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In vitro effect of (*E*)-5-(2-bromovinyl)-2′-deoxyuridine, 5′-amino-5-iodo-2′,5′-dideoxyuridine and 2-deoxy-D-glucose on latent ganglionic herpes simplex virus infection

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(*E*)-5-(2-Bromovinyl)-2′-deoxyuridine (BVDU) and 5′-amino-5-iodo-2′,5′-dideoxyuridine AIdUrd) blocked the reactivation of latent ganglionic herpes simplex virus in vitro. Furthermore, BVDU, but not AIdUrd, blocked the multiplication of reactivated latent virus and transiently suppressed emergence of reactivated virus from the sensory ganglia after removal of drug from the medium. 2-Deoxy-D-glucose (2-DG) neither prevented the in vitro reactivation of latent virus nor blocked the further multiplication of reactivated latent virus.

herpes simplex; ganglionic latency; antivirals

Introduction

Herpes simplex virus (HSV) infection establishes latent infections in the sensory or autonomic ganglia and the central nervous system both in humans [3,4,14] and in experimental animals [5,24,28]. Latent infection is characterized by the detection of infectious virus following culture of explanted nervous tissue but not by direct homogenization and assay [28]. The nature of latent HSV is, however, not yet known. Recent studies have shown that the whole HSV genome is present in its latent form, but it might be different from that in infectious virus particles [26]. Elimination of this latent HSV constitutes one of the optimal therapeutic objectives in preventing the recurrent herpetic disease which follows reactivation of the ganglionic virus.

Several in vivo studies have focused on the effect of systemic acyclovir on latent trigeminal ganglionic and central nervous system HSV infections. The use of acyclovir

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conferred an effective prophylactic effect in preventing the establishment of latency but little or no benefit once latency was established [12,18,22,29].

Wohlenberg et al. [30] have shown that phosphonoacetic acid and vidarabine both blocked the reactivation of latent ganglionic HSV infection in vitro but did not affect the persistence of latent infection. Klein et al. [17] have reported similar studies in which acyclovir blocked the reactivation of latent virus in vitro but did not eliminate the latent HSV from the ganglia of mice. More recently we have shown that both acyclovir and bromovinyldeoxyuridine (BVDU) blocked the reactivation and the multiplication of reactivated latent virus, and transiently suppressed but did not eliminate latent virus from the sensory ganglia of mice [21].

In the present study we have investigated the in vitro effects of three antiviral agents on the reactivation of ganglionic latent HSV in mice and multiplication of ganglionic HSV which had been reactivated through preincubation in drug-free medium. These drugs are (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU), 5′-amino-5-iodo-2′,5′-dideoxyuridine (AIdUrd), and 2-deoxy-D-glucose (2-DG). BVDU has been described as a potent and selective antiviral agent against HSV type 1 (HSV-1) [9]. The selective activity of BVDU is dependent on a preferential phosphorylation by herpes virus-induced thymidine kinase. It is converted to mono- and diphosphate by the herpes virus encoded thymidine kinase [8,11] 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 is also incorporated into DNA, but this incorporation would primarily be restricted to the virus infected cells [1]. AIdUrd also selectively inhibits HSV-1 replication in vitro [6], and the basis of this selectivity is that phosphorylation of AIdUrd occurs only in HSV-1 infected cells. After conversion to the triphosphate derivative this thymidine analogue is incorporated into viral DNA which is responsible for the antiviral activity of AIdUrd [7,13]. 2-DG works by a totally different mechanism in that this drug interferes with viral glycoprotein synthesis resulting in production of non-infectious particles with altered envelopes [19].

Materials and methods

Antiviral drugs

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. [16]. AIdUrd was obtained from Dr. W.H. Prusoff (Yale University School of Medicine, New Haven, CT), and 2-DG was purchased from Sigma Chemical Co. (St. Louis, MO).

Virus

HSV type 1 (HSV-1), F strain, from the American type Culture Collection (Rockville, MD) was grown in primary rabbit kidney cells and the viral titer was adjusted to yield 6×10^6 plaque-forming units (PFU)/ml. It was stored at -75°C until used.

Animals

Five-week-old inbred male albino mice of the BALB/c strain (Charles River Breeding Lab, Wilmington, MA) were housed 10 to a cage in the vivarium of the Eye Research Institute of Retina Foundation, Boston, MA. They were fed high-energy, ultradigestible mouse chow (Ralston Purina Co., St. Louis, MO). The average weight of the mice was 18 g when the experiment was initiated.

Establishment of latent HSV in trigeminal ganglia of mice

One hundred and forty mice were injected intradermally with 10% saline into their upper lips under pentobarbital anesthesia (50 mg/kg, intraperitoneal injection). Four hours after the saline injection HSV-1 solution was applied topically over the saline-injected area after careful scarification with a No. 15 Bard-Parker blade. The amount of viral solution applied was 20 μl/lip (1.2×10^5 PFU/lip). Our previous study indicates that this procedure induces latent HSV infection in the trigeminal ganglia of 100% of the inoculated mice, without mortality, when examined 4–8 weeks post-inoculation [23].

Determination of ganglionic viral titers

Four weeks post-inoculation (p.i.) the mice were killed and the trigeminal ganglia removed by sterile technique and placed in explant culture in the presence or absence of antiviral drugs as shown in Tables 1 and 2. After the initial 72 h of culture all ganglia were washed 3 times with phosphate buffered saline (PBS) and the medium was changed to drug-free. At the time intervals indicated in the tables, five ganglia from each group were washed three times in PBS and homogenized by sonification (Branson Sonic Power Co., Plainview, NY) and centrifuged for 10 min at 2 000 rpm at 4°C. The titers of reactivated HSV in the cell-free supernatants were determined by plaque assay in green monkey kidney cell (CV-1) monolayers [25].

Results

Effect of antivirals on in vitro reactivation of latent HSV-1

Ganglia were excised and placed immediately into medium containing no drug or medium containing 20 μM BVDU, 20 or 200 μM AIdUrd, or 500 or 1 000 μM 2-DG for three days before changing to drug-free medium as shown in Table 1. On day 0 post-drug removal (culture day 3) no virus was found in the 20 μM BVDU or 200 μM AIdUrd-treated groups. 2-DG at 1 000 μM showed some suppression compared to controls but the other two groups (20 μM AIdUrd and 500 μM 2-DG) had mean titers similar to controls. By day 1 post-drug removal (culture day 4), the BVDU group was still entirely negative but virus had started to appear in all other drug groups (Table 1).

TABLE 1

Effect of BVDU, AIdUrd and 2-DG on in vitro reactivation of HSV in trigeminal ganglia[a]

Groups	Antivirals	Antivirals in explant culture medium (days)	Viral titer (PFU/ganglion)[b] – Days post-removal of drug from medium			
			0	1	2	3
A	0	3	1.2×10^5	1.6×10^6	1.4×10^6	9.0×10^5
B	BVDU, 20 μM	3	0[c]	0[c]	1.2×10^2	1.8×10^3
C	AIdUrd, 20 μM	3	1.3×10^5	1.5×10^6	1.5×10^6	1.0×10^6
D	AIdUrd, 200 μM	3	0[c]	2.5×10^3	3.9×10^5	1.0×10^6
E	2-DG, 500 μM	3	4.9×10^4	1.0×10^6	1.5×10^6	1.0×10^6
F	2-DG, 1000 μM	3	2.3×10^3	3.0×10^5	1.3×10^6	8.0×10^5

[a] Following the establishment of latent HSV infection in mice, the trigeminal ganglia were incubated in culture medium containing 20 μM BVDU, 20 or 200 μM AIdUrd, or 500 or 1000 μM 2-DG for 3 days. Then the ganglia were washed × 3 in PBS and placed in drug-free media for another 0 to 3 days.

[b] Titers were determined by a plaque assay in green monkey kidney cells (CV-1) monolayers. Each titer represents the mean of 5 ganglia assayed individually.

[c] No virus was detected in undiluted ganglionic homogenates.

TABLE 2

Effect of BVDU, AIdUrd and 2-DG on the reactivated ganglionic latent HSV in vitro[a]

Groups	Preincubation period in the absence of antivirals (reactivation period)	Antivirals and period of in vitro treatment after reactivation	Virus content in trigeminal ganglia[b] (PFU/ganglion) - Days post-removal of drug from medium				
			0	1	2	3	4
A	1 day	None, 3d	1.6×10^6	1.4×10^6	9.0×10^5	8.0×10^5	9.7×10^4
B	1 day	BVDU (20 μM), 3d	0[c]	0[c]	0[c]	9(2/5)[d]	1.4×10^2
C	1 day	AIdUrd (200 μM), 3d	1.1×10^4	1.0×10^6	8.9×10^5	9.2×10^5	1.5×10^4
D	1 day	2-DG (1000 μM), 3d	5.0×10^4	1.5×10^6	9.0×10^6	7.2×10^5	8.8×10^4
E	2 days	BVDU (20 μM), 3d	0[c]	0[c]	0[c]	5(1/5)[d]	8.0×10^1
F	2 days	AIdUrd (200 μM), 3d	3.0×10^2	5.0×10^3	9.0×10^5	6.9×10^5	5.0×10^5
G	2 days	2-DG (1000 μM), 3d	6.1×10^2	1.0×10^4	1.0×10^6	4.8×10^5	5.5×10^4

[a] The latently infected trigeminal ganglia were incubated in drug-free media for 1–2 days in order to reactivate the latent HSV. Then the ganglia were placed in the culture media containing BVDU, AIdUrd or 2-DG for 3 days. After washing 3 times with PBS, the ganglia were cultured in the drug-free media for another 0 to 4 days.

[b] Titers were determined by a plaque assay in green monkey kidney cells (CV-1) monolayers. Each titer represents the mean of 5 ganglia assayed individually.

[c] No virus detected in undiluted ganglionic homogenates.

[d] Number of ganglia with reactivated HSV/Number of ganglia examined.

Effect of antivirals on reactivated latent HSV-1 in vitro

Excised ganglia with latent HSV-1 were cultured in drug-free medium for 1 or 2 days to initiate active viral replication. As shown in Table 2, the addition of BVDU to the medium for 3 days after 1 day of preincubation resulted in complete inhibition of viral growth for days 0 through 2 post-drug removal. An initial inhibitory effect was seen in the 200 μM AIdUrd and 1 000 μM 2-DG treated groups on day 0 post-drug removal but this suppressive effect was rapidly lost after drug removal.

As shown in Table 2, when two days preincubation were allowed before drug treatment, BVDU again effected a total suppression of viral growth on days 0 through 2 post-drug removal. AIdUrd at 200 μM and 2-DG at 1 000 μM also conferred an initial suppression of viral growth compared to the one day preincubation group and controls, but this effect was lost over the ensuing two days and titers remained high thereafter.

Discussion

Of the three antiherpetic drugs studied here, two, BVDU and AIdUrd are activated by virus-specific thymidine kinase [6,11,15,19]. A direct relationship was found between the amount of incorporation of AIdUrd into HSV type 1 DNA and inhibition of virus replication [7]. The incorporation of AIdUrd into HSV DNA produced single and, to a lesser extent, double-strand breaks in a dose dependent manner [13]. The exact mechanisms of action of BVDU are not fully established although it is known that this drug also inhibits viral DNA polymerase [2] and is incorporated into viral DNA [1]. The incorporation of BVDU into viral DNA leads to a dose dependent increase in single-strand breaks and a concomitant reduction in virus yield [20]. 2-DG works by a totally different mechanism in that this drug interferes with viral glycoprotein synthesis resulting in production of non-infectious particles with altered envelopes [19]. The decreased infectivity of HSV grown in the presence of 2-DG is due to either an inability to penetrate into the cell following adsorption to the cell surface or a deficiency in the process of uncoating after penetration [27]. All three drugs are relatively non-toxic to the host cell system and, therefore, good candidates for in vitro study of drug effect on herpetic latency.

BVDU has been shown to have very high levels of therapeutic efficacy at relatively low doses [9]. In an earlier in vitro study, we found 20 μM concentrations of this drug to be effective both in blocking reactivation of latent HSV and in inhibiting the multiplication of reactivated virus [21]. The same concentration, 20 μM of BVDU was, therefore, used as a positive control in the present study. AIdUrd, while therapeutically effective at non-toxic doses has, in our hands, not shown the high efficacy that is characteristic of BVDU in vivo [9]. It was used in the present study in concentrations of 20 μM and 200 μM. 2-DG has been shown to be effective in vitro against herpes virus at a concentration of 500 μM [19]. In the present study it was used at 500 μM and 1 000 μM.

Our results indicate that in vitro incubation of ganglia carrying latent HSV-1 results

in the rapid reactivation of virus within 24 h and the production of high virus titers which are sustained for at least 9 days (Tables 1 and 2). All three antiviral drugs studied here showed the ability to suppress reactivation of latent virus in vitro, with 20 μM BVDU and 200 μM AIdUrd totally inhibiting virus reactivation on the first day after drug removal. AIdUrd at 20 μM had no notable effect and 2-DG both at 500 and 1 000 μm had only transient and slight inhibitory action. These data indicate that both BVDU and AIdUrd are capable of blocking reactivation of latent virus but that their removal from the medium allows the latent virus to be reactivated. The data are in keeping with the in vitro findings of Klein and Wohlenberg using ACV and phosphonoacetic acid respectively, and in keeping with our own in vivo study indicating that the suppressive effect of systemic ACV on latent ganglionic HSV is transient and ceases once therapy is discontinued [28]. The present study also indicates that 2-DG has no meaningful suppressive effect on ganglionic virus in vitro even at very high concentrations. This difference in efficiency between BVDU and AIdUrd on the one hand and 2-DG on the other may reflect differences in the mode of action, antiviral potency, transport into the ganglion, or metabolic alteration.

As the present studies on the effect of the drugs on virus multiplication after reactivation show, 2-DG still has little antiviral effect compared to BVDU and AIdUrd, regardless of the preincubation time. Of the latter two drugs, BVDU has a more marked antiviral effect at a lower molar concentration. This result may be due to the potent antiviral activity of BVDU compared to AIdUrd: ID_{50} (50% inhibitory dose) of BVDU for HSV-1 is 0.008 μg/ml, whereas ID_{50} of AIdUrd is 26 μg/ml [10]. However, suppression is transient once the drugs are removed from the medium. Again, allowing greater time for viral reactivation through preincubation in drug-free medium did not reveal any greater or lesser effect of BVDU or AIdUrd on ganglionic titers of reactivation virus.

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