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Antiviral activity of *cyclo*saligenyl prodrugs of the nucleoside analogue bromovinyldeoxyuridine against herpes viruses

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Abstract

A series of 42 lipophilic bromovinyldeoxyuridine monophosphates (BVDUMPs) are presented as potential prodrugs of the antiviral agent (E)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU). The 5′-*cyclo*Sal-masking group technique has been applied to this cyclic nucleoside analogue to achieve delivery of the monophosphate of BVDU inside the target cells. The new substances have been tested for their antiviral activity against herpes simplex virus types 1 and 2 (HSV-1 and -2), thymidine kinase-deficient (TK^-) HSV-1, varicella-zoster virus (VZV), human cytomegalovirus (HCMV) and Epstein–Barr virus (EBV). The XTT-based tetrazolium reduction assay EZ4U (for HSV), the plaque inhibition test (for VZV and HCMV) and a DNA hybridisation assay (for EBV) were used to assess antiviral activity. The results indicate that *cyclo*Sal-BVDUMP triesters proved to be potent and selective inhibitors of HSV-1 comparable with aciclovir. VZV replication was inhibited by very low concentrations, and two substances had a slightly better anti-VZV activity than the parent compound BVDU. No antiviral effect could be demonstrated against TK^--HSV-1, HSV-2 and HCMV, most likely owing to the lack of phosphorylation to BVDU diphosphate. Most remarkably, several *cyclo*Sal-BVDUMP triesters yielded promising anti-EBV activity whereas the parent compound BVDU was entirely inactive.

Keywords: Antivirals; Brivudin; *cyclo*Sal-prodrugs; HSV; VZV; EBV

1. Introduction

Herpes viruses are common human pathogens producing a wide variety of diseases. After primary infection, all herpes viruses persist in the organism in a latent stage from which they can be reactivated.

For herpes simplex virus (HSV), varicella-zoster virus (VZV) and human cytomegalovirus (HCMV) infections, very effective antiviral compounds are available. Aciclovir, the first selective antiviral agent [1], can be regarded as the standard drug for the treatment of severe HSV and VZV infections since its introduction in 1982 [2]. In the mean time, new and improved drugs have been developed, e.g. famciclovir, valaciclovir, brivudin and foscarnet [3]. Regarding the treatment and prophylaxis of HCMV infections, the indications for the use of ganciclovir [3] and its oral prodrug valganciclovir [4] are limited. Besides, the phosphonate analogue cidofovir is mainly reserved for HCMV retinitis in acquired immune deficiency syndrome (AIDS) patients [5].

Epstein–Barr virus (EBV), the causative agent of infectious mononucleosis, is associated with several human malignancies such as Burkitt's lymphoma and nasopharyngeal carcinoma. With increasing relevance, EBV-induced post-transplant lymphoproliferative diseases may occur [6]. Despite the ability of several antiviral agents to inhibit EBV replication in vitro [7–9], no standard therapeutic regimens exist for the clinical management of EBV-associated diseases to date.

Problems regarding antiviral therapy with different antivirals consist of side effects such as bone marrow toxicity of

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ganciclovir as well as the appearance of drug-resistant virus strains. Particularly in immunosuppressed patients, aciclovir-resistant HSV strains were observed up to 5–6% [10–12]. In a study by Limaye et al. [13], resistance to ganciclovir could be detected in nearly 10% of lung transplant recipients. The general mode of action of nucleoside analogues is through inhibition of viral DNA polymerases by acting as competitive inhibitors and/or DNA chain terminators. For these actions, intracellular conversion of the nucleoside analogues into their monophosphates, diphosphates and triphosphates is required after cell penetration. However, efficient anabolism to the corresponding triphosphates can be limited due to altered or deficient viral enzymes necessary for phosphorylation as well as structural differences from the natural nucleosides. Resistance to nucleoside analogues is mostly mediated by deficiency of HSV or VZV thymidine kinase (TK) or by mutations in the HCMV-UL97 phosphotransferase [14].

Pronucleotides represent a promising alternative to improve the biological activity of nucleoside analogues. The principle of this 'pronucleotide approach' consists of direct administration of the nucleotides, thus bypassing the limiting phosphorylation steps [15]. To circumvent the polarity of the molecules, lipophilic carrier groups are linked to the phosphate moiety leading to neutral, membrane-permeable prodrugs. The *cyclo*saligenyl (*cyclo*Sal) pronucleotide system developed by Meier and colleagues [16–18] was designed to release the nucleotide and the masking group selectively by chemically induced hydrolysis. Using this concept, a series of pronucleotide derivatives of the pyrimidine nucleoside analogue bromovinyldeoxyuridine (brivudin) was synthesised by Meier et al. [19–21]. The present study was aimed at evaluating the in vitro activity of these novel substances against HSV, VZV, HCMV and EBV.

2. Materials and methods

2.1. Chemicals

Synthesis of pronucleotides on the basis of *cyclo*Sal-phosphotriesters of the pyrimidine nucleoside analogue (E)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU) was described in detail by Meier et al. [19]. Fig. 1 illustrates the general structure of the *cyclo*Sal-BVDU monophosphates (BVDUMPs). The incorporation of different substituents led to molecular weights varying from 515 to 776. In addition to the free 3′-hydroxyl compounds, the 3′-hydroxyl function has been masked as different 3′-*O*-esters of long aliphatic carboxylic esters and α-amino acids having natural and non-natural C-α-configuration. In total, a series of 42 BVDUMP derivatives has been tested for their anti-herpetic activity in vitro. Aciclovir, ganciclovir and BVDU served as reference compounds. BVDU was kindly provided by Berlin-Chemie/Menarini Group (Berlin, Germany).

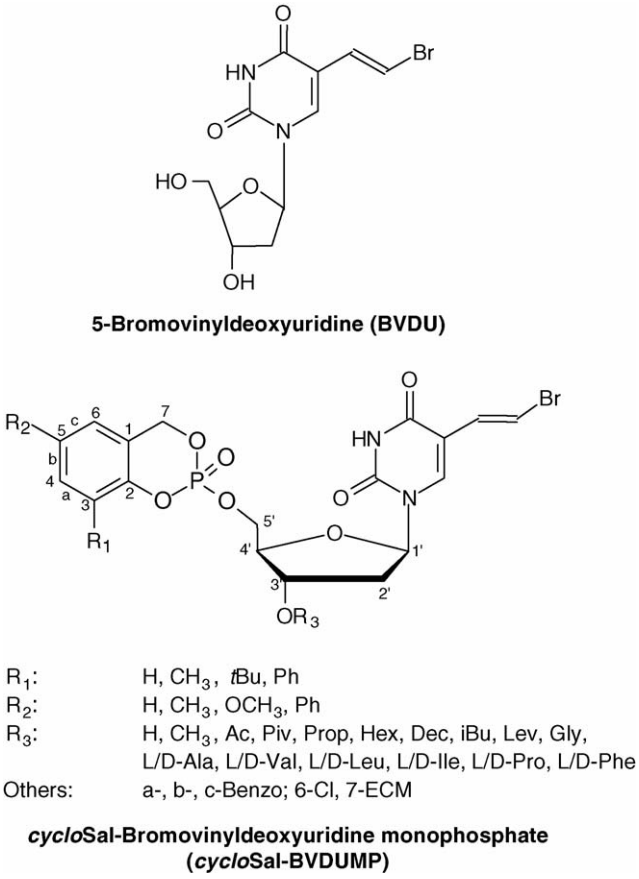

Fig. 1. Chemical structure of bromovinyldeoxyuridine (BVDU) and *cyclo*Sal-BVDU monophosphates.

2.2. Detection of anti-HSV-1 activity

2.2.1. Cells and virus strains

Vero cells were cultivated in Eagle's Minimal Essential Medium (EMEM) with Earle's balanced salt solution and 25 mM HEPES (Cambrex, Verviers, Belgium) supplemented with 2 mM L-glutamine (Cambrex), 5% newborn calf serum (PAA Laboratories, Linz, Austria), 100 U/mL penicillin (Cambrex) and 100 μg/mL streptomycin sulfate (Cambrex). TK-positive (TK^+) HSV-1 strain Kupka originates from the German National Reference Centre for α-herpes viruses. The TK-negative strain TK^--HSV-1 B2006 and the TK^+-HSV-2 strain G were obtained from E. De Clercq (Leuven, Belgium). All experiments for demonstration of antiviral activity and cytotoxicity were carried out in Vero cells.

2.2.2. Antiviral screening assay

Antiviral screening was performed in 96-well flat-bottomed microtitre plates by means of an XTT-based tetrazolium reduction assay (EZ4U; Biomedica GmbH, Vienna, Austria). The method described by Klöcking et al. [22] allows determination both of the inhibition of viral cytopathogenicity and substance-induced cytotoxic effects at the same time. Briefly, Vero cells were seeded at a density of 75 000/mL and grown for 2 days. After virus infection with $10^{3.2}$ tissue