[10] Bradstreet CMP, Dighero MW, Mitchell DN. The Kveim test: analysis of results of tests using colindale (K_{12}) materials. *Ann NY Acad Sci* 1976;**278**:681-6.
[11] Siltzbach LE. Qualities and behaviour of satisfactory Kveim suspensions. *Ann NY Acad Sci* 1976;**278**:665-9.
[12] Zavala DC. Pulmonary haemorrhage in fibreoptic transbronchial biopsy. *Chest* 1976;**70**:584-8.
[13] Knight RK, Clarke SW. An analysis of the first 300 fibreoptic bronchoscopies at the Brompton Hospital. *Br J Dis Chest* 1979;**73**: 113-20.
[14] Koerner SK, Sakowitz AJ, Appelman RI, Becker NH, Schoenbaum SW. Transbronchial lung biopsy for the diagnosis of sarcoidosis. *N Eng J Med* 1975;**293**:268-70.
[15] Tierstein AS, Chuang M, Miller A, Siltzbach LE. Flexible bronchoscope biopsy of lung and bronchial wall in intrathoracic sarcoidosis. *Ann NY Acad Sci* 1976;**278**:522-7.
[16] Turiaf J. Bronchial sarcoidosis. Special reference to bronchial biopsy. *Acta Med Scand* [*Suppl*] 1964;**425**:228-9.
[17] Ross JK, Mikhail JR, Drury RAB, Levis RD, Mitchell DN. Mediastinoscopy. *Thorax* 1970;**25**:312-6.
[18] Widstrom A, Schnurer LB. The value of mediastinoscopy—experience of 374 cases. *J Otolaryngol* 1978;**7**(2):103-9.
[19] Mitchell DN, Scadding JG. Sarcoidosis. State of the art. *Am Rev Resp Dis* 1974;**110**:774-802.
[20] Israel HL. Granulomatous hepatitis: relationship to sarcoidosis. In: Levinsky L, Macholda F, eds. *Fifth international conference on sarcoidosis, Prague, June 16-21, 1969*. Prague: Universita Karolva, 1971:563-6.
[21] Karma A, Sutinen S. Conjunctival biopsy in sarcoidosis. *Acta Ophthalmol* [*Suppl*] (*Kbh*) 1975;**125**:52-3.
[22] Nosal A, Schleissner LA, Mishkin FS, Lieberman J. Angiotensin-I-converting enzyme and Gallium scan in non-invasive evaluation of sarcoidosis. *Ann Int Med* 1979;**90**:328-31.
[23] Turton CWG, Grundy E, Firth G, Mitchell DN, Rigden BG, Turner-Warwick M. Value of measuring serum angiotensin I converting enzyme and serum lysozyme in the management of sarcoidosis. *Thorax* 1979;**34**:57-62.
[24] Stanislas-Leguern G, Marsac J, Arnoux A, Lecossier D. Serum angiotensin-converting enzyme and broncho-alveolar lavage in sarcoidosis. *Lancet* 1979;i:723.

(*Accepted 2 January 1980*)

Successful treatment of experimental B virus (Herpesvirus simiae) infection with acyclovir

E A BOULTER, B THORNTON, D J BAUER, A BYE

Summary and conclusions

The efficacy of the new nucleoside analogue acyclovir against B virus (Herpesvirus simiae) was investigated in rabbits and Vero cells infected with 2-136 and 0·3-1·0 TCD_{50} of the virus respectively.

In the Vero cells 1 mg of acyclovir/l reduced the yield of virus by 90%, which was slightly less than the effect on herpes simplex virus. Results in the rabbits varied with the interval between doses, duration of treatment, and delay before starting treatment. Acyclovir controlled an otherwise lethal infection when given not less than eight-hourly for 14 days. Withdrawing treatment after 9-10 days resulted in late-onset fatal disease in some rabbits. Treatment begun within 24 hours after infection gave complete protection, and rabbits first treated up to five days after infection showed a significant reduction in mortality ($p < 0·001$).

The plasma half life of acyclovir is twice as long in man as in rabbits and progression of the disease is much slower. Hence acyclovir may be useful for post-exposure prophylaxis against B virus infection in man and possibly also for treatment of the disease.

Introduction

Infection with B virus (*Herpesvirus simiae*) resulting from contact with macaque monkeys or their tissues rarely occurs but is almost invariably fatal; out of 24 reported cases, only one patient made a reasonable recovery,[1] though three others survived with severe neurological sequelae. The Medical Research Council has therefore established a special unit at the Microbiological Research Establishment to investigate the disease, including possible approaches to prophylaxis and treatment. Several antiviral agents have been tested both in vitro and in vivo. For the in-vivo studies rabbits have been used; rabbits respond to subcutaneous injection of small amounts of virus with a fatal ascending encephalomyelitis that is closely similar to the disease in man after a bite from an infected monkey. Adenine arabinoside (vidarabine) was the most active antiviral compound in early in-vitro tests, but pronounced depression of viral replication occurred only with high concentrations of the drug. Thus, as expected, vidarabine had little effect on mortality in rabbits infected with B virus, though survival was prolonged if treatment was begun immediately after infection.

The new nucleoside analogue acyclovir (acycloguanosine; 9-(2-hydroxyethoxymethyl) guanine; Burroughs Wellcome Co) is highly active against several herpesviruses both in vitro and in various animals.[2-6] It was also effective in human corneal ulcers caused by herpes simplex virus[7] and in pneumonia that developed in an immunocompromised boy a few days after the onset of severe and extensive herpes labialis.[8] We describe experiments suggesting that acyclovir will be useful in post-exposure prophylaxis against B virus infection in man and possibly for treating clinically evident disease.

Materials and methods

All manipulation of materials infected with B virus was carried out in completely enclosed class III safety cabinets. Culture flasks and other apparatus were disinfected by swabbing with 2% sodium hypochlorite solution (Chloros) before removal from the cabinet for incubation or other procedures. Culture medium was buffered with HEPES (N-2-hydroxyethylpiperazine-N¹-2-ethanesulphonate) to avoid the need to flush cultures with carbon dioxide.

Yield-reduction test—Confluent monolayers of Vero cells in 25 cm^2 plastic flasks were infected with 0·3-1·0 TCD_{50} of B virus/cell. The infected monolayers were washed and covered with 3 ml of medium containing appropriate concentrations of antiviral compound. After

Microbiological Research Establishment, Porton Down, Salisbury, Wiltshire

E A BOULTER, MB, BS, medical officer, research (now at the Chemical Defence Establishment, Porton Down)
B THORTON, PHD, senior microbiologist (now at the PHLS Centre for Applied Microbiology and Research, Porton Down)

Wellcome Research Laboratories, Beckenham, Kent

D J BAUER, FRCP, FRCPATH, head of antiviral chemotherapy department
A BYE, PHD, senior scientist

incubation for 18 hours the contents of each flask were harvested and titrated in 96-well, flat-bottomed microplates, using 10 wells for each ten-fold dilution of virus. The wells were examined three days later for viral cytopathic effect; the titre was taken as that producing cytopathic effect in 50% of the wells and was calculated as described.[9]

Animal studies—Half-lop rabbits were inoculated subcutaneously in the right thigh with 0·1 ml of suitably diluted virus; the inoculum was titrated as above. The freeze-dried sodium salt of acyclovir was reconstituted in sterile distilled water and inoculated into a suitable ear vein. Repeated injections tended to induce thrombosis of these veins, which was overcome to a large extent by inserting a 22-gauge Abbocath-T catheter fitted with a male adaptor plug (Abbott Laboratories) under anaesthesia induced with alphaxalone-alphadolone acetate (Althesin). After each dose of drug the catheter was flushed with phosphate-buffered saline followed by saline containing 20×10^3 units of heparin/l. Catheters remained open for several days; when thrombosis occurred they were removed and fresh ones inserted into another vein. Occasionally, when no suitable vein was available, the drug was administered intraperitoneally. Although the drug was well tolerated by this route despite its high pH, repeated intraperitoneal injections led to several deaths from trauma to the intestines; these animals were excluded from the experiments and account for the small numbers in some groups in table I.

Acyclovir assay—Plasma acyclovir concentrations were determined by radioimmunoassay.[10]

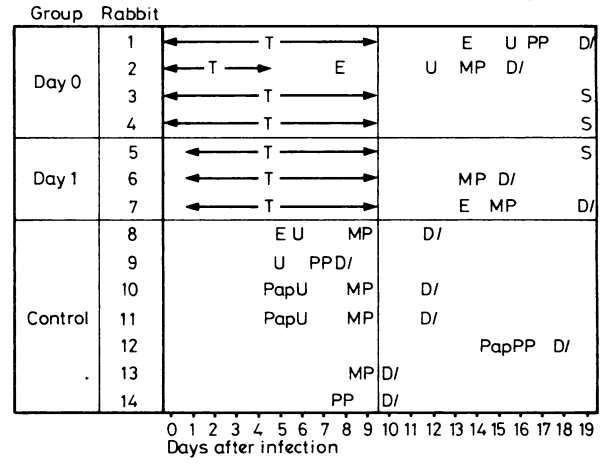

FIG 2—Experiment 3. Delayed disease seen when acyclovir given to rabbits with experimental B virus infection was stopped on day 9. ← T →=Duration of treatment with acyclovir. E=Erythema at inoculation site. Pap=Papule. U=Ulcer. MP=Monoplegia. PP=Paraplegia. D=Died. S=Survived.

Results and discussion

Figure 1 shows the mean depression of titres when virus was grown in different concentrations of acyclovir. Results of similar experiments with vidarabine are shown for comparison. Acyclovir was clearly more active than vidarabine; as little as 1 mg/l reduced the 18-hour yield of B virus by 90%. B virus was not quite as sensitive as herpes simplex virus to acyclovir.

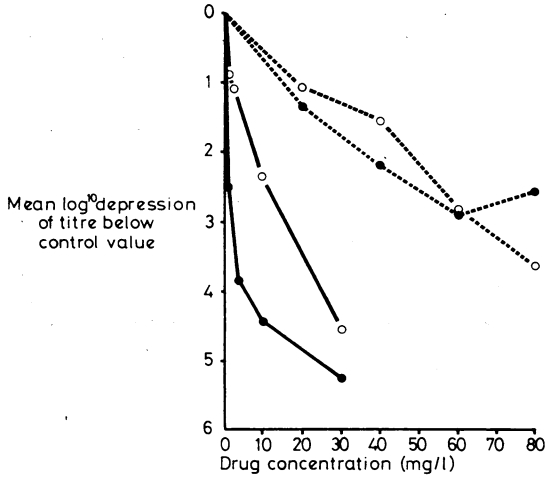

FIG 1—Comparative activities of acyclovir (———) and vidarabine (— — — —) against herpes simplex (●) and B (○) viruses.

Before the highly soluble sodium salt of acyclovir became available a few experiments were conducted on rabbits with the poorly soluble parent compound. A suspension containing 5 g/l was inoculated intraperitoneally over five days, beginning immediately after infection. A daily dose of 40 mg/kg given in two divided doses during the working day reduced the mortality slightly but significantly ($p < 0.05$) from 79% in 14 untreated controls to 40% in 20 treated rabbits. The mean survival time of rabbits that died increased from a control value of 10·6 days to 16·9 days.

Table I gives the results of seven experiments with different treatment schedules of the sodium salt of acyclovir. In all experiments except the first, in which a low dose of virus was used, all untreated control rabbits died. Three important factors emerged in the treatment schedules—namely, the interval between doses, the duration of treatment, and the delay before starting treatment. In the first two experiments the regimen adopted with the parent compound was used, and the results were similar to those of the earlier experiments despite increasing the dosage to 100 mg/kg/day. This schedule included alternate intervals of seven and 17 hours between doses, with the probability that the circulating drug would fall below a therapeutic concentration for a large part of the longer interval. In experiment 3 the treatment interval was reduced to six hours and the duration extended to 10 days; the dosage in this and subsequent experiments was also increased to 200 mg/kg/day. Although not evident from table I, the effects of these changes were remarkable. Figure 2 gives the details of this experiment. When treatment was stopped on day 9, six of the seven treated rabbits were apparently normal, whereas all but one of the control rabbits were either dead or severely paralysed. Rabbit 2 had slight erythema at the inoculation site, but treatment of this animal was stopped on day 4 when it removed the cannula from its last suitable ear vein. The other six treated rabbits remained well until day 14, when three of them developed the first signs of an ultimately fatal disease. Interestingly the five-day gap between the end

TABLE I—*Numbers of deaths in rabbits beginning different treatment regimens with sodium salt of acyclovir up to five days after infection with B virus. (Figures in parentheses are mean survival times in days of rabbits that died)*

Experiment No	Virus dose (TCD_{50})	Treatment regimen: Daily dose (mg/kg)	Dose interval (hours)	Duration (days)	Time of beginning treatment (days after infection): 0	1	2	3	4	5	Untreated controls
1	2	20	*	5	3/5 (16·7)‡	0/5					5/9 (11·5)
2	40	100	*	5	6/9 (17·5)			8/10 (14·8)			9/9 (12·8)
3	96	200	6	10	2/4 (17·5)‡	2/3 (17·0)					7/7 (11·7)
4	115	200	8	14	0/4	0/3		0/3			5/5 (10·2)
5	65	200	8	14				2/5 (17·0)		2/5 (13·0)	6/6 (10·5)
6	136	200	8	14				3/4 (14·7)	4/4 (11·3)	2/4 (13·0)	6/6 (10·0)
7	120	200	8, 12†	14	1/3 (19·0)	0/2	2/2 (23·0)				5/5 (11·4)
		Summation of experiments 4-7			1/7§ (19·0)‖	0/5§	2/2 (23·0)§	5/12§ (15·6)‖	4/4 (11·3)	4/9§ (13·0)	22/22 (11·0)

*Twice daily, at 0900 and 1600.
†Treated every eight hours for 10 days, then every 12 hours (see text).
‡Significantly different from controls ($p < 0.05$).
§Very highly significantly different from controls ($p < 0.001$).
‖Highly significantly different from controls ($p < 0.01$).

TABLE II—*Rabbit plasma acyclovir concentrations at various times after intravenous doses of 200 mg/kg daily given either eight-hourly or 12-hourly*

Dosage given eight-hourly						Dosage given 12-hourly					
Rabbit A (weight 3·8 kg; dose 253 mg)		Rabbit B (weight 3·7 kg; dose 247 mg)		Rabbit C (weight 2·8 kg; dose 187 mg)		Rabbit D (weight 2·6 kg; dose 260 mg)		Rabbit E (weight 2·8 kg; dose 280 mg)		Rabbit F (weight 2·8 kg; dose 280 mg)	
Time after dose (min)	Drug concentration (mg/l)	Time after dose (min)	Drug concentration (mg/l)	Time after dose (min)	Drug concentration (mg/l)	Time after dose (min)	Drug concentration (mg/l)	Time after dose (min)	Drug concentration (mg/l)	Time after dose (min)	Drug concentration (mg/l)
1	499	1	564	1	45	1	555	1	653	1	73
28	89	23	96	30	568	19	175	15	156	30	95
67	64	62	81	60	23	58	131	55	97	60	35
99	41	92	59	90	22	90	82	87	56	90	35
125	24	119	44	120	18	116	68	114	40	120	25
182	15	Died		180	23*	Died		167	22	180	16
302	6·3			300	4·5			286	9·5	300	12
417	3·6			420	2·5			401	4·3	420	5·9

*Sample haemolysed.

of treatment and the first signs of illness was identical with the incubation period in four of the seven control rabbits. Acyclovir had apparently suppressed viral replication during the 10-day course but not eliminated the virus.

In subsequent experiments extending treatment to 14 days prevented the late-onset disease seen in experiment 3. There was an apparent exception in experiment 7, in which one of the three rabbits treated from the time of infection and two rabbits treated from 48 hours after infection had late-onset disease. In this experiment, however, there was an enforced change in the treatment regimen: for the first 10 days treatment was given eight-hourly but, because of illness in one of the investigators, subsequent doses were given every 12 hours. As discussed below, probably circulating acyclovir was maintained above effective antiviral concentrations only during the first 10 days, so that conditions resembled those in experiment 3. Since with the exception of experiment 7 the treatment regimen was standardised in the last four experiments their results are collated in table I. Acyclovir was highly effective against B virus infection when treatment began within 24 hours after infection. Except for four rabbits in experiment 6 whose treatment was delayed until the fourth day of infection there was a pronounced reduction in mortality, even with delays of up to five days before starting treatment. The mean survival time of rabbits that died was also significantly prolonged when treatment was begun within three days after infection, but not beyond this point.

The radioimmunoassay for acyclovir[10] became available after the above experiments were completed. Table II shows the plasma concentrations at intervals after single doses of acyclovir corresponding to the eight-hourly and 12-hourly schedules of 200 mg/kg/day. The drug was rapidly distributed into an apparent mean volume of 1·7 l/kg, which exceeded the total body water volume. Pharmacokinetic data suggested a terminal half life of about two hours. We do not know the concentration of acyclovir needed to control viral replication in vivo, but 1-10 mg/l greatly depressed viral replication in vitro (fig 1). Plasma concentrations of 10 mg/l would have been present for about four hours in rabbits treated eight-hourly and for about five hours in those treated 12-hourly. Despite the initially higher plasma concentrations observed with the 12-hourly schedule they would be below 10 mg/l for periods nearly double that occurring with an eight-hourly schedule. The late-onset disease occurring in experiment 7 suggests that circulating acyclovir falls below an effective antiviral concentration in a 12-hourly schedule and that sustaining an effective plasma concentration of the drug is important in controlling viral replication.

The rapid progression through paresis and paralysis to death, once signs of illness develop, makes this rabbit model of B virus infection unsuitable for therapeutic studies in which treatment is begun after the onset of clinical signs. Nevertheless, evidence that acyclovir can arrest the progression of virus within the central nervous system was obtained in experiment 5. Two of the three surviving rabbits in the group treated five days after infection developed an ascending myelitis that was arrested at the paraplegic stage. Despite their paraplegia, these animals regained vigour, could move around their cages, and ate and drank normally. Such arrest of viral infection of the central nervous system has not been observed by us with any other agent.

The minimum daily dose required for successful treatment was not established in our experiments, as the dose was progressively increased along with other alterations to the treatment regimen. The 200 mg/kg/day used in later experiments was large compared with the 50 mg/kg/daily[4] and 100 mg/kg daily[3] used for herpes simplex virus in mice and with the 15 mg/kg daily used to treat pneumonia due to herpes simplex virus in an immunocompromised boy.[8] Although B virus is more resistant than herpes simplex virus to acyclovir, probably daily doses below 200 mg/kg would be effective. Acyclovir is remarkably free from toxicity, and daily doses of 450 mg/kg given to mice for 30 days had no apparent toxic effects.[3]

Conclusion

Our results suggest that successful treatment with acyclovir depends on three aspects of the treatment schedule. Firstly, an antiviral concentration must be maintained in the plasma by giving the drug at suitably short intervals, or even by constant intravenous infusion after a loading dose. Secondly, treatment must continue for at least 14 days. Finally, the earlier treatment is begun the better the results are likely to be, though in rabbits some benefit was evident even with delays of up to five days. Results of late treatment would probably be even better in man, since, compared with rabbits, the plasma half life is almost twice as long,[11] progression of the disease is much slower, and chemotherapy would be supplemented with intensive care and, when indicated, cerebral decompression.

This study was supported by a grant from the Medical Research Council.

Requests for reprints should be sent to Dr A Bye.

References

1 Bryan BL, Espana CD, Emmuns RW, Vijayan N, Hoeprich PD. Recovery from encephalomyelitis caused by *Herpesvirus simiae*. *Arch Intern Med* 1975;**135**:368-70.

2 Elion GB, Furman PA, Fyfe JA, De Miranda P, Beauchamp L, Schaeffer HJ. Selective action of an antiherpetic agent, 9-(2-hydroxyethoxymethyl) guanine. *Proc Natl Acad Sci USA* 1975;**74**:5716-20.

3 Schaeffer HJ, Beauchamp L, De Miranda P, Elion GB, Bauer DJ, Collins P. 9-(2-hydroxyethoxymethyl) guanine activity against viruses of the herpes group. *Nature* 1978;**272**:583-5.

4 Field HJ, Bell SE, Elion GB, Nash AA, Wildy P. Effect of acycloguanosine treatment on acute and latent herpes simplex infections in mice. *Antimicrob Agents Chemother* 1979;**15**:544-61.

5 Crumpacker CS, Schnipper LE, Zaia JA, Levin MJ. Growth inhibition by acycloguanosine of herpesviruses isolated from human infections. *Antimicrob Agents Chemother* 1979;**15**:642-5.

6 Klein RJ, Friedman-Kienst AE, De Stefano E. Latent herpes simplex virus infections in sensory ganglia of hairless mice prevented by acycloguanosine. *Antimicrob Agents Chemother* 1979;**15**:723-9.

7 Jones BR, Coster DJ, Fison PN, Thompson GM, Cobo LM, Falcon ME. Efficiency of acycloguanosine (Wellcome 248 U) against herpes-simplex corneal ulcers. *Lancet* 1979;i:243-4.

8 Goldman JM, Chipping PM, Agnarsdottir G, Brigden D. Acycloguanosine for viral pneumonia. *Lancet* 1979;i:820.

9 Reed LJ, Muench H. A simple method of estimating fifty per cent endpoints. *American Journal of Hygiene* 1938;**27**:493-7.

10 Quinn RP, De Miranda P, Gerald L, Good SS. A sensitive radioimmunoassay for the antiviral agent BW 248U [9-(2-hydroxyethoxymethyl) guanine]. *Anal Biochem* (in press).

11 De Miranda P, Whitley RJ, Blum MR, *et al.* Pharmacokinetics of the antiviral drug acyclovir (Zovirax™) in humans following intravenous infusion. *Clin Pharmacol Ther* (in press).

(*Accepted 10 January 1980*)