compounds into the DNA of virus- and mock-infected cells

DNA was isolated from virus- or mock-infected cells by trichloroacetate precipitation or phenol extraction (Chou et al., 1987a,b). The samples were digested with RNase A, RNase T_1, and RNase T_2. The DNA was precipitated by adding 3 volumes of 95% ethanol containing 2% of KAC and was kept at −20°C overnight followed by centrifugation at 4°C for 20 min at 10000 rpm to remove the RNAs. The DNAs were further digested with snake venom phosphodiesterase and hydrolyzed with bacterial alkaline phosphatase. The resulting deoxyribonucleosides were separated by reverse-phase HPLC (Waters, model 510) and analyzed using model 441 fixed wavelength detector. A Brownlee Lab RP-18 column (100 × 4.6 mm) connected to a Brownlee lab guard column (30 × 4.6 mm) was used for all reverse-phase analyses, with a flow rate of 1.2 ml/min and a column temperature of 40°C. Absorbance of the eluted material was monitored at 254 mm. The mobile

phase used consisted of a mixture of various concentrations (3–15%) of acetonitrile and 0.02 M potassium phosphate buffer, pH 3.8, during a 15 min period (Grant et al., 1982). The radioactivity was quantitated by liquid scintillation spectrophotometry.

Separation of viral and cellular DNA from virus- and mock-infected cells by CsCl isopycnic centrifugation

To study viral and cellular DNA synthesis in the presence of these antiviral compounds, DNAs isolated using proteinase K and sodium dodecyl sulfate followed by phenol/chloroform extraction were added to saturated CsCl solution and subjected to isopycnic gradient centrifugation for 65–75 h at 35000 rpm at 20°C, using a Beckman Ti-80 rotor in a Sorval model OTD preparative ultracentrifuge. Gradients were fractionated and radioactivity was quantitated by liquid scintillation spectrophotometry (Beckman Model LS 3801).

Results

HSV-1-infected and mock-infected Vero cells were exposed to radiolabeled pyrimidine analogs (10 μM), labeled DNA was purified and digested with the appropriate enzymes and was subjected to HPLC analysis. Of particular interest is that 83.5% [^{3}H]FEAU was incorporated as parent compound in the DNA extracted from HSV-1-infected cells (Table 2), i.e. 3.0 pmole in DNA per 10^6 cells as major component (Table 3) but no radioactivity above background was detectable in DNA from mock-infected cells (Fig. 2C). Approximately 98% of [2-^{14}C]FMAU was incorporated as FMAU in the DNA of both the HSV-1-infected cells and mock-infected cells. The amount of unchanged moiety incorporated into DNA was 4.2 pmole per 10^6 HSV-1-infected cells and 1.6 pmole per 10^6 mock-infected cells. The ratio of the incorporation in virus-infected cells vs mock-infected cells was 2.6-fold higher (Table 3). In contrast, 90% of [2-^{14}C]FIAC radioactivity in DNA from HSV-1-infected cells was recovered as 2′-fluoro-5-iodo-ara-U (FIAU), i.e. 42 pmole/10^6 cells. 61% of [2-^{14}C]FIAC radioactivity in DNA from mock-infected cells was found to be 2′-fluoro-ara-C (FAC) (2.95 pmole/10^6 cells). Only 13% of labeled FIAC (0.63 pmole/10^6 cells) was metabolized to FIAU and 17.5% was unidentified. Less than 1% of FIAC was recovered as FIAC in both viral-infected and mock-infected cells. The ratio of the incorporation in virus-infected cells vs mock-infected cells was 14.3 (Table 3). In fact, this ratio reflected the amounts of major components of FIAC detected in DNAs and did not result from a selective incorporation of FIAC or its metabolites, since the metabolites of FIAC in HSV-1-infected cells and mock-infected cells were totally different. The ratio, therefore, was 66.8 when comparing the incorporation of FIAU in viral-infected cells vs mock-infected cells. This observation is consistent with the results we previously reported on metabolism of FIAC in mammalian DNA (Grant et al., 1982). In DNA from HSV-1-infected cells, 31% of [2-^{14}C]F_2FMAU (1.1 pmole/10^6 cells) was detected as unchanged compound (Table 2). The peak in 5–6 min frac-

TABLE 2

Metabolic components in DNA digest from HSV-1-infected and mock-infected Vero cells[a] exposed to labeled nucleoside analogs

Labeled Compounds	Vero Cells	Total dpm[b]	Percentage present as									
			FAC	FAU	FMAU	FIAC	FIAU	F_2FMAU	FEAU	EdU	dThd	Misc.
[^{3}H]dThd	mock	68 280									98.4	1.6
	infected	112 180									99.2	0.8
[2-^{14}C]FIAC	mock	580	61.0	1.4	2.1	0.1	12.9					17.5
	infected	8 200	6.5	1.5	0.1	0.2	90.2					1.5
[2-^{14}C]F_2FMAU	mock	714			98.3							1.7
	infected	1 980			97.3							2.7
[Ethyl-^{3}H]FEAU	mock	ND										
	infected	248							83.5			16.5
[2-^{14}C]F_2FMAU	mock	66	6.1					63.6				30.3
	infected	1 930	0.2		0.6			31.0				68.3
[2-^{14}C]EdU	mock	8 100								96.7		3.3
	infected	34 140								99.6		0.4

[a] Vero cells were treated 12 h postinfection with labeled 2′-fluoro-5-substituted arabinosyl pyrimidine analogs (10 μM) for 4 h. For specific radioactivity, see Materials and Methods. The DNA was extracted, digested and subjected to HPLC analysis as described in Materials and Methods.

[b] Total dpm in DNA from 2×10^7 cells.

ND, not detectable.

TABLE 3

Major components in DNA digest from HSV-infected and mock-infected Vero cells[a]

Labeled Compounds	Vero cells	Major component detected[b]	pmole of major component in DNA per 10^6 cells[c]	Ratio of incorporation[d] (infected/mock-infected)
[^{3}H]dThd	mock	dThd	12.4	1.7
	infected	dThd	20.9	
[2-^{14}C]FIAC	mock	FAC	3.0	14.3[e]
	infected	FIAU	42.1	
[2-^{14}C]FMAU	mock	FMAU	1.6	2.6
	infected	FMAU	4.2	
[Ethyl-^{3}H]FEAU	mock	FEAU	ND[f]	-
	infected	FEAU	3.0	
[2-^{14}C]F_2FMAU	mock	F_2FMAU	0.1	10.1
	infected	F_2FMAU	1.1	
[2-^{14}C]EdU	mock	EdU	3.3	4.3
	infected	EdU	14.2	

[a] For experimental procedures, see legend for Table 1.
[b] Percentages of the major components detected, see Table 1.
[c] The data are the means of two experiments.
[d] pMole in DNA/10^6 cells.
[e] This ratio actually represents incorporation of FIAU in viral-infected cells over that of FAC in mock-infected cells. The ratio of incorporation of FIAU was 66.8 because 61% of FIAC was found as FAC and only 12.9% of FIAC (0.63 pmole/10^6 cells) was found as FIAU in mock-infected cells.
[f] Not detectable. No counts over background (18±2 cpm) were detectable at a concentration of labeled FEAU up to 100 μM in both mock-infected Vero and L1210 cells under the same conditions.

tions of radioactive chromatography was in the vicinity of the retention time of FMAU (5.85–6 min) but the peak did not co-elute with FMAU. This peak and the rest of the radioactivity that appeared in the front of the HPLC profile were unidentified (Fig. 2B); however, 64% of [2-^{14}C]F_2FMAU was found as F_2FMAU in DNA from mock-infected cells (0.11 pmole/10^6 cells). The ratio of F_2FMAU incorporation into the DNA of virus-infected cells vs mock-infected cells was 10.1. In contrast, [2-^{14}C]EdU was incorporated to the same extent in both HSV-1-infected and mock-infected cell DNA. No metabolites from EdU were found in the DNA digests under the experimental condition used.

In control experiments, HSV-1-infected cells and mock-infected cells were incubated with [^{3}H]dThd; 98.4% of the tritium in mock-infected cells and 99.2% in infected cells were detected as dThd, suggesting that the metabolism of this natural nucleoside was the same in virus-infected and mock-infected cells.

To determine selectivity of these nucleoside analogs with regard to the replication of HSV-1 DNA, isopycnographic analysis was performed to separate HSV-1 DNA from cellular DNA in HSV-1-infected cells incubated with or without these agents (Fig. 3). In the absence of antiviral nucleoside analogs, two distinct peaks of DNA were discernable corresponding to cellular DNA (lower density) and viral DNA (higher density), respectively (Fig. 3A). By calculating the total number of counts in the peaks corresponding to viral and cellular DNA, the incorporation of

[^{3}H]dThd into HSV-1-DNA and cellular DNA can be quantitated. When 10 μM ACV was used as a positive control, the incorporation of [^{3}H]dThd into HSV-1 DNA and cellular DNA was inhibited by 99.9% and 54.2%, respectively (data not shown). In the presence of 10 μM FEAU, viral synthesis was blocked while cellular DNA synthesis continued. The incorporation of [^{3}H]dThd into viral and cellular DNA was inhibited by 98% and 53%, respectively (Fig. 3B). Similar results were observed when 1 μM of FEAU was used (data not shown). In the presence of 2 μM FMAU, viral DNA and cellular DNA synthesis were inhibited by 90% and 61%, respectively (Fig. 3C). When 10 μM F_2FMAU was used, viral DNA synthesis was virtually eliminated (92% inhibition) but cellular DNA synthesis was

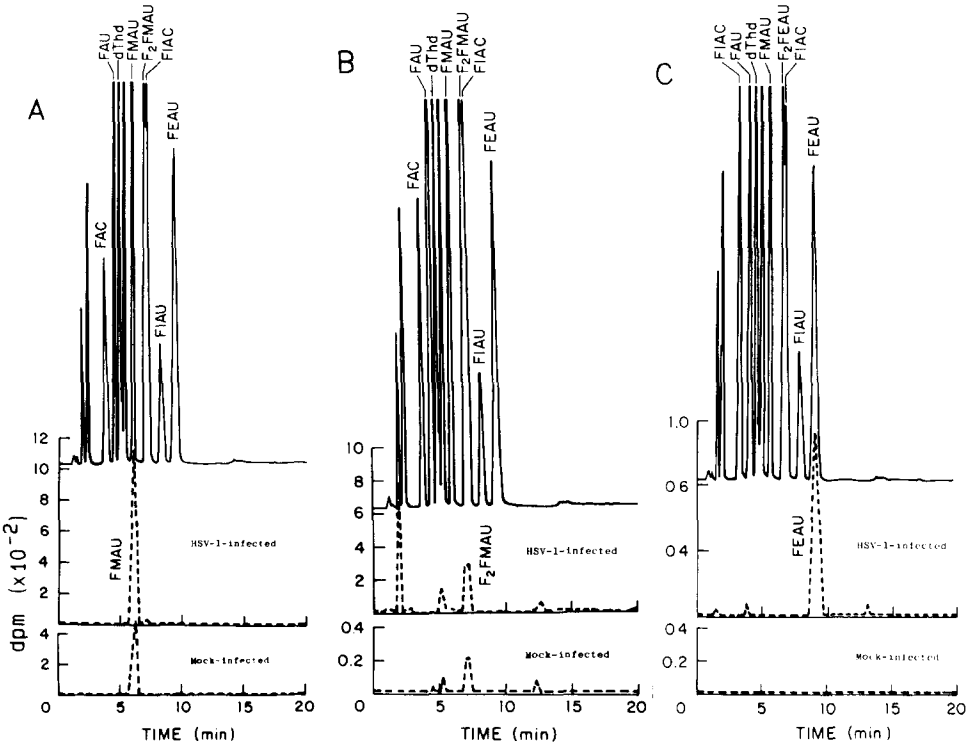
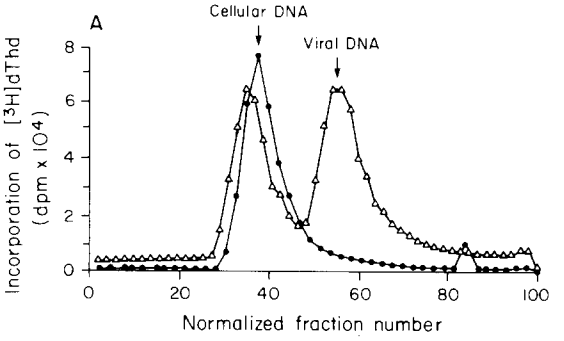


Fig. 2. Reverse phase HPLC chromatogram of enzyme-degraded DNA from HSV-1-infected and mock-infected Vero cells incubated with radiolabeled 2′-fluoro-5-substituted arabinosyl pyrimidine analogs (10 μCi/ml) for 4 h. Analyses were carried out in the presence of nonlabeled markers, FAC, FAU, dThd, FMAU, F_2FMAU, FIAC, FIAU and FEAU. For (A)-(C), absorbance of the standards is shown in the top panel, the middle panel is HSV-1-infected cells, and the lower panel is mock-infected cells. Solid line, absorbance at 254 nm; and dashed line, radioactivity in dpm. (A) mock-infected cells and HSV-1-infected cells treated with [2-^{14}C]FMAU; (B) mock-infected cells and HSV-1-infected cells treated with [2-^{14}C]F_2FMAU; (C) mock-infected cells and HSV-1-infected cells treated with [Ethyl-^{3}H]FEAU. Similar results were obtained in two other experiments.

still observable (44% inhibition) (Fig. 3D). In contrast, both viral and cellular DNA synthesis were inhibited to similar extents (80 and 66%, respectively) by 50 μM EdU (Fig. 3E). When the concentration of FMAU was increased to 10 μM, both viral and cellular DNA synthesis were completely inhibited (data not shown). The incorporation of [^{3}H]dThd into cellular DNA in mock-infected cells was inhibited by 1% for FMAU, and 7% for F_2FMAU. No inhibition was observed for ACV, FEAU or EdU.

Discussion

In this study, we found that most of the radiolabeled FIAC was incorporated into the DNA of HSV-1-infected cells as the deaminated metabolite FIAU, which has been shown to be a very potent anti-HSV agent (Schinazi et al., 1986). Our previous studies on the metabolism of 2′-fluoro-5-substituted arabinosyl pyrimidine nucleosides demonstrated that FIAC was incorporated into normal mammalian DNA primarily as the deiodinated compound FAC (Grant et al., 1982). FAC is a very weak anti-HSV agent but it is a potent cytotoxic agent against P815, L1210 and HL-60 cell lines in vitro. FAU is also a very weak metabolite of 2′-fluoro-5-substituted nucleoside analogs in terms of its anti-HSV or anticancer effects (Chou, Kong, Watanabe, Fox and Fanucchi, unpublished data). Our early studies on FIAC showed that the incorporation of [2-^{14}C]FIAC in Vero cells was more potently inhibited by dCyd. In HSV-1-infected Vero cells, however, the incorporation of [2-^{14}C]FIAC was more potently inhibited by dThd or dUrd (Chou et al., 1984). This difference in metabolism between HSV-1-infected and mock-infected cells may be more relevant to the selectivity of antiviral effect of this analog than to the mechanism of its antiviral effect. FMAU was incorporated into the DNAs of both HSV-1-infected cells and mock-infected cells as parent compound. In contrast, FEAU was only incorporated into DNA from HSV-1-infected cells under the experimental conditions used. Previous studies in this laboratory and other laboratories have reported that FEAU is a very poor substrate for the host HL-60 derived cytosol TK and an excellent substrate for HSV-1 and HSV-2 TKs (Chou et al., 1987a,b), and has a very low affinity for Vero cell TK but a high affinity toward HSV-1 and HSV-2 encoded TKs (Mansuri et al., 1987). Taken together, these findings explain why FEAU is less toxic than FIAC and FMAU, and therefore has a better therapeutic index as shown in Table 1. F_2FMAU, a newly synthesized pyrimidine analog with two fluoro groups in 5-substituted methyl side chain of FMAU (Matulic-Adamic et al., 1988), has less antiherpetic activity than FMAU but its therapeutic index is very close to that of FMAU. Yet its ratio of incorporation into virus-infected cell DNA over uninfected cell DNA is approximately 4-fold higher than that of FMAU. It has also been shown that F_2FMAU has a more selective inhibitory effect on the viral DNA synthesis than FMAU. Of specific interest was the finding that only 31% of [2-^{14}C]F_2FMAU was incorporated into DNA from HSV-1-infected cells in an unchanged form, while the incorporation of the parent compound was 2-fold (64%) greater in DNA from mock-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Viral DNA
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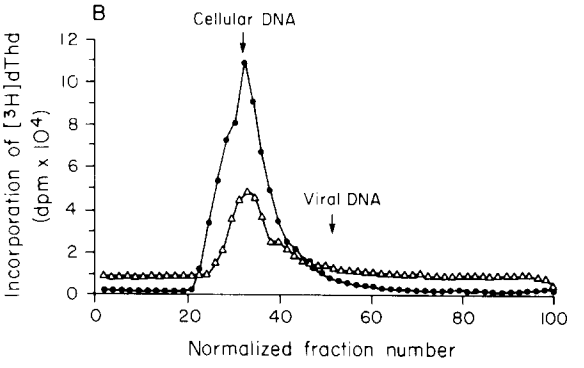
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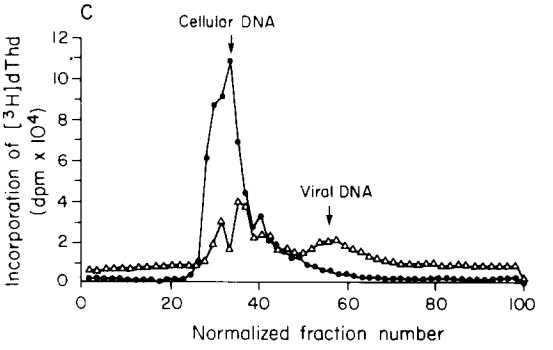
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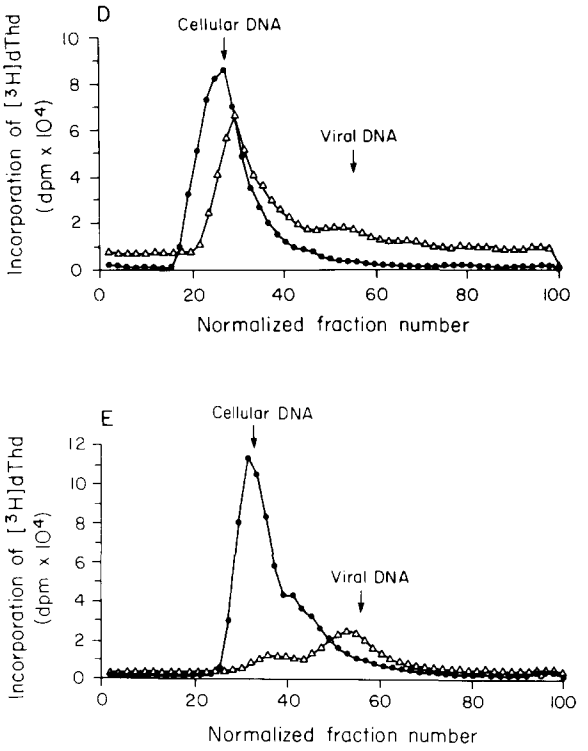

Fig. 3. Separation of viral and cellular DNA from HSV-infected and mock-infected Vero cells by isopycnic centrifugation in CsCl gradients. The amount of [^{3}H]dThd incorporation into viral and cellular DNA was determined in the absence of drug (A) and the presence of 10 μM FEAU (B), 2 μM FMAU (C), 10 μM F_2FMAU (D), 50 μM EdU (E). •—•, mock-infected cells; △—△, HSV-1-infected cells. Similar results were obtained in two other experiments and the minor differences observed in the migration of the cellular DNA in panels A and D are not significant.

infected cells. The metabolites from F_2FMAU were not identified, but clearly, they were not FMAU. These data indicate that F_2FMAU may have better selectivity than its parent compound FMAU and, unlike FMAU and FEAU, F_2FMAU was less stable and may have different metabolic behavior in virus-infected cells and mock-infected cells. The antiviral activity of the unidentified metabolites from F_2FMAU is worthwhile of examining further. The nature of the unidentified metabolite(s) will be determined in this laboratory.

The mechanisms of antiherpetic effects for the 2′-fluoro-5-substituted-ara-pyrimidines are still poorly understood. The selectivity of these antiherpes compounds is based on two assumptions: primarily, that specific phosphorylation of these nucleoside analogs occurs by the virus-encoded dThd kinase and, secondly, that the triphosphates of these compounds cause selective inhibition of the virus-

encoded DNA polymerase. Cheng and Allaudeen have reported that FIAC and FMAU have much greater affinity for HSV-1-encoded dThd kinase and DNA polymerase than the cellular dThd kinase and DNA polymerase (Cheng et al., 1981; Allaudeen et al., 1982). Ruth and Cheng (1981) have used an in vitro assay to show that FIAC triphosphate (FIACTP) and FMAU triphosphate (FMAUTP) can be used as substrate by the HSV-1-encoded DNA polymerase, but not by the cellular DNA polymerase. In their assay, these phosphorylated analogs were used half as efficiently as the normal substrate dTTP. Thus FIACTP and FMAUTP can serve as alternate substrates for viral DNA polymerase and hence be incorporated into DNA (Ruth and Cheng, 1981).

In this report, we confirmed that labeled 2′-fluoro-5-substituted-ara-pyrimidines were incorporated into the DNA of HSV-1-infected cells. We also found that HSV-1 DNA synthesis was inhibited to a greater degree than cellular DNA synthesis by 10 μM FEAU and F_2FMAU. Clearly, the replication of viral DNA was more susceptible to the inhibitory effect of FEAU and F_2FMAU than that of cellular DNA. This is most likely due to the greater amount of FEAU and F_2FMAU phosphorylation by HSV-1 thymidine kinase and to the selective inhibition of the HSV-1-specified DNA polymerase. Whether the antiherpetic activity of these compounds is related principally to incorporation of these compounds into viral DNA or to the ability of the triphosphates of these compounds to inhibit the viral DNA polymerase remains to be determined. We postulate that the triphosphates of these compounds may interact with viral DNA polymerase and eventually be incorporated into DNA, acting as a chain terminator.

In this study, we observed that all the compounds tested inhibited, to some extent, cellular DNA synthesis in HSV-infected cells but had little or no inhibitory effect on cellular DNA synthesis in mock-infected cells. A possible explanation is that in virus-infected cells, the compounds are phosphorylated by virus-encoded dThd kinase and in the triphosphate form they inhibit both viral and cellular DNA polymerase. In the absence of the virus-encoded dThd kinase (mock-infected cells), very little of the triphosphate forms of these compounds are produced. In addition, early steps in the replication cycle of HSV-1 may inhibit cellular DNA synthesis; therefore, the inhibition of cellular DNA synthesis in HSV-1-infected cells observed during the experiments described in this paper may actually involve both drug and virus effects.

In summary, the data presented herein suggest that FEAU and F_2FMAU may be the effective, relatively nontoxic antiviral agents on the basis of their selectivity. These two agents appear to be the most promising nucleoside analogs among the 2′-fluoro-arabinosyl pyrimidines tested thus far. Further toxicological studies of these two agents in animals are warranted and clinical trials may be considered.

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References

Allaudeen, H.S., Descamps, J., Sehgal, R.K. and Fox, J.J. (1982) Selective inhibition of DNA replication in herpes simplex virus infected cells by 1(2'-deoxy-2'-fluoro-β-D-arabinofuranosyl)-5-iodocytosine. J. Biol. Chem. 257, 11879–11882.

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., Lopez, C., Colacino, J.M., Feinberg, A., Watanabe, K.A., Fox, J.J. and Philips, F.S. (1984) Metabolic competition studies of 2'-fluoro-5-iodo-1-β-D-arabinofuranosylcytosine in Vero cells and herpes simplex type 1-infected Vero cells. Mol. Pharmacol. 26, 587–593.

Chou, T.-C., Kong, X.-B., Fanucchi, M.P., Cheng, Y.-C., Takahashi, K., Watanabe, K.A. and Fox, J.J. (1987a) Synthesis and biological effects of 2'-fluoro-5-ethyl-1-β-D-arabinofuranosyluracil. Antimicrob. Agents Chemother. 31, 1355–1358.

Chou, T.C., Kong, X.B., Scheck, A.C., Fanucchi, M.P., Watanabe, K.A., Fox, J.J. and Price, R. (1987b) Incorporation and metabolism of 2'-fluoro-5-substituted arabinosyl pyrimidines into DNA in herpes simplex virus type 1 (HSV-1) infected and uninfected Vero cells. Proc. Am. Assoc. Cancer Res. 28, 306.

De Clercq, E. and Shugar, D. (1975) Antiviral activity of 5-ethyl pyrimidine deoxynucleosides. Biochem. Pharmacol. 24, 1073–1078.

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.

Fourel, I., Hantz, O., Watanabe, K.A., Fox, J.J. and Trepo, C. (1987) FEAU therapeutic potential against HBV demonstrated in woodchuck hepatitis carriers. Abs. Am. Assoc. Study Liver Diseases, Cleveland, Ohio.

Fox, J.J., Lopez, C. and Watanabe, K.A. (1981a) 2'-Fluoro-arabinosyl pyrimidine nucleosides: chemistry, antiviral and potential anticancer activities. In: F. De Las Heras and S. Vega (eds.), Medicinal Chemistry, pp. 21–24, Pergamon Press, New York.

Fox, J.J., Watanabe, K.A., Lopez, C., Philips, F.S. and Leyland-Jones, B. (1981b) Chemistry and potent antiviral activity of 2'-fluoro-5-substituted arabinosylpyrimidine nucleosides. In: H. Shiota, Y.-C. Cheng and W.H. Prusoff (eds.), Herpes Virus. Clinical, Pharmacological and Basic Aspects, pp. 135–147, Excerpta Medica, International Congress Series 571, Amsterdam.

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.), Herpes Viruses and Virus Chemotherapy, pp. 53–61, Excerpta Medica, International Congress Series 667, Amsterdam.

Fox, J.J., Watanabe, K.A., Chou, T.-C., Schinazi, R.F., Soike, K.F., Fourel, I., Hantz, O. and Trepo, C. (1987) Recent studies on the antiviral activities of certain 2'-fluorinated arabinosyl-pyrimidine nucleosides. Am. Chem. Soc. Symposia Monograph (in press).

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, 1103–1111.

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.

Matulic-Adamic, J., Watanabe, K.A. and Price, R.W. (1986) Synthesis and biologic activity of α-monofluoro- and α,α-difluoro-thymidine nucleosides. Chem. Scripta 26, 127.

Matulic-Adamic, J., Takahashi, K., Chou, T.-C., Gadler, H., Price, R.W., Reddy, A.R.V., Kalman, T.I. and Watanabe, K.A. (1988) Nucleosides. 150. Synthesis and some biological properties of 5-monofluoromethyl, 5-difluoromethyl, and 5-trifluoromethyl derivatives of 2′-deoxyuridine and 2′-deoxy-2′-fluoro-β-D-arabinofuranosyluracil. J. Med. Chem. 31, 1642–1647.

Ruth, J.L. and Cheng, Y.-C. (1981) Nucleoside analogues with clinical potential in antivirus chemotherapy. Effect of several thymidine and 2′-deoxycytidine analogue 5′-triphosphates on purified human (α, β) and herpes simplex virus (types 1, 2) DNA polymerases. Molec. Pharmacol. 20, 415–422.

Schinazi, R.F., Fox, J.J., Watanabe, K.A. and Nahmias, A.J. (1986) Activities of 1-[2-deoxy-2-fluoro-β-D-arabinofuranosyl]-5-iodocytosine and its metabolites against herpes simplex virus types 1 and 2 in cell culture and in mice infected intracerebrally with herpes simplex type 2. Antimicrob. Agents Chemother. 29, 77–84.

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.