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Critical determinants of antiherpes efficacy of buciclovir and related acyclic guanosine analogs*

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Summary

Buciclovir is an example of an antiherpes, acyclic guanosine analog activated by the viral thymidine kinase and inhibiting viral DNA synthesis in infected cells. An investigation of closely related buciclovir-analogs with similar antiherpes activities in cell cultures and similar, or identical, modes of action but with disparate effects in vivo, revealed the following critical determinants of antiherpes efficacy. (1) The accumulation of guanosine analog-triphosphates in infected cells, which is cell-type-specific and analog-dependent. (2) The potencies of the triphosphates as inhibitors of the viral DNA polymerase. (3) The plasma kinetics of the analogs, which are widely different despite the similar structures. (4) The penetration into nervous tissue relative to penetration into non-nervous tissues, of importance in connection with the neurotropic behavior of the virus. (5) The concentration of the antagonist thymidine in certain tissues. (6) The difference in pathogenesis between primary infections and recurrent infections, exemplified in the different efficacies of topically applied drugs in cutaneous and genital HSV-2 infections in guinea pigs.

Herpes simplex virus; Acyclic guanosine analog; Buciclovir; Phosphorylation rate; DNA synthesis; Pharmacokinetics; Pathogenesis

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Introduction

One established practice of improving the design of potential antiherpes drugs is the 'targeting' of these drugs to virus-induced enzymes [22]. Soon, however, the need emerges to predict antiviral efficacy in particular animal models of viral infection. The prediction will be based on a limited number of parameters obtained from biochemical, pharmacological and virological studies. The kind of study that should be performed to obtain the critical parameters depends on the nature of the compound, the target enzyme, and the disease manifestation to be treated. One of the goals of antiherpes chemotherapy is to provide an effective treatment of recurrent genital herpes. A notable target enzyme is the herpes simplex virus (HSV) DNA polymerase [8,10,37]. Enzyme inhibitors used to date have been pyrophosphate analogs or analogs of a nucleoside triphosphate generated intracellularly from a nucleoside analog.

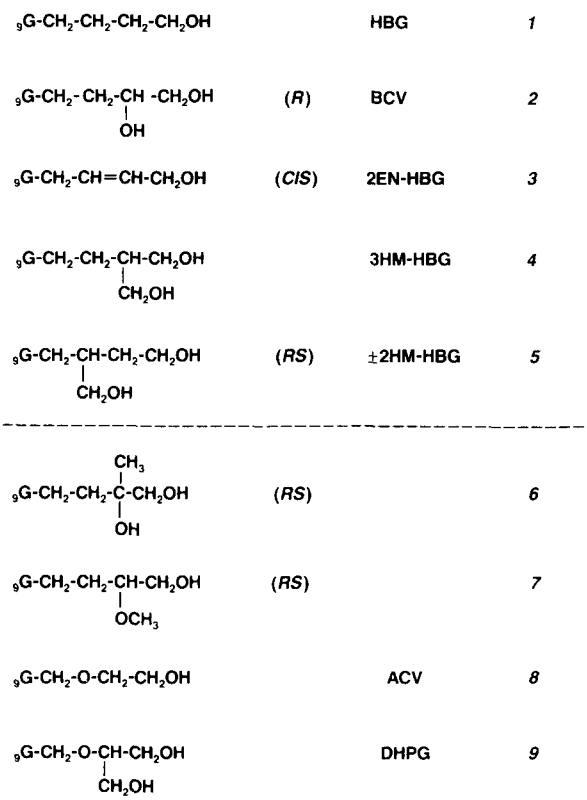

Fig. 1. Chemical structures of the acyclic guanosine analogs discussed. The guanine ring is substituted in the 9 position with different side chains. HBG = 9-(4-hydroxybutyl)guanine; BCV = buciclovir [*R*-9(3,4-dihydroxybutyl)guanine]; 2EN-HBG = *cis*-9-(hydroxy-2-butenyl)guanine; 3HM-HBG = 9-[4-hydroxy-3-(hydroxymethyl)butyl]guanine; ±2HM-HBG = *RS*-9-[4-hydroxy-2-(hydroxymethyl)butyl] guanine; ACV = acyclovir; DHPG = 9-[(1,3-dihydroxy-2-propoxy)methyl]guanine.

Therefore, the question arises, which properties of a specific inhibitor of herpes virus DNA synthesis are important in determining its in vivo potential for the treatment of recurrent genital herpes? To obtain some insight into mechanisms underlying efficacy of these inhibitors, we investigated a series of related antiherpes guanosine analogs with closely similar antiherpes effects in cell culture, but with widely disparate effects in vivo. The guanosine analogs studied have the deoxyribose moiety of guanosine replaced by different hydroxyalkyl side-chains. Some of these analogs (see Fig. 1 for structures) show antiherpes activity in cell culture [22], coincident with inhibition of viral DNA synthesis [28,30,31].

Efficacies in vitro

Approximately 50 guanosine analogs with hydroxyalkyl side-chains instead of the deoxyribose moiety were tested for antiviral activity, using Vero cells infected with a clinical isolate of HSV-1, strain C42. In addition, the same compounds were screened for cytotoxic effects, that is effects on growth of human embryo cells. Compounds inhibiting virus plaque formation at concentrations of 10 μM or less ($IC_{50} \leq 10$ μM), but not cell growth at concentrations of 100 μM or more ($IC_{50} \geq 100$ μM), were competitive inhibitors of the viral thymidine kinase with respect to thymidine: 0.6 μM $\leq K_i < 7.0$ μM [28,30,31 and unpublished data]. Furthermore, these drugs (1–5 in Fig. 1) inhibited viral DNA synthesis in the infected cells at those concentrations giving at least 50% inhibition in the plaque reduction tests [28,30,31]. These results suggested: (a) that inhibition of herpes virus multiplication was due to inhibition of viral DNA synthesis and (b), that the specificity of this effect involved phosphorylation by the HSV-induced thymidine kinase. Indeed, not only did the active compounds bind to the isolated viral thymidine kinase, they were also phosphorylated, although the phosphorylation rates differed from 9–73% of the rates measured for thymidine [28,30,31]. However, not all the guanosine analogs that proved to be good substrates for the viral thymidine kinase (K_i values and phosphorylation rates as above) showed antiherpes activity in cell culture ($IC_{50} \leq 10$ μM); for example, compound *6* (unpublished results). This observation shows that to exert antiviral effects, specific phosphorylation of acyclic guanosine analogs by viral thymidine kinase is not sufficient (see also below). We also observed that compounds which inhibit the viral thymidine kinase (K_i values as above) but were poorly phosphorylated ($\leq 5\%$ of the rate of thymidine) did not give prominent antiherpes effects in cell cultures ($IC_{50} \geq 50$ μM); for example, compound *7* (unpublished results). This result was not surprising since TK-defective variants of HSV multiply well in cell culture [9].

To establish which factors are important in the determination of the antiviral effects in vitro, the biochemical properties of some guanosine analogs were studied in detail. The relative substrate efficiencies of the *R*- and *S*-enantiomers of 9-(3,4-dihydroxybutyl)-guanine for the viral (HSV-1) thymidine kinase are almost identical [11; see also Table 1]. However, the 4′-monophosphate of the *S*-form is a 30-fold poorer substrate than the 4′-monophosphate of the *R*-form for guanylate ki-

nase [11,44] (Table 1), an enzyme that may be involved in diphosphate formation [36]. In addition, the triphosphate of the *S*-enantiomer is 11 times less active an inhibitor of the HSV-1 DNA polymerase than the triphosphate of the *R*-form (Table 1). It is therefore not surprising that the *S*-enantiomer is less active than the *R*-enantiomer in inhibiting viral DNA synthesis or plaque formation.

HBG (*1*) and BCV (*2*) (buciclovir, the *R*-enantiomer of 9-(3,4-dihydroxybutyl)guanine) have similar affinities for HSV-1 thymidine kinase, but are phosphorylated at different rates, BCV being the better substrate (Table 1). Also, the monophosphates of HBG and BCV formed by the action of the viral thymidine kinase, have similar affinities for guanylate kinase, but differ in their V_{max}, BCV-MP being again the better substrate ([44]; Table 1). These results may explain, in part, the finding (our unpublished data) that the pool size of HBG-TP (HBG-4'-triphosphate) in cells* expressing viral thymidine kinase is only 1.6% of the pool-size of BCV-TP (286 pmol/10^6 cells). Nevertheless, BCV is not more active than HBG in inhibiting viral DNA synthesis or viral plaque formation [6,11,28] (Table 1). Presumably, the low pool of HBG-TP is compensated by HBG-TP being a better inhibitor of the viral DNA polymerase than BCV-TP [32] (Table 1). A comparison of ACV (*8*) and HBG gives a similar picture. Thus, the ACV-TP pool* is not different from the HBG-TP pool, but ACV is approximately 10 times more active than HBG in inhibiting viral DNA synthesis or viral plaque formation [11,31]. Indeed, ACV-TP is an excellent inhibitor of the viral polymerase (K_i = 0.0014 μM for ACV-TP and 0.12 μM for HBG-TP; Table 1).

It is not clear why ACV-TP is a better polymerase inhibitor than HBG-TP, which

TABLE 1

Relevant biochemical parameters for antiherpes effect of guanosine analogs in vitro.

Compound	TK[a]		GMP-kinase[b]		DNA-polymerase[c]	HSV-1, C42[d]	
	K_i (μM)	Rate (%)	K_m (μM)	V_{max} (μmol/min/mg)	K_i (μM)	IC_{50} DNA synthesis (μM)	IC_{50} plaque formation (μM)
HBG (*1*)	2.1	10	50	0.048	0.12	2.3	3.0
R-DHBG (BCV)(*2*)	1.5	73	62	1.5	0.76	3.6	2.3
S-DHBG	1.5	46	210	0.17	8.63	11.0	13.0
3HM-HBG (*4*)	1.5	9	260	1.0	–	0.2	0.3
ACV (*8*)	173.0	27	460	0.10	0.0014	0.3	0.2
dT (K_m)	0.4	100	–	–	–	–	–
dG (K_m)	–	–	120	3.2	0.14	–	–

[a] HSV-1, C42, thymidine kinase.

[b] For the monophosphates, using hog-brain enzyme.

[c] For the triphosphates, using HSV-1, C42, DNA polymerase.

[d] Inhibition of viral DNA synthesis measured by the spot-hybridization test [31]. Data from [30–32].

*LM-TK_1^--cells, which are 'transformed' with the HSV-1, thymidine-kinase gene [27], and treated with 10 μM of the drug for 4 h.

differs from ACV-TP only in that the ether oxygen of the acyclic side-chain has been replaced by a methylene group [2]. In view of (a) the similar mechanisms of inhibition and (b) lack of solid evidence for incorporation of ACV-MP or HBG-MP into DNA, or for formation of irreversible enzyme-(template-)inhibitor complexes [15,32,35], Larsson et al. [32] proposed that reversible formation of an enzyme-(template-)inhibitor complex seen with either inhibitor (and with BCV-TP) is accompanied by a conformation change in this complex, which prevents elongation of the growing DNA chain, and that ACV-TP causes this conformation change at a lower concentration than HBG-TP (as reflected by the differences in K_i).

It is well known that the antiviral effect of guanosine analogs in cell cultures is dependent on the host cell used for the test [7,13,19,21]. One hundred-fold differences in IC_{50} values have been observed. An analysis of this phenomenon using green-monkey kidney (GMK) cells, in which high IC_{50} values are obtained, and a cell-line in which low IC_{50} values are obtained (human lung fibroblast: HL cells), showed that cell-type dependence of thymidine metabolism accounts for the differences: GMK cells maintain larger pools of the nucleoside thymidine than HL cells [17,18]. As intracellular thymidine competes with the phosphorylation of acyclic guanosine analogs in HSV-infected cells, the concentration of BCV-TP in GMK cells (196 pmol/10^6 cells) is much lower than in HL cells (6380 pmol/10^6 cells) treated with 10 μM BCV [23]. Whether, in addition, differences occur in the pool sizes of (deoxy)nucleoside mono- and diphosphates is not known, but the pool size of dGTP, competing with the guanosine analog triphosphates at the DNA polymerase level, is similar in infected HL and GMK cells [23].

The replication of HSV in guinea pig embryo cells is inhibited by the guanosine analogs *1–5* at concentrations 150–500 times higher than those which inhibit virus replication in mouse embryo cells to the same extent [31]. Yet, the thymidine pools in guinea pig cells are comparable to those in mouse cells. The reasons for the low intracellular concentrations of guanosine analog triphosphates found in infected guinea pig cells (Harmenberg, J., personal communication) remain to be elucidated.

In summary, we have seen that the antiviral effects in vitro of the guanosine analogs discussed are determined by the extent to which the compounds inhibit viral DNA synthesis. For some compounds it could be shown that the extent of inhibition was determined by the intracellular concentrations of the triphosphates and the potency of the triphosphates as inhibitors of the viral DNA polymerase (see also [4]).

Efficacies in vivo: systemic treatments

Compounds *1–5* (Fig. 1) differ only slightly in the IC_{50} values for HSV-1 or HSV-2 replication in a number of cell-lines. However, in vivo using a number of different experimental infection systems only BCV (*2*) and 3HM-HBG (*4*) show good therapeutic effects [31]. Following intra-peritoneal infection of mice with HSV, the

TABLE 2

Correlation between pharmacokinetic parameters and efficacy of guanosine analogs in a systemic HSV-1 infection in mice.

Compound[a]	IC_{50} plaque reduction in mouse cells (μM)	AUC (μmol h l^{-1})	Activity rank
HBG (*1*)	0.1	10	0
2EN-HBG (*3*)	0.2	18	+
(±) 2HM-HBG (*5*)	0.1	24	++
3HM-HBG (*4*)	0.1	27	+++
BCV (*2*)	0.2	50	++++

[a] For structures, see Fig. 1. AUC = Area Under the plasma-Concentration-time curve following one injection i.v. with 63 μmol/kg of drug. 0 = no effect on mean day to death (MDD) or survival when given at a dose of 100 mg/kg/day, in twice daily injections. + = 100 mg/kg/day increases the MDD, but no effect on final mortality (twice daily injection). ++ = 20–50 mg/kg/day needed to increase MDD, but 50 mg/kg/day or more needed to reduce final mortality (twice daily injection). +++ = 10 mg/kg/day needed to increase MDD; 10–20 mg/kg/day needed to obtain 100% survival (twice daily injections). ++++ = 5 mg/kg/day needed to increase MDD, 10 mg/kg/day or less needed to obtain 100% survival (twice daily injections).

virus replicates initially in visceral organs and then spreads to the central nervous system and the animals die [25]. When treated p.o. or i.p. with HBG, BCV, 3HM-HBG, 2EN-HBG or ±2HM-HBG, HBG was inactive, whereas the order of the activity of the other compounds was BCV>3HM-HBG>±2HM-HBG>2EN-HBG [11,31]. We could show that BCV inhibited virus replication in the spleen of the infected animals, whereas HBG did not have this effect [11]. This was true even with more frequent administration of higher doses of HBG than used for BCV. Differences in the pharmacokinetics of these guanosine analogs can, at least in part, explain the differences in efficacy. Thus, HBG, 2EN-HBG, and ±2HM-HBG (all of which are inactive, or only show limited activity) persist in the body for only a short time due to short half-lives and rapid plasma clearances [11,31]. In fact, in this infection model the efficacy of therapy is directly related to the amount of drug obtained in blood over a period of time (Table 2).

Nevertheless, the plasma kinetics of the guanosine analogs cannot account for the activity in vivo of BCV or 3HM-HBG. For example, the twice daily i.p. administration of 5 mg/kg of BCV to mice systemically infected with an HSV-1 strain is sufficient to give a complete protection, i.e. no mortality. Yet, serum levels of BCV exceed the IC_{90} (0.7 μM for this HSV-1 strain replicating in mouse cells) for a period not exceeding 3 h after injection (calculated from data in [11]). The apparent persistence of the antiviral effect in vivo after decline of the serum levels of BCV may be due to an intracellular trapping of the antivirally active agent (BCV triphosphate). Indeed, the rapid phosphorylation of BCV to the triphosphate shown to occur in vitro results in high intracellular concentrations of BCV-TP, which persist when the drug is removed from the medium [44]. Presumably because of the trapping of BCV triphosphate, the antiviral effect in vitro persisted when BCV was removed [44]. A similar trapping mechanism occurs in infected cells treated with DHPG (*9* in Fig. 1; see [39]), and presumably 3HM-HBG [31], but

less effectively in infected cells treated with ACV, presumably because of the low initial phosphorylation rate of ACV [39]. It is likely that because of this trapping effect, BCV and ACV have comparable effects in the treatment of systemic HSV infections [11] despite the fact that BCV is less active than ACV in cell culture.

BCV administered p.o. or i.p., however, is not effective in preventing death after intravaginal inoculation of mice with HSV-2 (Fig. 2) despite the facts that (a) the method of BCV administration ensured that serum levels of BCV constantly exceeded the IC_{50} for this HSV-2 strain by at least tenfold and (b) one-fifteenth of the dose used to treat the vaginal infection, effectively prevented death following i.p. inoculation with the same virus strain [34]. In the intravaginally infected mice the virus spread rapidly to the CNS, and this spread was not affected by BCV (Fig. 3). As the systemically administered BCV does not affect initial intravaginal viral replication [34], the lack of effect of BCV may be due to poor penetration of nervous tissues by BCV.

To investigate whether BCV penetrates nervous tissues less effectively than non-nervous tissues, and that BCV is, therefore, less active in inhibiting virus replication in nervous tissues than in non-nervous tissues the following experiments were carried out. Mice were infected intranasally with HSV-2 (strain 91075) and given BCV, ACV or left untreated. Following this route of inoculation virus replicates in both lungs and CNS (see below), and a comparison of viral titers and drug levels in brain and lungs provides a comparison of drug effects in nervous versus non-nervous tissues in the same mouse.

The intranasal infection of mice with 10^5 or 10^4 pfu of HSV-2 (91075) per mouse caused severe disease resulting finally in 100% mortality by day 6, although the mean time to death was different with the two inocula (Table 3). High virus titers were obtained from lungs, trigeminal ganglia, and brain stem (Fig. 4A, B). The virus was first found in the lungs (days 1, 2 post infection) and later (from day 3

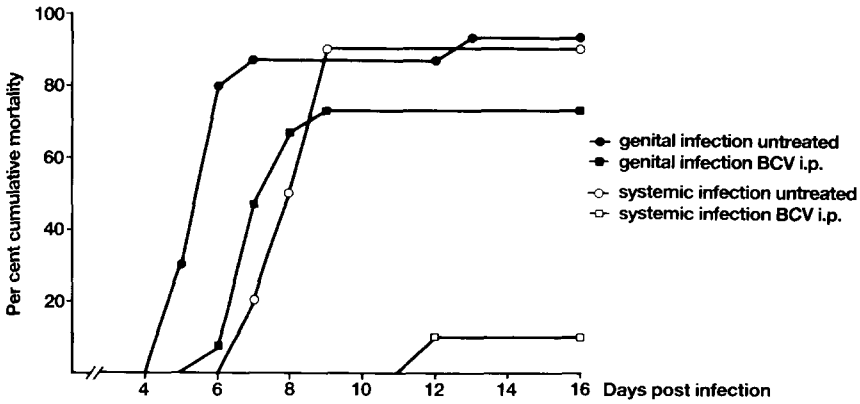

Fig. 2. Effects of i.p. administered BCV on mortality of mice infected intravaginally or intraperitoneally with HSV-2, strain 91075. Mice were infected with 7×10^4 pfu (genital infection) or 10^4 pfu (systemic infection) of HSV-2, strain 91075, as described [11,34]. Starting 1 h after infection, BCV was administered in a daily dose of 150 mg/kg, given four times daily.

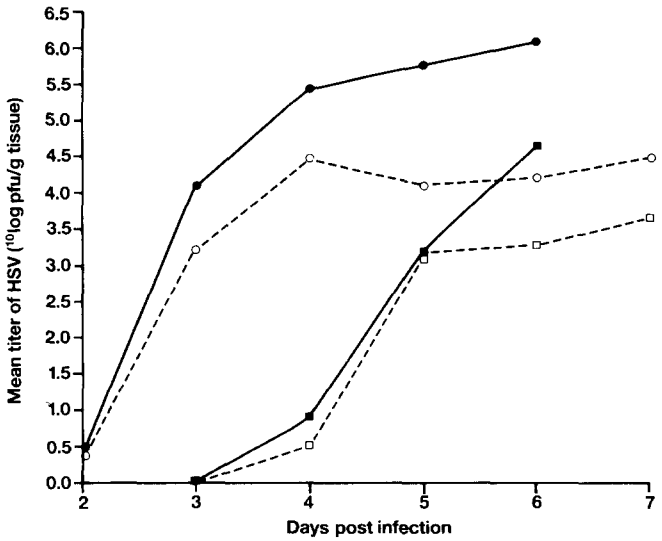

Fig. 3. Lack of effect of BCV on spread of virus to the spinal cord and to the brain following intravaginal infection of mice with HSV-2, strain 91075. Mice were infected intravaginally [34] with HSV-2, strain 91075, and treated (open symbols), or not treated (filled symbols) with BCV supplied in the drinking water (2 mg/ml), given from 1 h post infection onwards. Viral titers were determined as described [11,34] in spinal cord (-●-, --○--) and in brain (-■-, --□--). Mean titers were determined for 5 different tissues.

TABLE 3

Efficacy of BCV and ACV in intranasally infected mice, and drug levels in tissues of the mice.

Treatment[a]	Mortality[b]		Concentration of drug in[c]			
	A pct (MDD)	B pct (MDD)	serum (μmol/l)	lungs (μmol/kg)	brain (μmol/kg)	ear (μmol/kg)
None	100 (3.0±0)	100 (6.0±0.5)	–	–	–	–
0.5 mg/ml BCV	–	–	1.1±0.3	1.6±0.4	0.4±0.1	1.5±0.5
1.0 mg/ml BCV	100 (6.6±0.7)	100 (7.2±3.1)	2.7±0.6	2.2±0.9	0.7±0.2	2.2±0.2
2.0 mg/ml BCV	–	93 (8.3±1.1)	3.6±1.5	4.7±2.8	0.8±0.2	–
1.0 mg/ml ACV	100 (9.9±1.8)	50 (10.8±1.8)	5.2±1.6	5.5±2.5	2.5±0.7	–

[a] Treatment was achieved by dissolving the drugs in the drinking water, and started 1 h post infection.

[b] In exp. A mice were inoculated with 10^5 pfu/mouse, and 10 mice were used in each group; in exp. B infection was with 10^4 pfu/mouse, and 15 mice were used in each group; pct = percentage of the animals that died; MDD = mean day to death.

[c] Drug levels were determined in extracts from tissues of infected mice. Tissues were rinsed in phosphate-buffered saline and extracted with PCA. The extracts were neutralized with KOH before analysis by HPLC [11]. The results shown are average values for tissues of 5 (0.5 or 2.0 mg/ml) or 10 (1.0 mg/ml) mice. Tissue levels from infected mice were not different from uninfected mice. The tissues were taken 3 days after start of treatment.

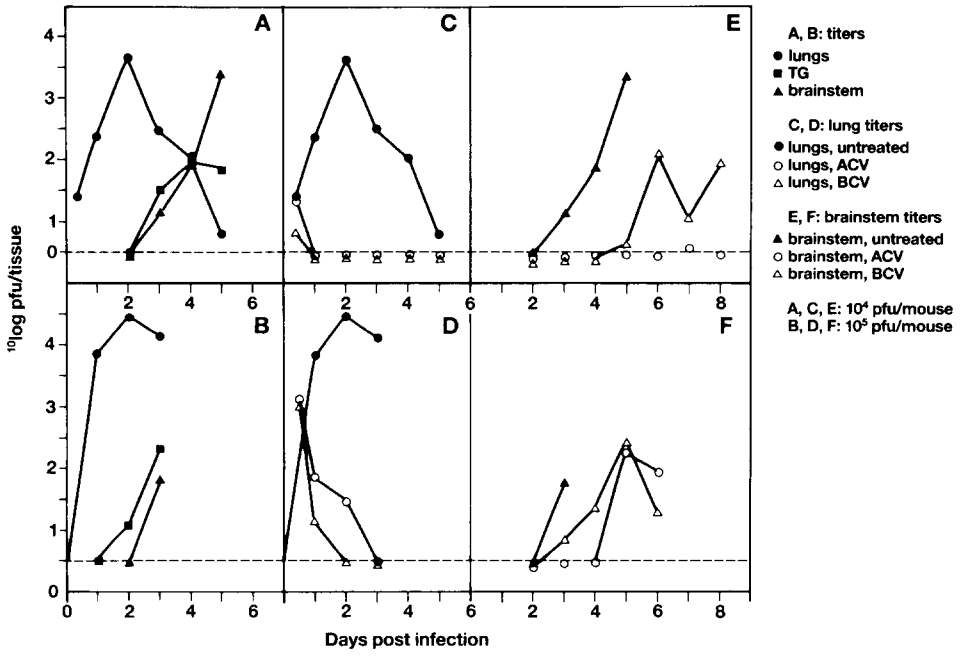

Fig. 4. Effects of BCV and ACV on the spread of virus to the lungs and the brain stem following intranasal infection of mice with HSV-2, strain 91075. Mice (male, weighing 15 g – 16 g) were infected with 10^4 pfu (A, C, E) or 10^5 pfu (B, D, F) of HSV-2, strain 91075, (see Table 3 for effects on mortality) and given either ACV (1 mg/ml) or BCV (1 mg/ml) in the drinking water, or left untreated. Viral titers were determined in lungs, brain stem and trigeminal ganglia (TG) as described [11,34]. Symbols used for treatment regimens and for the different organs are shown in the Figure. Mean titers were determined for 4 different tissues.

post infection) in the nervous tissues. Treatment of the infected animals by providing the drugs (ACV or BCV) in the drinking water (1 mg/ml) rapidly decreased the virus titer in the lungs (Fig. 4C, D), both regimens being equally effective. Although treatment with BCV delayed the occurrence of the virus in the brain stem, it did not prevent it (Fig. 4E, F). Treatment with ACV (Fig. 4E, F) prevented the virus from occurring in the brain stem (10^4 pfu/mouse) or delayed spread more effectively than BCV (10^5 pfu/mouse). Similar results as for brain stem were obtained when analyzing virus from trigeminal ganglia (data not shown). The mortality data obtained from these experiments were consistent with the result of the virus titers: (a) treatment with BCV did not decrease the final mortality with either inoculum size but it did increase the time to death, (b) treatment with ACV decreased mortality with the lower inoculum size, but not with the higher one, and (c) treatment with ACV was more effective than treatment with the same dose of BCV.

Supplying drugs in the drinking water leads to a more even drug intake than regular i.p. injections, and plasma levels can be maintained at a certain level following this oral therapy (data not shown, see also [23]). In addition, infected mice

appeared not to stop drinking (determined by measuring the daily intake of water) until at least one day before they died. Finally, tissue levels of ACV and BCV from infected mice were not different from levels in uninfected mice as determined by HPLC analysis of perchloric acid extracts (data not shown). Table 3 shows that the average serum levels of BCV were similar to the average lung levels of BCV and the levels of BCV in the ear flap. In addition, the drug concentrations in serum and lung were dose-dependent. Serum levels of ACV were higher than serum levels of BCV, when mice were supplied with drinking water containing the same concentration of either drug. This is probably due to the higher oral bioavailability of ACV (40%) than of BCV (20%). The lung levels of ACV and BCV (Table 3) were apparently sufficient to eliminate the virus (Fig. 4C, D), and ACV and BCV penetrated lung tissues equally well. However, BCV penetrated the CNS less effectively than ACV: the serum to brain ratio of BCV and ACV concentrations was 3.9 and 2.1, respectively (Table 3). Higher brain levels of ACV than of BCV* can explain why ACV is more efficacious than BCV in this experimental infection, because the BCV-treated animals died (probably of encephalitis) as BCV was apparently unable to prevent virus replication in brain (Figs. 4E,F).

Apparently, guanosine analogs differ in the extent to which they can penetrate nervous tissues. In the systemic treatment of symptomatic genital herpes in guinea pigs BCV had a limited effect (in contrast to ACV; see [24]), unless high doses of the drug were used [34]. Indeed, genital herpes in guinea pigs has a zosteriform character [42,43], i.e. symptom development in genital herpes is preceded by spread and/or replication of virus in the nervous system. As it is possible that zosteriform spread of virus resembles recrudescence [38], nervous tissue penetration of a drug may be a relevant parameter when considering the systemic treatment of recurrent genital herpes in humans. The lack of effect (unpublished data) on mortality of BCV, 3HM-HBG and (±)2HM-HBG after intranasal infection of mice with HSV-2 (strain 91075) suggests that in mice infected systemically with this virus (in which the drugs prevented mortality), the analogs prevented viral replication before spread of virus to brain and, also, that 3HM-HBG and (±)2HM-HBG poorly penetrate nervous tissue. Vice versa, the potent effects of the very similar analog DHPG (*9* in Fig. 1) in herpes-induced encephalitis [12,40] suggest that this analog penetrates nervous tissue easily. (The potencies of BCV and DHPG are similar in systemically HSV-2 infected mice.)

In summary, we have seen that for efficacy of the systemically administered guanosine analogs (a) plasma kinetics (b) intracellular trapping of the triphosphates and (c) nervous tissue penetration may be important parameters.

*The thymidine levels in brains and lungs are not significantly different from each other, and similar to plasma levels of thymidine (approximately 1 μM; Harmenberg, J., personal communication).

Efficacies in vivo: topical treatments

Intuitively, efficacy of viral DNA polymerase inhibitors applied topically should be determined by (a) the extent and rate at which the inhibitors penetrate the skin and (b) the extent and timing of viral DNA synthesis in the skin, relative to onset of treatment. It has been convincingly shown in intradermally infected guinea pig skin that efficacy of topical drugs is correlated with skin penetration of the drug [14]. However, two drugs that penetrate skin at near equal rates (at least through isolated skin), namely HBG (*1*) and BCV (*2*), and showing similar anti-HSV activity in cell cultures, differ in their efficacy when applied topically to guinea pigs infected on their back with HSV-1: HBG was inactive, whereas BCV was active [11].

One possible explanation has been proposed [6]. Guinea pig skin is rich in thymidine (ca. 25 μM; see [20]), and thymidine more easily antagonizes the antiviral effect of HBG than of BCV [29]. This was demonstrated experimentally by the rapid decrease in the antiviral effect of HBG observed in cell cultures when thymidine was added to the medium [27]. This prevention of the antiviral effect was thought to be caused by the low phosphorylation rate of HBG by viral thymidine kinase (7% of the rate of thymidine for the HSV-1 enzyme). However, the extrapolation, that guanosine analogs, which are only slowly phosphorylated by viral thymidine kinase and cellular guanylate kinase show poor efficacy in the topical treatment of HSV infection of guinea pig skin, does not hold. For example, 3HM-HBG (*4* in Fig. 1) is phosphorylated as poorly as HBG, at least by isolated enzymes, but is efficacious [31]. Apparently, a simple biochemical parameter for efficacy upon topical application is not at hand.

The relationship between efficacy and the time after infection at which topical treatment is started has not been extensively analyzed. Yet, it is well known that delaying topical treatment of genital herpes in guinea pigs to the time when at least 50% of the animals show the first symptoms (erythema) results in a drastic reduction in the efficacy of the treatment [34,40]. Interestingly, this is not observed in the treatment of the primary intradermal infection with the same virus in the guinea pig skin model (our unpublished results). A possible explanation for this difference may lie in the difference in pathogenesis of the two herpes infections, particularly the 'zosteriform' character of genital herpes infections in guinea pigs. This means that lesion development is preceded by spread and/or replication of the virus in the nervous system, and that adjacent skin sites are infected via sensory nerves. In the infection of the skin of the guinea pig back, the virus replicates at the original site of inoculation, and translocation through the peripheral nervous system may not be required for the involvement of contiguous skin.

A careful analysis of efficacy of topical antiherpes drugs in zosteriform herpes virus infections seems to be required, since topical ACV or foscarnet (phosphonoformic acid, also a viral DNA polymerase inhibitor) have shown only limited [26,45,46] or no efficacy [1,5,33,41] in the treatment of recurrent genital herpes infections in humans. For foscarnet it could be shown that the topically applied drug can inhibit lesion formation and virus replication in experimental infections

of human skin maintained on nude mice, suggesting that replication of virus in human skin can be suppressed by this viral DNA polymerase inhibitor [16].

In summary, the efficacy of viral DNA polymerase inhibitors applied topically is limited (a) by their skin penetration rates; (b) by the presence of antagonists, for example thymidine in the case of the guanosine analogs and (c) by particular features of recurrent or zosteriform herpes infections, which are not present in primary infections. Until these features are understood, it will be difficult to predict the efficacy of topical herpes virus DNA polymerase inhibitors applied when symptoms start. However, by gathering information on antiviral and pharmacological effects from several different infection models in animals, together with studies of drug effects on isolated enzymes and virus replication in cultured cells, it may be possible to make rational predictions for the likely efficacy in man, and help in selection and design of future analogs to be pursued as antiviral agents.

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