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Short Communication

Baseline sensitivity of HSV-1 and HSV-2 clinical isolates and defined acyclovir-resistant strains to the helicase–primase inhibitor pritelivir

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ABSTRACT

Fifty-nine US isolates of HSV-1 and HSV-2 obtained between 1998 and 2004 were tested for sensitivity to the helicase–primase inhibitor, pritelivir (AIC316, BAY 57-1293) by plaque-reduction assay. All isolates, which were collected prior to any clinical use of primase–helicase inhibitors, were sensitive and showed mean EC_{50} values of 0.026 and 0.029 μM for HSV-1 and HSV-2, respectively. Furthermore, several laboratory-selected acyclovir-resistant HSV mutants were also sensitive to pritelivir. These data provide a baseline for HSV sensitivity to pritelivir in general population before it is introduced and broadly used to treat HSV infection. The data also validate pritelivir as an appropriate therapy for nucleoside-resistant HSV infections.

1. Introduction

Helicase–primase inhibitors (HPI) comprise a new class of potent inhibitors of herpesviruses (Kleymann et al., 2002; Biswas and Field, 2011). The thiazolylphenyl derivative pritelivir (also known as AIC316 or BAY 57-1293) is an HPI that recently commenced phase II clinical trials for treatment of herpes simplex virus (http://www.aicuris.com/10d72/News_Publications.htm. Last accessed 24 April, 2013). The 50% effective concentration (EC_{50}) of pritelivir in tissue culture is reported to be 0.01–0.03 μM based on cytopathic effect or plaque-reduction assays (Kleymann et al., 2002). Using plaque reduction assay on several laboratory isolates of HSV-1 and HSV-2 and a small series of UK clinical HSV-1 isolates we confirmed the EC_{50} was 0.05 μM (Biswas et al., 2007). The present report concerns a larger series of clinical isolates that had been obtained from North America during a period of approximately ten years before the first clinical use of pritelivir or any other known HPI. Furthermore, a well-characterised group of acyclovir and penciclovir-resistant mutants was obtained from the Rega Institute, Leuven, Belgium. These mutants have defined resistance mutations in either the HSV thymidine kinase or DNA polymerase genes (Andrei et al., 2005, 2007).

Since HSV susceptibility to acyclovir measured by plaque-reduction assay correlated to clinical outcome (Bacon et al., 2003), the assay has become the standard procedure to measure viral drug susceptibility. We used this assay to measure the susceptibility of HSV isolates to the drug, pritelivir.

2. Materials and methods

2.1. Origin of virus strains

The clinical isolates were obtained from primary cultures of herpes lesion swabs from patients with labial or genital herpes and sampled during the period 1998–2004 in the Seattle region of USA. The majority of the patients had no obvious immunological abnormality. The isolates were typed by monoclonal antibody-staining of infected cells. After a single passage in cell culture, the isolates were stored at −80 °C in 1 ml aliquots.

The laboratory strain HSV-1 KOS, and seven acyclovir-resistant strains (KT1, KT2, KT3, KT4, KD1, KD2, and KD3) all derived from KOS were gifts from Dr. Graciela Andrei, Rega Institute for Medical Research, Leuven. Strains KT1, KT2, KT3 and KT4 contained resistance mutations in the *thymidine kinase* (TK) gene (Andrei et al., 2005, 2013) and the mutants KD1, KD2 and KD3 contained resistance mutations in the *DNA-polymerase* gene, respectively (Andrei et al., 2007, 2013). These mutants had shown various levels of resistance to ACV and penciclovir (Andrei et al., 2005, 2007,

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2013) and we confirmed their resistance in our laboratory prior using them for this study.

2.2. Passage of infectious virus from clinical isolates

To prepare viral stocks for plaque-reduction assay, 10^7 Vero cells in a 75 cm^2 flask were inoculated with 100 μl of clinical isolates received. The infected Vero cell monolayers were checked daily and when they reached 100% cytopathic effect, the infected cell cultures were harvested (usually 48–72 h p.i.). The virus isolates were harvested by centrifuging the culture at 550*g* for 10 min. The cell pellet was re-suspended in 1 ml Dulbecco's modified Eagle's medium (DMEM) containing 1% fetal calf serum (FCS) and sonicated for 1 min. This lysate was then divided into 10 aliquots of 125 μl each and frozen at −80 °C. One of the aliquots was used to determine the plaque formation units (p.f.u.) of the stock on Vero cells.

2.3. Plaque-reduction assays (PRA)

PRAs were carried out using Vero cells cultured in DMEM (containing 10% FCS) in 12-well plates at 37 °C in 5% CO_2 until they were 80–90% confluent. The overlying medium was decanted from each well; an estimated 100 p.f.u. of the HSV-1 or HSV-2 strains in 100 μl of DMEM (containing 1% FCS) were then added onto the well. After 45 min to 1 h at 37 °C, cells were overlaid with CMC–DMEM (DMEM containing 10% high-density carboxymethyl cellulose) with increasing concentrations of pritelivir (typically 0.001, 0.01, 0.03, 0.1, 1, 10 μg/ml) in multiple wells. Control wells were overlaid with CMC–DMEM without drug. After two days incubation at 37 °C the plates were stained and fixed using 0.1% w/v crystal violet dissolved in 20% ethanol. The number of plaques in each well was counted under the microscope. The percentage reduction of the number of plaques was calculated by comparing the number of plaques in the treatment wells with the number of plaques in the control wells. The percentage reduction of plaque number of each treatment was then plotted against drug concentration (μg/ml) on a log10 scale.

3. Results

Of 59 clinical isolates tested by PRA, all were sensitive to pritelivir. For the 27 HSV-1 isolates, the range of EC_{50} values was 0.011–0.042 μM with a mean of 0.026 μM (SD = 0.005 μM) (Fig. 1). The corresponding data for 32 HSV-2 isolates showed a greater range of EC_{50} from 0.009 to 0.065 μM with a mean value of 0.029 μM (SD = 0.018 μM). To confirm that the different susceptibility observed amongst the isolates was not due to experimental variation,

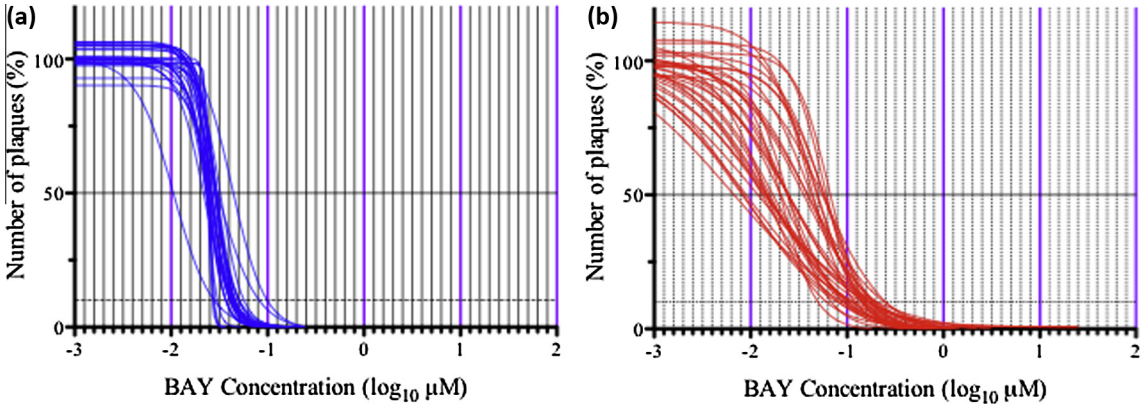

Fig. 1. A graph of PRA data used to determine the EC_{50} values. The pritelivir concentration scale has been transformed to Log10 values and the data has been analysed by means of a sigmoidal dose–response with variable slope. (a) HSV-1 isolates; (b) HSV-2 isolates.

Table 1
Pattern of cross-resistance/sensitivity amongst nucleoside analogues and HPI.

Resistant mutant	Inhibitor:	ACV		PCV		Pritelivir	
	Resistance gene	(μM)	Fold	(μM)	Fold	(μM)	Fold
KOS (Parent)	None	0.11	n/a[a]	1.07	n/a	0.04	n/a
[1]KT1	TK	2.50	22.7	2.30	2.1	0.03	0.8
[2]KT2	TK	183.33	1666.6	177.70	166.1	0.04	1.0
[3]KT3	TK	90.33	821.2	55.70	52.1	0.04	1.0
[4]KT4	TK	89.33	812.1	99.00	92.5	0.03	0.8
[5]KD1	pol	106.00	963.6	122.70	114.7	0.04	1.0
[6]KD2	pol	1.40	12.7	0.90	0.8	0.04	1.0
[7]KD3	pol	3.50	31.8	1.70	1.6	0.03	0.8

The data are EC_{50} concentrations of the respective inhibitors (μM) obtained in the plaque-reduction assay and the -fold change in sensitivity in comparison with the parental virus (HSV-1 KOS). The assay method was the same as for the clinical isolates shown in Fig. 1.

[1] Kos 2000/4 (09.07.04) TK mutant 430–436 insert G/146 frameshift.
[2] Kos 2000/15 (07.06.04) TK mutant 548–553 insert C/185 frameshift.
[3] Kos 2000/30 (07.06.04) TK mutant 548–553 deletion C/185 frameshift.
[4] Kos 2000/37 (01.08.01) TK mutant 430–436 deletion G/146 frameshift (Andrei et al., 2007, 2013).
[5] Kos PFAr clone 1014 (19.06.02) DNA Pol mutant A719V.
[6] Kos PFAr clone 1034 (06.08.03) DNA Pol mutant T821M.
[7] Kos PFAr clone C (22.10.02) DNA Pol mutant S724N (Andrei et al., 2007, 2013).
[a] Not applicable.

the most and least sensitive isolates were retested several times and the differences remained.

In addition to the clinical isolates, we also tested the pritelivir susceptibility of seven acyclovir resistant HSV-1 isolates, derived from KOS, with known mutations in either DNA-pol or TK. All these seven strains were uniformly sensitive to pritelivir and showed no change in EC_{50} compared to the parental strain, KOS. (Table 1).

4. Discussion

The results for clinical isolates obtained from the Seattle region of North America concurred with previous published sensitivity data of HSV strains to a representative HPI (Kleymann et al., 2002). Furthermore, the results are also consistent with a survey of ten clinical isolates of HSV-1 obtained from the Cambridge region (UK) during 2005–2006 where the EC_{50} was reported to be <0.05 μM (Biswas et al., 2007). Thus, it appears that all clinical isolates tested are susceptible to pritelivir at the concentration readily sustained in patients following a therapeutic dose. Intriguingly, the dose–response curves did show some differences between HSV-1 and HSV-2 (Fig. 1). The Hill slopes of HSV-1 strains seemed to be steeper than HSV-2, and HSV-2 showed a broader range of EC_{50} values. Nevertheless, all isolates were found to be sensitive to pritelivir with a similar EC_{50}. Furthermore, several laboratory-defined acyclovir-resistant mutants were also shown to be sensitive to pritelivir.

Although it has been shown that HSV variants harbouring HPI-resistance mutations located in the helicase or primase genes can be selected in the laboratory culture system (Biswas et al., 2007; Field and Biswas, 2011; Field and Mickleburgh, 2013), it remains to be seen whether HPI- resistant mutants will emerge following the widespread usage of HPIs to treat HSV infections. To date, no emergence of such resistant variants has been detected in swabs collected from patients in clinical trials treated with pritelivir, even at low doses (Huang et al., 2011). However, the trial was conducted in immunocompetent patients with genital herpes which is a population where the emergence of resistance to nucleoside analogues is not prevalent.

Overall, the data presented in this communication present a useful baseline for the sensitivity of HSV isolates to pritelivir and provide a definitive benchmark for monitoring drug resistance in the future. Based on a limited number of defined mutants, this work also suggests that pritelivir may be useful as an efficacious treatment for patients with nucleoside-resistance HSV infection.

Conflicts of interest

HJF was in receipt of funds from a research-collaboration with AiCuris GmbH who are developing pritelivir. AB and HZ are both employees of the same company.

Previous presentation

Some data in this manuscript were presented at the 25th International Conference on Antiviral Research, ISAR Japan, April 16–19, 2012.

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