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Inhibition of B Virus in Cell Culture by 5-Iodo-2'-Deoxyuridine

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The antimetabolite 5-iodo 2'-deoxyuridine (IDU) interferes with B virus replication in monolayers of cynomolgous monkey kidney, KB and HEp 2 cells. To determine the effect of this clinically active nucleoside in laboratory antiviral screening systems, several methods were compared. Most of the methods used were capable of detecting the activity of IDU at low levels.

Herpes virus simiae (B virus) causes a highly fatal ascending myelitis in humans infected by monkey bites (10). This infectious agent isolated from monkeys and grown in cell culture is used in our deoxyribonucleic acid (DNA) virus spectrum for chemotherapy testing.

The pyrimidine nucleosides have been shown to be effective in cell culture against several viruses having a DNA core (1, 4, 5, 7, 8). Kaufman first used 5-iodo 2'-doexyuridine (IDU) in clinical cases of herpes simplex keratitis with reported success (6).

The possibility of B virus infections in biological laboratories is of appreciable concern, and it seemed of interest to determine the effect of IDU on B virus in cell cultures with the possibility of eventual clinical application

Clinically active antivirals are rare, and it was felt that this nucleoside also could be used to determine the comparative sensitivity of various cell lines and test methods to a clinically effective antiviral agent. By use of B virus as the infectious agent, its sensitivity to IDU was determined by several cell culture systems. The following is a report of this study.

MATERIALS AND METHODS

The virus used was the Yale strain of B virus which is a monkey isolate and which has been through three passages in monkey kidney cells in our laboratory.

Cynomolgous monkey kidney (MK) monolayers were prepared in 16 by 150 mm glass tubes with medium 199 containing 10% calf serum and grown for 6 days at 37 C. Virus assays in these monolayers were performed with medium 199 containing no serum.

Monolayers of KB and HEp 2 established human, fermentor-adapted cell lines were grown in 120-ml prescription bottles.

The cells were planted at 250,000/ml with 20 ml of Eagle's Basal Medium containing 15% Tryptose Phosphate Broth (TPB) and 10% calf serum, and were incubated for 48 hr with one medium change. Primary MK cell suspension was planted in both 4-oz bottles and 22.9 by 30.5 cm Pyrex dishes with medium 199 containing 15% TPB and 10% calf serum. The medium was changed at 24 hr and the cells were incubated for an additional 6 days. The medium again was changed, and the monolayers were ready for use on the 7th day. All tests were performed at 37 C. The virus adsorption period in the plaque and disc-zone tests was 2 hr.

The nutrient agar overlay used in both the 120-ml bottles and the Pyrex dishes was previously described (9). The IDU (from Nutritional Biochemicals Corp., Cleveland, Ohio) which was prepared in warm Hank's balanced salt solution at 5 mg/ml did not go into solution completely. The saturated solution was distributed in 2-ml amounts in screw-cap vials and maintained frozen for stock.

The three test systems used for assay are outlined in Table 1.

The tube test system A was performed by adding the drug before the virus and grading the cytopathic effect (CPE) on a subjective scale of no cell involvement to complete cell destruction (0-4).

The system B used with three cell lines was based on the reduction of the number of plaque-forming units (PFU) by the drug in comparison with that of the untreated controls. This was considered to be a rigorous test for the drug, since the virus was adsorbed onto the cell monolayer for 2 hr before the drug was added. The drug was allowed to remain in contact for about 20 min before the agar overlay was added. Thus, the virus-infected cells were in contact with a high concentration of drug for a short period of time, and then, after overlaying with the nutrient agar, the cells were in contact with a lower concentration of the drug for the remainder of the test period. The concentration of drug reported in the tables was based on the final dilution obtained after overlaying.

System C was a modification of the agar diffusion method of Herrman et al. (3) and has been previously described (9).

This system is considered to be a less sensitive

TABLE 1. *Cell culture testing systems*

System	Cell	Container	Inoculum	Drug vol (ml)	Method[a]
A	MK[b]	Tube, 2 ml	10–100 $TCID_{50}$	0.5	CPE grade, 4 days
B	KB	120-ml bottle, 20 ml	50–300 PFU	0.5	Plaque reduction, 11 days
	HEp 2	120-ml bottle, 20 ml	50–300 PFU	0.5	Plaque reduction, 11 days
	MK	120-ml bottle, 20 ml	50–300 PFU	0.5	Plaque reduction, 6 days
C	MK	22.9 by 30.5 cm Pyrex dish, 500 ml	20,000–100,000 PFU	0.08, on disc	Zone size (mm), 6 days

[a] Procedure used for determining antiviral activity.
[b] MK = monkey kidney.

TABLE 2. *Comparative B virus titrations[a] in cell culture with two assay systems*

System	Cell line	PFU/ml		$TCID_{50}$ (0.5 ml)	
		Titration 1	Titration 2	Titration 1	Titration 2
B, plaque	MK	10^{6}	$10^{6.1}$	—	—
	KB	$10^{6.1}$	$10^{6.3}$	—	—
	HEp 2	$10^{5.9}$	$10^{5.7}$	—	—
A, CPE	MK (tubes)	—	—	$10^{-6.5}$	$10^{-6.2}$

[a] Duplicate titrations.

TABLE 3. *IDU activity against B virus determined by plaque count reduction in MK cell monolayers*

No. of plaque-forming units	Percentage of plaque reduction[a] at various levels of IDU (μg/ml)			
	250	78	25	7.8
300	100	100	75	64
150	100	100	62	43

[a] Average of three bottles

TABLE 4. *IDU activity against B virus determined by plaque counts in HEp 2 cell monolayers*

IDU	Plaque count	Per cent reduction
μg/ml		
0	300[a]	
7.8	149	54
25	23	93
78	0	100

[a] Control value. Average number of plaques per bottle in five bottles.

method than the plaque assay procedure for the following reasons: (i) a smaller volume of drug is used (0.08 ml); (ii) the drug is added to the disc and not directly to the infected monolayer; and (iii) a much

TABLE 5. *IDU activity against B virus determined by plaque counts in KB cell monolayers*

IDU	Plaque count	Per cent reduction
μg/ml		
0	226[a]	
2.5	157	31
7.8	117	49
25	29	88
78	12	95

[a] Control value. Average number of plaques per bottle in three bottles.

TABLE 6. *IDU activity against B virus in MK cell culture tubes[a]*

$TCID_{50}$[b]	Graded CPE[c] at various levels of IDU (μg/ml)													
	2,500		780		250		78		25		7.8		Virus control	
10	0	0	0	0	1	1	1	1	1	1	2	2	3	3
100	1	1	1	1	2	1	2	2	2	2	2	3	3	3
Control	0	0	0	0	0	0	0	0	0	0	0	0		

[a] VR = 5.8
[b] Values of 10 and 100 $TCID_{50}$ are equivalent to 12 and 120 PFU per tube, respectively.
[c] Scale of 0-4 represents no CPE to complete cell destruction.

TABLE 7. *B virus versus IDU in MK cells with agar-diffusion disc-zone method[a]*

Drug dilution	Test	Zone size (mm)
Undiluted	1	35–40
	2	35–38
1:3.2	1	Negative, no zone
	2	Negative, no zone

[a] Volume of drug, 0.08 ml per disc, and the inoculum was 20,000 PFU per tray. See Table 1, system C, for other details.

higher virus infecting dose is used. However, for a drug known to show antiviral activity this is an excellent system.

RESULTS AND DISCUSSION

Table 2 shows the comparative results of two titrations of B virus in three different cell lines with two assay systems. Very little difference was observed in the three plaque assays, although the HEp 2 results appeared to be slightly lower.

The MK plaque assays were read at 6 days. The plaques tended to increase in size and to coalesce so that the readings had to be completed before this occurred. In the KB and HEp 2 plaque tests, the optimal period appeared to be about 11 days, and the plaques did not coalesce except at very high PFU concentrations.

All three assay systems showed activity that was considered to be significant. A plaque reduction of at least 50% was used as the level indicating significant drug activity. Activity in the tube test was determined by the virus rating (VR) which was based on a grading of CPE, toxicity, and virus level (2).

The MK and HEp 2 plaque assays showed complete virus plaque inhibition at IDU concentrations of 78 μg/ml (Tables 3 and 4). The KB cell test was nearly as sensitive (Table 5). The control PFU count in the chemotherapy test results shown in Tables 3 to 5 compared closely, making these results more significant.

At a concentration of 78 μg of IDU per ml in the tube test shown in Table 6, the highest level of 100 $TCID_{50}$ (equal to 120 PFU per tube) showed a subjective reading of at least 50% cells still infected. The calculated VR value of 5.8 shows that IDU is a highly active drug against B virus, based on our criterion that a VR value of 1.0 or greater is significant.

Duplicate test results of the agar-diffusion disc-zone system are shown in Table 7. This method detected IDU activity at 400 μg per disc but not at 125 μg per disc. The monolayer was seeded with 20,000 PFU of B virus. Based on these results, this system appears to show low sensitivity for IDU.

A comparison of the tube and plaque test (Table 3 to 6) shows that the plaque system gives complete virus suppression at a lower drug level. It is also interesting to note that the three plaque titrations with different cell lines show an agreement within approximately one-half log dilution. The plaque assay was also used to determine whether the percentage of reduction in plaques varies with the number of virus PFU used. According to these results (Table 3), there was no significant difference within the range of PFU tested.

Because of the basic differences in the assay systems, it may not be meaningful to compare the results based on the number of virus PFU employed. It is interesting to note, however, that in the tube test, for which the calculated number of PFU seeded was 120 at 100 $TCID_{50}$ (Table 6) and for which the MK lowest level plaque count was 150 (Table 3), the levels of activity of IDU compared within one-half log.

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