NOTES

Differential Effect of Nucleoside Analog Triphosphates on Ribonucleotide Reductases from Uninfected and Herpes Simplex Virus-Infected HeLa Cells

KOJI NAKAYAMA, JERRY L. RUTH, AND YUNG-C. CHENG*

Department of Pharmacology, School of Medicine, University of North Carolina, Chapel Hill, North Carolina 27514

Received 30 December 1981/Accepted 8 March 1982

Effects of the triphosphates of eight pyrimidine nucleoside analogs (5-substituted, 2'-fluoroara-, and acyclonucleosides) and acycloguanosine were examined on the ribonucleotide reductases prepared from uninfected and herpes simplex virus (types 1 and 2)-infected HeLa cells. Of the analogs tested, *E*-5-propenyl- and *E*-5-(2-bromovinyl)-dUTP were more potent inhibitors than dTTP of the enzymes from virus-infected cells, whereas only the former compound showed this effect on the uninfected HeLa enzyme.

Ribonucleotide reductase (EC 1.17.4.1) from eucaryotic cells catalyzes the reduction of ribonucleoside diphosphates to 2'-deoxynucleoside diphosphates, similar to the *Escherichia coli* enzyme which has been extensively studied (12). This enzyme, which is involved in a balanced formation of dCDP, dUDP, dGDP, and dADP, is allosterically regulated in a complex manner by ribo- and deoxyribonucleoside triphosphates. Since the pathways involving ribonucleotide reductase comprise the major de novo source of four deoxynucleoside triphosphates for DNA synthesis, and since intracellular deoxynucleoside triphosphate pools are usually too small to sustain DNA synthesis for more than a few minutes, the possibility of influencing the enzyme has implications in both antiviral and antineoplastic chemotherapy. Close correlations have been observed between rate of tumor growth and amount of reductase activity (6). Other groups (8, 9) have reported three- to ninefold increases in reductase activity after infection with herpes simplex virus (HSV). The enzyme specificity in HSV-infected cells also differs in regulatory response to dTTP and dATP from uninfected cells (8, 9), although there is no direct evidence which indicates the virus-induced ribonucleotide reductase activity is actually encoded on the viral genome.

We extracted the reductase activity from HeLa cells, HSV type 1 (HSV-1)-infected HeLa cells, and HSV-2-infected cells and examined the behavior of the triphosphates of acycloguanosine (7), *E*-5-(2-bromovinyl)-2'-deoxyuridine

(BV-dUrd) (5), 2'-fluoro-5-iodo-1- β -D-arabino-cytosine (FIAC) (13), *E*-5-propenyl-2'-deoxyuridine (propenyl-dUrd) (4), and several other analogs on the enzyme preparations. Assessing any effects of the analog nucleoside triphosphates on ribonucleotide reductase could prove useful, since the molecular site of the antiviral-anticancer action is still unclear for the nucleoside analogs.

Analog triphosphates were chemically synthesized by a general procedure described earlier (11) and were greater than 96% homogeneous upon anion-exchange high-pressure liquid chromatography. Ribonucleotide reductase preparations were obtained by a modification of the method of Ponce de Leon et al. (10): HeLa S₃ cells grown in suspension were either uninfected or infected with HSV-1 (KOS) or HSV-2 (333) at 5 to 10 PFU per cell for 10 h. Cell pellets were disrupted in 5 volumes of homogenizing buffer (50 mM HEPES [N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid] buffer [pH 7.5], 2 mM dithiothreitol, 1 mM MgCl₂) by using a glass homogenizer and then centrifuged for 30 min at 20,000 $\times$ g. Streptomycin sulfate (final concentration, 1%) was added to the supernatant. After centrifugation, ammonium sulfate (45% saturation) was added to the supernatant. The precipitate which formed was collected by centrifugation, dissolved in homogenizing buffer, dialyzed against additional buffer, and used as the enzyme. Purification of ribonucleotide reductase to high purity was not accomplished since attempts at further purification caused loss of

FIG. 1. Effect of dUTP analogs on CDP reduction. Enzyme was prepared from (A) mock-, (B) HSV-1-, and (C) HSV-2-infected HeLa cells. CDP reduction is expressed as the percentage of dCDP formation in the control. Symbols: (○) dTTP; (●) 5-propyl-dUTP; (▲) *E*-5-propenyl-dUTP; (■) *E*-5-(2-bromovinyl)-dUTP.

activity. This lability of virus-induced reductases has been observed by others (8). Although it is unlikely that the host enzyme exists for 10 h postinfection without any modification (1), it is not clear to what degree the reductase activity obtained from HSV-infected cells reflects true virus-induced activity. Therefore, the data presented here should be considered preliminary pending further purification and characterization of the enzymes.

Ribonucleotide reductase assays, at 0.1 scale, were performed as described earlier (2, 3), using [^{14}C]CDP.

The enzymatic reduction of CDP to dCDP is allosterically inhibited by dTTP, dGTP, and dATP (3, 12). By using the crude enzyme preparations described above, it was found that ribonucleotide reductase from either HSV-1- or HSV-2-infected cells was less sensitive to inhibition by dTTP and dGTP than was the enzyme

from uninfected HeLa cells and equally sensitive to inhibition by dATP. These observations are in partial agreement with other studies, which have reported that reductases from HSV-1-infected KB cells (10) and HSV-infected Vero cells (9) are less sensitive to both dTTP and dATP.

With formation of dCDP from CDP as a measure of reductase activity, the triphosphates of three selective antiviral 5-substituted nucleosides were tested as allosteric inhibitors of the enzyme and compared with dTTP (Table 1; Fig. 1). *E*-5-Propenyl-dUTP was more inhibitory than dTTP for all three enzyme preparations. *E*-5-(2-Bromovinyl)-dUTP had less effect than dTTP on reductase from uninfected cells but was more inhibitory than dTTP on enzymes from either HSV-1- or HSV-2-infected cells. 5-Propyl-dUTP was a very poor inhibitor ($I_{50} > 400 \mu\text{M}$) of all of the enzyme preparations. (I_{50} is the concentration inhibiting the reduction of CDP by 50%, relative to control.)

Several 2'-deoxy-2'-fluoroarabinoside and acyclonucleoside triphosphates were also tested as inhibitors of CDP reduction (Table 1). The triphosphate of FIAC was a very poor inhibitor (I_{50} , 360 to 720 μM) of reductase activity, although it was slightly more effective against the reductase from HSV-infected cells. Both 2'-fluoro-araTTP and 2'-fluoro-5-methyl-araCTP were mild inhibitors. Acyclo-GTP, acyclo-CTP, and acyclo-TTP were also poor inhibitors ($I_{50} > 200 \mu\text{M}$) of all of the ribonucleotide reductase preparations.

Several of these nucleoside analogs are potent

TABLE 1. Effect of nucleoside triphosphate analogs on ribonucleotide reductase

Inhibitor	I_{50}^a (μM)		
	Mock infected	HSV-1 infected	HSV-2 infected
dTTP	45	88	220
5-Propyl-dUTP	>400	>400	>400
<i>E</i> -5-Propenyl-dUTP	18	56	92
<i>E</i> -5-(2-Bromovinyl)-dUTP	95	49	66
2'-Fluoro-araTTP	80	175	160
2'-Fluoro-5-iodo-araCTP	730	430	360
2'-Fluoro-5-methyl-araCTP	175	235	160
Acyclo-TTP ^b	>200	ND ^c	ND
Acyclo-CTP ^b	200	ND	ND
Acyclo-GTP ^b	>200	>200	>200

^a I_{50} represents the concentration of a drug which inhibited the reduction of CDP by 50%, relative to control (mean of duplicates).

^b Acyclonucleotides refer to analogs in which the sugar moieties are replaced by a (2-hydroxy-ethoxy)methyl group.

^c ND, Not determined.

and quite selective antiviral agents, especially BV-dUrd, acycloguanosine, FIAC, propenyl-dUrd, and propyl-dUrd. Most of them depend on phosphorylation by the HSV-encoded thymidine kinase for their selectivity (4, 7, 11). Although it appears that these analogs exert virucidal action after phosphorylation, the actual sites of the action are still unclear. The results presented here strongly suggest that the antiviral activities for the analogs, including propyl-dUrd, acycloguanosine, and FIAC, cannot be mediated to any significant extent through ribonucleotide reductase. However, the triphosphates of propenyl-dUrd and BV-dUrd appear to exert a significant effect on the ribonucleotide reductases, a factor which could contribute to the overall antiviral activities of the two analogs. In particular, BV-dUTP showed more inhibitory effect on reductase from HSV-infected cells than on that from uninfected cells, in contrast with *E*-5-propenyl-dUTP and dTTP. Based on the apparent differences in response to the analog triphosphates, ribonucleotide reductases from HSV-1 and HSV-2-infected cells are similar to each other, and both exhibit altered specificity from the enzyme in uninfected cells.

We thank Sue P. Grill for her excellent technical assistance. We are grateful to Jack J. Fox (2'-fluoroaraT, FIAC, and 2'-fluoro-5-methyl-araCTP), Eric De Clercq (BV-dUrd), Gertrude Elion (acycloguanosine), Alex Bloch (acycloctosine), and Shih-Hsi Chu (acyclothymine) for their gifts of nucleoside analogs.

This work was supported by Public Health Service grant CA 27448 from the National Institutes of Health and grant CH-29 from the American Cancer Society.

LITERATURE CITED

1. Caradonna, S. J., and Y.-C. Cheng. 1981. Induction of uracil-DNA glycosylase and dUTP nucleotidohydrolase activity in herpes simplex virus infected cells. *J. Biol. Chem.* **256**:9834-9837.
2. Chang, C.-H., and Y.-C. Cheng. 1979. Demonstration of two components and association of adenosine diphosphate-cytidine diphosphate reductase from cultured human lymphoblast cells (Molt-4F). *Cancer Res.* **39**:436-442.
3. Chang, C.-H., and Y.-C. Cheng. 1979. Effects of nucleoside triphosphates on human ribonucleotide reductase from Molt-4F cells. *Cancer Res.* **39**:5087-5092.
4. Cheng, Y.-C., S. Grill, J. Ruth, and D. E. Bergstrom. 1980. Anti-herpes simplex virus and anti-human cell growth activity of *E*-5-propenyl-2'-deoxyuridine and the concept of selective protection in antiviral chemotherapy. *Antimicrob. Agents Chemother.* **18**:957-961.
5. DeClercq, E., J. Descamps, P. DeSomes, P. J. Barr, A. S. Jones, and R. T. Walker. 1979. *E*-5-(2-Bromovinyl)-2'-deoxyuridine: a potent and selective antiherpes agent. *Proc. Natl. Acad. Sci. U.S.A.* **76**:2947-2951.
6. Elford, H. L., M. Freese, E. Passaman, and H. P. Morris. 1970. Ribonucleotide reductase and cell proliferation. I. Variations of ribonucleotide reductase activity with tumor growth rate in a series of rat hepatomas. *J. Biol. Chem.* **245**:5228-5233.
7. Ellison, G. B., P. A. Furman, J. A. Fyfe, P. de Miranda, L. Beauchamp, and H. J. Schaeffer. 1977. Selectivity of action of an antiherpetic agent, 9-(2-hydroxyethoxy)methylguanine. *Proc. Natl. Acad. Sci. U.S.A.* **74**:5716-5720.
8. Huszar, D., and S. Bacchetti. 1981. Partial purification and characterization of the ribonucleotide reductase induced by herpes simplex virus infection of mammalian cells. *J. Virol.* **37**:580-588.
9. Langelier, Y., M. Déchamps, and G. Buttin. 1978. Analysis of dCMP deaminase and CDP reductase levels in hamster cells infected by herpes simplex virus. *J. Virol.* **26**:547-553.
10. Ponce de Leon, M., R. J. Eisenberg, and G. H. Cohen. 1977. Ribonucleotide reductase from herpes simplex virus (types 1 and 2) infected and uninfected KB cells: properties of the partially purified enzymes. *J. Gen. Virol.* **36**:163-173.
11. Ruth, J. L., and Y.-C. Cheng. 1981. Nucleoside analogues with clinical potential in antiviral chemotherapy. The effect of several thymidine and 2'-deoxycytidine analogue 5'-triphosphates on purified human (α,β) and herpes simplex virus (type 1, 2) DNA polymerase. *Mol. Pharmacol.* **20**:415-422.
12. Thelander, L., and P. Reichard. 1979. Reduction of ribonucleotides. *Annu. Rev. Biochem.* **48**:133-158.
13. Watanabe, K. A., U. Reichman, K. Hirota, C. Lopez, and J. J. Fox. 1979. Nucleosides. 110. Synthesis and anti-herpes virus activity of some 2'-fluoro-2'-deoxyarabino-furanosylpyrimidine nucleosides. *J. Med. Chem.* **22**:21-24.