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# Topical effects of cidofovir on cutaneous rabbit warts: treatment regimen and inoculum dependence

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**Abstract**

The present study examined topical effects of cidofovir on cutaneous rabbit warts. Based on an inoculum-dependency study, each New Zealand White rabbit was inoculated with a high and low titer of cottontail rabbit papillomavirus (CRPV) at four sites on each dorsolateral area. Inoculation with 50 $ID_{50}$ induced papillomas at 100% of the inoculation sites within $16 \pm 1$ days, and the wart growth curve plateaued within $\sim 7$ weeks. With an inoculum of 5 $ID_{50}$, 80% of the inoculated sites developed papillomas within $21 \pm 1$ days and their size plateaued at a later time. Cidofovir was applied topically twice daily on the inoculated sites at a concentration of 1% for 18 days, starting at three different time points. In the first experiment, treatment was initiated 7 days post-inoculation. One of the inoculated sides received cidofovir or the vehicle, PBS, while the other side was left untreated. With this treatment regimen, cidofovir significantly delayed the time of onset and the growth rate of papillomas induced with the high titer of inoculum. It completely prevented papilloma-induction on the sites inoculated with the low titer of CRPV. Reversible side-effects of cidofovir were observed on the directly treated area including erythema, necrosis, and flaking. Both therapeutic and side-effects were limited to the sites of direct exposure. In the second experiment, one of the two sides in each group of rabbits received cidofovir or vehicle starting on day 29 post-inoculation. With this treatment regimen, cidofovir significantly reduced wart growth against the low titer only. Topical treatment initiated on day 49 post-inoculation was not effective on warts initiated with either viral titer. These results demonstrated that topical cidofovir could be very effective against papillomavirus-induced wart growth if it is initiated early during the infection, especially against low titers of inoculum. © 2000 Elsevier Science B.V. All rights reserved.

*Keywords:* Cidofovir; Cottontail rabbit papillomavirus (CRPV); Rabbits; Papilloma; Warts

## 1. Introduction

Human papillomaviruses (HPVs) are small DNA viruses that mediate proliferative epithelial lesions (Pfister, 1984; Howley, 1990; Laimins,

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1993; Majewski and Jablonska, 1997). All HPV genomes consist of ~8000 base pairs of circular DNA that encode nine to ten open reading frames (Howley, 1990; Laimins, 1993; Majewski and Jablonska, 1997). As highly species-specific pathogens, HPVs also exhibit strict tissue tropism (Laimins, 1993; Gangemi et al., 1994; Majewski and Jablonska, 1997). Upon entering basal cells, HPVs link their life cycles to the differentiation program of the host cell (Howley, 1990; Laimins, 1993; Gangemi et al., 1994; Majewski and Jablonska, 1997). Although they encode several necessary proteins regulating their genomic replication, HPV genomes do not contain the gene coding for their own DNA polymerase (Laimins, 1993; Majewski and Jablonska, 1997). Therefore, HPVs have to use the host cellular machinery for DNA replication (Majewski and Jablonska, 1997). The absence of good cell culture and animal models of HPV replication and disease (Ostrow et al., 1992, 1994; Cirelli and Tyring, 1994; Gangemi et al., 1994; Stanley et al., 1997), as well as limited genomic targets (Phelps et al., 1998), has represented a significant challenge for the discovery and development of effective therapeutics (Kreider et al., 1990; Gangemi et al., 1994; Stanley et al., 1997). Although more than 70 different genotypes of HPVs have been identified, current treatments against HPV disease remain unsatisfactory (Phelps and Alexander, 1995; Gross, 1997). Variable and limited efficacy, high recurrence rates and undesired side-effects sustain the search for other therapeutic options. As a potent inhibitor of DNA polymerase, cidofovir is active against a broad range of DNA viruses including herpesviruses, poxiviruses, hepadnaviruses, polymaviruses, adenoviruses and papillomaviruses (Naesens et al., 1996; De Clercq, 1997). However, potential toxicity of cidofovir limits its systemic application and requires pre-dose monitoring of renal function and concomitant administration of hydration and probenecid (Hitchoock et al., 1996). Therefore, trials of cidofovir treatment in clinical and experimental settings against papilloma disease have been limited to topical therapy (Snoeck et al., 1995, 1998b; Zabawski and Cockerell, 1998). Following an abstract that reported the topical efficacy of cidofovir in the cottontail rabbit papillomavirus (CRPV)-infection model (Kurtzman et al., 1993), several clinical studies have demonstrated in vivo activity of this compound against human papilloma disease (Snoeck et al., 1995, 1998a,b; Van Cutsem et al., 1995; Zabawski and Cockerell, 1998). Nevertheless, there is a large variability in terms of the therapeutic as well as adverse effects, from patient to patient, and from study to study (Snoeck et al., 1995, 1998a,b; Van Cutsem et al., 1995). One potential explanation for this variability may be related to the time when treatment is initiated, and/or the infection status of the patient receiving the treatment (Cirelli and Tyring, 1994). Therefore, the therapeutic potential and limitations of topical cidofovir may not be easily evaluated unless these variables are controlled. In the present study, we used the highly reproducible CRPV-infection model in rabbits (Kreider and Bartlett, 1981; Kreider et al., 1992) to study the treatment regimen and infection inoculum-dependence of topical cidofovir therapy. Our results demonstrate that it is critical to treat papillomas as early as possible, at least for this therapeutic effect.

## 2. Materials and methods

### 2.1. Compounds

Cidofovir was prepared in-house and the purity was more than 97% by HPLC. The compound was dissolved in phosphate buffered saline at a final concentration of 1%.

### 2.2. Animals

New Zealand White rabbits (female, 2 kg from Charles River, St Constant, Québec, Canada) were used for all experiments. Animals were maintained in plastic cages with access to Purina laboratory rabbit chow and water ad libitum. All experiments and animal handling were carried out according to protocols approved by the Canadian Council on Animal Care.

### 2.3. Virus and animal inoculation

Cottontail rabbit papillomavirus was kindly supplied by Dr Janet Brandsma, Yale University. Rabbits were anesthetized with a combination of xylazine and ketamine, 5 and 50 mg/kg, respectively, by the intramuscular route. Hair was removed from each side of the laterodorsal area using an electric clipper. The residual hair and superficial keratin were treated with a commercial depilatory (Nair). Then four evenly spaced 1-$cm^2$ regions of skin surface on each side were scarified with a No. 10 surgical blade and inoculated with 70 μl of CRPV suspension containing the designated dilutions of the initial stock. The scarification depth was controlled up to the minimal bleeding level ($\sim 1$ mm in depth with some adjustment dependent on the thickness of the inoculation sites). Scarification lines were made as crosses separated at a distance of $\sim 1$ mm. In an inoculum-dependency test, an $ID_{50}$ value was determined as the inoculum dilution of the initial stock (15 000-fold) that induced papillomas at 50% of the inoculation sites, and two titers of inoculum were chosen for papilloma induction. At 50 $ID_{50}$ (300-fold dilution of the initial stock), 100% of the inoculation sites developed papillomas within $16 \pm 1$ (mean $\pm$ S.E.M.) days, and the wart growth curve plateaued within $\sim 7$ weeks. At 5 $ID_{50}$ (3000-fold dilution of the initial stock), 80% of the inoculated sites developed papillomas within $21 \pm 1$ days and their size plateaued at a later time.

### 2.4. Drug treatment

In the first experiment, eight rabbits were allocated into two groups randomly. Only one side of each inoculated rabbit received topical treatment with the vehicle PBS or cidofovir. A total volume of 0.15 ml of compound or vehicle was delivered directly on the skin surface over the inoculation site, followed by gentle massage with the smooth bottom of a 5-ml test tube. The other side of both experimental groups was left untreated to test whether systemic effects could be observed. Compounds or vehicles were applied topically, twice daily for 18 days, starting 7 days post-inoculation. After seeing no systemic effects of vehicle or cidofovir, the second experiment was carried out using eight rabbits randomized into two groups. One side of the inoculated sites in the vehicle or cidofovir group received 18 days of treatments starting on day 29, while the other side received 18 days of treatments starting on day 49.

### 2.5. Histological study

Rabbit skin or wart tissues were collected from anesthetized animals, and immediately immersed in 10% formalin for fixation. The fixed samples were trimmed from the epidermal side through the specimen to the subcutis in three areas representing the entire tissue mass. The specimens were desiccated, processed through xylene, and perfused with paraffin. Sections were cut at 5 μm, stained with hematoxylin and eosin, examined and photographed microscopically.

### 2.6. Data collection and statistical analysis

Antiviral effects were evaluated by the onset, growth or regression rate of cutaneous papillomas. Papilloma sizes were measured weekly in three dimensions (length $\times$ width $\times$ height) and the geometric mean diameters (GMD) were calculated as the cubic root of the aggregated products for each inoculation site. Experiment 1 was terminated on day 49 when the non-treated wart growth curve, in the high titer inoculated sites, reached a plateau phase and the samples were collected for histological studies from euthanized rabbits. Experiment 2 was terminated later to allow a similar period of observation following the delayed treatment. Further prolongation of the experiment was difficult since the big warts started to have bleeding problems. Experimental data were presented as mean $\pm$ S.E.M. Effect of treatment on papilloma onset and wart size at each time point was analyzed using the standard single factor (treatment) one-way analysis of variance (Zar, 1984a) followed by Student–Newman–Keuls multiple comparison (Zar, 1984b) procedure supplied in SAS software (SAS Institute, Cary, NC, USA). $P < 0.05$ was considered statistically significant.

## 3. Results

In preliminary experiments, the inoculum-dependency study of CRPV-induced papilloma formation in New Zealand White rabbits was established in order to choose the most reproducible and sensitive titer for drug evaluation. Under our experimental conditions, an inoculum of 50 $ID_{50}$ reproducibly induced papillomas at 100% of inoculation sites with very small variability in terms of papilloma onset ($16.1 \pm 0.6$ days, $n = 8$) and growth rate (Fig. 1a and Fig. 2a). Without treatment, the size of papillomas reached a plateau within $\sim 7$ weeks. Further increased viral titers (decreased dilution) led to papillomas growing too quickly, creating a short time span for drug evaluation. On the other hand, further decrease of viral titers (increased dilution factor) led to increased time span for papilloma onset and plateau time, as well as increased variability. Based on these results, we chose 50 $ID_{50}$ as the

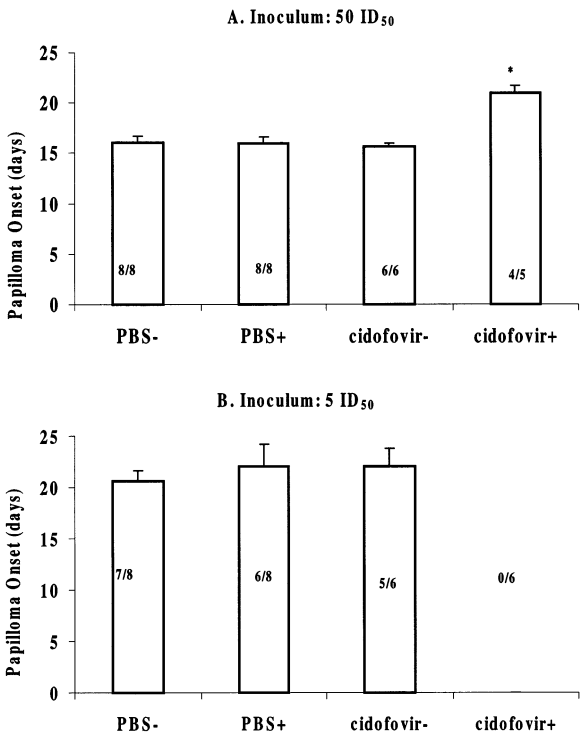



Fig. 1. Topical effects of cidofovir on CRPV-induced papilloma onset. One group of rabbits was treated for 18 days starting on day 7 post-inoculation with vehicle (PBS + ) on one side of the inoculated areas, while the other side was left as non-treated control (PBS − ). Another group of rabbits was treated with cidofovir (cidofovir + ) on one side of the inoculated areas, leaving the other side as non-treated control (cidofovir − ) in this group. Numbers inside the histogram indicate the induction frequency of papilloma over the total number of inoculation sites. Panels (A) and (B) represent papilloma onset induced by an inoculum of 50 or 5 $ID_{50}$. Asterisk * indicates $P < 0.05$ compared to other treatments analyzed using ANOVA followed by SNK multiple comparisons as detailed in the text.

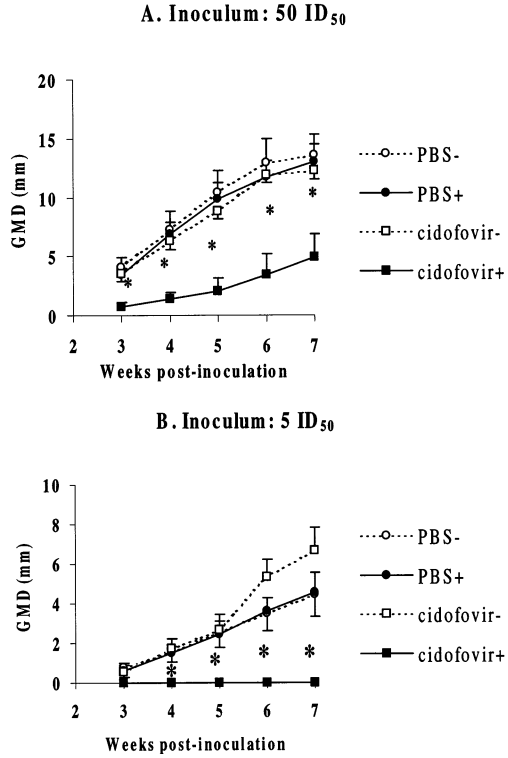
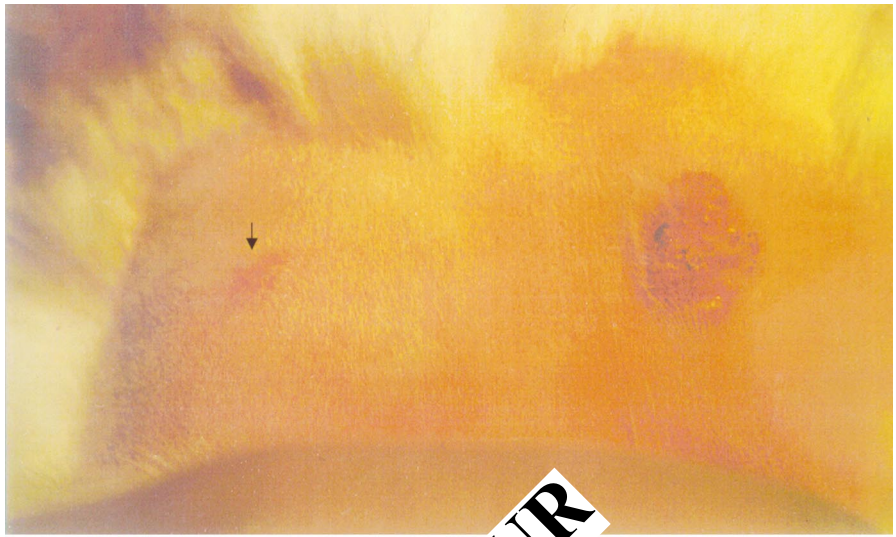



Fig. 2. Topical effects of cidofovir on CRPV-induced papilloma growth. One group of rabbits was treated for 18 days starting on day 7 post-inoculation with vehicle (PBS + ) on one side of the inoculated areas, while the other side was left as non-treated control (PBS − ). Another group of rabbits was treated with cidofovir (cidofovir + ) on one side of the inoculated areas, leaving the other side as non-treated control (cidofovir − ) in this group. Geometric mean diameters (GMD) were calculated as the cubic root of the products of three dimensions of the papilloma. Panels (A) and (B) represent papilloma induced by an inoculum of 50 or 5 $ID_{50}$. Asterisk * indicates $P < 0.05$ compared to other treatments analyzed by ANOVA followed by SNK multiple comparisons as detailed in the text.



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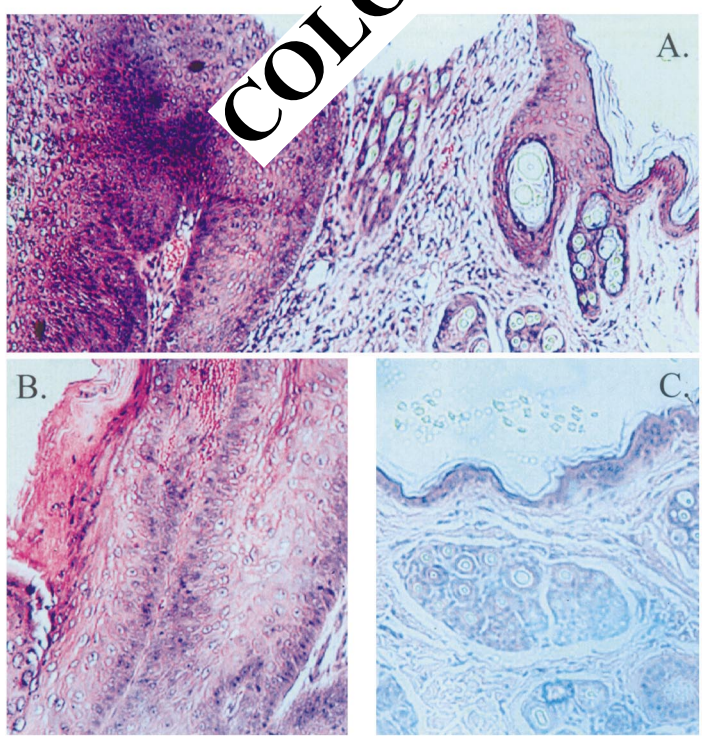


Fig. 4

Fig. 3. Topical effects of cidofovir. As indicated by the arrow, cidofovir prevented papilloma growth only at the infected sites that were directly exposed to the drug treatment. This therapeutic effect was associated with local irritation. The picture was taken 4 days following the termination of the treatment.

Fig. 4. Typical examples of histological features of CRPV-induced papillomatosis and cidofovir effects. Panels (A) and (B) show the histology at the border and the center area of the same papilloma, demonstrating profound epidermal proliferation, hyperkeratosis, parakeratosis, koilocytosis, and acanthosis. Panel (C) shows profound inhibition of papillomatosis by 18 days of cidofovir treatment started on day 7 post-inoculation.

high titer of inoculum that gave us 100% papilloma induction rate and highly reproducible growth rate. We choose 5 $ID_{50}$ as the low titer of inoculum that induced papillomas at $\sim 80\%$ of the inoculation sites, with reasonable variability in terms of papilloma onset ($20.6 \pm 1.0$ days, $n = 7$) and growth rate (Fig. 1b, and Fig. 2b).

Topical vehicle treatment for 18 days starting on day 7 post-inoculation did not affect papilloma induction rate and time to onset at all inoculation sites with the inoculum of 50 or 5 times $ID_{50}$ (Fig. 1A,B). However, 1% topical cidofovir significantly delayed the time to onset of papillomas induced by the high titer of inoculum, although four of the five directly treated sites did develop papillomas. It was more effective in preventing papilloma development on the sites inoculated with the low titer of inoculum, resulting in complete inhibition of papilloma induction. The inhibitory effects of cidofovir on CRPV-induced papilloma growth are summarized in Fig. 2. It is clear that papilloma growth rate was highly reproducible and overlapping in all the sites receiving the same titer of inoculum except those directly exposed to cidofovir treatments. One of the inoculation sites receiving the high titer of inoculum missed direct exposure to topical cidofovir by a distance of $\sim 1.5$ cm, leading to a complete loss of anti-papilloma effects. Obvious side-effects including reversible erythema (reaching up to $\sim 1$ $cm^2$ in size within the 2nd week of treatment, and starting to subside 1 week after the termination of treatment) were observed with all sites (16/16) directly treated with cidofovir. Some sites were associated with flaking and minor ulceration (Fig. 3). One of the rabbits developed necrosis and behavioral abnormalities. This rabbit was euthanized for ethical reasons.

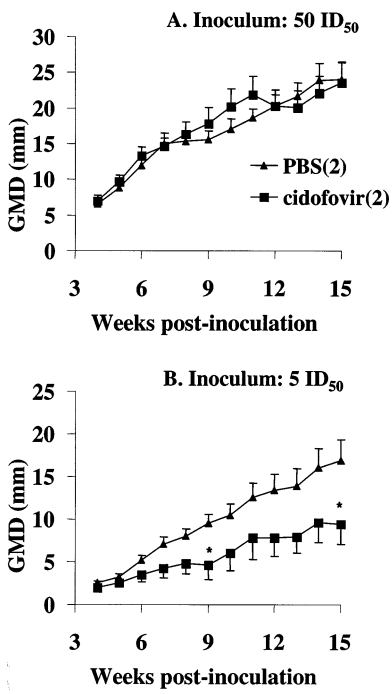


Fig. 5. Topical effects of cidofovir on CRPV-induced papilloma growth. Animals were treated for 18 days starting on day 29 post-inoculation, with vehicle (PBS, 2), or cidofovir (cidofovir, 2), respectively, as detailed in the text. Geometric mean diameters (GMD) were calculated as the cubic root of the products of three dimensions of the papilloma. Panels (A) and (B) represent papilloma induced by an inoculum of 50 or 5 $ID_{50}$. Asterisk * indicates $P < 0.05$ compared to other treatments analyzed by ANOVA followed by SNK multiple comparisons as detailed in the text.

Fig. 4 shows typical examples of histological features of CRPV-induced papillomatosis and cidofovir effects. Panel A was taken from the border zone of the inoculation area. Compared to the right side where almost normal epidermis could be seen, profound proliferative papillomatosis is observed on the left side of the panel. More typical features of cutaneous warts can be observed in panel B, which was taken from the center area of the same wart, demonstrating profound epidermal proliferation, hyperkeratosis, parakeratosis, koilocytosis, and acanthosis. Cidofovir treatment for 18 days starting on day 7 post-inoculation profoundly inhibited papillomatosis (Fig. 4C).

Complete absence of systemic effects of topically applied cidofovir made it possible to evaluate drug effects on individual sides of the rabbit to reduce the number of animals required. In the second experiment, one side of the rabbit was treated for 18 days starting on day 29 post-inoculation, while the other side was treated starting on day 49. It is clear that cidofovir treatment only affected the side of direct exposure in the second experiment as well. As shown in Fig. 5, cidofovir (1% for 18 days) starting on day 29 post-inocula-

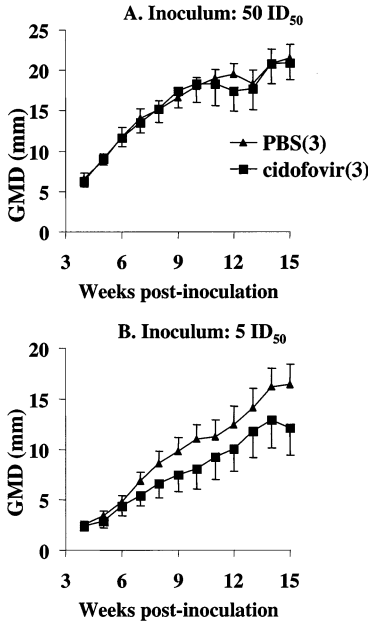


Fig. 6. Topical effects of cidofovir on CRPV-induced papilloma growth. Animals were treated for 18 days starting on day 49 post-inoculation, with vehicle (PBS, 3), or cidofovir (cidofovir, 3), respectively, as detailed in the text. Geometric mean diameters (GMD) were calculated as the cubic root of the products of three dimensions of the papilloma. Panels (A) and (B) represent papilloma induced by an inoculum of 50 or 5 $ID_{50}$. The effect of cidofovir was not statistically significant.

tion did not significantly affect papilloma growth against that mediated by high titer of inoculum (Fig. 5A). However, it did reduce papilloma growth significantly against the low titer of inoculum (44% reduction in GMD at the end of experiment, $P < 0.05$; Fig. 5B). Further delay of cidofovir treatment initiation to day 49 post-inoculation was associated with a complete loss of anti-papilloma effects against both high and low inoculum. Although there seems to be a trend of some reduction in papilloma growth rate in cidofovir-treated sites against the low titer of inoculum, the effects never reached statistical significance at any of the time points examined (Fig. 6).

## 4. Discussion

The present study provides the first inoculum and treatment regimen dependent effects of cidofovir against CRPV in a highly reproducible papilloma disease model. Our results demonstrated that topically applied cidofovir inhibits or prevents papilloma development in a titer-dependent manner based on the inoculum and treatment regimen. Therefore, initiation of early treatment of cidofovir can be used as an effective therapy against papilloma disease without much concern for systemic toxicity. However, local side-effects may still be a limitation for this treatment.

Highly reproducible papilloma induction and growth rate in the CRPV-infection model offers the possibility to test the treatment regimen and inoculum dependence of cidofovir. Unlike the highly variable papilloma onset and growth rates observed in the HPV xenograft model and those seen in the clinic (Cirelli and Tyring, 1994), CRPV infection in the rabbit model offers the advantage of high reproducibility (Kreider et al., 1992; Ostrow et al., 1992, 1994). This advantage makes it more efficient for the evaluation of drug activity and treatment regimen. Although several differences exist between HPV and CRPV diseases, this model shares certain similarities with HPV infection. These include high homology in genomic structure of CRPV and HPV, especially within the E1 and L1 reading frames (Giri et al., 1985), epidermis tissue tropism, high incidence of spontaneous regression and possible neoplastic progression (Kreider and Bartlett, 1981; Kreider et al., 1990). Therefore, this model remains one of the widely used disease models for in vivo drug evaluations (Kreider et al., 1992; Ostrow et al., 1992, 1994). Indeed, comparable activity of several therapeutics has been demonstrated in this model as those observed in the clinic. These include podofilox, photodynamic therapy with dihemotoporphyrin ether, and ribavirin (Shikowitz et al., 1986; Kreider et al., 1992; Ostrow et al., 1992; Shikowitz, 1992). Topical activity of cidofovir has been mentioned briefly in a previously published abstract and book chapter (Kurtzman et al., 1993; Christensen and Kreider, 1999). The current study demonstrated that depending on the

infection status and treatment regimen, cidofovir treatment may range from extremely effective (complete cure of papilloma) to no effect at all. These results may offer an explanation why highly variable responses were observed in clinical studies, where the patient population receiving the same treatment varies in terms of extent and time of infection, stage of papilloma development, and other factors including the immune status of the patients (Cirelli and Tyring, 1994). The data demonstrated the importance of early treatment for a better therapeutic outcome. This observation with cidofovir was consistent with that observed for ribavirin (Ostrow et al., 1992) where early treatment was critical, but different to that obtained with topical podofilox (Kreider et al., 1992), where delayed treatment was almost similarly effective against papilloma growth. It is interesting to note that the effective dose of podofilox in that study was tenfold higher than that recommended for patients. In our current study, the concentration of cidofovir was similar to that used in the clinic.

The current experimental design made it possible to separate the potential topical effects of cidofovir from those acting via the systemic routes. Cidofovir is a potent DNA polymerase inhibitor with a broad spectrum of antiviral activity against several different DNA viruses (De Clercq, 1997; Lalezari, 1997). However, a big concern for cidofovir is its systemic toxicity (Hitchoock et al., 1996). As reported in a previous study, systemic delivery of 9-(2-phosphonylmethoxy)ethylguanine (PMEG, 1 mg/kg) reduced papilloma growth in the CRPV-infection model when the treatment was started on the same day of inoculation. The effective dose was associated with a significant systemic toxicity, as indicated by a significant loss of animal body weight (Kreider et al., 1990). Although topically applied therapeutics may mediate systemic effects, our present study demonstrated that topically applied cidofovir only affected the area which was directly exposed to drug treatment. Therefore, it is an advantage that topical cidofovir treatment is not associated with systemic toxicity. On the other hand, it is a disadvantage that its therapeutic effect is limited to the directly treated area only. Any latently infected sites will not benefit from the treatment applied at distance.

Severe local irritation and flaking did occur with topical treatment of cidofovir. This observation is consistent with that observed in the clinic (Snoeck et al., 1998a). Since cidofovir non-selectively inhibits DNA polymerase activity, its mechanism of action against papilloma development may be through non-specific apoptosis (Andrei et al., 1998). Therefore, the normal epidermis may also be damaged by local high concentrations of the compound. It is possible that lower concentrations of the compound may be associated with less severe side-effects. However, the moderate therapeutic effects of cidofovir with delayed treatment, as shown in the second experiment, argue against further reduction of the dosage.

It is possible that the hyperkeratinization associated with established warts may be a barrier for drug penetration into highly proliferating cells, thus reducing the effects of delayed treatment. Therefore, improved compound delivery may increase the therapeutic activity of cidofovir. As demonstrated in a recent study (Cundy et al., 1997), topical bioavailability of cidofovir was increased by tenfold with the addition of propylene glycol to the gel formulation. Therefore, it is possible to increase local delivery of cidofovir to the infected epidermis to achieve better therapeutic effects without changing systemic concentration dramatically (Cundy et al., 1997). However, care has to be taken since compound applied topically on the abraded skin did lead to high bioavailability (41%; Cundy et al., 1997). Apparently, the potential of improving therapeutic efficacy with optimized formulation remains to be studied.

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## References


Andrei, G., Snoeck, R., De Clercq, E., 1998. Detection of soluble nuclear matrix protein (NMP) released from apoptotic nuclei of human papillomavirus (HPV) positive cell lines treated with cidofovir. Antiviral Res. 37, A47 (Abstract 26).

Christensen, N.D., Kreider, J.W., 1999. Animal models of papillomavirus infections. In: Zak, O., Sande, M.A. (Eds.), Handook of Animal Models of Infection. Academic Press, New York, pp. 1039–1047 Chapter 125.

Cirelli, R., Tyring, S.K., 1994. Interferons in human papillomavirus infections. Antiviral Res. 24, 191–204.

Cundy, K.C., Lynch, G., Lee, W.A., 1997. Bioavailability and metabolism of cidofovir following topical administration to rabbits. Antiviral Res. 35, 113–122.

De Clercq, E., 1997. Acyclic nucleoside phosphonates in the chemotherapy of DNA virus and retrovirus infections. Intervirology 40, 295–303.

Gangemi, J.D., Pirisi, L., Angell, M., Kreider, J.W., 1994. HPV replication in experimental models: effects of interferon. Antiviral Res. 24, 175–190.

Giri, I., Danos, O., Yaniv, M., 1985. Genomic structure of the cottontail rabbit (Shope) papillomavirus. Proc. Natl. Acad. Sci. USA 82, 1580–1584.

Gross, G., 1997. Therapy of human papillomavirus infection and associated epithelial tumors. Intervirology 40, 368–377.

Hitchoock, M.J.M., Jaffe, H.S., Martin, J.C., Stagg, R.J., 1996. Cidofovir, a new agent with potent anti-herpesvirus activity. Antiviral Chem. Chemother. 7, 115–127.

Howley, P.M., 1990. Papillomavirinae and their replication. In: Fields, B.N., et al. (Eds.), Virology. Raven, New York, pp. 1625–1648.

Kreider, J.W., Bartlett, G.L., 1981. The shope papilloma-carcinoma complex of rabbits: a model system of neoplastic progression and spontaneous regression. Adv. Cancer Res. 35, 81–110.

Kreider, J.W., Baloogh, K., Olson, R.O., Martin, J.C., 1990. Treatment of latent rabbit and human papillomavirus infections with 9-(2-phosphonylmethoxy)ethylguanine (PMEG). Antiviral Res. 14, 51–58.

Kreider, J.W., Christensen, N.D., Christian, C.B., Pickel, M.D., 1992. Preclinical system for evaluating topical podofilox treatment of papillomas: dose-response and duration of growth prior to treatment. J. Invest. Dermatol. 99, 813–818.

Kurtzman, G., Pickel, M., Christensen, N., Kreider, J., 1993. Phosphonate nucleoside analogues are potent anti-papillomavirus agents in animal models. In: 33rd Interscience Conference on Antimicrobial Agents and Chemotherapy. (Abstract No. 1593).

Laimins, L.A., 1993. The biology of human papillomaviruses: from warts to cancer. Infect. Agents Dis. 2, 74–86.

Lalezari, J.P., 1997. Cidofovir: a new therapy for cytomegalovirus retinitis. J. Acquired Immune Defic. Syndr. Hum. Retrovirol. 14 (suppl. 1), S22–S26.

Majewski, S., Jablonska, S., 1997. Human papillomaviruses — associated tumors of the skin and mucosa. J. Am. Acad. Dermatol. 36, 659–685.

Naesens, L., Snoeck, R., Andrei, G., Balzarini, L., Neyts, J., De Clercq, E., 1996. HPMPC (cidofovir), PMEA (adefovir) and related acyclic nucleoside phosphonate analogues: a review of their pharmacology and clinical potential in the treatment of viral infections. Antiviral Chem. Chemother. 8, 1–23.

Ostrow, R.S., Forslund, K.M., McGlennen, R.C., Shaw, D.R., Schlievert, P.M., Ussery, M.A., Huggins, J.W., Faras, A.J., 1992. Ribavirin mitigates wart growth in rabbits at early stages of infection with cottontail rabbit papillomavirus. Antiviral Res. 17, 99–113.

Ostrow, R.S., Coughlin, S., McGlennen, R.C., Lu, Z., Zelterman, D., Faras, A.J., 1994. Topical CTC-96 accelerates wart growth in rabbits infected with cottontail rabbit papillomavirus. Antiviral Res. 24, 27–35.

Pfister, H., 1984. Biology and biochemistry of papillomaviruses. Rev. Physiol. Biochem. Pharmacol. 99, 112–181.

Phelps, W.C., Alexander, K.A., 1995. Antiviral therapy for human papillomaviruses: rational and prospects. Ann. Intern. Med. 123, 368–382.

Phelps, W.C., Barnes, J.A., Lobe, D.C., 1998. Molecular targets for human papillomaviruses: prospects for antiviral therapy. Antiviral Chem. Chemother. 9, 359–377.

Shikowitz, M.K., 1992. Comparison of pulsed and continuous wave light in photodynamic therapy of papilloma: an experimental study. Laryngoscope 102, 300–310.

Shikowitz, M.K., Steinberg, B.M., Abramson, A.L., 1986. Hematoporphyrin derivative therapy of papillomas. Arch. Otolaryngol. Head Neck Surg. 112, 42–46.

Snoeck, R., Van Ranst, M., Andrei, G., De Clercq, E., De Wit, S., Poncin, M., Clumeck, N., 1995. Treatment of anogenital papillomavirus infection with an acyclic nucleoside phosphate analogue. New Engl. J. Med. 333, 943–944.

Snoeck, R., Bossens, M., Parent, D., Delaere, B., De Greef, H., De Clercq, E., Safrin, S., McGuire, B., Jaffe, H.S., 1998a. A double-blind, placebo-controlled study of cidofovir gel for human papillomavirus (HPV) -associated genital warts. Antiviral Res. 37, A47 (Abstract 27).

Snoeck, R., Wellens, W., Desloovere, C., Van Ranst, M., Naesens, L., De Clercq, E., Feenstra, L., 1998b. Treatment of severe laryngeal papillomatosis with intralesional injections of cidofovir [(*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine]. J. Med. Virol. 54, 219–225.

Stanley, M.A., Masterson, P.J., Nicholls, P.K., 1997. In vitro and animal models for antiviral therapy in papillomavirus infections. Antiviral Chem. Chemother. 8, 381–400.

Van Cutsem, E., Snoeck, R., Van Ranst, M., Fiten, P., Opdenakker, G., Geboes, K., Janssens, L., Rutgeerts, P., Vantrappen, G., De Clercq, E., 1995. Successful treatment of a squamous papilloma of the hypopharynx-esophagus by local injections of (*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine. J. Med. Virol. 45, 230–235.

Zabawski, E.J. Jr, Cockerell, C.J., 1998. Topical and intralesional cidofovir: a review of pharmacology and therapeutic effects. J. Am. Acad. Dermatol. 39, 741–745.

Zar, J.H., 1984a. Multisample hypotheses: the analysis of variance. In: Zar, J.H. (Ed.), Biostatistical Analysis, second ed. Prentice-Hall, Englewood Cliffs, NJ, pp. 162–184.

Zar, J.H., 1984b. Multiple comparisons. In: Zar, J.H. (Ed.), Biostatistical Analysis, second ed. Prentice-Hall, Englewood Cliffs, NJ, pp. 185–205.