Antiviral Research, 4 (1984) 187–199
Elsevier

AVR 00123

Inhibition of human and woodchuck hepatitis virus DNA polymerase by the triphosphates of acyclovir, 1-(2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)-5-iodocytosine and *E*-5-(2-bromovinyl)-2′-deoxyuridine

O. Hantz[1], H.S. Allaudeen[2], T. Ooka[1], E. De Clercq[3] and C. Trepo[1]

[1]*Laboratoire des Hépatites, INSERM U 45 et Laboratoire d'Immunovirologie et Epidémiologie des Tumeurs/CNRS, Faculté de Médecine Alexis Carrel, Rue Guillaume Paradin, 69372 Lyon Cedex 3, France,* [2]*The Smith Kline and French Laboratories, 1500 Spring Garden Street, P.O. Box 7929, Philadelphia, PA 19101, U.S.A., and* [3]*Rega Institute for Medical Research, Katholieke Universiteit Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium*

(Received 10 October 1983; accepted 23 December 1983)

Summary

The triphosphates of acyclovir (ACV), 1-(2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)-5-iodocytosine (FIAC) and *E*-5-(2-bromovinyl)-2′-deoxyuridine (BVdU) have been examined for their inhibitory effects on the endogenous DNA polymerase reactions of human hepatitis B virus (HBV) and woodchuck hepatitis virus (WHV). All three triphosphates (ACVTP, FIACTP and BVdUTP) inhibited the HBV and WHV DNA polymerases by competing with the corresponding natural substrates. FIACTP was the most potent inhibitor of HBV and WHV DNA polymerase while ACVTP was the least effective inhibitor. The inhibitory properties of these compounds were compared with those of the 5′-triphosphates of 1-β-arabinofuranosylcytosine (ara-CTP) and 1-β-arabinofuranosylthymine (ara-TTP). The 50% inhibitory doses for HBV and WHV DNA polymerases were in the following order: FIACTP < BVdUTP < ara-TTP < ACVTP < ara-CTP. BVdUTP appeared to be an efficient alternate substrate to dTTP for HBV DNA polymerase while FIACTP was much less efficient when substituted for dCTP. ACVTP did not act as an alternate substrate to dGTP and appeared to prevent DNA chain elongation.

hepatitis B virus; woodchuck hepatitis virus; DNA polymerase; acyclovir; FIACTP; BV dUTP

Introduction

Hepatitis B virion (HBV), also known as the Dane particle [7] is a 42 nm complex spherical particle composed of an outer lipoprotein coat (hepatitis B surface antigen, HBsAg) and an inner core (hepatitis B core antigen, HBcAg). This core contains a circular partially double-stranded DNA and a DNA polymerase [15,22]. In vitro, the DNA polymerase generates from the large single-stranded region of the genome a fully double-stranded DNA [18,24].

So far, the nature of the DNA particle-associated DNA polymerase remains uncertain. Selective inhibition of the HBV DNA polymerase by intercalating agents [14], pyrophosphate analogs [20] and arabinofuranosyl nucleotides [13] have been reported and offer the ability to inhibit hepatitis B virus replication in individuals suffering from chronic hepatitis B [26,27]. In the present report, we describe the inhibition of HBV DNA polymerase by the triphosphate derivatives of three recently developed anti-herpesvirus agents, the 9-(2-hydroxyethoxymethyl)guanine (ACV) [10], E-5-(2-bromovinyl)-2′-deoxyuridine (BVdU) [8] and 1-(2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)-5-iodocytosine (FIAC) [17].

An agent very closely related to HBV was recently identified in woodchucks [25]: the woodchuck hepatitis virus (WHV). Both agents belong to the same family of viruses, now designated hepadnaviridae [23]. As previously suggested, HBV and WHV DNA polymerase share the same basic features [11]. The WHV DNA polymerase activity was therefore studied in parallel with that of HBV DNA polymerase.

Materials and Methods

Preparation of virus particles

HBV particles containing DNA polymerase activity were purified from the serum of an immunosuppressed patient, positive for HBsAG. WHV was obtained from a serum pool of 5 woodchucks (*Marmota monax*) which were all chronic carriers of the virus. The woodchucks were initially imported from Pennsylvania (Dutchland Lab., PA, U.S.A.) and were followed for over 1 year by serological tests [12]. Virus was purified by centrifugation on sucrose and isopycnic CsCl gradients as described elsewhere (O. Hantz et al., submitted for publication).

Chemicals

Unlabelled nucleoside triphosphates were obtained from Sigma Chemical Company (St. Louis, MO, U.S.A.). Tritiated deoxyribonucleoside triphosphates, [^{3}H]dTTP (30 Ci/mmol), [^{3}H]dATP (24 Ci/mmol), [^{3}H]dCTP (17 Ci/mmol) and [^{3}H]dGTP (9.5 Ci/mmol), were obtained from Amersham (France). ACV was obtained from Burroughs Wellcome (Research Triangle Park, NC, U.S.A.) [10]; BVdU was described by De Clercq et al. [8] and FIAC was a gift from Dr. J.J. Fox (Sloan-Kettering Institute for Cancer Research, New York, NY, U.S.A.) All three nucleoside analogs were converted to their corresponding 5′-triphosphates, as described by Allaudeen et al. [3]. The purity of the triphosphates was examined by NMR spectroscopy with a bruker

HX-270 spectrometer and by high pressure liquid chromatography with an Altex Model 332 gradient liquid chromatography. 1-β-D-Arabinofuranosylthymine-5'-triphosphate (ara-TTP) was a gift from Dr. G.A. Gentry (Mississippi Medical Center, Jackson, MS, U.S.A.). 1-β-D-Arabinofuranosylcytosine-5'-triphosphate (ara-CTP) and 9-β-D-arabinofuranosyladenine-5'-triphosphate (ara-ATP) were purchased from P.L. Biochemicals (Milwaukee, WI, U.S.A.).

DNA polymerase assay

DNA polymerase was assayed as described by Kaplan et al. [15] with minor modifications. The assay was performed in a 50 µl reaction mixture containing 50 mM Tris-HCl pH 7.6, 40 mM $MgCl_2$, 60 mM NH_4Cl, 100 µM each of dATP, dCTP and dGTP, 0.2–0.7 µM [^{3}H]dTTP (30 Ci/mmol), 10 mM 2-mercaptoethanol and 0.5% Nonidet P-40. The reaction was started by addition of the virus particles. Incubation was at 37°C for 1 h. Acid-insoluble radioactive material was collected on a glass fiber filter (GF/C Whatman). Filters were washed 5 times with cold 5% trichloroacetic acid containing 10 mM sodium pyrophosphate, then with 95% ethanol. Radioactivity of the dried filter was measured in a liquid scintillation counter.

Agarose gel electrophoresis of [^{32}P]DNA

The [^{32}P]DNA product of the endogenous DNA polymerase reaction was analyzed by agarose gel electrophoresis as described by Summers et al. [25]. The reaction was carried out in 15 µl of the standard DNA polymerase mixture with 1 µM of [^{32}P]dCTP (500 Ci/mmol) as labelled nucleotide. After 2 incubations at 37°C, 20 µl of 10 mM Tris, 10 mM EDTA, 0.1 mg/ml of proteinase K, and 0.1% (w/v) sodium dodecyl sulfate were added, followed by another incubation for 1 h at 37°C. Then, 5 µl of 5% (w/v) sucrose, and 1% bromophenol blue were added, the entire mixture was heated for 5 min at 65°C, immediately cooled to 4°C and then analyzed by electrophoresis on an horizontal slab gel of 1% agarose. After electrophoresis, the gel was dried and developed by autoradiography.

Results

Products of the HBV and WHV DNA polymerase reactions

The specificity and the efficiency of the DNA polymerase in the HBV and WHV particle preparations were monitored by analysis of the DNA product synthesized during the endogenous reaction. The DNA polymerase assay was run as described in Materials and Methods with [^{32}P]dCTP as the labelled nucleotide. The reaction product was then analyzed by agarose gel electrophoresis and autoradiography. The results in Fig. 1 show the progressive synthesis of HBV DNA and WHV DNA after 15, 30, 120 and 180 min. For HBV, a complete 3.3 kb long DNA was obtained, as well as smaller forms, ranging from 1.8 to 2.8 kb and corresponding to an incomplete duplication of the single-stranded region of the viral genome. The product of the WHV DNA polymerase reaction migrated slightly faster; the longer DNA formed after 180 min incubation was 3.1 kb long.

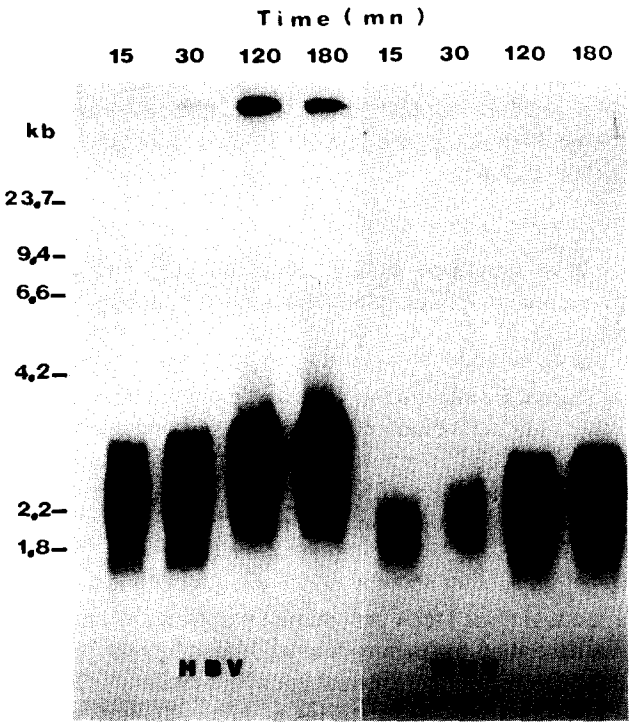

Fig. 1. Agarose gel electrophoresis of the [^{32}P]DNA synthesized by HBV and WHV particles in the endogenous DNA polymerase reaction. The reaction was performed as described in the text with [^{32}P]dCTP as the labelled substrate. At indicated times, the reacton was stopped and the DNA product analyzed by agarose gel electrophoresis and autoradiography. Hind III fragments obtained from λ DNA served as markers (kb = kilobases).

Relative sensitivities of HBV and WHV DNA polymerases to ACVTP, FIACTP and BVdUTP

The relative sensitivities of the HBV and WHV DNA polymerases to ACVTP, FIACTP and BVdUTP were compared using an excess of nucleotides. For each inhibitor, the concentration of the corresponding radioactive triphosphate was maintained at 2–3 times the K_m value, while the remaining nucleotides were in great excess (50 μM). Fig. 2 shows that FIACTP was the most efficient inhibitor of HBV DNA polymerase, whereas ara-CTP and ACVTP were the least efficient; BVdUTP and ara-TTP showed an intermediate inhibitory effect.

Similar results were obtained with WHV DNA polymerase. The concentrations of triphosphates required to inhibit the HBV and WHV DNA polymerases by 50% are presented in Table 1. The lowest ID_{50} values were recorded for FIACTP. The ID_{50} values obtained for BVdUTP and ACVTP exceeded those of FIACTP by a factor of 3–5-fold and 7–18-fold, respectively.

Kinetics of inhibition

The kinetics of inhibition of HBV and WHV DNA synthesis by ACVTP, FIACTP

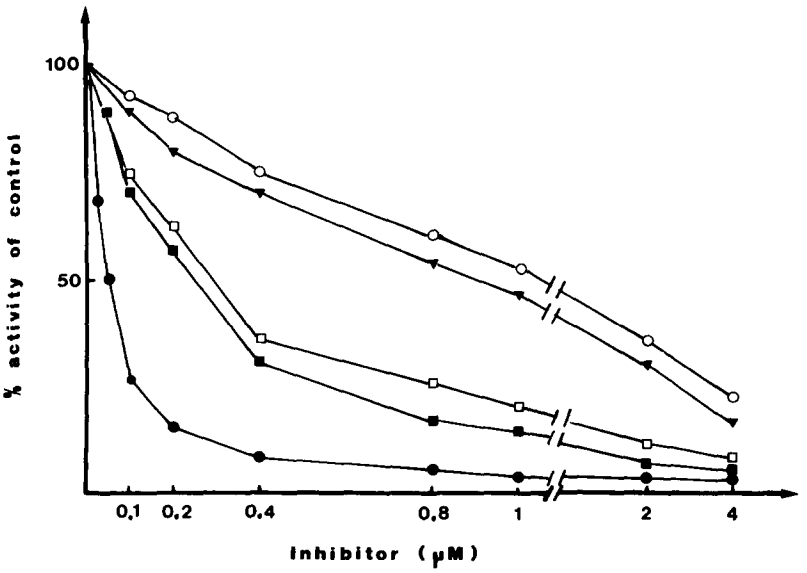

Fig. 2. Inhibition of HBV DNA polymerase by the nucleotide analogs. The polymerase activities were determined by measuring the incorporation into DNA of the radioactive triphosphate precursor that corresponded to the inhibitor examined: 0.2 μM of [^{3}H]dCTP for FIACTP (●——●) and ara-CTP (○——○), 0.3 μM of [^{3}H]dTTP for BVdUTP (■——■) and ara-TTP (□——□), 0.8 μM of [^{3}H]dGTP for ACVTP (▲——▲). The other 3 triphosphates were in excess.

TABLE 1

Comparative inhibitory activities of nucleoside triphosphate analogs on HBV and WHV polymerases

Inhibitor	ID_{50} (μM)	
	HBV DNA polymerase	WHV DNA polymerase
FIACTP (1)	0.05	0.10
ara-CTP (1)	1.10	1.20
BVdUTP (2)	0.25	0.30
ara-TTP (2)	0.30	0.40
ACVTP (3)	0.90	0.70

Results are expressed as ID_{50}, i.e., the concentration of inhibitor giving a 50% inhibition of the DNA polymerase activity. The tritiated nucleosides (1) [^{3}H]dCTP, (2) [^{3}H]dTTP, (3) [^{3}H]dGTP, were used at a concentration 2–3 times higher than the K_m value for the enzyme.

and BVdUTP were examined by determining the extent of inhibition with increasing concentrations of the substrates. In assays with ACVTP, [^{3}H]dGTP was the rate-limiting substrate, while the other 3 nucleotides were in excess. Similarly, [^{3}H]dCTP was the rate-limiting substrate for FIACTP and [^{3}H]dTTP was the rate-limiting substrate for BVdUTP. Lineweaver-Burk plots [16] show that the inhibition of HBV DNA

polymerase reaction by ACVTP, FIACTP and BVdUTP was competitive with dGTP, dCTP and dTTP, respectively (Fig. 3). Similar results were obtained for WHV DNA polymerase (data not shown). The K_m value of dGTP, dCTP, dTTP and the K_i value of ACVTP, FIACTP and BVdUTP for the HBV and WHV DNA polymerases are presented in Table 2. WHV DNA polymerase showed a slightly lower affinity for dGTP (ACVTP), dCTP (FIACTP) and dTTP (BVdUTP) than did HBV DNA polymerase; however, the K_m/K_i ratios were comparable for both enzymes.

Effects of BVdUTP, FIACTP and ACVTP as alternate substrates for DNA synthesis in vitro

To examine the possibility that BVdUTP, FIACTP or ACVTP may act as an alternate substrate for DNA synthesis, a time-response experiment was performed with HBV DNA polymerase and [^{3}H]dATP as the labelled precursor. In the presence of all 4 nucleoside triphosphates, the reaction was linear for up to 60 min (Fig. 4).

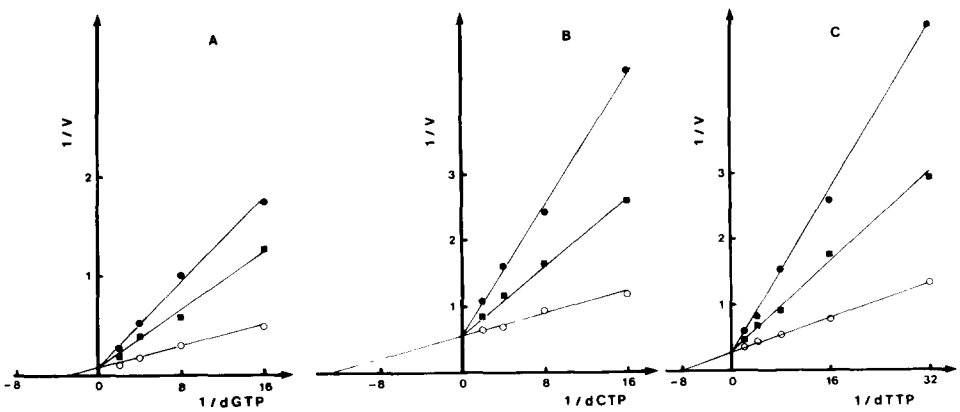

Fig. 3. A) Kinetics of inhibition of HBV DNA polymerase. A) Effect of ACVTP on DNA polymerase reaction in the presence of different concentrations of [^{3}H]dGTP. No inhibitor (○); 0.2 μM ACVTP (■), 0.4 μM ACVTP (●). B) Effect of FIACTP on DNA polymerase reaction in the presence of different concentrations of [^{3}H]dCTP. No inhibitor (○); 0.05 μM FIACTP (■); 0.1 μM of FIACTP (●). C) Effect of BVdUTP on DNA polymerase reaction in the presence of different concentrations of [^{3}H]dTTP. No inhibitor (○); 0.1 μM of BVdUTP (■); 0.2 μM of BVdUTP (●).

TABLE 2

Kinetics of inhibition of HBV and WHV DNA polymerase by BVdUTP, FIACTP and ACVTP

Substrate	Inhibitor	K_m (μM)	K_i (μM)	K_m/K_i
A. HBV DNA polymerase				
dGTP	ACVTP	0.31	0.11	2.8
dCTP	FIACTP	0.08	0.01	8
dTTP	BVdUTP	0.11	0.05	2.2
B. WHV DNA polymerase				
dGTP	ACVTP	0.50	0.13	3.8
dCTP	FIACTP	0.20	0.03	6.6
dTTP	BVdUTP	0.25	0.11	2.2

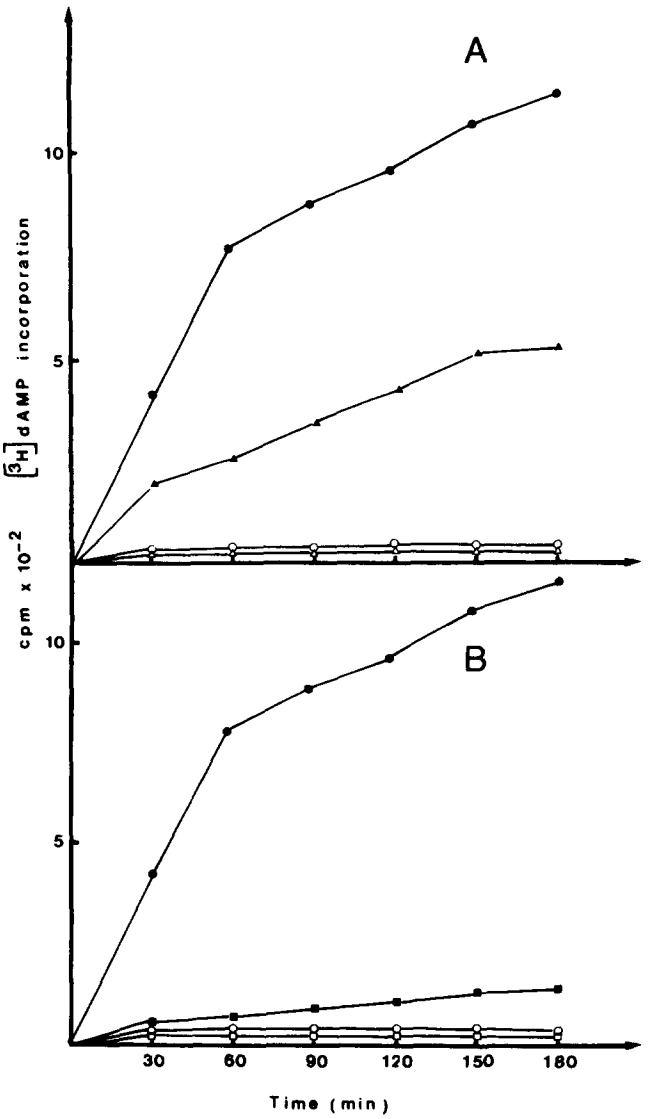

Fig. 4. Examination of BVdUTP, ara-TTP, FIACTP and ara-CTP as alternate substrates for HBV DNA polymerase. A) BVdUTP and ara-TTP as alternative substrates to dTTP. The assay was similar to that described in Materials and Methods except that 5 μM [^{3}H]dATP was the labelled substrate: assay with all 4 dNTPs (●), assay without dTTP (○), assay without dTTP in the presence of 10 μM BVdUTP (▲), assay without dTTP in the presence of 10 μM ara-TTP (△). B) FIACTP and ara-CTP as alternate substrates to dCTP. The assay was similar to that described in Materials and Methods except that 5 μM [^{3}H]dATP was the labelled substrate; assay with all 4 dNTPs (●), assay without dCTP (○), assay without dCTP in the presence of 10 μM FIACTP (■), assay without dCTP in the presence of 10 μM ara-CTP (□).

When dTTP, dCTP or dGTP were omitted from the reaction mixture, no significant DNA synthesis was observed. The substitution of dTTP by BVdUTP permitted viral DNA synthesis up to 46% of control after a 3 h incubation period (Fig. 4A). This value increased to 67% after 4 h (Table 3). Under similar conditions, no significant DNA synthesis was observed when dTTP was substituted by ara-TTP (Fig. 4A).

When a similar experiment was performed with FIACTP instead of dCTP, a small amount of [^{3}H]dAMP was incorporated (about 10% of control after 3 h incubation), while no significant incorporation was obtained if ara-CTP was used instead of dCTP (Fig. 4B). Neither did substitution of ACVTP for dGTP lead to the incorporation of any [^{3}H]dAMP. The efficiency of ACVTP, FIACTP, ara-CTP, BVdUTP and ara-TTP as alternate substrates for HBV and WHV DNA polymerases are summarized in Table 3. BVdUTP was the most efficient alternate substrate for both enzymes. The DNA synthesized with BVdUTP as alternate substrate was analyzed by agarose gel electrophoresis (Fig.5). This DNA product was smaller than the DNA product synthesized in the presence of dTTP, suggesting that the single-stranded region of HBV and WHV was only partially duplicated in the presence of BVdUTP.

The effect of ACVTP on HBV DNA synthesis was also studied in a time-response experiment and the results are shown in Fig. 6. [^{3}H]dTTP served as the labelled substrate. When the reaction was initiated in the absence of dGTP or with 5 μM ACVTP instead of dGTP, no incorporation of [^{3}H]dTMP was detected. If 100 μM dGTP was added at 120 min, DNA synthesis occurred as expected. However, when the assay mixture contained 5 μl ACVTP instead of dGTP from time zero, no DNA synthesis was observed upon addition of 100 μM dGTP at 120 min (Fig. 6A). In

TABLE 3

Endogenous HBV and WHV DNA synthesis in the presence of different nucleoside triphosphate analogs, as measured after a 4 h incubation period

Non-radioactive dNTP (100 μM)	dNTP analog (μM)	[^{3}H]dAMP incorporation (%)	
		HBV DNA polymerase	WHV DNA polymerase
dCTP, dTTP, dGTP		100	100
dCTP, dTTP		2	2
dCTP, dTTP	ACVTP (10)	0	0
dCTP, dTTP, dGTP	ACVTP (10)	85	82
dGTP, dTTP		4	2
dGTP, dTTP	FIACTP (5)	9.6	12
dGTP, dTTP	FIACTP (10)	11.5	9
dGTP, dTTP, dCTP	FIACTP (10)	34	34
dGTP, dTTP	ara-CTP (10)	3	0
dGTP, dCTP		4	0
dGTP, dCTP	BVdUTP (5)	67	59
dGTP, dCTP	BVdUTP (10)	63	45
dGTP, dCTP, dTTP	BVdUTP (10)	92	93
dGTP, dCTP	ara-TTP (10)	2	0
dGTP, dCTP, dTTP	ara-TTP (10)	89	87

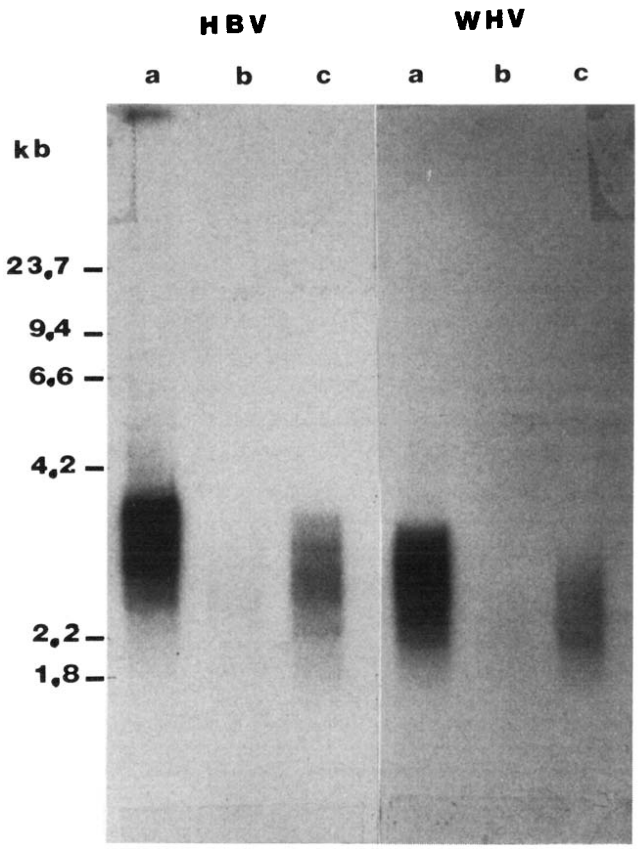

Fig. 5. Product of the HBV and WHV DNA polymerase reaction with BVdUTP as alternate substrate; the reaction was performed with [^{32}P]dCTP for 3 h and labelled DNA was analysed by agarose gel electrophoresis and autoradiography: a) all 4 natural triphosphates; b) 3 nucleotides only, without dTTP; c) 3 nucleotides only, with dTTP replaced by 5 μM BVdUTP. Hind III fragments obtained from λ DNA served as markers (kb = kilobases).

contrast, if the reaction was started with ACVTP (5 μM) and dGTP (100 μM) from time zero DNA synthesis occurred to a significant extent (Fig. 6A). Similar experiments were carried out with ara-ATP (Fig. 6B) and ara-CTP (Fig. 6C). In both cases addition of normal substrate (dATP or dCTP) at 120 min increased HBV DNA synthesis, even in the presence of ara-ATP or ara-CTP. The fact that dGTP failed to do so for reaction mixtures containing ACVTP suggests that ACVTP must have acted as a chain terminator.

Discussion

Nucleoside analogs demonstrating antiviral activity have to be converted to their triphosphate derivatives within the cell to interfere with viral DNA synthesis [9].

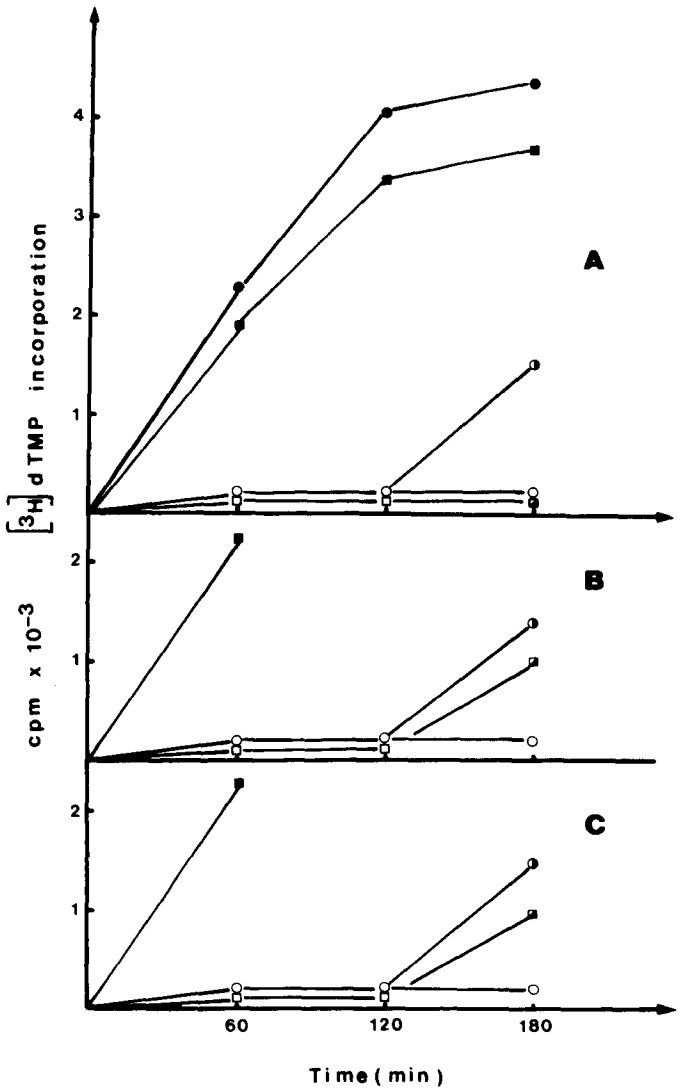

Fig. 6. HBV DNA synthesis in presence of ACVTP (A), ara-ATP (B) or ara-CTP (C). The reaction was performed as described in Materials and Methods with [^{3}H]dTTP as labelled substrate. Control: all 4 nucleotide triphosphates present from time zero (●). A) Three nucleotides only, without dGTP, present from time zero (○); 100 μM dGTP added at 120 min to the latter reaction mixture (◑); 3 nucleotides and 5 μM ACVTP present from time zero (□); 100 μM dGTP added at 120 min to the latter reaction mixture (◪); all 4 nucleotides and 5 μM ACVTP present from time zero (■). B) Three nucleotides only, without dATP, present from time zero (○); 100 μM dATP added at 120 min to the latter reaction mixture (◑); 3 nucleotides and 5 μM ara-ATP present from time zero (□); 100 μM dATP added at 120 min to the latter reaction mixture (◪); all 4 nucleotides and 5 μM ara-ATP present from time zero (■). C) Three nucleotides only, without dCTP, present from time zero (○); 100 μM dCTP added at 120 min to the latter reaction mixture (◑); 3 nucleotides and 5 μM ara-CTP present from time zero (□); 100 μM dCTP added at 120 min to the latter reaction mixture (◪); all 4 nucleotides and 5 μM ara-CTP present from time zero (■).

ACVTP [10], BVdUTP [3] and FIACTP [2] preferentially inhibit herpes simplex virus DNA polymerase and this may explain in part the selective antiviral activity of ACV, BVdU and FIAC. The present results show that all 3 compounds inhibit the DNA polymerases associated with HBV and WHV and that this inhibition is competitive with respect to the corresponding natural nucleoside triphosphate. The inhibitory activities of ACVTP, BVdUTP, FIACTP were compared with those of two other nucleotide analogs, ara-CTP [13] and ara-TTP [5]. The inhibitory effect on HBV and WHV DNA polymerase decreased in the following order: FIACTP > BVdUTP > ara-TTP > ACVTP > ara-CTP. The conditions used to measure the endogenous HBV and WHV DNA synthesis without addition of foreign template DNA did not allow us to compare our results with those obtained previously for other systems. However, the mode of inhibition of HBV and WHV DNA polymerases by ACVTP, BVdUTP and FIACTP appears to be similar to that observed for herpes simplex virus type-1 (HSV-1) DNA polymerase and cellular DNA polymerase α [1–3]. As noted for HSV-1 DNA polymerase [1], our results indicate that either ACVTP is not an alternate substrate for HBV DNA polymerase or it prevents chain elongation. Like HSV-1 DNA polymerase [2,3,5], the HBV and WHV DNA polymerases are strongly inhibited by BVdUTP, ara-TTP and FIACTP. In contrast, EBV DNA polymerase is inhibited by ara-TTP [21] but not by ACVTP or BVdUTP [1,3].

BVdUTP can serve as an alternate substrate to dTTP for both viral and cellular DNA polymerases [3,4] and the extent of substitution of thymidine by BVdU into HSV-1 DNA correlates closely with its potent antiviral activity [19]. Our results show that BVdUTP also acts as an alternate substrate to dTTP for HBV and WHV DNA polymerases. However, the polymerase reaction generated by BVdUTP only leads to an incomplete duplication of the single-stranded DNA. FIACTP differs from BVdUTP in that it has been shown to be an alternate substrate to dCTP for herpes virus -DNA polymerase but not for cellular DNA polymerase [2]. Only very low HBV and WHV DNA polymerase activity is observed with FIACTP as an alternate substrate to dCTP. Thus, BVdUTP is a more efficient substrate for the HBV and WHV DNA polymerase than FIACTP.

In vivo inhibition of HBV replication by ACV has been demonstrated in patients with chronic hepatitis B [26,28]. Whether the in vivo effect of ACV can be related to the in vitro inhibitory action of ACVTP on HBV DNA polymerase remains uncertain, since the role of the endogenous DNA polymerase in HBV replication within the infected cells has not been established. Moreover, an important condition for the antiviral action of anti-herpes agents such as ACV, FIAC and BVdU is the selective phosphorylation of these analogs by the herpes virus-encoded thymidine kinase [6,10]. Such virus-specified thymidine kinase activity has not been reported for HBV or WHV.

However, considering the lack of any cell culture system to support the growth of HBV, potential anti-HBV agents can be detected only by inhibition of the endogenous HBV DNA polymerase. Our in vitro studies should therefore be extended to the in vivo situation. The woodchuck may be the model of choice for a preliminary evaluation of potential anti-HBV agents. Indeed, the striking similarities in the affinity of WHV and HBV DNA polymerase for nucleotide analogs such as ACVTP, BVdUTP

and FIACTP, as demonstrated here, point to the validity of the WHV model system for such in vivo studies.

Acknowledgements

We thank Mrs. Marie-Pierre Scanella for assistance in the preparation of the manuscript. The work was supported by grants from INSERM, CNRS, UER LYON SUD, Biologie Humaine and UER Alexis Carrel.

References

1 Allaudeen, H.S., Descamps, J. and Sehgal, R.K. (1982) Mode of action of acyclovir triphosphate on herpesviral and cellular DNA polymerases. Antiviral Res. 2, 123–133.
2 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-iodo-cytosine J. Biol. Chem. 257, 11879–11882.
3 Allaudeen, H.S., Kozarich, J.W., Bertino, J.R. and De Clercq, E. (1981) On the mechanism of selective inhibition of herpes-virus replication by (*E*)-5-(2-bromovinyl)-2′-deoxyuridine. Proc. Natl. Acad. Sci. U.S.A. 78, 2698–2702.
4 Allaudeen, H.S., Chen, M.S., Lee, J.J., De Clercq, E. and Prusoff, W.H. (1982) Incorporation of E-5-(2-halovinyl)-2′-deoxyuridine into deoxyribonucleic acids of herpes simplex virus type 1-infected cells. J. Biol. Chem. 257, 603–606.
5 Aswell, J.F., Allen, G.P., Jamieson, A.T., Campbell, D.E. and Gentry, G.A. (1977) Antiviral activity of arabinosylthymine in herpesviral replication: mechanism of action in vivo and in vitro. Antimicrob. Agents Chemother. 12, 243–254.
6 Cheng, Y.-C., Dutschman, G., De Clercq, E., Jones, A.S., Rahim, S.G., Verhelst, G. and Walker, R.T. (1981) Differential affinities of 5-(2-halogenovinyl)-2′-deoxyuridines for deoxythymidine kinases of various origin. Mol. Pharmacol. 20, 230–233.
7 Dane, D.S., Cameron, C.H. and Brigs, M. (1970) Virus like particles in serum of patients with Australia antigen associated hepatitis. Lancet 1, 695–697.
8 De Clercq, E., Descamps, J., De Somer, P., Barr, J.P., Jones, A.S. and Walker, R.T. (1979) (*E*)-5-(2-Bromovinyl)-2′-deoxyuridine: a potent and selective anti-herpes agent. Proc. Natl. Acad. Sci. U.S.A. 76, 2947–2951.
9 De Clercq, E. (1979) New trends in antiviral chemotherapy. Arch. Int. Physiol. Biochim. 87, 353–395.
10 Elion, G.B., Furman, P.A., Fyfe, J.A., de Miranda, P., Beauchamp, L. and Schaeffer, H.J. (1977) Selectivity of action of an antiherpetic agent, 9-(2-hydroxyethoxymethyl)guanine. Proc. Natl. Acad. Sci. U.S.A. 74, 5716–5720.
11 Hantz, O., Vitvitski, L., Pichoud, C., Ooka, T. and Trepo, C. (1983) Immunological and pharmacological comparison between the woodchuck hepatitis virus (WHV) and the human hepatitis B virus (HBV). In: Proceedings of the 2nd International Max V. Pettenkofer Symposium on Viral Hepatitis, Munich 19–22 October 1982, in press.
12 Hantz, O., Pichoud, C., Vitvitski, L. and Trepo, C. (1983) Use of the cross-reactivity with hepatitis B virus antigens and antibodies for the demonstration of a woodchuck hepatitis virus e antigen-antibody system. J. Virol. Methods 7, 45–55.
13 Hess, G., Arnold, W. and zum Büschenfelde, K.H.M. (1981) Inhibition of hepatitis B virus deoxyribonucleic acid polymerase by the 5′-triphosphates of 9-β-D-arabinofuranosyladenine and 1-β-D-arabinofuranosylcytosine. Antimicrob. Agents Chemother. 19, 44–50.
14 Hirschman, S.Z. and Garfinkel, E. (1978) Inhibition of hepatitis B DNA polymerase by intercalating agents. Nature 271, 681–683.

15 Kaplan, P.M., Greenman, R.L., Gerin, J.L., Purcell, R.H. and Robinson, W.S. (1973) DNA polymerase associated with human hepatitis B antigen. J. Virol. 12, 995–1005.
16 Lineweaver, H. and Burk, D. (1934) The determination of enzyme dissociation constants. J. Am. Chem. Soc. 56, 658–666.
17 Lopez, C., Watanabe, K.A. and Fox, J.J. (1980) 2'-Fluoro-5-iodo-aracytosine, a potent and selective anti-herpes virusagent. Antimicrob. Agents Chemother. 17, 803–806.
18 Lutwick, L.I. and Robinson, W.S. (1977) DNA synthesized in the hepatitis B Dane particle DNA polymerase reaction. J. Virol. 21, 96–104.
19 Mancini, W.R., De Clercq, E. and Prusoff, W.H. (1983) The relationship between incorporation of (*E*)-5-(2-bromovinyl)-2'-deoxyuridine into herpes simplex virus type 1 DNA with virus infectivity and DNA integrity. J. Biol. Chem. 258, 792–795.
20 Nordenfelt, E., Oberg, B., Helgstrand, E. and Miller, E. (1980) Inhibition of hepatitis B Dane particle DNA polymerase activity by pyrophosphate analogs. Acta Pathol. Microbiol. Scand. Sect. B 88, 169–175.
21 Ooka, T., Calender, A., de Turenne, M. and Daillie, J. (1983) Effect of arabinosylthymine on the replication of Epstein-Barr virus and relationship with a new induced thymidine kinase activity. J. Virol. 46, 187–195.
22 Robinson, W.S., Clayton, D.A. and Greenman, R.L. (1973) DNA of a human hepatitis B virus candidate. J. Virol. 14, 384–391.
23 Robinson, W.S., Marion, P., Fertelson, M. and Sidiqui, A. (1981) The Hepa-DNA-virus group: hepatitis B and related virus. In: Viral Hepatitis International Symposium, Szmuness, W., Alter, J. and Maynard, J., Eds. The Franklin Institute Press, Philadelphia, PA, pp. 57–68.
24 Summers, J., O'Connell, A. and Millman, I. (1975) Genome of hepatitis B virus: restriction enzyme cleavage and structure of DNA extracted from Dane particles. Proc. Natl. Acad. Sci. U.S.A. 72, 4597–4601.
25 Summers, J., Smolec, J.M. and Snyder, R. (1978) A virus similar to hepatitis B virus associated with hepatitis and hepatoma in woodchucks. Proc. Natl. Acad. Sci. U.S.A. 75, 4533–4537.
26 Weller, I.V.D., Carreno, V., Fowler, M.J.F., Monjardino, J., Makinen, D., Thomas, H.C. and Sherlock, S. (1982) Acyclovir inhibits hepatitis B virus replication in man. Lancet 1, 273.
27 Weller, I.V.D., Banendine, M.F., Craxi, A., Fowler, M.J.F., Monjardino, J. and Thomas, M.C. (1982) Successful treatment of HBs and HBe Ag positive chronic liver disease. Prolonged inhibition of viral replication by highly soluble adenine arabinoside 5'-monophosphate (ara-AMP). Gut 29, 717–723.
28 Weller, I.V.D., Carreno, V., Fowler, M.J.F., Monjardino, J., Makinen, D., Varghese, Z., Sweny, P., Thomas, H.C. and Sherlock, S. (1983) Acyclovir in hepatitis B antigen-positive chronic liver disease: inhibition of viral replication and transient renal impairment with iv bolus administration. J. Antimicrob. Chemother. 11, 223–231.