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ANTIVIRAL ACTIVITY OF 5-METHYLTHIOMETHYL-2′-DEOXYURIDINE AND OTHER 5-SUBSTITUTED 2′-DEOXYURIDINES

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Abstract—Of a series of eight 5-substituted 2′-deoxyuridine (dUrd) derivatives, which were evaluated for their antiviral and antimetabolic activities in primary rabbit kidney or human skin fibroblast cell cultures, five dUrd derivatives, 5-dimethylaminomethyl-dUrd, 5-chloroacetamidomethyl-dUrd, 5-iodoacetamidomethyl-dUrd, 5-pyrrolidinylmethyl-dUrd and 5-*N*-methylpiperazinylmethyl-dUrd, showed little, if any, activity. The three others, 5-formyl-dUrd, 5-azidomethyl-dUrd and 5-methylthiomethyl-dUrd, were found to inhibit the replication of various HSV (herpes simplex virus) strains (whether type 1 or 2) at a concentration of approximately 1–10 μg/ml. The antiviral activity of 5-formyl-dUrd may be accounted for by an inhibition of thymidylate synthetase. The inhibitory effect of 5-formyl-dUrd on HSV multiplication was readily reversed by adddition of 2′-deoxythymidine (dThd), and, in analogy with other established thymidylate synthetase inhibitors, 5-formyl-dUrd blocked the incorporation of [2-^{14}C]dUrd into cellular DNA to a markedly greater extent than the incorporation of [methyl-^{3}H]dThd. Unlike 5-formyl-dUrd, 5-azidomethyl-dUrd and 5-methylthiomethyl-dUrd did not preferentially inhibit the incorporation of [2-^{14}C]dUrd. Antiviral indexes, defined as the ID_{50} for [2-^{14}C]dUrd incorporation divided by the ID_{50} for HSV (type 1, strain KOS) replication, were 0.25, 43 and > 100 for 5-formyl-dUrd, 5-azidomethyl-dUrd and 5-methylthiomethyl-dUrd, respectively. The latter two compounds may therefore be considered as rather selective anti-herpes agents.

As recently reviewed by De Clercq and Torrence [1], various 5-substituted 2′-deoxyuridines (dUrd), including 5-fluoro-, 5-chloro-, 5-bromo-, 5-iodo-, 5-nitro-, 5-cyano-, 5-thiocyano-, 5-vinyl-, 5-ethynyl-, 5-allyl-, 5-ethyl-, 5-propyl-, 5-trifluoromethyl-, 5-methylamino-, 5-ethylamino-, 5-methoxymethyl-, 5-mercaptomethyl-, 5-methylmercapto-, 5-hydroxymethyl-, 5-hydroxy-, 5-allyloxy- and 5-propynyloxy-2′-deoxyuridine, have been shown to inhibit the replication of DNA viruses such as herpes simplex virus (HSV) and vaccinia virus. Among these dUrd analogues, 5-propyl-dUrd [2], 5-methylamino-dUrd [3,4], 5-ethylamino-dUrd [4], 5-methoxymethyl-dUrd [5,6] and 5-propynloxy-dUrd [7] specifically inhibit the replication of HSV. More recently, two new dUrd derivatives, *E*-5-(2-bromovinyl)-dUrd and *E*-5-(2-iodovinyl)-dUrd, have been added to the growing list of selective antiherpes agents [8–10]. Both compounds were found to inhibit the multiplication of HSV (type 1) at a concentration which was nearly 10,000-fold lower than the concentration required to suppress normal cell growth or metabolism. In marked contrast with the 5-(2-halogenovinyl)derivatives, the 5-(1-halogenovinyl)derivative

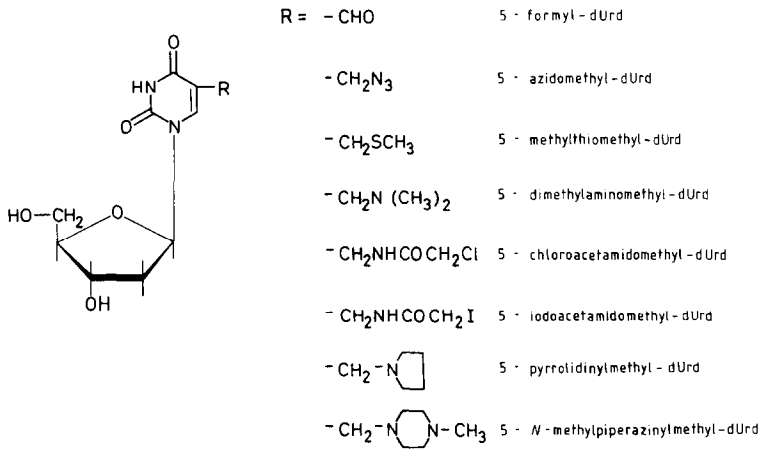

Fig. 1. Structural formulae of 5-substituted 2′-deoxyuridines.

5-(1-chlorovinyl)-dUrd did not exhibit any selectivity in its anti-herpes action [10].

We now report on the antiviral activity of a new series of 5-substituted 2′-deoxyuridines, 5-formyl-, 5-azidomethyl-, 5-methylthiomethyl-, 5-dimethylaminomethyl-, 5-chloroacetamidomethyl-, 5-iodoacetamidomethyl-, 5-pyrrolidinylmethyl- and 5-*N*-methylpiperazinylmethyl-dUrd (Fig. 1). Of this series, only 5-formyl-, 5-azidomethyl- and 5-methylthiomethyl-dUrd were endowed with antiviral properties. For 5-formyl-dUrd the active concentrations coincided closely with the cytotoxic concentrations. 5-azidomethyl-dUrd and 5-methylthiomethyl-dUrd were more selective in their antiherpes activity: they inhibited the replication of HSV (whether type 1 or 2) at concentrations which were considerably lower than those that affected normal cell metabolism.

MATERIALS AND METHODS

Compounds. The synthesis and physical characteristics of 5-formyl-dUrd, 5-azidomethyl-dUrd, 5-chloroacetamidomethyl-dUrd, 5-iodoacetamidomethyl-dUrd, 5-dimethylaminomethyl-dUrd, 5-pyrrolidinylmethyl-dUrd and 5-*N*-methylpiperazinylmethyl-dUrd have been described previously [11,12].

The synthesis of 5-methylthiomethyl-dUrd was accomplished by treatment of the 3′, 5′-di-*O*-toluoyl ester of 5-chloromethyl-dUrd with methanethiol and base followed by deprotection using potassium carbonate. The spectral characteristics and elemental analyses were as expected.

5-Iodo-dUrd was obtained from Ludeco (Brussels, Belgium).

Viruses. Both vaccinia and HSV served as the challenge viruses. For HSV, various laboratory strains or clinical isolates of either type 1 (HSV-1) or type 2 (HSV-2) were employed. The differentiation between HSV-1 and HSV-2 was based upon several criteria: (a) origin (genital or oral), (b) plaque-forming ability in chick embryo cells and (c) virus yields attained in primary rabbit kidney (PRK) cell cultures [13]. The laboratory strains HSV-1 (F), HSV-1 (Mac Intyre) and HSV-2 (G) were obtained from the American Type Culture Collection (Rockville, MD), whereas the laboratory strains HSV-1 (KOS), HSV-2 (LYONS) and HSV-2 (196) were provided by Dr. W. E. Rawls (Baylor University College of Medicine, Houston, TX) through the aid of Dr. J. Desmyter of our Institute. The thymidine kinase deficient (TK^-) mutant of HSV-1 (B 2006) was kindly given by Dr. Y.-C. Cheng (Roswell Park Memorial Institute, Buffalo, NY). The clinical HSV-1 isolates resulted from either vesicle fluid (VEX 142, VEW 257, VDX 500), mouth swab (VEX 538) or conjunctival swabs (VEX 149, VEO 231, VDO 374, VDX 348). The clinical HSV-2 isolates (VCX 210, VCX 301, VCX 304, VDX 192) originated from cervical swabs. All HSV stocks were prepared in PRK cell cultures.

Inhibition of virus-induced cytopathogenicity (CPE, cytopathic effect). Confluent PRK or HSF (human skin fibroblast) cell cultures in Linbro microtiter trays were inoculated with 100 $CCID_{50}$ of virus, 1 $CCID_{50}$ being the virus dose required to infect

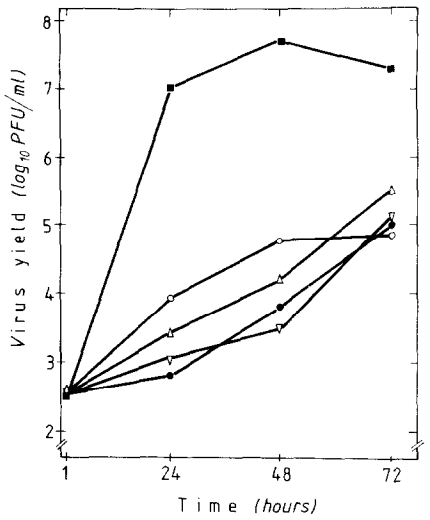

Fig. 2. Effect of 5-formyl-dUrd (—▽—), 5-azidomethyl-dUrd (—△—), 5-methylthiomethyl-dUrd (—○—) and 5-iodo-dUrd (—●—), all at a dose of 10 μg/ml, on the growth of HSV-1 (strain KOS) in PRK cell cultures. Virus yield was determined at different times after virus infection (as indicated on the abscissa). Control virus yields: —■—. The data represents average values for two separate determinations.

50 per cent of the cell cultures. After 1 hr of virus adsorption, residual virus was removed and the cell cultures were incubated with maintenance medium (Eagle's minimal essential medium supplemented with 3% calf serum) containing varying concentrations of the test compounds (400, 200, 100, . . . μg/ml). Viral CPE was recorded as soon as it reached completion in the control virus-infected cell structures.

Inhibition of virus growth. Confluent PRK cell monolayers in plastic Petri dishes were inoculated

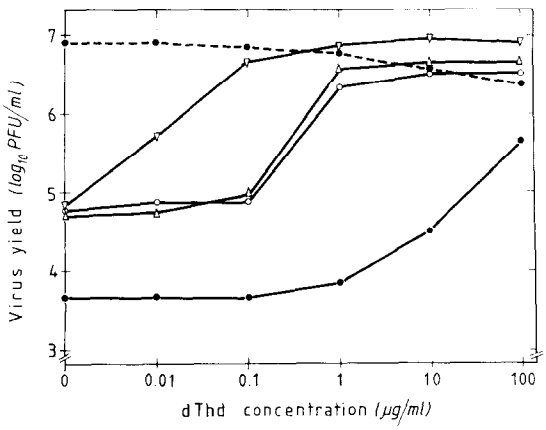

Fig. 3. Reversal of anti-herpes activity of 5-formyl-dUrd (—▽—), 5-azidomethyl-dUrd (—△—), 5-methylthiomethyl-dUrd (—○—) and 5-iodo-dUrd (—●—), all at a dose of 10 μg/ml, by varying concentrations of dThd (as indicated on the abscissa); dThd alone (---●---). Virus yield was determined 24 hr after infection of PRK cell cultures by HSV-1 (strain KOS). The data represents average values for two separate determinations.

with 30,000 PFU (plaque forming units) of HSV-1 (strain KOS) per Petri dish. After 1 hr of virus adsorption, residual virus was removed and the cell cultures were incubated with maintenance medium containing the indicated concentrations of the test compounds (see legends to Figs 2 and 3). Virus yields were determined at the indicated times (see legends to Figs. 2 and 3) by plaque formation in VERO cell cultures.

Inhibition of (methyl−^{3}H)dThd or (2−^{14}C)dUrd incorporation. The incorporation of [methyl−^{3}H]dThd and [2−^{14}C]dUrd into PRK cell DNA was monitored as described previously [14]. To this end, PRK cells were seeded in Linbro microwells in the presence of either [methyl-^{3}H]dThd (0.12 μCi/0.01 nmole/10^5 cells) or [2−^{14}C]dUrd (14 μCi/250 nmole/10^5 cells) and varying concentrations (400, 200, 100, . . . μg/ml) of the test compounds, and allowed to proliferate for 16 hr at 37° in a humidified, CO_2-controlled atmosphere.

RESULTS AND DISCUSSION

Of the eight 5-substituted dUrd derivatives which were examined for their inhibitory effects on HSV-1 (KOS) and vaccinia virus replication, and [methyl-^{3}H]dThd and [2-^{14}C]dUrd incorporation into cellular DNA, five compounds, 5-dimethylaminomethyl-dUrd, 5-chloroacetamidomethyl-dUrd, 5-iodoacetamidomethyl-dUrd, 5-pyrrolidinylmethyl-dUrd and 5-*N*-methylpiperazinylmethyl-dUrd, proved essentially inert as antiviral and antimetabolic agents (Table 1). As 5-iodoacetamidomethyl-dUMP has been previously described as a selective inhibitor of mammalian thymidylate synthetase [15], one may have expected this compound to preferentially inhibit the incorporation of [2-^{14}C]dUrd [14]. Yet, 5-iodoacetamidomethyl-dUrd did not markedly affect [2−^{14}C]dUrd incorporation (Table 1). Apparently, 5-iodoacetamidomethyl-dUMP is not sufficiently potent as thymidylate synthetase inhibitor (K_i/K_m = 3.5 for calf thymus thymidylate synthetase and 15 for the Ehrlich ascites tumor enzyme [15]) to achieve an appreciable inhibition of [2−^{14}C]dUrd incorporation *in vivo*.

The relatively low potency of 5-azidomethyl-dUMP as thymidylate synthetase inhibitor (K_i/K_m = 2.3 and 1.2 for the calf thymus and Ehrlich ascites tumor enzyme, respectively [16]) may also explain why 5-azidomethyl-dUrd exerted but a weak inhibitory effect on [2−^{14}C]dUrd incorporation (Table 1). This parallelism may be further extended to 5-methythiomethyl-dUrd which, on the one hand, did not inhibit [2−^{14}C]dUrd incorporation into PRK cell DNA (Table 1), and, on the other hand, demonstrated poor activity (in its 5′-monophosphate form) against *Lactobacillus casei*, calf thymus and Ehrlich ascites tumor thymidylate synthetase [K_i/K_m = 2, 5.8 and 6, respectively (unpublished observations)].

The results obtained for 5-formyl-dUrd contrasted markedly with those of 5-azidomethyl-dUrd and 5-methylthiomethyl-dUrd. 5-formyl-dUrd suppressed [2−^{14}C]dUrd incorporation at a concentration of 0.5 μg/ml, that is 100-fold lower than the concentration required to inhibit [methyl−^{3}H]dThd incorpor-

Table 1. Antiviral and antimetabolic activities of 5-methylthiomethyl-dUrd and related 5-substituted 2′-deoxyuridines in PRK and HSF cell cultures

Compound	ID^*_{50} (μg/ml)						Antiviral index†
	HSV-1 (KOS) PRK	Vaccinia PRK	HSV-1 (KOS) HSF	Vaccinia HSF	(methyl-^{3}H)dThd PRK	(2-^{14}C)dUrd PRK	
5-Formyl-dUrd	2	0.2	0.4	0.1	50	0.5	0.25
5-Azidomethyl-dUrd	0.7	7	1	7	60	30	43
5-Methylthiomethyl-dUrd	4	200	2	> 200	100	> 400	> 100
5-Dimethylaminomethyl-dUrd	> 200	> 200	> 200	> 200	> 400	> 400	
5-Chloroacetamidomethyl-dUrd	150	150	150	100	150	> 200	> 1.3
5-Iodoacetamidomethyl-dUrd	> 200	100	> 200	100	100	150	< 0.75
5-Pyrrolidinylmethyl-dUrd	> 200	≥ 200	> 200	≥ 200	> 200	> 200	
5-*N*-Methylpiperazinylmethyl-dUrd	> 200	≥ 200	> 200	≥ 200	> 200	> 200	
5-Iodo-dUrd	0.2	0.2	0.4	0.2	2.5	1.2	6

* Concentration required to reduce virus-induced CPE, or [methyl-^{3}H]dThd or [2-^{14}C]dUrd incorporation into DNA of uninfected PRK cells, by 50 per cent.

† Defined as the ratio of the ID_{50} for [2-^{14}C]dUrd incorporation to the ID_{50} for HSV-1 (KOS), both determined in PRK cells.

ation (Table 1). As premised [14], dThd analogues that inhibit dUrd incorporation to a significantly greater extent than dThd incorporation, may be assumed to selectively block thymidylate synthetase *in vivo*. Indeed, thymidylate synthetase is the sole metabolic step that distinguishes the pathways of dUrd and dThd incorporation into DNA dTMP (see Fig. 3 of ref. 14). Thus, 5-formyl-dUrd may be considered as a selective thymidylate synthetase inhibitor *in vivo*. Other examples of dThd analogues that may specifically act at the thymidylate synthetase level *in vivo* include 5-fluoro-dUrd, 5-trifluoromethyl-dUrd, 5-nitro-dUrd, 5-cyano-dUrd, 5-thiocyano-dUrd [14], 5-ethynyl-dUrd, 5-(1-chlorovinyl)-dUrd [10] and, possibly, 5-ethyl-dUrd [14]. That 5-formyl-dUrd may act as an inhibitor of thymidylate synthetase *in vivo* is not unexpected in view of previous studies which point to 5-formyl-dUMP as an extremely potent inhibitor of thymidylate synthetase from *Escherichia coli, Lactobacillus casei,* calf thymus and Ehrlich ascites tumor cells [16–19] (K_i/K_m values of 5-formyl-dUMP for these enzymes range from 0.0001 to 0.02 [16–19].)

As noted previously for other selective inhibitors of thymidylate synthetase such as 5-cyano-dUrd [20], 5-thiocyano-dUrd [21], 5-ethynyl-dUrd [10] and 5-nitro-dUrd [14], 5-formyl-dUrd inhibited the replication of both HSV and vaccinia virus (Table 1); and in PRK cells, vaccinia virus was inhibited at a 10-fold lower concentration than HSV-1 (KOS). 5-azidomethyl-dUrd and 5-methylthiomethyl-dUrd, two dThd analogues which did not selectively inhibit thymidylate synthetase *in vivo*, differed from 5-formyl-dUrd in antiviral behavior: these two compounds inhibited HSV replication at a significantly lower concentration than vaccinia virus replication (Table 1). For 5-methylthiomethyl-dUrd, there was even a $\geq$ 50-fold difference in the ID_{50} for vaccinia virus and the ID_{50} for HSV-1 (KOS). 5-methylthiomethyl-dUrd may therefore be considered as highly selective in its anti-herpes activity. This selectivity also appeared from toxicity studies which indicated that 5-methylthiomethyl-dUrd did not cause an inhibition of [2−^{14}C]dUrd incorporation (Table 1) or a microscopically detectable alteration of cell morphology (data not shown) at 400 μg/ml (100 times the anti-herpes concentration). For 5-azidomethyl-dUrd and 5-formyl-dUrd the minimum concentrations to produce evidence of microscopic cytotoxicity in confluent PRK cell monolayers were 40 and 4 μg/ml, respectively. Thus, antiviral indexes defined as the minimum dose at which cytotoxicity was observed microscopically divided by the ID_{50} for HSV-1 (KOS) replication were found to be > 100 for 5-methylthiomethyl-dUrd, ~ 50 for 5-azidomethyl-dUrd and 2 for 5-formyl-dUrd. These antiviral indexes correlated well with those based upon the ratio of the ID_{50} for [2−^{14}C]dUrd incorporation to the ID_{50} for HSV-1 (KOS) replication (Table 1). They further point to the lack of specificity in the antiviral action of 5-formyl-dUrd. In accord with previous findings [22], 5-formyl-dUrd inhibited normal cell metabolism and virus multiplication at approximately the same concentration (1–2 μg/ml).

Table 2. Susceptibility of different HSV-1 and HSV-2 strains to the inhibitory effects of 5-methylthiomethyl-dUrd, 5-azidomethyl-dUrd, 5-formyl-dUrd and 5-iodo-dUrd in PRK cell cultures

Herpes strain	ID^*_{50} (μg/ml) 5-methylthiomethyl-dUrd	5-azidomethyl-dUrd	5-formyl-dUrd	5-iodo-dUrd
HSV-1 strains				
KOS	4	0.7	2	0.15
F	10	2	2	0.15
Mac Intyre	4	1	0.7	0.15
VEX 142	10	7	2	0.1
VEX 149	20	2	0.4	0.15
VEX 538	20	10	2	0.1
VEO 231	10	10	1	0.15
VEW 257	20	2	1	0.15
VDO 374	2	4	0.4	0.1
VDX 348	40	7	1	0.1
VDX 500	20	2	0.4	0.1
Average for all HSV-1 strains	14.5	4.3	1.2	0.13
HSV-2 strains				
LYONS	4	7	2	0.2
196	4	10	1	0.2
G	4	10	1	0.4
VCX 210	20	1	4	0.3
VCX 301	4	4	1	0.3
VCX 304	4	7	1	0.4
VDX 192	10	4	0.4	0.4
Average for all HSV-2 strains	7.1	6.1	1.5	0.31
HSV-1 TK$^-$ mutant (B 2006)	> 200	≥ 40	2	> 200

* Concentration required to reduce virus-induced CPE by 50 per cent.

The anti-herpes activity of 5-methylthiomethyl-dUrd, 5-azidomethyl-dUrd and 5-formyl-dUrd was not restricted to HSV-1 (KOS). Their antiviral spectrum also encompassed various other laboratory strains of HSV-1 (F, Mac Intyre) and HSV-2 (LYONS, 196, G) as well as clinical HSV-1 and HSV-2 isolates (Table 2). Neither 5-methylthiomethyl-dUrd, 5-azidomethyl-dUrd nor 5-formyl-dUrd discriminated between HSV-1 and HSV-2: they proved equally effective against both types. The standard anti-herpes compound 5-iodo-dUrd, however, was slightly more inhibitory for HSV-1 than for HSV-2 (Table 2). As noted previously [23], HSV type 2 strains tend to be less sensitive to 5-iodo-dUrd than type 1 strains.

While 5-formyl-dUrd inhibited the replication of a dThd kinase deficient (TK^-) mutant of HSV-1 at a concentration of 2 μg/ml, thus close to the cytotoxic concentration, 5-methylthiomethyl-dUrd, 5-azidomethyl-dUrd and 5-iodo-dUrd did not effect the replication of the TK^- mutant of HSV-1 (Table 2; for azidomethyl-dUrd the lack of activity could only be established at concentrations up to 40 μg/ml, since the compound was cytotoxic from 40 μg/ml). The latter results suggest that, as previously shown for several other 5-substituted 2′-deoxyuridines [24,25], 5-methylthiomethyl-dUrd and 5-azidomethyl-dUrd must be phosphorylated by the HSV-induced dThd kinase to exert a selective anti-herpes effect.

That the inhibitory effects of 5-formyl-dUrd, 5-azidomethyl-dUrd and 5-methylthiomethyl-dUrd on virus-induced CPE (Table 1) actually reflected an inhibition of virus multiplication was ascertained by measuring the effects of 5-formyl-, 5-azidomethyl- and 5-methylthiomethyl-dUrd on HSV-1 (KOS) growth. When assayed at 100 μg/ml, all three compounds as well as 5-iodo-dUrd brought about a significant reduction in virus yield (Fig. 2). For the 24 hr yield reduction in virus titer amounted to 3–4 $\log_{10}$, and the relative order of (decreasing) activity was 5-iodo-dUrd > 5-formyl-dUrd > 5-azidomethyl-dUrd > 5-methylthiomethyl-dUrd. This is the same order of activity as that obtained for the CPE-inhibition experiments (Table 2).

In agreement with the postulated mode of action of 5-formyl-dUrd, that is an inhibition of thymidylate synthetase (see above), the antiviral activity of 5-formyl-dUrd was readily reversed by dThd: at a concentration 100-fold lower than that of 5-formyl-dUrd, dThd annihilated the inhibitory effect of 5-formyl-dUrd on HSV-1 (KOS) growth (Fig. 3). Similar results have been reported previously for 5-thiocyano-dUrd [21] and 5-nitro-dUrd [14]. These dThd analogues have also been assumed to act as selective inhibitors of thymidylate synthetase [14], and, consequently, they lost their antiviral potency upon addition of extremely low concentrations of dThd (100-fold lower than the concentration at which 5-thiocyano-dUrd and 5-nitro-dUrd were employed) [14, 21]. To abolish the antiviral activity of 5-iodo-dUrd, much higher dThd concentrations were required: even at a 10-fold higher concentration than that of 5-iodo-dUrd, dThd did not completly reverse the inhibitory effect of 5-iodo-dUrd on HSV-1 (KOS) growth (Fig. 3). The relative resistance of the antiviral potency of 5-iodo-dUrd to reversal by dThd, as noted herein (Fig. 3) and previously [14], would seem compatible with the purported mode of action of 5-iodo-dUrd, that is at the DNA replication or transcription level, thus subsequently to its incorporation into DNA [26].

The inhibitory effects of 5-azidomethyl-dUrd and 5-methylthiomethyl-dUrd on herpes virus multiplication could also be reversed by dThd (Fig. 3). This reversal was achieved at a dThd concentration that was intermediate between the dThd concentrations required to abolish the antiviral activities of 5-formyl-dUrd and 5-iodo-dUrd. The fact that the antiviral activity of 5-azidomethyl-dUrd and 5-methylthiomethyl-dUrd resisted the reversing effect of dThd more effectively than 5-formyl-dUrd, would strengthen the hypothesis that 5-azidomethyl-dUrd and 5-methylthiomethyl-dUrd, unlike 5-formyl-dUrd, do not specifically act at the thymidylate synthetase level. Neither do they seem to act like 5-iodo-dUrd. Otherwise their antiviral activity should have been more resistant to reversal by dThd. 5-azidomethyl-dUrd and 5-methylthiomethyl-dUrd may interfere at one or several stages of DNA biosynthesis. Their selective anti-herpes activity seems to depend on the conversion of the nucleoside to the 5′-monophosphate by the virus-induced dThd kinase. Whether the 5′-monophosphate is the active form of 5-methylthiomethyl-dUrd and 5-azidomethyl-dUrd, or whether the compounds must be further converted to the 5′-di- and triphosphate forms to exert their specific anti-herpes action is a matter of further study.

CONCLUSION

Whereas 5-dimethylaminomethyl-dUrd, 5-chloroacetamidomethyl-dUrd, 5-iodoacetamidomethyl-dUrd, 5-pyrrolidinylmethyl-dUrd and 5-*N*-methylpiperazinylmethyl-dUrd were almost inert as antiviral and antimetabolic agents, 5-formyl-dUrd, 5-azidomethyl-dUrd and 5-methylthiomethyl-dUrd exhibited a characteristic biologic activity. 5-Azidomethyl-dUrd and 5-methylthiomethyl-dUrd proved to be specifically active against herpes simplex virus. In this aspect, 5-methylthiomethyl-dUrd is reminiscent of 5-methoxymethyl-dUrd which has also been accredited with a therapeutic index of > 100 [6] and which has recently shown efficacy in the treatment of experimental herpes simplex keratitis in rabbits [27]. In view of its selective activity against herpes simplex virus in cell culture, 5-methylthiomethyl-dUrd could be pursued as an anti-herpes drug.

In marked contrast with 5-azidomethyl-dUrd and 5-methylthiomethyl-dUrd, 5-formyl-dUrd was not specific in its antiviral action. 5-Formyl-dUrd inhibited the replication of both herpes simplex and vaccinia virus, but, at the concentrations that were required to inhibit virus replication, 5-formyl-dUrd also inhibited normal cell metabolism and proliferation [22]. Its antiviral and antimetabolic properties can at least partially be attributed to an inhibition of thymidylate synthetase. Akin to other specific inhibitors of thymidylate synthetase such as 5-fluoro-dUrd and 5-trifluoromethyl-dUrd [28], 5-formyl-dUrd should be pursued as an antitumor agent.

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