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Penciclovir Is a Selective Inhibitor of Hepatitis B Virus Replication in Cultured Human Hepatoblastoma Cells

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Penciclovir [9-(4-hydroxy-3-hydroxymethylbut-1-yl)guanine], an effective antiherpesvirus agent, was found to be a potent and selective antiviral agent against intracellular hepatitis B virus (HBV) replication (drug concentration at which a 10-fold decrease in HBV DNA from the average level in an untreated culture was observed [EC_{90}], 1.6 μM) and extracellular virion release (EC_{90}, 0.7 μM) by cultured human hepatoblastoma (2.2.15) cells. Acyclovir and three other related 9-alkoxypurines with activity against either herpesviruses or human immunodeficiency virus were uniformly inactive against HBV. The activity of penciclovir is discussed in relation to recent findings related to its mode of action against HBV.

Hepatitis B virus (HBV) is a causative agent of both acute and chronic hepatitis, a major etiologic factor of primary hepatocellular carcinoma, and a major global health problem with more than 300 million estimated chronically infected individuals. Although there are several promising antiviral agents currently in clinical trials, alpha interferon remains the only licensed treatment for chronic HBV infection.

Penciclovir (PCV) [9-(4-hydroxy-3-hydroxymethylbut-1-yl) guanine] is an effective antiviral agent against herpes simplex virus types 1 (HSV-1) and 2 (HSV-2) and varicella-zoster virus (VZV) (2) and, in its orally available form (famciclovir [FAMVIR]), is licensed for the treatment of herpes zoster and acute recurrent genital herpes. It is also highly active against the duck HBV in both primary hepatocyte culture and in chronically infected Peking ducks (21–23). Famciclovir controlled HBV replication effectively in orthotopic liver transplant patients (11) and inhibited HBV replication in a small, single-center clinical trial against chronic HBV infection (13). Larger, multicenter trials of famciclovir against chronic hepatitis B are currently in progress.

The HBV-producing human hepatoblastoma cell line, 2.2.15, has been shown to be an accurate model of chronic cellular viral replication and a predictive model of antiviral response for in vivo hepadnaviral infection (9, 10, 20). This study demonstrates that penciclovir is an active and selective agent against intracellular HBV replication and extracellular virion release by cultured human hepatoblastoma 2.2.15 cells and that penciclovir compares well in potency to another nucleoside analog, 3TC [lamivudine, (−)β-L-2′,3′-dideoxy-3′-thiacytidine], which is also in clinical trials against chronic HBV infection.

Confluent cultures of 2.2.15 cells were maintained on 24-well flat-bottomed tissue culture plates in RPMI 1640 medium with 2% fetal bovine serum (Biofluids, Inc., Gaithersburg, Md.) and were treated with nine consecutive daily doses of test compounds as previously described (9). Confluent cultures of 2.2.15 cells represent the culture state necessary to support stable, high levels of HBV replication (9, 10, 20). An analysis of toxicity using uptake of neutral red dye was performed in 96-well flat-bottomed tissue culture plates following 9 days of drug treatment under experimental conditions which were identical to those used for the antiviral analyses (9). Antiviral effects were determined by the analysis of either intracellular HBV DNA replicative forms or HBV virion DNA levels in culture medium by blot hybridization methods (Southern or dot blot) (9, 10). For the antiviral analyses, a total of four individual cultures on two plates were used for each drug concentration (four concentrations per test compound). For the toxicity analyses, a total of three separate cultures were used for each drug concentration (four concentrations per test compound). A total of four or nine untreated (control) cultures were used for each antiviral or toxicity analysis, respectively.

PCV, 3TC, and 2′,3′-dideoxyguanosine (2′,3′-ddG) represent distinct, clinically relevant classes of nucleoside analogs which have activity against hepadnavirus replication both in vitro and in vivo (3, 9, 12, 18, 21, 23). All three compounds were found to be selective agents against the extracellular production of HBV virions and against intracellular DNA replication of HBV genomes in 2.2.15 cells following 9 days of treatment (Table 1). PCV was >10-fold more potent against HBV replication and >2-fold less cytotoxic than 2′,3′-ddG (Table 1). PCV was approximately 3-fold less potent than 3TC, the most selective antihepadnaviral agent identified in this culture system that is also in clinical trials against chronic HBV infection (3, 18).

BRL44385, an isomer of acyclovir, and BRL45148, an isomer of ganciclovir, are 9-alkoxypurines (7), and BRL47923 is a 9-[2-(phosphonomethoxy)alkoxy]purine (4) (Fig. 1). BRL44385 is about 3 times more active than acyclovir against HSV-1 and HSV-2 and about 5 times more active against VZV. BRL45148 and acyclovir exhibit similar activity against herpesviruses (7). BRL47923 is a potent and selective agent against human immunodeficiency virus (HIV) with a 50% inhibitory concentration (IC_{50}) of 0.3 μM (15). All of these compounds had no effect on HBV replication in this culture system at concentrations up to 30 μM (Table 1). The contrast between the potent activity of PCV against HBV and the lack of activity of its 9-alkoxy analog, BRL45148, emphasizes the influential effect of the carbon-oxygen linkage at the N-9 position (Fig. 1), which appears to be incompatible with activity against HBV but fully compatible with activity against herpesviruses. This concept is reinforced by the inactivity of both BRL44385 and BRL47923 against HBV and their potent activity against herpesviruses and HIV, respectively. Famciclovir also had no activity against HBV replication in this study (Table 1). This observation is

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TABLE 1. Antiviral activities of test compounds against HBV replication in 2.2.15 cells[a]

Compound[b]	CC_{50} (μM)[c,d]	EC_{50} (μM)[d]		EC_{90} (μM)[d]		Selectivity index (CC_{50}/EC_{90})[e]	
		Virion[f]	HBV RI[g]	Virion	HBV RI	Virion	HBV RI
PCV	448 ± 30	0.20 ± 0.02	0.6 ± 0.05	0.7 ± 0.05	1.6 ± 0.1	630	280
2′,3′-ddG	203 ± 15	2.3 ± 0.2	4.3 ± 0.4	7.2 ± 0.6	14 ± 1.1	28	14
3TC	1,722 ± 102	0.05 ± 0.005	0.2 ± 0.02	0.2 ± 0.01	0.6 ± 0.05	8,200	2,870
Acyclovir	680 ± 50	108	>100	>100	>100		
BRL44385	ND	>30	>30	>30	>30		
BRL45148	ND	>30	>30	>30	>30		
BRL47923	ND	>30	>30	>30	>30		
Famciclovir	ND	>30	>30	>30	>30		

[a] Analysis of HBV DNA was performed 24 h after the ninth day of treatment (8). Cultures were treated and analyzed as described in the text.
[b] PCV, famciclovir, BRL44385, BRL45148, and BRL47923, were obtained from SmithKline Beecham Pharmaceuticals, under code, for analysis by the National Institute of Allergy and Infectious Diseases in vitro HBV Antiviral Screening Program conducted at Georgetown University (contracts N01-AI-45195 and N01-AI-72623). 3TC and acyclovir were purchased from Moravek Biochemicals, Inc., and 2′,3′-ddG was purchased from Calbiochem, Inc.
[c] CC_{50}, drug concentration at which a twofold decrease of neutral red dye uptake from the average level in untreated cultures was observed. ND, not determined.
[d] Values presented (± standard deviations) were calculated by linear regression analysis using data combined from all treated cultures; standard deviations were calculated by using the standard error of regression generated from the linear regression analyses. A notation of ">30" or ">100" indicates that no significant decrease of HBV DNA was observed at the highest concentration tested (30 or 100 μM, respectively).
[e] EC_{90}s were used for the calculation of the selectivity indices, since at least a threefold decrease of HBV RI levels is typically required to achieve statistical significance in this assay system (8).
[f] Extracellular HBV virion DNA.
[g] Intracellular HBV DNA replication intermediates.

consistent with previous observations which demonstrated that famciclovir is not metabolized to PCV in cell culture (24) and suggests that the appropriate esterase and/or oxidative enzyme is also not present in this assay system.

The lack of a significant or selective anti-HBV activity in 2.2.15 cells for acyclovir (drug concentrations at which a 10-fold decrease in HBV DNA from the average level in an untreated cultures was observed [EC_{90}], >100 μM [Table 1]) (9) is consistent with the overall lack of efficacy of acyclovir against chronic HBV infections in clinical trials. Although some of the numerous clinical studies with this drug reported a modest degree of anti-HBV activity for intravenous treatment with high doses of acyclovir (which was often associated with unacceptable degrees of nephrotoxicity after prolonged administration), oral administration of acyclovir had little, if any, effect on HBV replication and is no longer considered to have any clinical benefit for the management of HBV disease in patients with established chronic infections (see references 1 and 19 for examples).

Comparisons of the activity of PCV against HBV in 2.2.15 cells (drug concentrations at which a twofold decrease in HBV DNA from the average level in untreated cultures were observed [EC_{50}s], 0.2 and 0.6 μM [Table 1]) and its activity against clinical strains of HSV-1, HSV-2, and VZV in MRC-5 cells (EC_{50}s, 1.6, 7.2, and 29.4 μM, respectively) (2) show that PCV is at least as active against HBV as it is against members of the herpesvirus family. The EC_{50}s and EC_{90}s for PCV against HBV fall well within the peak plasma levels of PCV (3.4, 6.4, and 13.4 μM) following single oral doses of 125, 250, and 500 mg of famciclovir, respectively (16).

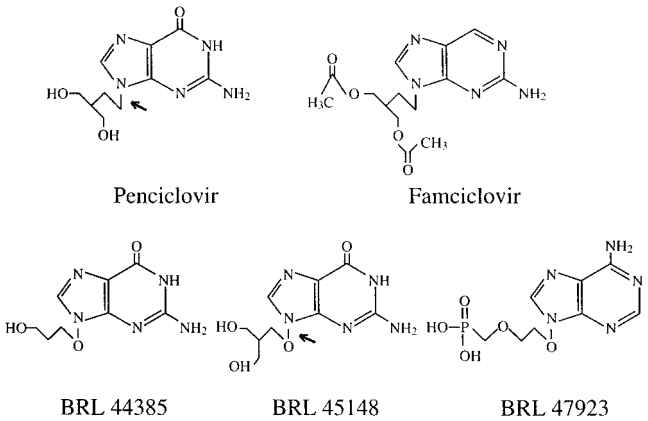

FIG. 1. Structures of PCV, famciclovir, BRL44385, BRL45158, and BRL47923. The arrows denote the position of the N-9 linkage discussed in the text.

Recent findings on the mode of action of PCV against HBV help to explain its potent activity. It is well established that, in herpesvirus-infected cells, the viral thymidine kinase efficiently phosphorylates penciclovir to the monophosphate form, and cellular enzymes thereafter produce high concentrations of PCV triphosphate (PCV-TP) (24). The K_is of the herpesvirus DNA polymerases for (*S*)-PCV-TP are high (8.5, 5.8, and 1.6 μM for HSV-1, HSV-2, and VZV, respectively), and high concentrations of PCV-TP are necessary for the inhibition of herpesvirus replication (6, 24). In contrast, the K_i of HBV DNA polymerase for (*R*)-PCV-TP, the more active enantiomer against HBV, is 0.03 μM, and thus, very low intracellular concentrations of PCV-TP would inhibit HBV replication (14). The intracellular concentration of PCV in 2.2.15 cells under dosing regimens similar to those used in the current study has been estimated to be 0.44 μM, about 10-fold higher than the K_i for inhibition of HBV DNA polymerase (14).

There is no known HBV-encoded enzyme comparable to the thymidine kinase of the herpesviruses which could initiate the phosphorylation of PCV. However, since the concentrations of PCV-TP in HBV-transfected and untransfected cells were similar (14), it appears that the very limited phosphorylation brought about by cellular enzymes is sufficient to inhibit HBV replication. Investigation into those cellular enzymes that may be responsible for the anabolism of PCV is therefore required. At present, the enantiomeric specificity of PCV-TP formed in HBV-infected or uninfected cells is unknown. The recent demonstration that (*R*)-PCV-TP inhibited hepadnaviral reverse transcription by inhibiting the synthesis of the short DNA primer and that both *R* and *S* enantiomers inhibit primer

extension (IC_{50} of <1.0 μM for each enantiomer) gives further insight into the mode of action of PCV against HBV and the stereospecificity of its triphosphate ester (25). The high selectivity index for PCV in the present study is a further key feature of the properties of the compound and is well supported by the ratio of more than 4,000:1 between the K_i of HBV DNA polymerase for (*R* or *S*)-PCV-TP (0.04 μM) and the corresponding K_i of human DNA polymerase α (175 to 200 μM) (5, 8, 14). The high K_i value for PCV-TP (judged to be at least 70% *S* isomer) and DNA polymerase α is accompanied by a similarly high K_i for DNA polymerase γ (2a) and high IC_{50}s for DNA polymerases δ and ε (120 and 375 μM, respectively) (8), emphasizing the selectivity of PCV. The concentration of PCV-TP within 2.2.15 cells (0.44 μM) (14), is so much lower than these IC_{50}s as not to cause concern that cellular DNA synthesis would be adversely affected at the reported effective antiviral doses.

In summary, the potent and selective activity of PCV against HBV can be explained by the very high affinity of the HBV DNA polymerase and the very low affinity of cellular polymerases for PCV-TP. The low level of phosphorylation of PCV by cellular enzymes gives concentrations of PCV-TP which are sufficient to inhibit the HBV polymerase but are too low to inhibit cellular DNA replication. The effectiveness of famciclovir, the oral form of PCV, against duck HBV replication in vivo and its safety record in human clinical experience against herpesvirus infections in humans (17) indicate that famciclovir will be a successful treatment for chronic HBV infection.

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