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Short Communication

Treatment of latent rabbit and human papillomavirus infections with 9-(2-phosphonylmethoxy)ethylguanine (PMEG)

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Summary

The acyclic nucleotide PMEG was studied for effectiveness against Shope papillomavirus (CRPV) infection of rabbits and human papillomavirus type 11 (HPV-11) infections of human foreskin xenografts in athymic mice. PMEG given in the latent period strongly suppressed the subsequent growth rates of Shope papillomas. PMEG starting in the latent period and continuing for the duration of the experiment, inhibited HPV-11 infections of human skin, including condyloma growth, and synthesis of viral DNA and capsid antigen. Drug toxicity paralleled the therapeutic effects in rabbits but there was much less toxicity in athymic mice.

Human papillomavirus type 11; Cottontail rabbit papillomavirus; Athymic mouse; Domestic rabbit, Acyclic nucleotide; PMEG

Human papillomavirus (HPV) genital infections are rising in incidence (Kiviat et al., 1989; Reeves et al., 1989). Infection with several HPV types may contribute to the development of cancer of the uterine cervix (Kiviat et al., 1989; Reeves et al., 1989). Current treatments are only partially effective at eradicating HPV infections of the uterine cervix (Reichman and Strike, 1989). The most commonly employed modality is laser surgery. While this treatment usually results in eradication of visible lesions, follow-up studies reveal a high incidence of recurrences. Further,

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it is now clear that many HPV infections of the cervix may be 'inapparent', i.e. undetectable on routine examinations (Fuchs et al., 1988; Slater et al., 1987; Ferenczy et al., 1985). Surgical treatments are limited to detectable lesions and offer little for the eradication of inapparent, potentially recurrent infections. Drug treatments, especially if given by the systemic route, have the potential to eliminate latent or inapparent infections. Current drugs for the treatment of HPV infections are not very effective. The best results have been obtained with intralesional interferon (Reichman and Strike, 1989), but this route requires that the lesions be macroscopic. There is a need for drugs which could be given systemically and eliminate recurrent and inapparent HPV infections.

A class of drugs which might have treatment potential are the acyclic nucleotides (De Clercq et al., 1986). The prototype compound, HPMPA, was active against a number of DNA viruses including herpes simplex types 1 and 2 (Bronson et al., 1989), cytomegalovirus, varicella-zoster virus, Epstein-Barr virus, adenovirus, and Moloney sarcoma virus (De Clercq et al., 1987; Lin et al., 1987; Baba et al., 1987). PMEG is a member of this class of compounds (De Clercq et al., 1987). HPMPA is >50-fold more effective than acyclovir in the treatment of murine HSV-1 infections (De Clercq et al., 1986). The objective of our studies was to determine if PMEG treatment in the latent period could suppress the growth of subsequent papillomavirus infections. We used 2 complementary model systems, the Shope papilloma of rabbits (reviewed in Kreider and Bartlett, 1981), and the HPV-11 human foreskin xenograft system which we have recently described (Kreider et al., 1985b).

The Shope papillomavirus is a naturally-occurring infection of midwestern cottontail rabbits (reviewed in Kreider and Bartlett, 1981)). It is readily transmitted to domestic rabbits but comparatively little virion is produced in those hosts. About 2–3 months after infection, 10–40% of the infected rabbits experience spontaneous regression of all lesions, possibly secondary to an immune response to papilloma cells (Kreider, 1963). One to two years after infection, squamous cell carcinomas develop in >50% of the hosts. Thus, this animal system is analogous to human genital papillomavirus infections which are associated with a high incidence of spontaneous regression and possible neoplastic progression (Kreider and Bartlett, 1985a).

It is difficult to conduct experiments with human papillomaviruses since they are highly species-specific and no satisfactory culture systems have been developed. Therefore, antiviral agents cannot be readily tried against HPV infections because of the lack of pre-clinical models. We have recently developed an HPV model system which overcomes those difficulties (Kreider et al., 1985b). In that system, small fragments of human foreskin are infected with HPV-11 and transplanted beneath the renal capsule of the athymic female mouse. After three months, the infected cells produce condylomatous lesions which are identical in every respect to naturally-occurring human lesions induced by HPV-11. This system offers an opportunity for the experimental therapist to test the efficacy of antiviral agents against HPV-11 infected human cells.

New Zealand White rabbits (1.8–2.4 kg) of both sexes were purchased from

Hazleton Labs, Denver, PA. They were individually housed in stainless steel cages, and fed laboratory chow ad libitum supplemented occasionally with fresh kale. The CRPV stock used in these experiments was obtained from wild trapped Kansas cottontails. It was used at specified dilutions from a 10% extract (w/v) in phosphate-buffered saline. The stock was prepared in 1968 and has been stored at −70°C with no detectable loss of infectivity. Rabbits were shaved with electric clippers on the dorsum. Scarified sites (1–2 cm diameter) were produced by superficial abrasion with a #10 scalpel blade held perpendicular to the skin, with gentle scraping until slight oozing of serum occurred. Virus suspensions were administered to these sites in 50 μl aliquots and allowed to air-dry before the rabbits were returned to their cages. Shope papillomas were measured weekly in three dimensions and the geometric mean diameters calculated. For each treatment group, the mean ± sem was calculated and the significance of the differences observed determined with Student's *t*-test.

Methods for the production of HPV-11 stocks have been previously described (Kreider et al., 1987a). The HPV-11 virus stock used in this study was from the third laboratory passage. Athymic female mice (*nu/nu* on a Swiss background) were purchased from Harlan Sprague Dawley, Inc., Madison, WI. They were housed in flexible film isolators and provided with sterile air, water and laboratory chow. All surgery was conducted under anesthesia in sterile housings, through glove-box type sleeves. Human foreskins were obtained at routine neonatal circumcisions at hospitals in our region. They were placed in Minimum Essential Medium with gentamicin (800 μg/ml) and grafted within two days of excision. Grafts were cut and infected with HPV-11 by methods previously detailed (Kreider et al., 1987b). Grafts were placed beneath the renal capsule (Kreider et al., 1986) of athymic mice and allowed to grow for 90 days. PMEG was given (0.1 mg/kg) s.c.-five days per week for the full 90 days. At that time the mice were killed and the kidneys removed. Condylomas were measured in two dimensions and the geometric mean diameter of each calculated.

We determined the optimal dose of PMEG (provided by Bristol-Myers Co.) for treatment of latent infections with Shope papillomavirus. Rabbits were scarified on the dorsum at four sites, two on each side. The left and right anterior sites were infected with a 10^{-1} dilution of CRPV. The two posterior sites on each side received CRPV at 10^{-2} dilution. Rabbits were given PMEG at 0.01, 0.1, or 1.0 mg/kg, s.c., in the nape of the neck, each early morning and late afternoon. The injections were given for ten consecutive days when treatment of all groups was terminated because of toxicity noted in the animals receiving the highest dose.

Strong therapeutic effects were obtained with 1.0 mg/kg PMEG (Fig. 1). These effects were greatest on the papillomas induced by the lowest dose of CRPV. Modest growth inhibition was also obtained with 0.1 mg/kg, but only on the papillomas induced by the higher virus dose (upper graph).

Toxicity was evident as substantial epilation, lassitude, and bodyweight loss (Fig. 2). Body weight loss was most severe in the group which received the highest treatment dose (1.0 mg/kg). Despite cessation of treatment on day 10, significant weight regain was not observed in this group until day 30. The rate of gain when measured was similar to the rate of gain in the other groups. Weight gain in the

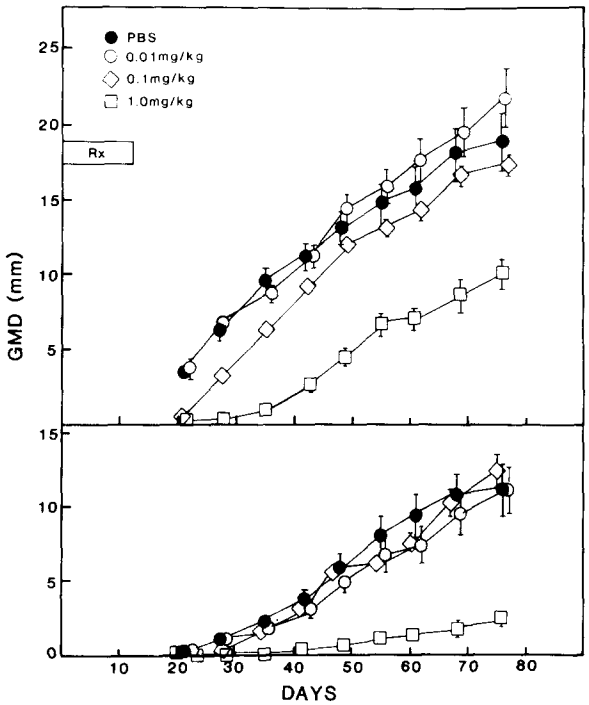

Fig. 1. Effect of PMEG treatment at various doses beginning on day 0 on Shope papilloma sizes (geometric mean diameter). PMEG was given twice daily for 10 consecutive days. Papillomas were induced in duplicate on each side of the back with dilutions of CRPV at 10^{-1} (upper graph) or 10^{-2} (lower graph). Each symbol represents the mean±SEM (vertical bars) of the weekly measurements on groups of ten rabbits.

next highest treatment dose (0.1 mg/kg) slowed beginning on day 20, but returned to the usual rate of gain on day 50. Peripheral leucocyte counts were conducted on days 14, and 40. These were unremarkable except for mild lymphocytosis on day 14 in the highest dose treatment group.

The objective of the next experiment was to determine if PMEG could inhibit the growth of human foreskin which had been infected with HPV-11 and transplanted to the subrenal capsular space of athymic mice. The results demonstrated significant suppression of human condylomas in all PMEG-treated groups (Fig. 3). In the first treatment group, PMEG (1.0 mg/kg) treatment was given s.c. 5 days per week, beginning on the day of virus infection and continuing for 90 days. Condyloma suppression was greatest in this group, but almost as great a suppression was obtained when the onset of PMEG treatment was delayed for 30 days. When only 10% of the PMEG dose was given in the last group, beginning on day 0, suppression was almost as great as in the second group which received 10 times the dose. No toxicity was evident in any of the treatment groups until the last few weeks of the experiment, when a mild bodyweight loss was observed. Peripheral leucocyte counts were not done on the mice.

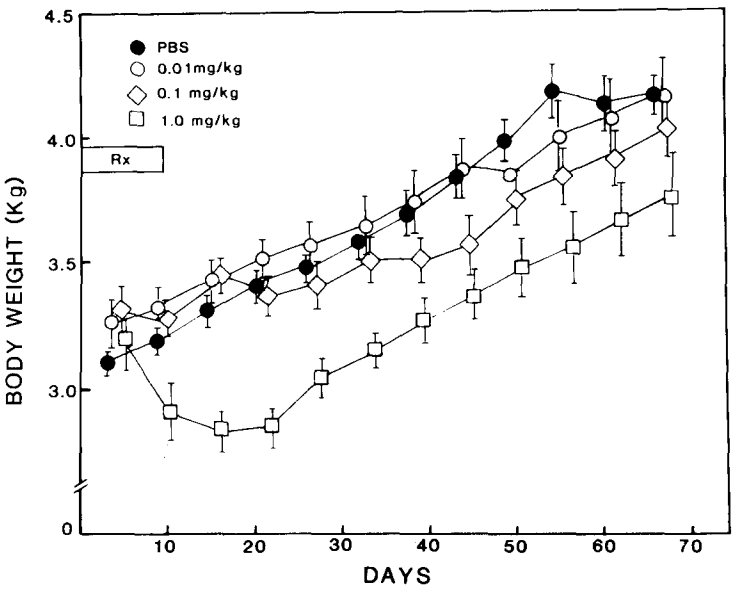

Fig. 2. Effect of PMEG treatment on rabbit bodyweights. Each symbol represents the mean±SEM (vertical bars) of the measurements on groups of ten rabbits, weighed weekly.

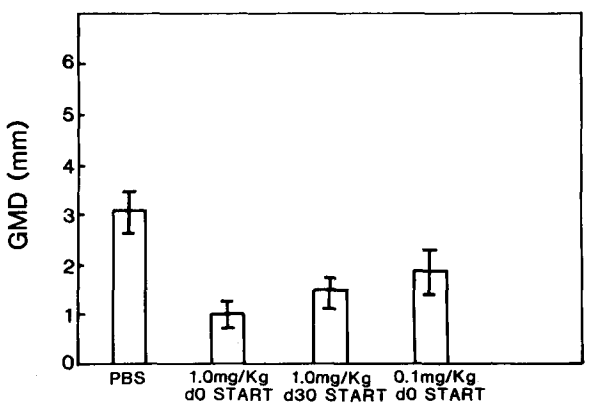

Fig. 3. Effect of PMEG treatment beginning on day 0 on condylomatous transformation of human foreskin by HPV-11. Athymic nude mice received bilateral, subrenal capsular xenografts of infected human foreskin. Animals were treated with either PBS (first bar), or 1.0 mg/kg PMEG beginning on day 0 (second bar), or the same dose of PMEG beginning on day 30 (third bar), or 0.1 mg/kg PMEG beginning on day 0 (fourth bar). Values represent the mean±SEM of 20 grafts per group.

We also examined the expression of HPV-11 in PMEG-treated and control experimental condylomas. Koilocytosis (perinuclear clear spaces and nuclear wrinkling and pyknosis) were the criteria for condylomatous transformation in hematoxylin and eosin (H&E) stained paraffin sections. Production of papillomavirus virion

TABLE 1
Influence of PMEG treatment in HPV-11 experimental condyloma virus expression

Treatment groups	No. grafted	No. condylomas/No. survived		
		H&E	GSA	HPV-II DNA
PBS	20	8/13	9/13	6/13
0.1 mg/kg	20	5/13	4/13	5/13
1.0 mg/kg	18	1/8	1/8	1/8
1.0 mg/kg[a]	16	0/8	4/8	3/8

[a]After 30 days.

group-specific antigen (GSA) was evaluated in the same paraffin sections (Jenson et al., 1980). In situ hybridization was conducted to demonstrate HPV-11 DNA in the paraffin sections. The results (Table 1) demonstrated that 1.0 mg/kg PMEG, begun on either day 0 or on day 30, slightly but not significantly reduced survival of the xenografts. However, condylomatous transformation was also strongly suppressed in H&E sections of the surviving grafts. Surviving grafts were also less likely to be positive for GSA and HPV-11 DNA by in situ hybridization. In the group where PMEG treatment was started at 30 days, there was GSA and HPV-11 DNA present in the keratin core apparently produced before drug treatment. The grafts were dead when the mice were killed at 90 days duration of condyloma growth. Thus, PMEG treatment killed some of the grafts but suppressed condylomatous transformation and viral antigen and DNA in the survivors.

The studies reported here are the first investigations of the effectiveness of PMEG against animal or human papillomavirus infections. The experiments were designed so that PMEG treatment began on the day of virus infections. Thus, we were treating *latent* infections, i.e. before grossly visible lesions developed. In the rabbits, treatment was terminated in the high dose group after 10 days because of severe toxicity. This was evident as weight loss, epilation, and lymphopenia. Despite cessation at day 10 of treatment in this group, there was substantial inhibition of subsequent papilloma growth. This suggests that PMEG interfered with the earliest stages of papilloma development, possibly by killing a proportion of the initially-infected cells. A major contribution to the therapeutic benefit might have been due to direct papilloma cell toxicity rather than antiviral effects. Alternatively, the drug might have altered virus–cell interactions essential to the papillomatous transformation, thus aborting infection in some of the cells. There is no data which would permit resolution of those possibilities, and they are not mutually exclusive. Suppression of Shope papillomas induced with the lower dose of virus was greater than that of the higher virus dose. The rate of growth and frequency of infection are known to be related to dose of Shope papillomavirus (Bryan and Beard, 1940), so it is not surprising that PMEG was most effective on the smaller, slower growing papillomas.

In the HPV-11 xenograft system, PMEG was started on either the day of virus infection or 30 days later, and continued in both groups for a total of 90 days. In this system, condylomatous transformation first appears at 40–50 days post-infection.

Thus, the drug was given starting at either the early or late latent phases of infection. It seems most likely that the drug suppressed the infected cells early in the treatment course, since this was clearly shown in the Shope papilloma experiment. However, since drug treatment was continuous for three months, it is likely that there was inhibition of papilloma cell growth during the entire treatment period. We also found that PMEG killed some of the xenografts, but it was not possible to conclude that this was a specific effect only on condylomas. Expression and replication of HPV-11 was suppressed in the surviving grafts. Toxicity for mice was much less than that observed in rabbits, so that sustained administration was possible in this experiment. Interspecies factors responsible for this differential toxicity are not known.

Thus, a relatively brief PMEG treatment during latency significantly retarded subsequent growth of Shope rabbit papillomas. PMEG treatment of human skin xenografts infected with HPV-11 also retarded the growth of resultant human condylomas. At effective dose levels in rabbits, PMEG was very toxic, but the toxicity was much less in mice. PMEG is an effective drug for treatment of latent papillomavirus infections.

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