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EFFICACY OF (*E*)-5-(2-BROMOVINYL)-2′-DEOXYURIDINE IN THE TREATMENT OF EXPERIMENTAL HERPES SIMPLEX VIRUS ENCEPHALITIS IN MICE

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Systemic treatment of mice with (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU) showed a significant therapeutic efficacy against herpes simplex type 1 virus (HSV-1) encephalitis. With treatment initiated 12 h after viral inoculation and continued for 10 consecutive days, BVDU administered intraperitoneally in daily doses of 100–500 mg/kg increased the 21-day survival rates from 30 to 100% and reduced brain virus titers by 3–4 $\log_{10}$ on day 6 post-infection. Furthermore, at doses of 300–500 mg/kg per day BVDU prevented the establishment of latent virus infection in the trigeminal ganglia following intracerebral HSV-1 inoculation.

herpes simplex virus; encephalitis; latency; bromovinyldeoxyuridine

INTRODUCTION

(*E*)-5-(2-Bromovinyl)-2′-deoxyuridine (bromovinyldeoxyuridine, BVDU) has been described recently as a potent and selective antiviral agent against herpes simplex type 1 virus (HSV-1) and varicella zoster virus (VZV) [10–12]. The selective activity of BVDU is dependent on a specific activation of BVDU by herpes virus-induced thymidine kinase [4]. It is converted to mono- and diphosphate by the HSV-1 encoded thymidine kinase [16,18] and further to the triphosphate (by a cellular kinase). BVDU triphosphate inhibits the viral DNA polymerase to a significantly greater extent than the cellular DNA polymerases α, β and γ [2]. BVDU can also be incorporated into DNA, but this incorporation would primarily be restricted to the virus infected cells [1].

Several studies have been reported on the therapeutic efficacy of BVDU in animal HSV-1 model infections. Maudgal et al. [23–25] and Hettinger et al. [20] showed that BVDU, when applied as 0.1–0.5% eye drops, was therapeutically effective against HSV-1 epithelial and stromal keratitis and iritis in rabbits. A significant therapeutic efficacy was

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also observed when BVDU was given systemically or topically to athymic-nude or hairless mice inoculated intracutaneously with HSV-1 [13,14,17]. BVDU was also found effective in the systemic or topical treatment of orofacial HSV-1 infection of hairless mice. The chemotherapeutic response to BVDU was dose-dependent and clearly evident even when the treatment was initiated during the clinical manifestation of HSV-1 infection at 72 h after inoculation. Initiation of therapy with BVDU at 3 or 24 h after inoculation significantly prevented the establishment of latent HSV-1 infection in the trigeminal ganglia of mice [27].

In the present study, we evaluated the therapeutic efficacy of BVDU in mice inoculated intracerebrally with HSV-1. The therapeutic responses were determined by measuring the capacities of BVDU to protect mice from mortality and to suppress the multiplication of HSV-1 in the brain. Moreover, the establishment of latent HSV-1 infection in the trigeminal ganglia of surviving mice was also examined. BVDU has been studied previously for its inhibitory effects on the mortality rate of mice inoculated intracerebrally with HSV-1 [15]. However, in this earlier study no attempts were made to evaluate the inhibitory effects of BVDU on either virus multiplication in the brain or establishment of latency in the trigeminal ganglia.

MATERIALS AND METHODS

Virus

HSV-1 (McKrae strain) was propagated on primary rabbit kidney cells and the viral titer was adjusted to yield 2.0×10^7 plaque-forming units per ml (PFU/ml). This virus stock was stored at -75°C until used in the present experiment.

Mice

Outbred albino male mice (CD-1 strain; purchased from Charles River Breeding Laboratories, Wilmington, MA) were maintained for at least 1 week in our animal laboratory at the Eye Research Institute, Boston, MA. They were housed 5 to a cage and fed high-energy, ultradigestible Mouse Chow (Ralston Purina Co., St. Louis, MO). The average weight of the mice was 20 ± 1 g when the experiments were initiated.

Compounds

BVDU was synthesized by Dr. R. Busson and Professor H. Vanderhaeghe (Rega Institute, University of Leuven, Belgium) following a procedure similar to that described by Jones et al. [21]. Appropriate suspensions of BVDU were prepared in sterile physiological saline.

Inoculation of HSV-1

Mice anesthetized with sodium pentobarbital were inoculated intracerebrally with 10 LD_{50} (50% lethal dose) of HSV-1 (5.0×10^2 PFU). Control mice were injected intracerebrally with 20 μl of Dulbecco's phosphate-buffered saline.

Determination of maximum tolerated dose and toxicity of BVDU

160 non-infected mice were divided into 8 groups of 20 mice and treated as follows: (1) physiological saline, 0.3 ml/day; (2) BVDU, 200 mg/kg per day; (3) BVDU, 300 mg/kg per day; (4) BVDU, 400 mg/kg per day; (5) BVDU, 500 mg/kg per day; (6) BVDU, 600 mg/kg per day; (7) BVDU, 800 mg/kg per day; (8) BVDU, 1000 mg/kg per day. Saline or BVDU suspension in a volume of 0.3 ml/day was given through intraperitoneal injection, three times a day for 10 consecutive days. Mice were examined every day to determine any side effects of mortality for a period of 21 days. Ten mice from each group were killed one day after the last day of drug treatment and the whole blood was collected to measure the number of white blood cells (WBC).

Survival studies

117 mice inoculated with HSV-1 were divided into 6 groups of 20 mice (17 mice for group 1); the treatment of the groups was as follows: (1) physiological saline, 0.3 ml/day; (2) BVDU, 100 mg/kg per day; (3) BVDU, 200 mg/kg per day; (4) BVDU, 300 mg/kg per day; (5) BVDU, 400 mg/kg per day; and (6) BVDU, 500 mg/kg per day.

20 mice inoculated with saline were divided into 2 equal groups which were treated as follows: (1) physiological saline, 0.3 ml/day; and (2) BVDU, 500 mg/kg per day. BVDU was given in suspension form in a volume of 0.3 ml/day.

Treatment was initiated 12 h after the inoculation of HSV-1 or saline and consisted of intraperitoneal injections of three divided doses per day for 10 consecutive days. The mice were observed daily for 21 days to document the survival rates and the mean survival time of mice that died. The brains of dead mice were removed and homogenized. The homogenates were centrifuged (2000 rpm), the supernatant was inoculated into green monkey kidney cell (CV-1, American Tissue Culture Collection, Rockville, MD) monolayers, and the cytopathic effects observed confirmed that the mice died of viral encephalitis.

Determination of the viral titers in brain

In order to determine the inhibitory effects of BVDU on HSV-1 replication in brain, 5 mice from each group were killed on day 6 post-infection. The brains of mice were aseptically removed, weighed and washed in sterile normal saline. After removal of the surface membranes, they were finely minced with scissors, homogenized, and made into a 10% suspension with sterile Hanks' balanced salt solution. The suspension was frozen and thawed three times and centrifuged at 2000 rpm for 5 min at 4°C. The supernatant was then serially diluted and assayed by an ordinary plaque technique [30].

Determination of ganglionic latent HSV infection

4–8 weeks post-infection, the surviving mice were killed and their trigeminal ganglia were removed. The ganglia were submitted to explantation-cocultivation for isolating latent HSV as described before [28].

RESULTS

Maximum tolerated dose of BVDU

In non-infected mice, BVDU up to 500 mg/kg per day for 10 days did not produce death of mice. Over 600 mg of BVDU/kg per day did cause some mortality of mice, most probably due to drug toxicity. The number of white blood cells was not significantly reduced when BVDU up to 300 mg/kg per day was injected intraperitoneally for 10 days. However, over 400 mg of BVDU/kg per day brought about a significant and dose-dependent reduction in the number of white blood cells in the whole blood (Table 1).

Survival rate and mean survival time

Within 11 days post-inoculation, all infected saline-treated mice were dead. The mean survival time was 5.8 days. BVDU at doses of 500, 400, 300, 200 and 100 mg/kg per day for 10 days yielded survival rates of 100, 80, 60, 50 and 30%, respectively. The mean survival time of mice that died was also significantly increased by a dose of 200–400 mg/kg per day (~ 8.0 days) compared with saline treated controls (5.8 days) (Table 2).

Virus titer in brain

Treatment with BVDU resulted in a marked reduction in infectious virus titer from brains (Table 3). BVDU at a daily dose of 100–300 mg/kg reduced the virus titer 5–60-fold compared to saline-treated controls on day 6 post-infection. Higher doses of BVDU

TABLE 1

Determination of maximum tolerated dose and toxicity of BVDU in mice

Treatment[a]	No. of dead mice/ No. of mice tested	% change of body weight after the cessation of treatment (10 days)[b]	% change of WBC number after the cessation of treatment (10 days)[c]
Physiological saline (0.3 ml/day)	0/10	+ 32	100
BVDU (200 mg/kg per day)	0/10	+ 28	98
BVDU (300 mg/kg per day)	0/10	+ 17[d]	93
BVDU (400 mg/kg per day)	0/10	+ 15[d]	88[d]
BVDU (500 mg/kg per day)	0/10	+ 10[d]	75[d]
BVDU (600 mg/kg per day)	1/10	- 4[d]	45[d]
BVDU (800 mg/kg per day)	1/10	- 10[d]	38[d]
BVDU (1000 mg/kg per day)	3/10	- 27[d]	30[d]

[a] Treatment was given intraperitoneally three times a day for 10 consecutive days. BVDU was given in suspension form (0.3 ml/day).

[b] Relative to the weight at the initiation of treatment.

[c] Relative to the physiological saline-treated group.

[d] Significantly different ($P < 0.05$) from physiological saline-treated group.

TABLE 2

Effect of BVDU on the survival rate of mice and mean survival time of mice that died

Treatment[a]	Inoculation with	Survival rate at 21 days (No. survival/No. tested)	Mean survival time (days) of mice that died
Physiological saline (0.3 ml/day)	HSV-1[b]	0/17	5.8 ± 0.61 (3–11)[d]
	saline[c]	10/10[e]	
BVDU (100 mg/kg per day)	HSV-1	6/20[e]	7.8 ± 0.67 (5–9)
BVDU (200 mg/kg per day)	HSV-1	10/20[e]	8.0 ± 0.40 (6–9)[f]
BVDU (300 mg/kg per day)	HSV-1	12/20[e]	8.1 ± 0.32 (7–9)[f]
BVDU (400 mg/kg per day)	HSV-1	16/20[e]	8.0 ± 0.20 (7–9)[f]
BVDU (500 mg/kg per day)	HSV-1	20/20[e]	
	saline	10/10[e]	

[a] Treatment was initiated 12 h after inoculation of HSV-1 or saline, and consisted of intraperitoneal injections given three times a day for 10 consecutive days. BVDU was given in suspension form (0.3 ml/day).

[b] 10 LD_{50} (50% lethal doses) of HSV-1 were inoculated by the intracerebral route.

[c] 20 μl of Dulbecco's phosphate-buffered saline were injected intracerebrally.

[d] Numbers in parentheses are ranges.

[e] Significantly different ($P < 0.05$) from the physiological saline-treated mice inoculated with HSV-1 (Fisher exact test, double-tail).

[f] Significantly different ($P < 0.05$) from the physiological saline-treated mice inoculated with HSV-1 (Student's t-test).

TABLE 3

Effect of BVDU on virus concentration in the brains of HSV-1 infected mice on day 6 post-infection

Treatment[a]	Average virus concentration (PFU/g of tissue)	Frequency of ganglionic latent infection (No. of mice with infection/ No. of survivors)
Physiological saline (0.3 ml/day)	3.3×10^5 ($2.1 \times 10^5 - 4.0 \times 10^5$)[b]	-
BVDU (100 mg/kg per day)	6.1×10^4 ($8.0 \times 10^2 - 4.0 \times 10^5$)	6/6
BVDU (200 mg/kg per day)	5.0×10^3 ($7.0 \times 10^2 - 9.5 \times 10^3$)[c]	8/10
BVDU (300 mg/kg per day)	6.7×10^3 ($4.9 \times 10^2 - 1.6 \times 10^4$)[c]	7/12
BVDU (400 mg/kg per day)	6.6×10^2 (0 $- 1.8 \times 10^3$)[c]	0/16
BVDU (500 mg/kg per day)	0.9×10^2 (0 $- 9.8 \times 10^2$)[c]	0/20

[a] Treatment was initiated 12 h after inoculation of 10 LD_{50} of HSV-1, and consisted of intraperitoneal injections given three times a day for 10 consecutive days. BVDU was given in suspension form (0.3 ml/day).

[b] Numbers in parentheses are ranges.

[c] Significantly different ($P < 0.05$) from the physiological saline-treated mice (Student's t-test).

decreased the level of virus in brains to a greater extent and resulted in brain virus titers approx. 3–4 $\log_{10}$ less than the titers in the saline-treated control groups on day 6 post-infection.

Ganglionic latent infection

BVDU, at a dose of 300 mg/kg per day, partially prevented the development of latent HSV-1 infection in the trigeminal ganglia when the treatment was initiated 12 h after intracerebral virus inoculation. Higher doses of BVDU (400 or 500 mg/kg per day) completely blocked the establishment of latent HSV-1 infection in the trigeminal ganglia of surviving mice (Table 3).

DISCUSSION

Much laboratory and clinical research has been focused on the development of an effective therapy for HSV encephalitis. Systemic therapy with iododeoxyuridine for the treatment of HSV encephalitis was initially met with some enthusiasm [7,26], but double-blind studies designed to evaluate the efficacy of iododeoxyuridine in HSV encephalitis were prematurely terminated; iododeoxyuridine not only failed to prevent morbidity and mortality, but also caused severe toxicity [3]. Vidarabine has been shown to be effective in the treatment of HSV encephalitis in hamsters [33], mice [19,34,35], and humans [5,36,37]. Although no serious acute toxicity has been reported in humans, anorexia, nausea and vomiting, weight loss, weakness, megaloblastic changes in erythroid elements of bone marrow, tremors, and thrombophlebitis have been encountered with systemic application of vidarabine in daily dose of 20 mg/kg [31]. Trifluorothymidine (5-trifluoromethyl-2′-deoxyuridine) [8], 5-ethyl-2′-deoxyuridine [9], and 1-β-D-arabinofuranosylthymidine (ara-T) [22] have also been credited with some potential for the management of HSV encephalitis in mice. More recently, ACV has shown significant therapeutic efficacy against intracerebral HSV infections in mice [6,29,32].

The present study indicates that BVDU is highly effective against HSV-1 encephalitis in mice and this activity, measured by increased survival rates or mean survival times, is dose-dependent. BVDU also diminished brain titers of HSV-1 in infected mice in a dose-dependent manner. Furthermore, BVDU prevented the development of latent infection in the trigeminal ganglia following intracerebral HSV inoculation, when treatment with BVDU at a sufficiently high dose ($\geqslant$ 300 mg/kg per day) was initiated shortly (12 h) post-HSV inoculation. These results indicate that BVDU attained a sufficient therapeutic level in brain to prevent the replication of HSV-1 during the treatment period. The maximum therapeutic dose administered (500 mg/kg per day) did not adversely affect the survival of treated uninfected controls (Table 2). On the basis of these observations, we believe that BVDU clearly merits further investigation as a highly effective antiviral agent for the therapy of HSV encephalitis in humans.

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