ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Dec. 1990, p. 2417–2419
0066-4804/90/122417-03$02.00/0

Vol. 34, No. 12

Inhibition of Human Herpesvirus 6 Replication by 9-[4-Hydroxy-2-(Hydroxymethyl)Butyl]Guanine (2HM-HBG) and Other Antiviral Compounds

ANNELIE ÅKESSON-JOHANSSON, JOHAN HARMENBERG, BRITTA WAHREN, AND ANNIKA LINDE*

Department of Virology, National Bacteriological Laboratory, S-105 21 Stockholm, Sweden

Received 8 June 1990/Accepted 25 September 1990

The in vitro susceptibilities of human herpesvirus 6 to foscarnet; the guanosine analogs acyclovir, ganciclovir, and two isomers of 9-[4-hydroxy-2-(hydroxymethyl)butyl]guanine; and the thymidine analogs 3′-azido-3′-deoxythymidine and 1-β-D-arabinofuranosyl-*E*-5-(2-bromovinyl)uracil were investigated. All compounds except 3′-azido-3′-deoxythymidine and 1-β-D-arabinofuranosyl-*E*-5-(2-bromovinyl)uracil inhibited human herpesvirus 6 replication. The highest in vitro selectivity was obtained for 9-[4-hydroxy-2-(hydroxymethyl)butyl]guanine.

Human herpesvirus 6 (HHV-6) is distinct from other known human herpesviruses biologically and immunologically and by molecular analysis (2). Sequence data indicate that it is most closely related to human cytomegalovirus (8). The virus is commonly spread, and at least 85% of the Swedish population has antibodies against HHV-6 (9). HHV-6 is the causal agent for a mild childhood disease, exanthema subitum (17), but it is possible that primary and reactivated HHV-6 infections may give severe symptoms in patients who have received organ transplants (16). High titers of immunoglobulin G (IgG) against HHV-6 were further described in patients with the chronic fatigue syndrome, lymphoproliferative disorders, and the acquired immunodeficiency syndrome (4).

HHV-6 was initially isolated from cultures of peripheral blood mononuclear cells from patients with lymphoproliferative disorders (13). The virus is predominantly trophic and cytopathic for $CD4^+$ T lymphocytes in vitro. Coinfection of $CD4^+$ cells by HHV-6 and human immunodeficiency virus (HIV) leads to enhanced cell degeneration, but the in vivo effects of HHV-6 in HIV-infected persons are unknown (6, 11). It has been suggested that dual infection with HHV-6 and HIV may aggravate the HIV-induced immunodeficiency (6), but other coinfection experiments indicate that HHV-6 replication can inhibit the growth of HIV in lymphocytes and produce false-negative HIV culture results (11).

We tested several antiviral compounds for inhibition of HHV-6. Foscarnet (phosphonoformic acid [PFA]), acyclovir [9-(2-hydroxyethoxymethyl)guanine (ACV)], and 9-[4-hydroxy-2-(hydroxymethyl)butyl]guanine (2HM-HBG) were gifts from Medivir, Stockholm, Sweden. Ganciclovir (9-[1,3-dihydroxy-2-propoxymethyl]guanine [GCV]) was obtained from Syntex Research, Palo Alto, Calif. Zidovidine (3′-azido-3′-deoxythymidine [AZT]) was from Sigma Chemical Co., St. Louis, Mo. 1-β-D-Arabinofuranosyl-*E*-5-(2-bromovinyl)uracil (BvAraU) was a gift from Squibb Laboratories.

HHV-6 (GS strain)-infected HSB-2 cells (the virus and the cell line were gifts from S. Z. Salahuddin and R. Gallo, National Institutes of Health, Bethesda, Md.) containing 40% cells stained by specific immunofluorescence (IF) (9) were used for infection of HSB-2 cells. Infected and uninfected cells (1:8) were added together with 0.2 ml of maintenance RPMI 1640 medium containing duplicate dilutions of the antiviral drugs in 24-well Costar plates (10^5 cells per ml). After 3 days of incubation at 37°C in 7% CO_2, when a 90% cytopathic effect and optimal specific fluorescence were obtained, the cells were stained by IF with anti-HHV-6-positive human serum and fluorescein-labeled sheep anti-human IgG. The concentration of antiviral drug which reduced the number of fluorescence-stained cells by 50% (IC_{50}) was determined. The effects of the antiviral substances on uninfected cells were determined by inhibition of proliferation. Uninfected cells were inoculated with medium containing the antiviral drugs. After 3 days, when control wells contained about 4×10^5 cells, all viable cells were counted in a volume distribution analyzer (VDA 140; Analysinstrument AB, Stockholm, Sweden). Control wells contained cells and medium but no antiviral compound. The concentration of antiviral compound required to inhibit cell proliferation by 50% (CIC_{50}) was determined. In vitro selectivity indices (SI; the ratio of the CIC_{50} to IC_{50}) were calculated for the compounds by using the CIC_{50}s and the IC_{50}s obtained in HSB-2 cells.

2HM-HBG, ACV, and GCV are guanosine analogs with antiherpesvirus activities. ACV is phosphorylated by viral thymidine kinase (TK), and its triphosphate inhibits viral polymerase. Similar mechanisms are likely to occur with 2HM-HBG and GCV against herpesviruses with active viral TKs. In cases in which no viral TK is present, as in cytomegalovirus and possibly HHV-6 (5), the compound might be activated by cellular enzymes. The guanosine analogs all inhibited HHV-6 replication at somewhat different concentrations, and the compounds showed low levels of toxicity toward uninfected HSB-2 cells (Table 1). Other investigators have found the sensitivity of HHV-6 to ACV to be in a range similar to that found in this study (3, 5, 7, 12, 15), but exact comparisons are impossible because of the use of different virus strains, different methodologies, and different ways of expressing results. Concerning the debate on the possibility of using ACV clinically against HHV-6 infections (7, 12), the low SI for ACV argues against this possibility (Table 1).

We found HHV-6 to be less susceptible to GCV than has been reported previously (3, 12). Variations between the different HHV-6 strains used in the studies may explain this

* Corresponding author.

TABLE 1. Anti-HHV-6 activities and cytotoxicities of antiviral compounds in HSB-2 cells[a]

Compound	IC_{50} (μM)	CIC_{50} (μM)	SI
(±)2HM-HBG	14 ± 2	640 ± 18	47 ± 8
ACV	59 ± 7	200 ± 32	4 ± 1
GCV	25 ± 4	200 ± 10	8 ± 2
AZT	>200	>200	NE[b]
BvAraU	>80	100 ± 6	NE
PFA	49 ± 2	1,500 ± 50	31 ± 2

[a] Values are means ± standard deviations. The results represent data from three experiments, each of which was performed in duplicate.
[b] NE, Not evaluated.

difference. It may also be that our use of whole human serum for the fluorescence assays allows for detection of HHV-6 early antigens, which may be expressed, although viral DNA replication is prevented.

The IC_{50} of HHV-6 to the (−)2HM-HBG enantiomer was 4 μM (Fig. 1), and that of the (+)2HM-HBG enantiomer was 9.3 μM; thus, the differences in the IC_{50}s between the two enantiomers were small. We consider the inhibitory concentrations of 4 and 9.3 μM (Fig. 1) and 14 ± 2 μM for the separated and racemic compounds, respectively, to be within the range for interassay variations, although unknown interferences between the isomers cannot be ruled out. The similar activities for (−)2HM-HBG and (+)2HM-HBG against HHV-6 are in sharp contrast to their inhibitory activities against varicella-zoster virus, in which (−)2HM-HBG is very potent and (+)2HM-HBG is inactive (1; G. Abele, Ph.D. thesis, Karolinska Institute, Stockholm, Sweden, 1988). This indicates that in the molecular interaction, the HHV-6 target is less discriminatory and that the two arms of the sugar side chain are more or less interchangeable, which can be used advantageously for the design of more potent inhibitors. 2HM-HBG was less toxic toward HSB-2 cells than ACV or GCV was; thus, the highest SI was obtained for 2HM-HBG (Table 1).

In analogy with what was reported by Agut et al. (3), the thymidine analog AZT did not inhibit HHV-6 replication (Table 1). The virus also was not affected by BvAraU. These findings indicate that there is no TK with a capacity to phosphorylate these substances or that the triphosphates are not inhibitory to a HHV-6 DNA polymerase.

PFA interacts with viral DNA polymerases at a site where PP_i is split off during polymerization of nucleoside triphosphates (10). It has been shown to be an effective inhibitor of several viral polymerases, inhibiting replication of herpesviruses, HIV, and hepatitis B virus (10). Results of this study demonstrate that PFA is a moderately effective inhibitor of HHV-6 replication, with an IC_{50} of 49 μM (Table 1) and with comparatively little toxicity to uninfected cells. Streicher et al. (15) found PFA to be active at a concentration of 20 μg/ml (67 μM), while Agut et al. (3) reported the IC_{50} to be 8.7 μg/ml. Shiraki et al. (14) found the closely related analog phosphonoacetic acid to inhibit HHV-6 in mononuclear cells at 2 to 5 μg/ml and to inhibit the HHV-6 DNA polymerase in cell-free assays at 2 to 5 μg/ml (14.3 to 35.7 μM). In those studies, variations of the different HHV-6 strains and methodologies were also apparently important.

Thus, 2HM-HBG, PFA, and perhaps, GCV appear to have selective inhibitory activities against HHV-6 replication in vitro.

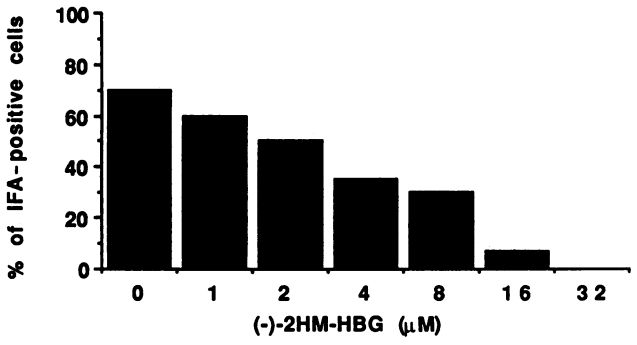

FIG. 1. Anti-HHV-6 activity of (−)2HM-HBG. The test was performed by an IF assay (IFA), and the means of two experiments were calculated. The IC_{50} for (−)2HM-HBG was 4 μM, while the IC_{50} for (+)2HM-HBG was 9.3 μM (data not shown).

We thank B. Öberg and N. G. Johansson, Medivir, Stockholm, Sweden, for fruitful discussions.
This study was supported by the Swedish Society of Medicine.

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