In vivo anti-papillomavirus activity of nucleoside analogues including cidofovir on CRPV-induced rabbit papillomas

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Abstract

A series of nucleoside analogues were tested for in vivo anti-papillomavirus activity using the cottontail rabbit papillomavirus (CRPV) domestic rabbit model. Compounds were delivered either topically, injected into growing papillomas, or delivered subcutaneously at a site remote from the papillomas. Compounds tested included cidofovir [(S)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine] (HPMPC); cyclic HPMPC (cHPMPC); cyclopentenylcytosine (CPE-C); lobucavir [1R(1α,2β,3α)]-9-[2,3-bis(hydroxymethyl)cyclobutyl]guanine; 9-((2-phosphonylmethoxy)propyl)adenine (PMPA); adefovir 9-((2-phosphonylmethoxy)ethyl)adenine(PMEA) and cyclopropyl 9-(2-phosphonylmethoxyethyl)-2,6-diaminopurine (cyclopropylPMEDAP). Dose response curves and time-course treatments were included for most compounds tested. Strong anti-viral activity was detected using cidofovir and cHPMPC when delivered either topically or by the intralesional route. Complete cures were obtained using 1% (w/v) topical cidofovir at dosing schedules of twice daily for 8 weeks beginning at 4 weeks after CRPV infection, which represents a time when papillomas were clearly visible. Complete cures of large established papillomas were obtained by intralesional injection of 1% cidofovir three times per week for 8 weeks. Topical treatments with adefovir had strong anti-viral activity, cyclopropyl PMEDAP had moderate anti-viral activity, and CPE-C, PMPA and lobucavir showed no effects. These data indicate that certain nucleoside analogues have strong in vivo anti-papillomavirus activity and that the CRPV/rabbit model is a good model for assessing clinical responses of anti-viral treatments for patients with HPV disease. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: CRPV; Papillomas; Rabbits; Cidofovir; Nucleoside analogues; Adefovir; Antiviral testing

1. Introduction

Human papillomaviruses (HPVs) are a group of DNA tumor viruses which can induce neoplastic proliferation of human epithelial cells. More than

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100 HPV types have been described, and life-threatening disease can result from increased risk of squamous cell carcinoma of human ano-genital tissues and skin, as well as from mass obstruction in juvenile laryngeal papillomatosis (reviewed in (Schiffman et al., 1993; zur Hausen, 1994)). Many new HPV types are still being characterized, and increased associations with additional human cancers such as non-melanoma skin cancers have been indicated (Shamanin et al., 1996; de Villiers et al., 1997; Harwood et al., 2000). The risk of initial infection, as well as the growth and progression to malignancy are markedly enhanced in patients with suppressed immunity.

Current clinical treatments for HPV infections involve lesion destruction. These procedures include excision using scalpel, lazer, freezing, or lesion ablation with toxic agents applied topically and/or intralesionally (trichloroacetic acid, phenol, salicylic acid, podophyllin, 5-fluorouracil, acyclic nucleotides and podofilox), as well as photo-dynamic therapy (Abramson et al., 1994). Additional strategies include locally applied immune modulators such as Imiquimod and interferon α (Gross, 1988; Sand Petersen et al., 1991; Cirelli and Tyring, 1994; Edwards et al., 1998; Arany et al., 1999). Recurrence rates are very high, usually greater than 50% (Auborn and Steinberg, 1990; Baker and Tyring, 1997). In addition, subclinical infections often go undetected and untreated. These latter infections may be reactivated by a variety of poorly characterized events and agents such as environmental carcinogens and/or co-factors, UV-irradiation, hormones, wounding, immune suppression and other STD agents to produce new active clinical disease. Thus, the current treatments are unsatisfactory, and there is an urgent need to develop drugs with greater efficacy and specificity.

Nucleoside analogues targeting DNA and RNA polymerases have been tested extensively for anti-viral activity (De Clercq, 1991, 1997; Naesens et al., 1997). We have used the cottontail rabbit papillomavirus (CRPV) domestic rabbit model (Shope and Hurst, 1933) for testing a variety of anti-viral compounds (Kreider et al., 1990, 1992; Kreider and Pickel, 1993; Kreider and Christensen, 1994; Okabayashi et al., 1993). Previously

tested compounds included 9-(2-phosphonyl-methoxy)ethyl)guanine (PMEG), podophyllin, podophyllotoxin, matrigel 5-fluorouracil, and several immune modulators. The rabbit model has also been tested by other investigators for anti-viral activity of ribavirin (Ostrow et al., 1992), bryostatin 1 (Bodily et al., 1999), CTC-96 (Ostrow et al., 1994), photodynamic activators (Shikowitz et al., 1986, 1988; Lofgren et al., 1994, 1995) and more recently, with cidofovir (Christensen and Kreider, 1999; Duan et al., 2000). The data indicated that the model is robust and reliable, and demonstrated clinical correlates for the treatment of HPV-related disease. We have tested anti-viral activity of a variety of nucleoside analogues including cidofovir in the CRPV/rabbit model over several years, and the goal of this report is to present a summary of these findings.

2. Materials and methods

2.1. Anti-viral compounds

Compounds assessed for anti-viral activity in the CRPV/rabbit model included; cidofovir [(*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine] (HPMPC); cyclic HPMPC (cHPMPC); cyclopentenylcytosine (CPE-C); lobucavir [1R(1α, 2β,3α)]-9-[2,3-bis(hydroxymethyl)cyclobutyl]guanine; 9-((2-phosphonylmethoxy)propyl)adenine (PMPA); adefovir 9-((2-phosphonylmethoxy)ethyl)adenine(PMEA); 9-((2-phosphonylmethoxy)ethyl)guanine (PMEG) and cyclopropyl 9-(2-phosphonylmethoxyethyl)-2,6-diaminopurine (cyclopropylPMEDAP). PMEG, PMEA, HPMPC, cHPMPC and cyclopropyl PMEDAP were obtained from Gilead Sciences, Foster City, CA, in saline (0.9% NaCl) solution. Lobucavir was obtained from Westwood-Squibb Pharmaceuticals, Buffalo, NY. Lobucavir and CPE-C were prepared in saline solution.

2.2. CRPV infection model

The CRPV/rabbit model (Kreider and Bartlett, 1981) was used to assess anti-viral activity. A standardized testing procedure was used as previ-

ously described (Kreider et al., 1990, 1992; Christensen and Kreider, 1999). Outbred NZW rabbits of both sexes were purchased from Covance Research Products, Inc., (Denver, PA), and maintained in the animal facility of the Pennsylvania State University College of Medicine. All the animal care and handling procedures were approved by the Institutional Animal Care and Use Committee of the Pennsylvania State University. Stocks of CRPV virus were prepared from CRPV-infected cottontail rabbit skin xenografts transplanted into athymic mice (Christensen and Kreider, 1990). The infectious titer of these stocks was determined biologically by infection of NZW rabbits with 10-fold dilutions of virus extract. A dilution between 1:1000 and 1:10 000 represented the EC_{50} infectious dose.

To assess anti-viral activity of compounds, 4 papillomas per rabbit were produced on the shaved surface of each rabbit. Briefly, rabbits were lightly anesthetized with a mixture of Ketamine HCl (40 mg/kg) and xylazine (5 mg/kg), and their backs shaved with an electric clippers. Two sites on each side of the flanks of the mid-dorsum, were scarified with a scalpel until abraded areas of approximately 1×1 cm were produced. The two anterior papillomas on each side were induced with a dilution of 10^{-1} CRPV extract, whereas the two posterior papillomas were induced with a dilution of 10^{-2}. Papillomas typically appeared 14 days after infection with 10^{-1} CRPV extract, and between days 21 and 28 with 10^{-2} extract. Each scarified site received 50 μl of papilloma extract, rubbed gently into the wound. The animals were observed, beginning at 3 weeks, for the development of papillomas. Papillomas were measured weekly, in three dimensions (length $\times$ height $\times$ width in mm) and the geometric mean diameter (GMD) in mm calculated. For the purposes of this study, a 'cure' was identified as the elimination of the clinical signs of papilloma. We do not imply that the lesions have been permanently cured.

2.3. Anti-viral testing

In our standard testing regime, animals were assigned to experimental groups, in groups of five.

Topical drugs were applied with an Eppendorf pipette in volumes of 100 μl, drop-wise, covering the papilloma surface. For the compounds that were tested topically, we treated the left-side sites, leaving the right-side sites untreated as integral controls. Placebo-treated rabbits were included in each experiment to compare possible systemic effects on the untreated control sites in the test groups. For systemic treatments, separate groups of rabbits were used for control treatments. Intralesional delivery consisted of direct injection of 100 μl of compound into the base of the papilloma in two to three places, depending upon the size of the papillomas under treatment. Systemic delivery consisted of subcutaneous injection of compounds in 100 μl doses at sites remote from the papillomas.

For all inoculations, rabbits were provided with plastic 'Elizabethan' collars to prevent licking or chewing of the papillomas. This prevented the possibility of severe toxicity and possible death by ingestion of topically applied compounds. In addition, 'noise' in the data due to chewing of papillomas was prevented even if the drug was given systemically.

2.4. Statistical analyses and data presentation

Groups of five rabbits were inoculated with CRPV at four sites per rabbit, and at two different concentrations of virus per rabbit (as described above). For each drug dilution there were 5 papillomas (five rabbits) that were measured weekly to assess anti-viral effects. Statistical analysis of mean papilloma size (GMD) over time was compared with the mean size of control-treated or untreated papillomas using Student's *t*-test. Statistical analyses and plots were prepared using SigmaPlot 4.0 software program. Experimental data were presented as the mean (of GMD values) $\pm$ S.E.M. of papilloma sizes for each dose of compound plotted against time after CRPV infection. An anti-viral effect was determined as a statistically significant reduction in papilloma size of treated versus untreated papillomas. Anti-viral effects that were presented in tabular form were described as the percentage reduction in mean papilloma size of treated versus untreated or

placebo-treated papillomas at the end of the experimental treatment phase.

We used a CRPV isolate (Rous and Beard, 1935; Salmon et al., 1997) that produced very low numbers of spontaneous regressions (4/220 or 2% of our rabbits have shown spontaneous regression-pooled data from several experiments). Low numbers of spontaneous regressions allowed us to use a smaller number of rabbits per drug treatment since almost all rabbits contained untreated or placebo-treated papillomas that continued to grow with time.

3. Results

3.1. Anti-viral activity of cidofovir and cHPMPC

The anti-papillomavirus activity of cidofovir (HPMPC) has been tested extensively in the CRPV rabbit model in our laboratory over several years, and a summary of these findings are presented in this report. Several experiments with cidofovir activity on CRPV-induced papillomas have been reported recently (Christensen and Kreider, 1999; Duan et al., 2000). An overview of the various experiments describing dosing schedules and routes of administration are presented in Table 1. Both cidofovir and cHPMPC showed strong anti-papillomavirus activity against CRPV-induced papillomas. In experiments where dosing schedules were identical, cidofovir was slightly more effective than cHPMPC.

Topical dosing schedules indicated that 1% cidofovir applied once daily for 8 weeks cured all papillomas if the treatments were started 15 days after infection (Fig. 1). Incomplete cures (two of the four sites with complete cures) were obtained with the lower dose of 0.1% cidofovir (Fig. 1B). At this time point for treatment initiation, papillomas are not usually visible or are just beginning to erupt. However, if the same treatment schedule was initiated at the later time point of 28 days after viral infection when the papillomas are clearly visible, growth suppression was weak (Table 1). In contrast, twice daily treatments with 1% cidofovir beginning at 28 days after infection led to the complete cure of papillomas (Fig. 2). At this later start time for treatment, 0.1% cidofovir was ineffective.

Systemic delivery of cidofovir at sites remote from the papilloma led to significant growth suppression without cure (Fig. 3A). The highest dose of cHPMPC (225 mg/kg per week) showed the greatest anti-viral effect. Significant toxicity as determined by general poor appearance of the rabbits together with weight loss was not observed with these systemic treatments, with the exception of a slight reduction in body weight gain ($P = 0.04$ for one time point only) for the highest dose of cHPMPC (Fig. 3B).

Intralesional 1% cidofovir three times per week for 6–9 weeks was especially effective, and cured large established papillomas (Fig. 4). For sites initiated with 10^{-1} CRPV extract, six of the eight sites were cured, but continuous treatment for 5 weeks was needed before lesion reduction began (Fig. 4A). In contrast, sites infected with 10^{-2} CRPV extract, seven of the eight sites were cured, most within a 4-week treatment period (Fig. 4B). Although the control sites were not injected with saline in these experiments, our previous studies have shown no reduction in papilloma growth with intralesional saline treatment (data not shown).

3.2. Anti-viral activity of other nucleoside analogues

We have tested topical applications of several other nucleoside analogues on the potential inhibitory activity against CRPV-induced papillomas (Table 2). Only adefovir and cyclopropyl PMEDAP showed anti-papillomavirus activity, but at the doses tested the responses were moderate with no cures (data not shown). Several compounds showed no anti-papillomavirus activity including lobucavir, PMPA and CPE-C. Lack of anti-viral activity was observed even when treatments were begun at 15–21 days after infection. This early treatment initiation was at a time when papillomas were either latent or very small and thus represented a minimal therapeutic challenge for the compound. One point of note here is that we did not check whether these compounds penetrated into the papilloma tissue. However, since

Table 1
Summary of experimental treatments of CRPV-induced papillomas with cidofovir and cHPMPC

Experiment #	Treatment regime	Compound(s)	Duration and frequency of treatment[a]	Outcome[b]
1	Topical	HPMPC	0.1 and 1.0% QD 5 days, 8 weeks, beginning day 15	0.1% — Hi (14% reduction); Lo (56% reduction) Fig. 1B 1.0% — Hi (76% reduction[c]); Lo (100% reduction[c]) Fig. 1C
2	Topical	HPMPC	0.1 and 1.0% BID 5 days, 8 weeks, beginning day 28	0.1% — Hi (29% reduction); Lo (68% reduction) 1.0% — Hi (87% reduction[c]); Lo (100% reduction[c])
3	Topical	HPMPC	0.1, 0.3 and 1.0% BID 5 days, 8 weeks, beginning day 28	0.1% — Hi (0% reduction); Lo (37% reduction) Fig. 2B 0.3% — Hi (60% reduction); Lo (62% reduction) 1.0% — Hi (100% reduction[c]); Lo (100% reduction[c]) Fig. 2C
4	Topical	HPMPC	1.0, 3.0 and 5% QW and BIW, 8 weeks, beginning day 28	1% — Hi (QW 0% reduction; BIW 44% reduction) 1% — Lo (QW 66% reduction; BIW 53% reduction) 3% — Hi (QW 4% reduction; BIW 60% reduction) 3% — Lo (QW 33% reduction; BIW 100% reduction[d]) 5% — Hi (QW 29% reduction; BIW 19% reduction) 5% — Lo (QW 82% reduction[c]; BIW 91% reduction[c])
5	Topical	HPMPC, cHPMPC	1% HPMPC and cHPMPC QD 5 days, 8 weeks, beginning day 28. 1% HPMPC and cHPMPC BIW, 8 weeks, beginning day 28	1% — QD, Hi (46% reduction[c], HPMPC; 63% reduction[c], cHPMPC) 1% — QD, Lo (61% reduction, HPMPC; 47% reduction, cHPMPC) 1% — BIW, Hi (42% reduction, HPMPC; 0% reduction, cHPMPC) 1% — BIW, Lo (38% reduction, HPMPC; 19% reduction, cHPMPC)
6	Sub—cutaneous (systemic)	HPMPC	1, 3 and 25 mg/kg MWF[e], 8 weeks, beginning day 21 (no body weight loss)	1 mg/kg — Hi (15% reduction[c]); Lo (30% reduction) 3 mg/kg — Hi (14% reduction); Lo (5% reduction) 25 mg/kg — Hi (45% reduction[c]); Lo (56% reduction[c])
7	Sub-cutaneous (systemic)	HPMPC and cHPMPC	25 mg/kg HPMPC and cHPMPC; 75 mg/ml and 225 mg/ml cHPMPC, QW, 8 weeks beginning day 21 (Fig. 4)	25 mg/kg — Hi (32% reduction[c], HPMPC; 33% reduction[c], cHPMPC) 25 mg/kg — Lo (50% reduction[c], HPMPC; 51% reduction[c], cHPMPC)

Table 1 (*Continued*)

Experiment #	Treatment regime	Compound(s)	Duration and frequency of treatment[a]	Outcome[b]
				75 mg/kg — cHPMPC; Hi (17% reduction; Lo 46% reduction[c])
				225 mg/kg — cHPMPC; Hi (30% reduction[c]; Lo 80% reduction[c])
8	Intra-lesional	HPMPC	1% MWF[e], 6 weeks, beginning day 35	Hi (6 cures/8 papillomas treated) Fig. 4A
				Lo (7 cures/8 papillomas treated) Fig. 4B
9	Intra-lesional	HPMPC	1% MWF[e], 9 weeks, beginning day 45	Hi (9 cures/10 papillomas treated)
				Lo (10 cures/10 papillomas treated)

[a] Duration of treatment: BID, twice-daily; QD, once daily; BIW, twice weekly, QW, once weekly.

[b] Impact of treatment on CRPV-induced papillomas. Either as % reduction in mean of GMDs for treated versus untreated or placebo-treated papillomas; or number of sites cured/number of sites treated. Hi, high-dose virus infected sites; Lo, low-dose virus infected sites.

[c] $P < 0.05$

[d] $P = 0.1$

[e] Monday, Wednesday, Friday.

cidofovir, cHPMPC, adefovir and cyclopropyl PMEDAP were delivered in an identical manner and formulation (saline) it appears that the latter three compounds have no significant in vivo antiviral effects in the CRPV/rabbit model.

3.3. Drug skin toxicities

Cidofovir (0.1 and 1.0%) and PMEG (0.1%) were applied in volumes of 100 μl per site BID 5 days per week for a period of 8 weeks to shaved normal rabbit back skin to assess local skin toxicities. This treatment represented typical dosing schedules for topical applications to papillomas with these compounds. Size (length × width) and induration of the treated sites were measured weekly, and general observations taken (data not shown). The data indicated that cidofovir at both doses induced more irritation than vehicle alone. The size of the irritated areas were similar for the cidofovir and PMEG treated sites. However, PMEG (0.1%) showed the most severe toxicities including significant tissue necrosis with less necrosis for cidofovir treatments. Histological assessments of the depth of tissue necrosis for the two treatments were not conducted. In sites that were cured by topical and/or intralesional cidofovir treatments, local skin blistering with necro-

sis was observed, and this has been reported by others (Duan et al., 2000). Normal skin epithelium was re-established within 1–2 weeks after treatment cessation for these sites (data not shown).

4. Discussion

Anti-viral activity of cidofovir and other nucleoside analogues were tested using the CRPV/rabbit model. Cidofovir is a cytidine nucleotide analogue with a wide spectrum of anti-viral activity in vitro and in vivo (Safrin et al., 1999; De Clercq, 1997). Recent clinical trials have been initiated to treat HPV infections including juvenile laryngeal papillomatosis (Van Cutsem et al., 1995; Snoeck et al., 1998; Wilson et al., 2000), ano-genital HPV infections (Snoeck et al., 1995, 2000; Hengge and Tietze, 2000; Schurmann et al., 2000) and lesions of patients with epidermodysplasia verruciformis (Preiser et al., 2000). Cidofovir delivered topically and intralesionally has shown encouraging but variable responses (Van Cutsem et al., 1995; Snoeck et al., 1995, 1998, 2000; Preiser et al., 2000; Wilson et al., 2000). Variation in clinical responses may be due in part
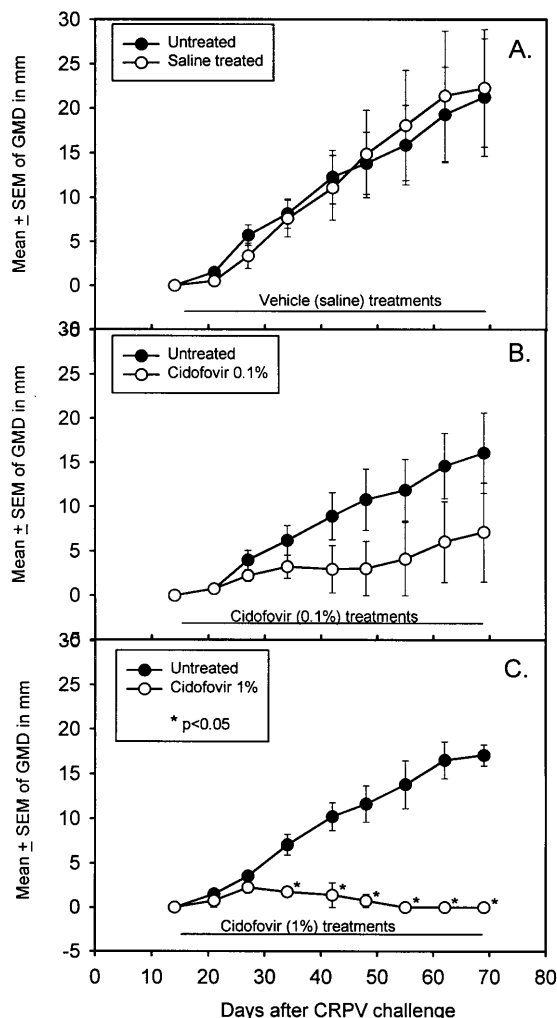
Fig. 1. Topical treatment of left-side papillomas initiated with 10^{-2} dilution of CRPV extract. Five papillomas from five rabbits per treatment group were given daily topical applications of saline (A), 0.1% cidofovir (B) or 1% cidofovir (C) for 5 days per week for 8 weeks beginning on day 15. Mean ± S.E.M. of papilloma size as determined from geometric mean diameters (GMDs) was plotted against time in days after CRPV infection. Treated papillomas for each group were compared with mean GMDs of untreated contralateral control papillomas by Student's t-test. For 0.1% cidofovir treatments, two of the four sites were cured; for 1% cidofovir treatments, five of the five sites were cured. Additional data are summarized in Table 1, experiment #1.

In the studies described here, a variety of delivery systems including systemic, topical and intralesional treatments at various doses and schedules were compared over the course of a number of experiments. Cidofovir showed very
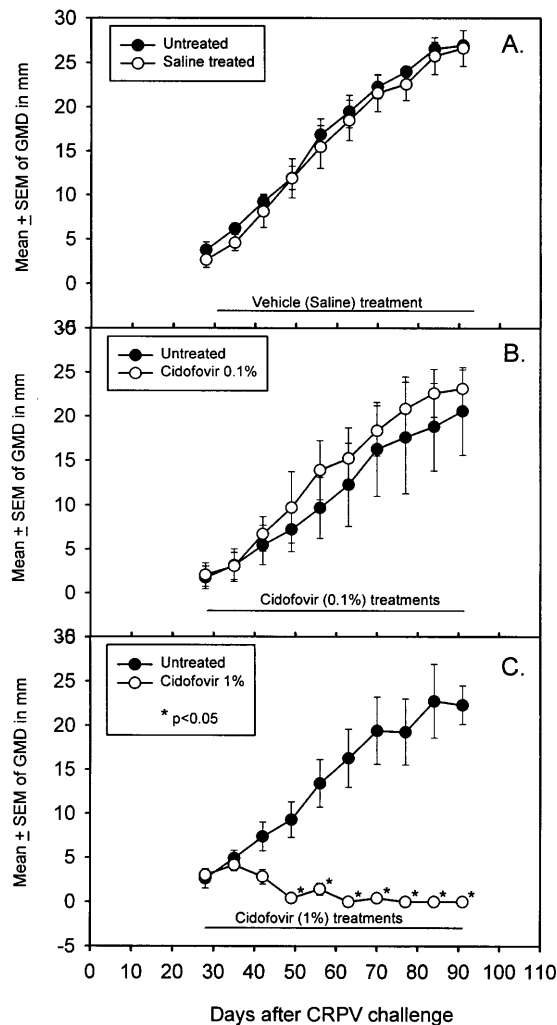

Fig. 2. Topical treatment of left-side papillomas initiated with 10^{-1} dilution of CRPV extract. Treatment schedules consisted of twice-daily topical applications of saline (A), 0.1% cidofovir (B) or 1% cidofovir (C) for 5 days per week for 8 weeks beginning on day 28. For 0.1% cidofovir treatments, none of the five sites were cured; for 1% cidofovir treatments, five of the five sites were cured. Similar growth suppressions and cure rates were obtained for papillomas initiated from 10^{-2} CRPV extract (data not shown). Additional data are summarized in Table 1, experiment #3.

to the variability in dosing schedules and to the uncertainties as to what is the best therapeutic dosing regimen for maximum efficacy.
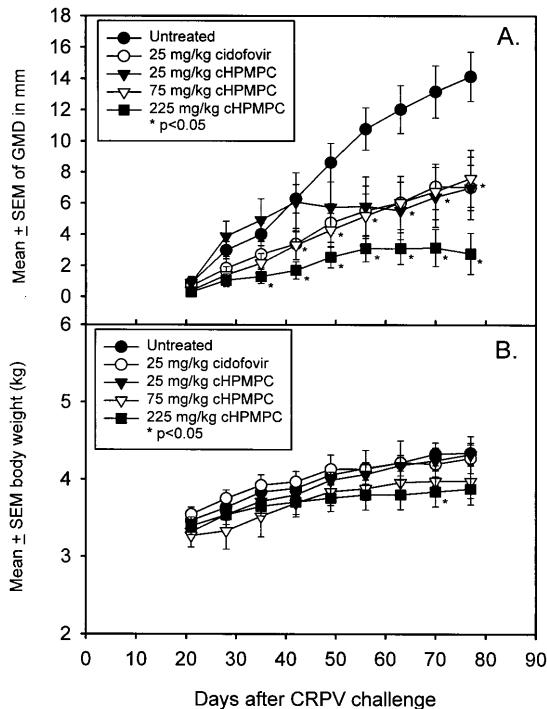
Fig. 3. Systemic treatment (once-weekly s.c. injections for 8 weeks beginning on Day 21) of rabbits infected with CRPV (10^{-2} extract, two sites per rabbit, five rabbits per group) followed by treatment with 25 mg/kg cidofovir (○); and cHPMPC at 25 mg/kg (▼), 75 mg/kg (▽), or 225 mg/kg (■); and untreated (●). Mean ± S.E.M. of papilloma size (determined from GMDs) of 10 papillomas on five rabbits was plotted against time after CRPV infection (A). Mean body weights of five rabbits for each treatment group were also determined (B). Significant reduction in papilloma size was observed for all treatment groups beginning 4 weeks after treatment initiation. Additional data are summarized in Table 1, experiment #7.

strong anti-papilloma effects leading to complete cures when delivered topically starting from when papillomas were small to moderate in size. Complete cures of large papillomas were possible by intralesional delivery of 1% cidofovir. Collectively, these studies demonstrated that systemic delivery was least effective followed by topical applications then intralesional delivery which was the most effective treatment modality. The most effective topical treatments were achieved when the twice-daily drug applications were placed onto small papillomas. This topical dosing regimen was less effective when started at a later time point

when the papillomas were larger and well-established. These data are in very good agreement with recent studies with cidofovir treatments of rabbit papillomas (Duan et al., 2000), and illustrate the utility and robustness of the CRPV/rabbit system as a preclinical model for antiviral testing. More frequent topical applications of low-dose cidofovir (1% BID) were also more effective than less frequent high-dose cidofovir (5% QW). We also noted that systemic toxicity following topical and intralesional delivery of cidofovir was minimal such that the treated rabbits remained in good health with body weight gain equivalent to
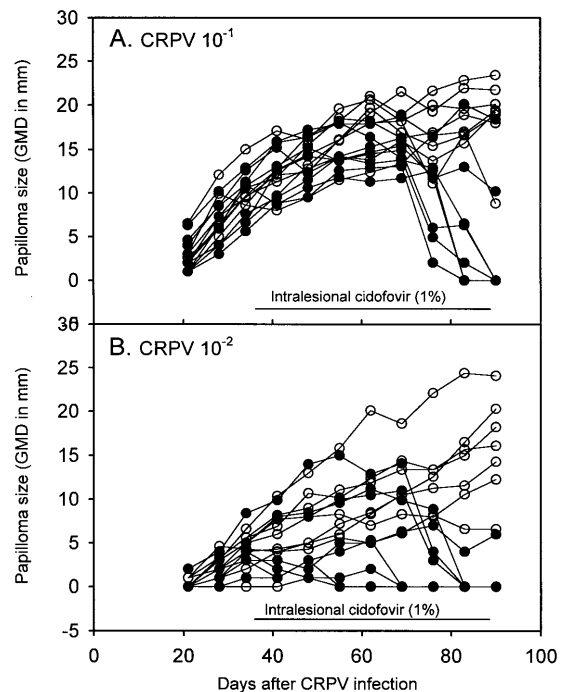

Fig. 4. Intralesional treatment of large established papillomas with 1% cidofovir (100 μl per treatment injected into the base of the papillomas) given three times per week for 8 weeks. Papillomas were initiated with either 10^{-1} extract of CRPV (A) or 10^{-2} CRPV extract (B), and intralesional treatments began 35 days after CRPV infection. At this time point, the papillomas were substantial in size, with mean diameters from 5 to 12 mm (maximum size of papillomas reached 20–25 mm in diameter). Individual sites on eight rabbits were treated intralesionally (●) of left untreated (○) and plotted against time after CRPV infection. For sites infected with 10^{-1} CRPV extract, six of the eight papillomas were cured, and for 10^{-2} sites, seven of the eight papillomas were cured.

Table 2
Summary of experimental treatments of CRPV-induced papillomas with nucleoside analogues other than cidofovir[a]

Expt #	Treatment regime	Compound(s)	Duration and frequency of treatment	Outcome
10	Topical	PMEA	5% QD 5 days, 8 weeks; 5% BIW 8 weeks, beginning day 21	Hi (QD 0% reduction; BIW 24% reduction) Lo (QD 12% reduction; BIW 76% reduction[d])
11	Topical	PMPA	1 and 5% PMPA 0.1%, 0.5 and 1.0%	PMPA 1.0% — Hi (0% reduction); Lo (0% reduction) 5.0% — Hi (0% reduction); Lo (0% reduction)
		cyclopropyl PMEDAP	Cyclopropyl PMEDAP QD 5 days, 8 weeks, beginning day 21	Cyclopropyl PMEDAP 0.3% — Hi (0% reduction); Lo (0% reduction) 1.0% — Hi (50% reduction); Lo (46% reduction) 5.0% — Hi (38% reduction); Lo (29% reduction)
12	Topical	Lobucavir	0.3, 1 and 5% BID 5 days, 8 weeks, beginning day 21	0.3% — Hi (0% reduction); Lo (5% reduction) 1.0% — Hi (4% reduction); Lo (18% reduction) 5.0% — Hi (2% reduction); Lo (0% reduction)
13	Topical	CPE-C	0.1 and 1% QD 5 days, 8 weeks, beginning day 15	0.1% — Hi (0% reduction); Lo (0% reduction) 1.0% — Hi (7% reduction); Lo (11% reduction)

[a] Treatment and outcomes as described in Table 1 legend.

the untreated rabbits. This series of experiments provide important information for dosing and scheduling for current and future clinical trials with cidofovir (Van Cutsem et al., 1995; Snoeck et al., 1995, 1998, 2000; Preiser et al., 2000; Wilson et al., 2000).

Several other nucleoside analogues were tested for anti-papillomavirus activity. In general weak to no papilloma growth suppression was obtained. We conclude that of the reagents tested so far in the CRPV/rabbit model, cidofovir is the most efficacious. Earlier studies with PMEG (Kreider et al., 1990) also showed strong antiviral activity in the CRPV/rabbit model, but the systemic and local toxicities of PMEG were severe, and therefore, may exclude this compound from clinical treatments of HPV infections.

In most experiments, papilloma measurements ended at the time of treatment cessation and the animals were euthanized. For systemic treatments, serum and tissue samples were taken for toxicity assessment when needed. Since topical treatments led to minimal toxicity, no tissue samples were collected from these studies. In a small number of experiments, continued monitoring of drug 'cured' sites after treatment cessation were conducted to assess recurrence rates. In these latter studies, papilloma recurrences were observed at rates of about 50% of cured sites (manuscript submitted) and were similar in frequency to clinical recurrence rates (Auborn and Steinberg, 1990; Baker and Tyring, 1997). Therapeutic strategies to reduce recurrence rates of CRPV-induced papillomas have been initiated and are described elsewhere (manuscript submitted).

The CRPV/rabbit model has been used by us and other investigators for testing anti-viral therapies for papillomavirus infections. The most effective compounds so far included PMEG (Kreider et al., 1990), podophyllotoxin (Kreider et al., 1992; Kreider and Pickel, 1993), cidofovir (Christensen and Kreider, 1999; Duan et al., 2000, this report) and photodynamic therapy (Shikowitz et al., 1986, 1988; Lofgren et al., 1994, 1995). Other compounds including ribavirin (Ostrow et al., 1992), CTC-96 (Ostrow et al., 1994), bryostatin-1 (Bodily et al., 1999) and several nucleoside analogues (this report) were less effective and/or had no effect. The in vivo antiviral mechanism of action of the nucleoside analogues is most likely a cellular cytotoxicity of the papilloma tissue, since papillomaviruses use host polymerases for their replication needs (Schiffman et al., 1993; zur Hausen, 1994). Some in vitro studies have shown that cidofovir triggers non-specific apoptosis (Andrei et al., 1998), but it is unclear whether this occurs in vivo. These combined studies indicate that the CRPV rabbit system is an effective model to assess anti-papillomavirus activity (Stanley et al., 1997) and is especially useful for determining dosing schedules and different treatment regimens.

In conclusion, a number of nucleoside analogues were assessed for papillomavirus anti-viral activity using the CRPV/rabbit model. Cidofovir and cHPMPC were the most effective, leading to complete cures with topical and intralesional dosing regimens. Dosing schedules for topical cures required twice-daily treatments of 1% cidofovir for 5 days per week for 6–8 weeks, whereas intralesional cures were achieved with 1% cidofovir, three times per weeks for 6–9 weeks.

Acknowledgements

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