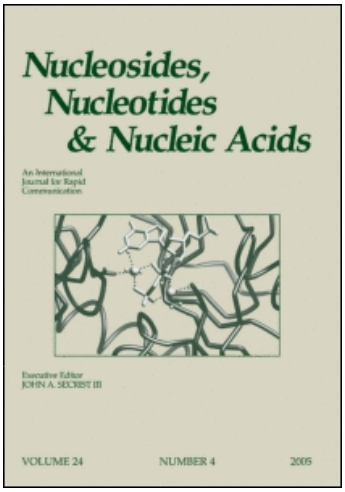

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HUMAN CYTOMEGALOVIRUS STIMULATES THYMIDYLATE SYNTHASE IN HUMAN EMBRYONIC LUNG CELLS: A POSSIBLE TARGET FOR ANTI-HCMV THERAPY?

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ABSTRACT: We have demonstrated that thymidylate synthase (TS) activity is enhanced by more than 10- to 20-fold in HCMV-infected HEL cells as compared to non-infected cells. The increase in TS activity was found (i) to parallel the progression of the viral cytopathic effect over a 5-days period, (ii) to be independent of viral DNA synthesis and to result from an early event in the viral replicative cycle. Several compounds known to be targeted at TS, such as 5-fluoro-dUrd, 5-trifluoromethyl-dUrd, and 5-formyl-dUrd, inhibited TS activity in HCMV-infected cells and, concomitantly, displayed HCMV activity. The exact impact of inhibition of TS activity in the overall anti-HCMV activity of the test compounds remains however to be determined.

INTRODUCTION

TS catalyzes the conversion of dUMP to dTMP. Regulation of TS activity in the cell is coupled with the synthesis of DNA. During the S-fase of the cell-cycle there is an increased need for nucleotides and the enzyme is stimulated. When DNA synthesis stops, the accumulation of dTMP, dTDP and dTTP results in inhibition TS activity. Since there is also an increased demand for nucleotides during viral replication, we examined whether HCMV infection of fibroblasts modulates cellular TS activity. We found that TS activity is markedly stimulated in HCMV-infected cells, that this process is independent of viral DNA synthesis, and that several inhibitors of TS-activity efficiently inhibit HCMV replication.

MATERIALS AND METHODS

Determination of TS activity in HCMV-infected and non-infected HEL cells. Confluent HEL cells in 24-well plates were infected with HCMV (Davis strain: at 1.8 x 10^3 PFU/ml MEM for 2 hr). Cultures were then further incubated for the indicated

period, at which time 0.5 μCi [5-^{3}H]dUrd [Specific radioactivity 20 Ci/mmol] in 250 μl MEM was added to each well. After 2, 4, 6 or 8 hr incubation at 37°C hr, 400 μl medium from 2 wells was pooled and the amount of released tritium determined as described before[1]. To determine the inhibitory effects of the test compounds on TS activity, they were added at different concentrations to the uninfected or infected (100% CPE) cultures in 24-well plates, together with [5-^{3}H]dUrd and the amount of released ^{3}H was measured 6 hr later.

To determine the inhibition of [6-^{3}H]dUrd incorporation (another measure of TS-activity) in HCMV-infected HEL cells, cultures exhibiting 100% CPE were incubated with [6-^{3}H]dUrd (Specific radioactivity 25 Ci/mmol) at 0.25 μCi/ml together with different concentrations of the compounds. After 24 hr incubation at 37°C, cells were washed twice with PBS, disrupted mechanically and cell-associated, acid-insoluble radioactivity determined.

Anti-HCMV activity. Anti-HCMV activity was determined by the CPE reduction assay as described before[2].

RESULTS AND DISCUSSION

Increased TS-activity in HCMV-infected HEL cells. A marked enhancement of TS-activity was observed in HCMV-infected HEL cell cultures as compared to uninfected cells (Fig. 1). Similar observations were recently made for varicella-zoster virus[3]. The increase in TS-activity was more pronounced at later times post infection when CPE became more prominent. At day 5 post infection, at which time the infected cultures exhibited 100% CPE, almost a ten-fold increase in TS activity was observed. To determine whether this increase in TS activity was coupled with viral DNA synthesis, cells were infected at a high multiplicity of infection (so as to obtain 100% CPE at 1 to 2 days p.i.) and HPMPC or PFA (two inhibitors of viral DNA synthesis) were added (at 50 μg/ml). Under this condition (i.e. early after infection and in the presence of inhibitors of viral DNA synthesis) TS activity was stimulated to a comparable level as in the virus control (data not shown). Thus stimulation of TS activity in HCMV-infected cells takes place early in the replication cycle and is not coupled with viral DNA synthesis

Inhibition of HCMV replication by inhibitors of TS. Several well known inhibitors of TS i.e. 5-iodo-deoxyuridine (IdUrd, IDU); 5-fluoro-deoxyuridine (FdUrd); 5-formyl-deoxyuridine (Formyl-dUrd); 5-trifluoromethyl-deoxyuridine (F_3dThd, TFT); *E*-5-(2-bromovinyl)-deoxyuridine (BV-dUrd, BVDU) and 5-fluorouridine (FUrd) were

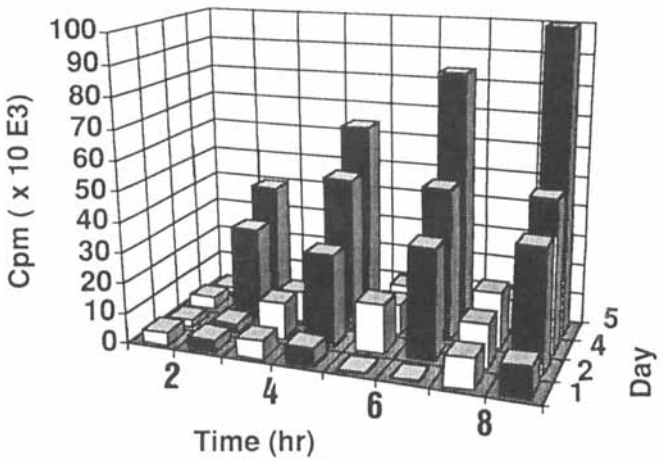

Fig 1. TS activity in HCMV-infected (black histograms) and uninfected HEL cells (white histograms) at 1, 2, 4 and 5 days post infection, and upon a 2, 4, 6 or 8 hr incubation with [5-^{3}H]dUrd.

Table 1. Anti-HCMV activity of TS inhibitors in combination with dThd

Compound	IC_{50} (μg/ml)			
	-	0.1 μg/ml dThd	1 μg/ml dThd	10 μg/ml dThd
FUrd	0.90	0.88	0.58	0.17
IDU	0.25	0.45	3.7	38
FdUrd	0.03	0.52	0.74	0.90
Formyl-dUrd	1.5	6.8	49	> 200
BVDU	≥20	> 200	> 200	> 200
TFT	0.11	1.3	35	> 50

Data are mean values for at least 2 separate determinations.

evaluated for their inhibitory effect on HCMV replication. The compounds inhibited HCMV replication at concentrations below those that caused a detectable alteration of normal cell morphology (Table 1). Addition of increasing concentrations of dThd resulted in a progressive increase of the IC_{50} values (Table 1), whereas addition of dUrd (at 10 μg/ml) had little or no effect (data not shown).

Inhibition of TS-activity in infected and non-infected cells IC_{50} values for inhibition of TS activity (as determined by ^{3}H release) by the different compounds were comparable for infected and non-infected cells (Table 2), FdUrd and TFT being the

Table 2. Inhibition of TS activity in HCMV-infected and non-infected HEL cells

Compound	IC_{50}[a] (μg/ml)		
	[^{3}H] release		[6-^{3}H]dUrd incorporation
	HCMV-infected cells	uninfected cells	HCMV-infected cells
FUrd	0.3	0.4	1.2
IDU	0.2	0.2	1.3
FdUrd	0.0001	0.0001	0.002
Formyl-dUrd	0.2	0.1	0.6
BVDU	0.2	0.2	0.04
TFT	0.005	0.007	0.021

Data are mean values for at least 2 separate experiments.

strongest inhibitors. When inhibition of TS activity was assessed by means of measuring [6-^{3}H]dUrd incorporation, FdUrd and TFT invariably elicited the most potent activity. BVDU, although equipotent to IDU, Formyl-dUrd and FUrd in inhibiting TS activity displayed little or no anti-HCMV activity. The exact impact of TS as a target for anti-HCMV therapy needs therefore further study.

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