Biochemical Pharmacology, Vol. 37, No. 9, pp. 1789–1790, 1988.
Printed in Great Britain.

0006-2952/88 $3.00 + 0.00
Pergamon Press plc

INTERACTIONS OF ANTIVIRAL AGENTS WITH VIRAL DNA SYNTHESIS

E. DE CLERCQ
Rega Institute for Medical Research, Katholieke Universiteit Leuven,
Minderbroedersstraat 10, B-3000 Leuven, Belgium

With very few exceptions, all antiviral compounds currently used, or considered for use, in clinical medicine belong to the class of the nucleoside analogues [1], and most of these agents owe their antiviral activity to an interaction with viral DNA synthesis. Typical examples [2] of nucleoside analogues that, following phosphorylation to the triphosphate, interact with viral DNA synthesis are acyclovir [ACV, 9-(2-hydroxyethoxymethyl)guanine] [3], DHPG [9-(1,3-dihydroxy-2-propoxymethyl)guanine] [4,5] and buciclovir [BCV, (R)-9-(3,4-dihydroxybutyl)guanine] [6]. ACV is incorporated at the 3'-end of DNA and thus acts as a chain terminator [3], DHPG may be able to enter both internal and terminal linkages [4,5] and BCV would not be incorporated at all [6].

Our own investigations have been mainly focussed on the following three classes of compounds: 5-substutited 2'-deoxyuridines and carbocyclic analogues thereof; phosphonylmethoxyalkylpurines and -pyrimidines; and 2',3'-dideoxynucleoside analogues (Table 1). EDU (5-ethyl-2'-deoxyuridine) is phosphorylated to a much greater extent by herpes simplex virus (HSV)-infected cells than mock-infected cells; and, within the HSV-1-infected cells, EDU is incorporated to a much greater extent into viral DNA than cellular DNA [7]. In fact, a close correlation has been found between the incorporation of EDU into viral DNA, the inhibition of viral DNA synthesis and the inhibition of virus progeny formation [7].

BVDU [(E)-5-(2-bromovinyl)-2'-deoxyuridine] and IVDU [(E)-5-(2-iodovinyl)-2'-deoxyuridine] are specifically phosphorylated by the HSV-1-infected cells [8,9], and, within the HSV-1-infected cells, incorporated into both viral and cellular DNA [9], albeit preferentially into viral DNA [10]. The incorporation of BVDU into HSV-1 DNA is closely correlated with the reduction in virus yield [10]. Apparently, incorporation of BVDU into DNA renders it more prone to single strand breakage [10] and less functional as a template for transcription to RNA [11], and both phenomena may obviously contribute to the antiviral effects observed with BVDU.

TABLE 1. Interaction of antiviral compounds with viral DNA synthesis

Compounds Prototype	Phosphorylated by ... kinases	Incorporated into DNA	Inhibition of viral DNA synthesis
5-Substituted 2'-deoxyuridines			
EDU	Viral	Interior	Yes
BVDU	Viral	Interior	Yes
IVDU			
C-BVDU	Viral	Interior	Yes
C-IVDU			
Phosphonylmethoxyalkylpurines and -pyrimidines			
HPMPA	Cellular	?	Yes
2',3'-Dideoxynucleoside analogues			
AZT (AzddThd)	Cellular	3'-Terminal	Yes
ddCyd			

C-BVDU and C-IVDU, the carbocyclic (cyclopentyl) derivatives of BVDU and IVDU, are equally well recognized as substrates by the HSV-1-encoded thymidine kinase as their parental compounds [12] and eventually incorporated into both viral and cellular DNA of the HSV-1-infected cell [9]. Yet, C-BVDUTP is a much poorer substrate of DNA polymerase than BVDUTP, so that it is incorporated into DNA to a limited extent [maximally 3.6 % in poly(dA-dT)] [13]. The primary action of C-BVDU would reside in an inhibitory effect of its triphosphate on the viral DNA polymerization reaction.

HPMPA [(S)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine] is the prototype of a new class of compounds which are active against a wide variety of DNA viruses, including adeno-, pox(vaccinia)-, and herpes (herpes simplex, varicella-zoster, cytomegalo, Epstein-Barr) viruses, and retroviruses [14]. HPMPA is equally well phosphorylated by virus- and mock-infected cells to its mono- and di-phosphoryl derivatives [15]. It is incorporated to a very low extent into DNA of both virus- and mock-infected cells; but, most remarkably, HPMPA inhibits viral DNA synthesis at a concentration which is several orders of magnitude lower than the concentration required for inhibition of cellular DNA synthesis, and the latter finding has been confirmed with HSV-1-infected [15] as well as Epstein-Barr virus-infected [16] cells. How HPMPA achieves this specific inhibition of viral DNA synthesis is an intriguing question that remains to be resolved.

The class of compounds which has recently drawn most attention as antiviral agents is that of the 2',3'-dideoxynucleosides. The prototypes of this class are AzddThd (AZT, azidothymidine, 3'-azido-2',3'-dideoxythymidine) and ddCyd (2',3'-dideoxycytidine), and, in addition to AzddThd and ddCyd, several other congeners, i.e. 2',3'-dideoxycytidinene [17,18], 2',3'-dideoxythymidinene [19], 2',3'-dideoxy-2,6-diaminopurineriboside [20] and 3'-azido-2',2'-dideoxyguanosine [21], have been found which are about as potent and selective as inhibitors of human immunodeficiency virus (HIV) as are AzddThd and ddCyd. These compounds are phosphorylated nonspecifically by cellular enzymes and, once they have been converted to their 5'-triphosphate, they are assumed to act as chain terminators of the HIV reverse transcriptase [22]. Apparently, AzddTTP has a 100-fold greater affinity for HIV reverse transcriptase than for cellular DNA polymerase α [22]. The molecular basis for this selective interaction remains to be elucidated. The selective anti-HIV activity of AzddThd, ddCyd and their congeners may be related not only to an intrinsically higher sensitivity of the HIV reverse transcriptase than of the cellular DNA polymerases to the inhibitory effects of the 2',3'-dideoxynucleoside 5'-triphosphates but also to the intracellular pool levels achieved by these triphosphates relative to the intracellular pool levels of the natural substrates (dTTP, dCTP, dGTP, dATP) [23]. These pool levels can in fact be modulated, i.e. by exogenous addition of dThd, which stimulates the conversion of ddCyd to ddCTP and thereby potentiates the anti-HIV activity of the latter [24].

REFERENCES

1. E. De Clercq, ISI Atlas of Science (Pharmacology) 1, 20 (1987).
2. E. De Clercq, Biochem. Pharmacol. 33, 2159 (1984).
3. D. Derse, Y.-C. Cheng, P.A. Furman, M.H. St. Clair and G.B. Elion, J. Biol. Chem. 256, 11447 (1981).
4. Y.-C. Cheng, S.P. Grill, G.E. Dutschman, K. Nakayama and K.F. Bastow, J. Biol. Chem. 258, 12460 (1983).
5. K.B. Frank, J.-F. Chiou and Y.-C. Cheng, J. Biol. Chem. 259, 1566 (1984).
6. K. Stenberg, A. Larsson and R. Datema, J. Biol. Chem. 261, 2134 (1986).
7. E. De Clercq and R. Bernaerts, J. Biol. Chem., in press (1987).
8. J. Descamps and E. De Clercq, J. Biol. Chem. 256, 5973 (1981).
9. E. De Clercq, R. Bernaerts, J. Balzarini, P. Herdewijn and A. Verbruggen, J. Biol. Chem. 260, 10621 (1985).
10. W.R. Mancini, E. De Clercq and W.H. Prusoff, J. Biol. Chem. 258, 792 (1983).
11. J. Sági, A. Czuppon, M. Kajtár, A. Szabolcs, A. Szemző and L. Ötvös, Nucleic Acids Res. 10, 6051 (1982).
12. E. De Clercq, J. Balzarini, R. Bernaerts, P. Herdewijn and A. Verbruggen, Biochem. Biophys. Res. Commun. 126, 397 (1985).
13. J. Sági, T. Kovács, E. De Clercq, A. Szemző, A.H. Csárnyi and L. Ötvös, Submitted for publication (1987).
14. E. De Clercq, A. Holy, I. Rosenberg, T. Sakuma, J. Balzarini and P.C. Maudgal, Nature 323, 464 (1986).
15. I. Votruba, R. Bernaerts, T. Sakuma, E. De Clercq, A. Merta, I. Rosenberg and A. Holý, Mol. Pharmacol., in press (1987).
16. J.-C. Lin, E. De Clercq and J.S. Pagano, Antimicrob. Agents Chemother., in press (1987).
17. J. Balzarini, R. Pauwels, P. Herdewijn, E. De Clercq, D.A. Cooney, G.-J. Kang, M. Dalal, D.G. Johns and S. Broder, Biochem. Biophys. Res. Commun. 140, 735 (1986).
18. T.-S. Lin, R.F. Schinazi, M.S. Chen, E. Kinney-Thomas and W.H. Prusoff, Biochem. Pharmacol. 36, 311 (1987).
19. M. Baba, R. Pauwels, P. Herdewijn, E. De Clercq, J. Desmyter and M. Vandeputte, Biochem. Biophys. Res. Commun. 142, 128 (1987).
20. J. Balzarini, R. Pauwels, M. Baba, M.J. Robins, R. Zou, P. Herdewijn and E. De Clercq, Biochem. Biophys. Res. Commun. 145, 269 (1987).
21. M. Baba, R. Pauwels, J. Balzarini, P. Herdewijn and E. De Clercq, Biochem. Biophys. Res. Commun., 145, 1080 (1987).
22. P.A. Furman, J.A. Fyfe, M.H. St. Clair, K. Weinhold, J.L. Rideout, G.A. Freeman, S. Nusinoff-Lehrman, D.P. Bolognesi, S. Broder, H. Mitsuya and D.W. Barry, Proc. Natl. Acad. Sci. USA 83, 8333 (1986).
23. J. Balzarini, G.-J. Kang, M. Dalal, P. Herdewijn, E. De Clercq, S. Broder and D.G. Johns, Mol. Pharmacol., in press (1987).
24. J. Balzarini, D.A. Cooney, M. Dalal, G.-J. Kang, E. De Clercq, S. Broder and D.G. Johns, Mol. Pharmacol., in press (1987).