Antiviral Research, 11 (1989) 275–284
Elsevier

AVR 00362

Oral chemotherapy of fatal B virus (herpesvirus simiae) infection

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(Received 11 January 1989; accepted 4 April 1989)

Summary

Acyclovir and ganciclovir, which were only about 10-fold less effective against B virus than herpes simplex virus type 1 in VERO cells, were tested in vivo in B virus-infected rabbits. Untreated control rabbits became paralysed from 8 days and died from 10 days. Oral acyclovir at a dose rate of 500 mg/kg/day for 21 days prevented death; acyclovir prevented disease at 700 mg/kg/day. In B virus-infected humans such a high dose of acyclovir could not be given by mouth. Nevertheless, high dose oral acyclovir is suggested for immediate prophylaxis when monkey handlers have been exposed to potentially fatal B virus infection. Should signs or symptoms of disease occur then high dose intravenous acyclovir has been recommended. Since ganciclovir was found to be more effective than acyclovir, intravenous ganciclovir might be preferred for the treatment of established infection.

B virus; Herpes simplex virus type 1; Acyclovir; Ganciclovir

Introduction

B virus in its natural host, e.g. *Macaca mulatta* (rhesus monkey), closely resembles herpes simplex virus in man. Both genital and oral infection occur (Zwartouw and Boulter, 1984), and the accumulated evidence favors venereal transmission between monkeys with secondary oral infection (Zwartouw et al., 1984). This secondary site of infection presents the main hazard to man since B virus is easily transmitted by an accidental bite from an infected monkey. The infected monkey

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may be asymptomatic during primary infection or during reactivation of latent virus. Although the disease in macaques is trivial, B virus infection in man progresses to a fatal encephalomyelitis (Palmer, 1987). Human cases are very infrequent but recent fatalities in Florida (Griffin et al., 1987) have highlighted the constant threat to all who have contact with infected monkeys of Asian origin. The virus causes a similar fatal disease in rabbits and this animal model has been used to study possible treatments. The fatal outcome was prevented by eight-hourly intravenous administration of acyclovir during at least 14 days (Boulter et al., 1980). This treatment was completely effective only when commenced within 24 h of the initial infection. A second method of treatment was infiltration of anti-B virus serum into the virus inoculation site within 6 h of the initial infection (Boulter et al., 1981). The antiserum was most effective when it originated from homologous species. As human anti-B virus serum is not generally available, it was proposed to prepare immunoglobulin from more readily available simian antiserum for post-exposure immunoprophylaxis of humans. Both of the above methods need to be initiated soon after virus inoculation or, in human terms, soon after an accidental infection from a monkey or laboratory accident. With the related and similarly fatal herpes simplex virus encephalitis, opinion has favored starting acyclovir treatment at the earliest possible indication of the disease (Nicholson, 1984; Jeffries, 1986). Intravenous administration is only practicable in the hospital (Timbury, 1982) and so is not a suitable treatment for all potential exposures when a handler is at risk after receiving a scratch or bite from a monkey.

Topical acyclovir, which according to some studies is beneficial in both herpes labialis (Fiddian et al., 1983) and genitalis (Kinghorn et al., 1983), would provide an ideal prophylaxis and treatment for monkey bites and scratches. However intensive application of acyclovir to superficial inoculation sites of B virus in rabbits did not prevent death although it slightly prolonged survival time (Boulter et al., 1982). Another practical solution would be oral administration of acyclovir, which has been used successfully to treat herpes simplex virus (Nilsen et al., 1982) and varicella zoster virus infections (McKendrick et al., 1986).

Methods

B virus and herpes simplex virus type 1 were grown in tissue culture and serum neutralising antibody was measured as described previously (Boulter et al., 1981). The B virus was isolated from a cynomolgus monkey and the HSV-1 was a gift from Prof. Fulton (Vizoso, 1975). Drugs were tested in vitro by serial half log dilutions in microtiter plates using 6 wells per dilution at 0.05 ml/well. Virus (0.05 ml, 100 $TCID_{50}$/well) and Vero cells (0.1 ml, 2×10^4/well) were then added and the plates sealed and incubated at 37°C. After 4 days the 50% inhibitory dose was calculated. Half lop rabbits (2.5–3 kg) were inoculated intradermally with 100 $CCID_{50}$ (cell culture 50% infective dose) of B virus on the shaved outer right thigh. Rabbits were subsequently observed for the appearance of a lesion at the inoculation site (usually a 2–3 cm raised inflammatory area with a central necrotic zone),

(Sabin, 1934), paralysis (sometimes first detected as paresis of the inoculated limb and progressing to monoplegia and paraplegia) and death. Blood was collected from treated survivors and virus-neutralizing antibody titer measured in the serum.

Acyclovir was dissolved in water by adding sufficient sodium hydroxide to achieve solution at room temperature. At the highest concentration used (1%) the solution had a pH of 10.35. Desciclovir (BW A515U, 6-deoxyacyclovir), which is oxidized by xanthine oxidase to acyclovir (Selby et al., 1984), was readily soluble in water. Ganciclovir (BW 759U, 2′-nor-deoxyguanosine (Ashton et al., 1982) or 9-(1,3-dihydroxy-2-propoxymethyl)guanine (DHPG) (Smee et al., 1983) or BIOLF-62 required small amounts of sodium hydroxide and at the highest concentration used (0.2%) the solution had a pH of 8.5.

Oral treatment with antiviral drugs was carried out by replacing drinking water with drug solution for the appropriate number of days. The rabbits were fed standard diet pellets (Special Diet Services Ltd) ad libitum and typically drank about 300 ml daily. Appropriate concentrations of drug were used to supply the intended doses but actual doses were calculated from the measured volumes drunk daily by each animal.

Results

Initial tests were carried out in cell culture on a number of compounds reported to inhibit replication of herpes simplex virus. The compounds were compared with acyclovir and tested against B virus and herpes simplex virus type 1 (Table 1). All the compounds were less inhibitory for B virus than herpes simplex virus type 1. The difference was especially marked with bromovinyldeoxyuridine. Ganciclovir inhibited B virus as effectively as acyclovir and both were selected for further tests in animals. The prodrug desciclovir was also included in animal tests.

In the first experimental oral treatment three antivirals were compared. The in-

TABLE 1

Inhibitory effect of antiviral compounds on B virus and herpes simplex virus type 1 in VERO cell cultures

Compound	50% Inhibitory dose (μg/ml)	
	B virus	Herpes simplex virus type 1
Acyclovir[a]	18	1.3
Ganciclovir	9	1.2
Bromovinyldeoxyuridine (BVDU)[b]	600	1.0
Phosphonoformic acid (PFA)[c]	380	120.0
Phosphonoacetic acid (PAA)[c]	220	100.0

[a]Desciclovir was not included in the in vitro tests as it has been shown to be ineffective in vitro (Krenitsky et al., 1984).
[b]Tricot et al. (1986).
[c]Helgstrand et al. (1978).

TABLE 2

Comparison of three antivirals for oral treatment of B virus-infected rabbits; drug treatment on days 0–21

Rabbit No.	Compound (mg/kg/day)	Day of observation			Surviving at day 150
		Lesion	Paralysis	Death	
1	None	6	8	12	no
2		6	9	17	no
3		6	11	19	no
4	Desciclovir (200)	6	9	12	no
5		6	10	17	no
6		15	11	28	no
7	Acyclovir (330)	–	–	–	yes
8		–	10	14	no
9		–	17	22	no
10	Ganciclovir (170)	–	–	–	yes
11		–	–	–	yes
12		–	–	–	yes

hibitors were supplied in the drinking water to groups of three rabbits for 21 days starting on the same day as virus inoculation (Table 2). Three infected but untreated control rabbits developed lesions at the inoculation site after 6 days (typically 6–8 days). Paresis of the infected limb progressing to monoplegia and par-

TABLE 3

Dose response of B virus-infected rabbits to oral acyclovir; drug treatment on days 1–22

Rabbit No.	Mean dose (mg/kg/day)	Day of observation			Surviving at day 47	Antibody titer
		Lesion	Paralysis	Death		
1	0	7	8	11	no	–
2	0	8	9	12	no	–
3	0	8	11	18	no	–
4	140	12	14	24	no	–
5	150	–	14	–	yes[a]	8
6	170	–	11	–	yes[a]	32
7	330	–	12	20	no	–
8	400	–	13	–	yes[a]	16
9	460	–	–	–	yes	<2
10	490	–	12[b]	–	yes	8
11	630	–	13[b]	–	yes	8
12	640	–	–	–	yes	16
13	650	–	–	–	yes	<2
14	700	–	–	–	yes	<2
15	760	–	–	–	yes	<2

[a]Monoplegic at end of experiment.

[b]Paralysis/paresis was transient and inapparent by day 30.

aplegia was evident after about 8–11 days. These control animals died between 12 and 19 days (slightly earlier (i.e. at 10 days) in some experiments). Treatment with desciclovir (200 mg/kg/day) was almost without effect. This is most likely due to poor conversion of the prodrug to the active form (acyclovir) in this species (unpublished data of Wellcome Research Laboratories).

Oral treatment with acyclovir (330 mg/kg/day) prevented lesions developing at the inoculation site and one out of the three rabbits survived without sign of the disease. However, the other two became monoplegic and progressed to paraplegia and death. Treatment with ganciclovir (170 mg/kg/day) was completely successful with no sign of disease in the group of three rabbits. Attempts were then made to explore the efficacy of higher doses of acyclovir and lower doses of ganciclovir. With acyclovir concentrations increased to 1% in the drinking water rabbits drank normal volumes in spite of the increased alkalinity required to keep the drug in solution. Table 3 shows the results of treatment for 21 days starting 1 day after virus inoculation. At the lower doses, up to 400 mg/kg/day, all 5 rabbits had severe disease and either died slightly later than untreated controls or remained monoplegic until the end of the experiment at 47 days.

In the middle dose group (460–640 mg/kg/day) all four rabbits survived. However two had transient paralysis of the inoculated hind limb, and another produced virus neutralizing antibody indicating that significant virus replication had occurred. Three rabbits at the highest doses of 650–760 mg/kg/day as well as one in the middle group remained free of all signs of disease. Ganciclovir was tested at lower doses with treatment reduced to 14 days starting one day after virus inoculation. Table 4 shows that at doses below 50 mg/kg/day only one of four treated rabbits survived. This animal had transient paralysis and produced a high antibody titer. Above 50 mg/kg/day all five rabbits survived and only one had signs of dis-

TABLE 4

Dose response of oral ganciclovir in B virus-infected rabbits; drug treatment on days 1–15

Rabbit No.	Mean dose (mg/kg/day)	Day of observation			Surviving at day 70	Antibody titer
		Lesion	Paralysis	Death		
1	0	6	7	11	no	–
2	0	7	8	11	no	–
3	0	8	8	12	no	–
4	8	–	11	22	no	–
5	10	–	9	18	no	–
6	10	12[a]	10[b]	–	yes	256
7	42	–	22	26	no	–
8	54	–	–	–	yes	<2
9	56	23[a]	–	–	yes	256
10	86	–	–	–	yes	2
11	130	–	–	–	yes	4
12	175	–	–	–	yes	<2

[a]Lesion healed at about day 30.

[b]Paralysis/paresis was transient and inapparent by day 22.

TABLE 5

Delayed treatment of B virus-infected rabbits with oral ganciclovir (170 mg/kg/day)

Rabbit No.	Treatment			Days of observation			Surviving at day 120	Antibody titer
	Duration (days)	Start day	Stop day	Lesion	Paralysis	Death		
1	0	0	0	6	7	10	no	–
2	0	0	0	7	8	10	no	–
3	0	0	0	7	10	10	no	–
4	14	1	15	–	–	–	yes	<2
5	14	2	16	23[b]	–	–	yes	8
6	14	3	17	23[b]	–	–	yes	32
7	35	4	39	8[b]	10[c]	–	yes	512
8	34	5	39	7[b]	21	–	yes[d]	256
9	32[a]	6	38	7	14	38	no	–
10	9[a]	7	16	7	9	16	no	–

[a]Duration of treatment limited by death.
[b]Lesion healed by about day 40.
[c]Paralysis transient and inapparent by day 27.
[d]Monoplegic survivor.

ease. This rabbit developed a late lesion at the inoculation site and also produced a high antibody titer.

In a further experiment with ganciclovir, treatment was delayed from 1 to 7 days after virus inoculation. Treatment was intended to last for 14 days using a high dose of 170 mg/kg/day. However, animals with longer delays before starting treatment had signs of disease at 14 days so their treatment was continued up to 33 days. The results, shown in Table 5, demonstrated that rabbits survived even when treatment was delayed for 5 days post inoculation. This point was halfway between initial inoculation and death in untreated controls.

We have recently discovered that a South American primate *Callithrix jacchus* (common marmoset) is highly susceptible to B virus with the disease progressing in a similar manner to that in the rabbit. After inoculation of as little as 10 $CCID_{50}$ of B virus in the skin of the thigh, these primates developed monoplegia at 7–8 days and died at 9–12 days. A limited test of 2 marmosets given a high oral dose of desciclovir (734 mg/kg/day) for 14 days following inoculation of 100 $CCID_{50}$ of B virus indicated that desciclovir provided an effective treatment in primates. The two treated animals showed no signs of the disease and remained healthy 40 days after virus inoculation.

Discussion

When tested in vitro all the antivirals were less effective against B virus than against herpes simplex virus. The difference was so great with bromovinyldeoxyuridine that this substance could provide a test to differentiate the two viruses. Acyclovir and ganciclovir were the most effective substances and required about

10 times higher concentrations to inhibit B virus than herpes simplex virus type 1.

Since animals infected with B virus are potentially dangerous to handle, we administered the drugs in the drinking water. In the case of acyclovir it was necessary to use a pH above 10 to get sufficient drug into solution. The rabbits drank normal volumes in spite of the high alkalinity. Preliminary results indicated that ganciclovir could be a very effective treatment for B virus infection. However, this drug has undesirable side effects and is reported to produce testicular damage and neutropenia (Wood and Geddes, 1987), whereas acyclovir is a clinically accepted drug with 'remarkably low apparent toxicity' (Jeffries, 1985).

For these reasons, we increased the oral dose of acyclovir until an effective level was achieved. At 500 mg/kg/day, acyclovir was demonstrably beneficial, but 700 mg/kg/day was required to prevent all signs of disease in the B virus-infected rabbit. This is about 10 times higher than the largest recommended human oral dose used for treatment of acute herpes zoster (McKendrick et al., 1986). Acyclovir is poorly adsorbed after oral administration and the high plasma levels needed to combat B virus infection would be achieved more satisfactorily with the well adsorbed prodrug desciclovir. It was recently demonstrated to be well tolerated in humans (Petty et al., 1987) and would appear to require only one-tenth of the oral acyclovir dose to achieve similar levels of acyclovir in the plasma. Although desciclovir is not readily converted to acyclovir in the rabbit, an experiment with a B virus-susceptible South American primate demonstrated successful treatment of the lethal infection with desciclovir. We believe that this drug would provide the best prophylaxis when accidents occur with attendant risks of possible human B virus infection. However, desciclovir is not available for use in man. Treatments proposed previously (Boulter et al., 1980, 1981) need to be started early for maximum effectiveness. This would be much easier in practice with the simpler oral route of chemotherapy. Supplies of oral acyclovir should be kept available at all locations where monkeys of Asian origin are handled so that prophylactic treatment can be started as soon as possible after accidental exposure to B virus. Intravenous acyclovir may be used if signs or symptoms of disease develop. Ganciclovir was more effective than acyclovir in the B virus infected rabbit and required only 50 mg/kg/day. However, treatment was not effective if delayed for more than five days after virus inoculation. Since progression of the disease appears slower in humans than in rabbits it could be assumed that the drug would be effective after a longer delay in human infection. Ganciclovir probably offers the best choice of effective treatment in humans when B virus infection has already progressed to the nervous system. Absorption in man after oral administration is poor, so the intravenous route is preferred. The drug has been used to treat cytomegalovirus infections in AIDS patients (Chachoua et al., 1987). It caused leucopenia and neutropenia, which was moderate in some patients and severe in a few cases but reversible. Gonadal function appeared normal but a cutaneous rash appeared in a small minority. In our animal experiments no obvious side effects were apparent in rabbits which were treated for 35 days or which had survived up to 150 days.

In response to requests from physicians concerned with the urgent treatment of recent human cases at Pensacola, Florida, we supplied them with the results of our

animal experiments at that time. Two cases, which were far advanced and needing artificial life support, were treated with ganciclovir but, not surprisingly, they failed to respond to treatment. Two others at earlier stages of infection were successfully treated with intravenous acyclovir (Griffin et al., 1987).

Acknowledgements

We thank Gisela Green and Janet Naish for their excellent technical assistance and Dr David Kelly for his encouragement and interest.

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