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Absence of Idoxuridine and Persistence of Herpes Simplex Virus In Brains of Patients Being Treated for Encephalitis (38966)

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Parenteral administration of idoxuridine (5-iodo-2′ deoxyuridine, IDU) in treatment of herpesvirus infections, particularly herpes simplex virus encephalitis (HSVE) has always been controversial (1–3). Initial trials were optimistic, and observations in animal models and man testing efficacy, limits of safety and pharmacokinetics were undertaken (3–5). When IDU was given to mice with HSVE, titers of virus decreased in several viscera, but did not diminish in brain (4). Evidence for efficacy did not become apparent (6, 7) and dangers of a nonjudicious use of IDU became evident (3).

A sensitive specific assay for IDU in body fluids was developed, and transient concentrations of antiviral activity were found in serum, urine, and cerebrospinal fluid (CSF) (5). We have adapted our method to tissues and have tested brains of patients with encephalitis dying during or after therapy. We report the results of this experiment.

Materials and Methods. Samples of brain were obtained from five patients at biopsy and ultimately at postmortem examinations. Each specimen was cut into two 5-g portions and half was quickly frozen (−20°) for later HSV and IDU assays, while the remainder was fixed in formalin for pathologic study. Four patients died while receiving intravenous IDU, and the final patient died 7 days later. Every patient received 60–80 mg/kg of IDU/day by intravenous infusion (4 mg/min) over the projected course of 5 days (3).

Methods for isolation and typing of herpes simplex viruses as well as assays of IDU in body fluids have been reported (5, 8, 9). In the antiviral assay of body fluids described earlier from this laboratory, virus is inoculated into vero renal tissue cultures in 30-ml flasks. After a 2-hr absorption, the fluid to be tested is added. Since neutralizing antibody inhibits virus at an extracellular locus, neutralizing antibody does not interfere with measures of idoxuridine (5). Vero renal cells are sensitive to human interferon, but the findings of these experiments obviate the necessity of excluding this possibility.

Prior to determinations of antiviral activity in brains, virus was inactivated (10, 11). At a distance of 33.3 cm from the specimen a G.E. Germicidal Lamp (G8T5) was used in laminar flow hood. Plastic Petri plates containing 5×10^4 PFU of HSV-1 (Ket. strain) in 2 ml of 5% fetal calf serum (FCS) in Eagle's medium (EM) were placed on a rotary shaker at 100 rpm, 23°. Similar plates contained HSV-1 (Ket.) with 10% suspensions of human brain in EM which had been prepared with a mortar and pestle. Covers of the Petri plates were removed to expose the contents to air during the procedure. For each series, controls were identical except that plates were not treated with ultraviolet light. After irradiations for 10, 15, or 20 min 0.2 ml aliquots were removed and 0.1 ml inoculated onto two 30-ml flasks containing vero renal cells in monolayer tissue cultures.

Specimens were allowed an absorption period of 120 min (37°), and washed twice with 2 ml of warm EM (37°). An overlay containing 2 ml each of 2 × EM–10% FCS and 0.6% agarose was finally added and flasks were incubated for an additional 96 hr in an incubator (37°). Thereafter, to each culture we added 2 ml of 10% formalin, and 20 min later, the overlay was poured away. Flasks were washed in running tap water, and 2 ml of a paragon–formalin stain added (8). After overnight incubation at room temperature, cells were washed with tap water. Using a stereoscopic microscope PFU of HSV-1 were counted (5).

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TABLE I. INACTIVATION OF HERPES SIMPLEX VIRUS, TYPE 1 IN SPECIMENS OF BRAIN BY TREATMENT WITH ULTRAVIOLET LIGHT

HSV-1 (5×10^4 PFU)	Irradiation (GE Germicidal Lamp, 33.3 cm, 23°)			
	Minutes			
	0	10	15	20
5% FCS, EM	TNTC[a] (x2)	O, PFU (x2)	O, PFU (x2)	O, PFU (x2)
10% Brain, EM	TNTC (x2)	TNTC (x2)	22, 9 PFU	O, PFU (x2)

[a] TNTC, PFU too numerous to count.

Nonirradiated samples at 10, 15, and 20 min contained confluent PFU which were too numerous to count. Irradiation for 10 min inactivated samples containing HSV-1 in FCS with EM, but a 20-min exposure to the germicidal lamp was necessary to inactivate the same quantity of virus in 10% suspensions from brain (Table I).

Therefore, HSV-1 positive specimens of brain were prepared as 10% suspensions, and irradiated for 30 min. Each was tested in vero cell cultures for virus, and was negative. Finally using the same procedure as that for body fluids (5), 0.2 ml of the treated suspensions of brain was tested for IDU. The limits of these methods are 5 μg/0.4 ml of specimens to be tested (5).

Results. CSF showed varying degrees of inflammation with proteins as high as 234 mg/100 ml; white blood cells, 266/ml and erythrocytes, 358/ml (Table II). Virus was not isolated from any of the CSFs but HSV-1 was found in three of five biopsy specimens. On microscopic section, brains obtained from biopsies or autopsies showed necrotizing encephalitis. At autopsy, each brain which was positive at biopsy retained virus, and in one of the three cases (Table II, case no. 2) the quantity of HSV-1 increased.

Sera, urines, and CSFs from one of our patients were assayed for IDU (Table III). All sera were negative, but one of two urines contained IDU (45 μg/ml), and one of the CSFs tested had antiviral activity (833 μg/ml, Table III). These data have been reported and suggest that when IDU is given by slow intravenous infusion (approx 4 mg/min), degradation of IDU to inactive metabolites (iodouracil, uracil, and iodide) exceeds the rate of administration, and serum, urine, or CSF only sporadically contain measurable IDU (5). However, none of the 32 samples of brain analyzed for IDU contained drug-related antiviral activity.

Moreover, four of the five patients died while still receiving IDU. Some of the specimens contained interferon (12).

Discussion. When IDU is given in maximal doses (60–80 mg/kg/day iv) and within the limits of our methods, significant concentration of antiviral activity is not found in brain. Iodothyronine deiodinase has been found in preparations of tissues from brain (13), but IDU is stable in serum at 37, 4°, and to freezing and thawing (5). The stability of IDU in freshly separated serum suggests that its inactivation results largely from intracellular events. Although enzymatic inactivation of IDU after biopsy or death is possible, this seems unlikely.

Breeden, Hall, and Tyler (1) failed to demonstrate an uptake of ^{131}I-labeled IDU in the brain of a patient with encephalitis. Likewise, in a murine model Kern, Overall, and Glasgow (4) did find IDU in blood and liver, but not in brain. Clarkson, Oppelt, and Byvoet (14) found IDU transiently in plasma and CSF of dogs. Using different methods, Calabresi *et al.* (15) and Lerner and Bailey (5) studied pharmacokinetics in human serum and urine with similar results. On the other hand Buckley and MacCallum (16) did not find IDU in CSF of a patient receiving IDU by intracarotid arterial infusion. A transient presence of IDU in the CSF as demonstrated by us does not prove that antiviral activity reaches the brain.

Our data and that of others indicate that IDU does not alter the ultimate mortality in HSVE (3, 6, 7, 17). Whether morbidity among surviving patients is changed, remains to be convincingly shown. The toxicity of

TABLE II. ABSENCE OF IDOXURIDINE IN BRAIN; HSV-1 AT BIOPSY AND AUTOPSY; AND CSF FINDINGS IN PATIENTS AFTER TREATMENT FOR SUSPECTED HSVE

Case no.	Age	Sex	Day(s) of treatment with IDU (60–80 mg/kg/day, iv)	CSF				Brain			Day(s) after onset of IDU at death
				wbc/mm³	rbc/mm³	Protein mg %	HSV[a]	Region	HSV (PFU or TCD_{50}/g)	IDU (μg/ml)	
1	27	F	0	0	20	110	0	L. temporal (biopsy)	3170	<6	12
			5	20	140	128	0	Occipital (autopsy)	0	<6	
								L. parietal (autopsy)	0	<6	
								R. parietal (autopsy)	0	<6	
								L. frontal (autopsy)	+	<6	
								R. frontal (autopsy)	+	<6	
								L. temporal (autopsy)	100	<6	
								R. temporal (autopsy)	+	<6	
								Olfactory nerves (2) (autopsy)	+	<6	
2	62	F	0	40	0	54	0	R. temporal (biopsy)	3000	<6	1
			1	54	0	70	0	R. temporal (autopsy)	20,000	<6	
				0	111	234	0				
3	40 (approx)	M	0	98	52	54	0	R. temporal (autopsy)	0	<6	4
			4				0	R. frontal (autopsy)	0	<6	
								L. frontal (autopsy)	0	<6	
4	18	M	0	266	0	79	0	L temporal (biopsy)	0	<6	5
			5	241	20	85	0	L. frontal (autopsy)	0	<6	
								R. frontal (autopsy)	0	<6	
								L. temporal (autopsy)	0	<6	
								R. temporal (autopsy)	0	<6	
								L. parietal (autopsy)	0	<6	
								R. parietal (autopsy)	0	<6	
								Sp. cord (autopsy)	0	<6	
								Olfactory nerves (2)	0	<6	
5	57	F	0	0	160	38	0	L. temporal (biopsy)	4000	<6	3
			3	10	358	124	0	L. frontal (autopsy)	100	<6	
				9	0	82	0	R. frontal (autopsy)	100	<6	
								L. cerebellum (autopsy)	0	<6	
								R. cerebellum (autopsy)	+	<6	
								L. temporal (autopsy)	100	<6	
								R. temporal (autopsy)	+	<6	

[a] +, present; 0, absent; PFU/g, plaque forming units/gram; TCD_{50}/g, 50% tissue culture doses/gram.

TABLE III. ASSAYS FOR IDU DURING (OR FOLLOWING THERAPY) IN BODY FLUIDS AND BRAIN OF A SINGLE PATIENT

				Idoxuridine (IDU)			
Case no.	Age	Sex	Dose mg/kg/day	Day of treatment	Body fluid	Brain	μg/ml (g)
1	27	F	60	−1	Serum	—	<6
				1	Serum		<6
				2	Serum		<6
				3	Serum		<6
				4	Serum		<6
				1	Urine	—	<6
				3	Urine		45
				1	CSF	—	833
				3	CSF		<6
				+7	—	9[a] Regions	<6

[a] Nine separate regions of the brain were tested at autopsy.

IDU is severe at dosages of >60 mg/kg/day. Its use can be lethal particularly when there is thrombocytopenia and tracheostomy is necessary.

Myelosuppressive effects produce leukopenias and life-threatening nosocomial infections occur (3). On account of these toxicities, systemic IDU is no longer used in HSVE (18). On the other hand another candidate antiviral nucleoside, adenine arabinosied, ara-A (9-β-D arabino-furanosyladenine) does penetrate the brain, has constant antiviral activity in serum and urine and a higher therapeutic index (19). Myelosuppressive effects of ara-A are less than those due to IDU. In view of these apparent advantages for ara-A, further testing of systemic IDU at lower dosages does not seem warranted.

Summary. Thirty-two specimens of brain from five patients with encephalitis suspected to be caused by herpes simplex virus, type 1 (HSV-1) were assayed for antiviral activity. Each patient received 60–80 mg/kg/day of idoxuridine (IDU) by intravenous infusion. The antiviral assay does not measure anti-HSV-1 antibodies. Biopsies of brain in every patient taken before IDU was used, and portions of several regions of the brain at autopsy were available during courses of treatment in four of the five patients. The last patient died 7 days after completing treatment. A significant concentration of IDU (833 μg/ml) was measured transiently in the cerebrospinal fluid of one patient. Meninges and brains showed inflammatory changes. Within the sensitivity of the test (≧ 6 μg/g) all specimens contained no IDU. As given, IDU does not achieve therapeutic concentrations in human brain. Further clinical use of IDU in therapy of herpes simplex virus encephalitis is not indicated.

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