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Differentiation of Herpes Simplex Virus Types 1 and 2 by Sensitivity to (*E*)-5-(2-Bromovinyl)-2′-Deoxyuridine†

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Selective inhibition of herpes simplex virus (HSV) type 1 replication but not of HSV type 2 replication by (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU) was utilized to differentiate clinical isolates of these viruses. BVDU (0.7 μg/ml) reduced HSV type 1 PFU by an average of 2.74 $\log_{10}$, but had no effect on the plaque-forming ability of HSV type 2 isolates. The incorporation of BVDU into the media of primary cell cultures used for virus isolation in the clinical laboratory allowed for the correct presumptive identification of HSV types.

Many antiviral compounds which possess specific antiherpetic activity in vitro have been described. De Clercq et al. (1) compared more than 20 antiherpetic drugs against several strains of herpes simplex virus (HSV) in rabbit kidney (RK) cells. Although several of the drugs are more effective against HSV type 1 (HSV-1) than HSV type 2 (HSV-2), the most significant differences are demonstrated by (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU) and (*E*)-5-(2- iodovinyl)-2′-deoxyuridine. These compounds inhibit HSV-2 strains at concentrations more than 100 times greater than those required to inhibit HSV-1 strains. The authors stated that this differential sensitivity can be a useful marker test for the differentiation of HSV-1 and HSV-2 strains. The present report compares the use of BVDU sensitivity as a marker with a selective cell culture system which has been used routinely in this laboratory for typing HSV strains (6). Included also are the results of the incorporation of BVDU directly into the culture media of cells inoculated with clinical specimens suspected to contain HSV.

Primary RK cells, guinea pig embryo (GPE) cells, and chicken embryo (CE) cells were prepared by standard laboratory procedures (4). The selective cell culture system for typing HSV strains has been described previously (6). Briefly, suspensions of CE and GPE cells were seeded into 24-well Costar microtest plates and incubated in the presence of 5% CO_2 at 34°C for 2 to 3 days. Virus assays were performed simultaneously in CE and GPE cells by inoculation of 0.1-ml aliquots of 10-fold dilutions. After a 1-h adsorption, plates were overlaid, incubated at 34°C for 4 days, and stained, and plaques were counted with a microscope. HSV-2 isolates produce plaques in both CE and GPE cells, whereas HSV-1 isolates produce plaques only in GPE cells.

Clinical specimens which had been submitted for virus isolation were stored at −70°C and used directly for HSV typing or were passaged once in RK or GPE cells. A few laboratory strains of HSV were provided by B. Francke, Yale University School of Medicine, New Haven, Conn.

BVDU was kindly provided by E. DeClercq, Rega Institute for Medical Research, Leuven, Belgium. The drug was suspended in sterile phosphate-buffered saline at a concentration of 500 μg/ml, dispersed in 1-ml aliquots, and stored at −70°C. The sensitivity of HSV isolates to BVDU was determined by incorporating 0.7 μg of BVDU per ml into the overlay medium of inoculated GPE cell monolayers titrated simultaneously with the cell selection system described above. For a test of the ability of BVDU to differentiate between HSV-1 and HSV-2 upon the initial isolation of the viruses, the same concentration of drug (0.7 μg/ml) was added to the culture medium of two RK or GPE cell tubes inoculated with clinical material from suspected HSV cases. These tubes were used in addition to RK or GPE cells without drug and to commercially available human embryo fibroblasts (WI-38 or MRC-5). All tubes were held for 2 weeks before being judged as negative. The appearance of characteristic herpes-like cytopathic effects (CPE) in RK or GPE cell tubes with and without the drug was considered a presumptive HSV-2 isolation, whereas the appearance of characteristic CPE only in tubes without the drug was a presumptive HSV-1 isolation. All isolates were

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TABLE 1. Sensitivity of HSV-1 and HSV-2 to inhibition by BVDU

Virus type[a]	Clinical isolate no. or laboratory strain	Infectivity titers (log_{10} PFU/0.1 ml) in:			
		CE cells	GPE cells		Log $_{10}$ reduction
			No drug	Plus BVDU[b]	
HSV-1	1389	<1	5.72	3.22	2.50
	1745	<1	6.35	3.30	3.05
	1762	<1	3.25	0.70	3.18
	1806	2.18	5.24	2.75	2.49
	1833	<1	3.65	1.30	2.35
	1859	<1	6.13	3.89	2.24
	1877	<1	5.35	2.81	2.54
	1897	<1	5.76	3.76	2.00
	1935	<1	5.90	3.24	2.66
	1960	<1	6.17	2.80	3.37
	1967	<1	5.50	2.00	3.50
	1968	<1	5.34	2.18	3.16
	1978	<1	6.00	3.78	2.22
	2018	<1	4.76	2.06	2.70
	2020	2.15	4.83	2.70	2.13
	2021	<1	4.92	2.15	2.77
	2054	<1	6.54	2.88	3.66
	2059	<1	7.26	3.98	3.28
	2103	<1	5.35	3.14	2.21
	2113	<1	4.93	1.57	3.36
	2128	<1	5.27	1.90	3.37
	2189	<1	4.31	2.10	2.21
	2192	<1	5.71	3.31	2.40
	KOS	<1	6.11	4.12	1.99
	17	<1	6.35	3.81	2.54
	NYU 78	<1	6.27	3.20	3.07
HSV-2	1714	3.09	3.14	3.01	0.13
	1781	2.94	3.19	3.07	0.12
	1783	2.83	2.98	3.06	−0.08
	1800	1.83	2.05	2.14	−0.09
	1805	1.81	1.99	2.22	−0.23
	1815	1.54	1.80	1.98	−0.18
	1837	3.74	4.55	4.48	0.07
	1838	3.12	3.24	3.43	−0.19
	1867	4.52	4.62	4.66	−0.04
	1868	3.16	3.39	3.33	0.06
	1890	4.65	4.72	4.63	0.09
	1943	3.06	2.98	3.41	−0.43
	1945	6.45	6.30	6.21	0.09
	2015	4.33	4.59	4.44	0.15
	2016	4.26	4.38	4.31	0.07
	2040	4.06	4.15	4.19	−0.04
	2041	5.20	5.73	5.64	0.09
	2073	3.84	4.40	4.41	−0.01
	2098	2.32	2.20	2.12	0.08
	2102	2.81	2.85	2.81	0.04
	2138	4.44	5.30	5.02	0.28
	WT-186	5.32	5.94	6.08	−0.14
	HG52	4.18	5.32	4.89	0.43

[a] Typed by the selective cell culture system (see text).
[b] 0.7 μg/ml.

subsequently confirmed by cell selectivity and BVDU sensitivity.

The incorporation of BVDU into the overlay medium of the titrations of 23 HSV-1 isolates resulted in a reduction of plaque formation varying from 2.00 to 3.66 log_{10} (Table 1). The mean reduction of PFU was 2.74 log_{10}, and the median was 2.66 log_{10}. The laboratory strains were not

TABLE 2. Presumptive identification of HSV-1 and HSV-2 by sensitivity to BVDU[a]

Clinical isolate no.	Specimen source	Day of appearance of CPE		Preliminary identification
		No drug	Plus BVDU	
1960	Facial	2	—[b]	HSV-1
1967	Nasopharynx	4	—	HSV-1
1968	Tracheal suction	4	—	HSV-1
1978	Facial	1	—	HSV-1
2015	Genital	4	4	HSV-2
2018	Facial	4	—	HSV-1
2020	Facial	2	—	HSV-1
2021	Facial	2	—	HSV-1
2040	Genital	2	2	HSV-2
2041	Arm	1	1	HSV-2
2054	Facial	1	—	HSV-1
2059	Facial	1	1	HSV-2[c]
2073	Genital	6	5	HSV-2
2102	Hand	2	1	HSV-2
2103	Facial	1	—	HSV-1
2113	Facial	3	—	HSV-1
2128	Facial	1	—	HSV-1
2189	Facial	1	—	HSV-1

[a] BVDU (0.7 μg/ml) was added directly to culture media of cells used for the primary isolation of HSV from clinical specimens (see text).

[b] —, no CPE during the entire observation period.

[c] Misidentified preliminarily; subsequently typed as HSV-1 (see Table 1).

included in these calculations but were added only for reference. Table 1 also shows the results of the titrations of 21 HSV-2 strains under the identical conditions used for the HSV-1 experiments. There was no significant effect of BVDU on the plaque-forming ability of any of the HSV-2 strains. All strains were identified as HSV-1 or HSV-2 by titration in the selective cell culture system which was run in parallel with the drug studies. In addition, approximately 50% of each HSV type was analyzed for its ability to induce deoxypyrimidine triphosphatase, an HSV-1-specific enzyme (8). Only HSV-1 induces the enzyme (F. Wohlrab, D. R. Mayo, G. D. Hsiung, and B. Francke, Abstr. Int. Workshop Herpesviruses, Bologna, Italy, p. 181, 1981).

The great difference in sensitivity of HSV-1 and HSV-2 to BVDU prompted an investigation into the possibility of identifying HSV as HSV-1 or HSV-2 upon the first appearance of CPE in cell cultures inoculated with clinical specimens. All lesion swabs or other specimens suspected of containing HSV were inoculated into two additional tubes of RK or GPE cells containing 0.7 μg of BVDU per ml of medium. Based on the appearance or absence of CPE in cells with and without BVDU, the presumptive identification of 18 HSV isolates was made (Table 2). In 17 of 18 isolations, the presumptive identification was correct when the isolates were subsequently tested by the selective cell culture and BVDU sensitivity methods. The isolate which was initially misidentified is the only specimen in Table 2 that was collected directly from the patient while in the clinical virology laboratory and inoculated undiluted into appropriate cell cultures within seconds of collection. This suggests, quite expectedly, that the effects of a limited amount of drug can be overcome by very high titers of virus. The problem can be overcome by the dilution of specimens suspected to be of high titers, such as those collected in the laboratory or inoculated at the bedside, into the appropriate cell cultures.

Herpes simplex viruses are the most frequently isolated viruses in this laboratory (5) and in the majority of clinical virology laboratories (7). The identification of these viruses as HSV-1 or HSV-2 is important for patient management and counseling purposes (5, 6). Typing of HSV isolates may assume even greater importance when antiviral agents with varying effectiveness against HSV-1 or HSV-2 (1) become more widely available. In the present studies, all isolates of HSV whose ability to form plaques was inhibited by at least 2 $\log_{10}$ by BVDU were identified as HSV-1 by the selective cell method. Similarly, the isolates of HSV which were not inhibited by BVDU were all identified as HSV-2 by this CE-GPE method.

The question of the emergence of BVDU-resistant HSV-1 strains must be raised since this would lead to the misidentification of HSV strains. No BVDU-resistant HSV-1 strains have been described. The search for naturally occurring drug-resistant mutants to another more widely used and investigated antiviral drug, acycloguanosine [9-(2-hydroxyethoxymethyl)gua-

nine], has been unsuccessful. Experimentally, it has not yet been possible to induce acycloguanosine-resistant HSV mutants in vivo, apparently because such mutants that may arise do not thrive (2). However, treatment-resistant strains of HSV have been isolated from patients who have undergone prolonged therapy with 5-iodo-2′-deoxyuridine (3).

All of the BVDU sensitivity determinations done in this study used GPE cells; however, several virus strains were also typed using RK cells. As expected, the results were identical since the work which described the specific effect of BVDU on HSV-1 was performed in RK cells (1). More recently, basic studies of the effects of BVDU on HSV have also been performed in RK cells (B. L. Wigdahl, H. Isom, E. D. De Clercq, and F. Rapp, Virology, in press; B. L. Wigdahl, H. Isom, and F. Rapp, Proc. Natl. Acad. Sci. U.S.A., in press). Several virus strains were also correctly identified by this method as HSV-1 or HSV-2 with tubes of commercially available human embryonic lung cells. It is therefore possible that any cell line that is used for the isolation of HSV can also be used for typing by the BVDU method. However, each laboratory should test its own cell lines for suitability.

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ADDENDUM IN PROOF

Since this paper was submitted for publication, 25 more HSV isolates have been correctly identified in this laboratory with the described system, and an additional 30 isolates have been correctly identified in another laboratory (E. DeClercq, personal communication).

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