The Laryngoscope
Lippincott Williams & Wilkins, Inc.

Local and Systemic Effects of Intralaryngeal Injection of Cidofovir in a Canine Model

Dinesh K. Chhetri, MD; Babak Jahan-Parwar, MD; Stephen D. Hart, MD; Sunita M. Bhuta, MD; Gerald S. Berke, MD; Nina L. Shapiro, MD

Objective: **The safety of intralaryngeal injection of cidofovir remains a concern. Our goal was to evaluate local and systemic effects of intralaryngeal injection of cidofovir.** ***Study Design:*** **Animal study using a canine model.** ***Methods:*** **Two groups of three young beagle dogs (6 vocal folds in each group) were used. Subepithelial vocal fold injections were performed in each group biweekly for 6 months with 0, 2.5, 5, 10, 20, and 37.5 mg cidofovir in a 0.5 mL volume. Direct laryngoscopy was performed at each injection interval. Complete blood cell count and renal parameters were measured at baseline and monthly thereafter. Histopathologic examination of the vocal folds was performed after the 6-month injection period in one group of animals and after an additional 6-month observation period in the second group.** ***Results:*** **Endomysial edema with muscle fiber separation and dose-dependent atrophy and scarring of the vocal folds was present. Onset of atrophy and scarring was observed after 3, 7, and 11 injections in the vocal folds injected with 37.5, 20, and 10 mg cidofovir, respectively. After the 6-month observation period, recovery of histologic abnormalities was complete in the low-dose (0, 2.5 mg) vocal folds, near complete in the intermediate-dose (5, 10 mg) vocal folds, and no apparent recovery was seen in the high-dose (20, 37.5 mg) vocal folds. Leukocyte count and renal parameters remained unchanged at up to 4.26 mg/kg body weight of systemic dose of cidofovir.** ***Conclusions:*** **Intralaryngeal cidofovir leads to dose-dependent scarification of the vocal folds that appears irreversible at higher doses. Lower concentrations of this drug should be used in intralesional intralaryngeal use.**
Key Words: **Larynx, cidofovir, drug toxicity.**
Laryngoscope, **113:1922–1926, 2003**

INTRODUCTION

Cidofovir (Gilead Sciences, Foster City, CA) is a nucleoside analogue antiviral drug approved for the treatment of cytomegalovirus retinitis in patients with acquired immune deficiency syndrome. It has also been shown to be effective against a broad range of DNA viruses, including human papillomas virus (HPV).[1,2] Several case reports have found dramatic resolution of laryngeal papillomas after intralesional injection of cidofovir.[1–7] This drug is therefore being increasingly used by otolaryngologists as adjunctive therapy in the treatment of aggressive recurrent laryngeal papillomatosis.

The safety of intralaryngeal injection of cidofovir has not been studied. When administered intravenously, the major toxicity of cidofovir is renal impairment. To reduce possible nephrotoxicity, the manufacturer recommends intravenous prehydration with normal saline and administration of probenecid with each intravenous drug infusion. Neutropenia is the other major side effect of cidofovir therapy, and monitoring of the leukocyte count is also recommended during therapy. The renal and hematologic effects of intralaryngeal injection of cidofovir are unknown. Finally, laryngeal tissue reaction to injections of cidofovir has not been studied.

The primary focus of published reports on intralesional treatment of laryngeal papillomas with cidofovir has been control of papillomas. However, despite its increasing use, the role of this drug in the treatment of laryngeal papillomas remains to be established. In light of the increasing use of cidofovir for laryngeal papillomas, our goal was to study the local (tissue reaction) as well as systemic (renal and hematologic) effects of repeated intralaryngeal cidofovir injections at various predetermined doses.

MATERIALS AND METHODS

The institutional animal research committee approved this study. A canine model was used because the canine laryngeal structure closely resembles the human larynx. Two groups of three young healthy beagle dogs (6 vocal cords per group) were used. Experimental injection parameters were identical for each group. The first group (group 1) was killed immediately at the conclusion of the 6-month injection period. The second group (group 2) was killed after a 6-month observation period.

This article was presented at the Western Section Meeting of the Triological Society, Indian Wells, California, January 31–February 2, 2003.

From the Division of Head and Neck Surgery (D.K.C., B.J.P., G.S.B., N.L.S.); and the Department of Pathology and Laboratory Medicine (S.D.H. and S.M.B.), University of California Los Angeles School of Medicine, Los Angeles, California, U.S.A.

Editor's Note: This Manuscript was accepted for publication June 9, 2003.

Address Correspondence to Dr. Dinesh K. Chhetri, 62-132 CHS, Head and Neck Surgery, Mail Code 162418, UCLA School of Medicine, Los Angeles, CA 90095, U.S.A. E-mail: dchhetri@ucla.edu

During each injection, anesthesia was induced using intravenous pentobarbital, and the animal was placed on the operating table for direct laryngoscopy. Vital signs were continuously monitored. A rigid 0° endoscope attached to a Charged Coupled Device (CCD) camera (Karl Storz, Telecam 20 2101 20, Culver City, CA) with illumination from a stroboscopic light source (Kay Elemetrics, Model RLS 9100, Lincoln Park, NJ) was inserted into the hypopharynx. The appearance of the larynx was noted and recorded on a 3/4 inch videocassette recorder (Sony, Model VO9850, Park Ridge, NJ). Vocal cord injection was then performed, and the animal was allowed to wake up from anesthesia under close monitoring. Each procedure took approximately 5 minutes. Injections were performed every 2 weeks for a total of 12 injections.

Injections were made into both vocal folds of each animal. The drug is supplied as a clear and colorless solution in clear glass vials, each containing 375 mg of anhydrous cidofovir in 5 mL aqueous solution, at a concentration of 75 mg/mL. Cidofovir was diluted to the desired concentration in normal saline. Each vocal fold was injected with 0.5 mL volume of the drug at predetermined concentrations. One animal from each group received control saline injection to the left vocal fold and 2.5 mg cidofovir (5mg/mL) to the right vocal fold. A second animal received 5 mg (10 mg/mL) and 10 mg (20mg/mL), and the third animal received 20 mg (40mg/mL) and 37.5 mg (75mg/mL) to the left and right vocal folds, respectively. The injection volume was delivered at the midcord in the subepithelial layer using a 25 gauge needle on a 3 mL syringe.

Venipuncture was performed at baseline and every 4 weeks, and a complete blood cell count, renal parameters (blood urea nitrogen [BUN] and creatinine), electrolytes, and liver function tests were measured. At the end of the 6-month treatment period, one group was humanely killed per established protocol. The second group was observed for another 6 months and then killed. Laryngectomy was performed after death. The larynges were fixed in formaldehyde, decalcified using EDTA, and coronally cut at 0.5 cm slices from the anterior commissure to the vocal process of the arytenoid and embedded in paraffin blocks. Microscopic sections 3 μm thick were then cut from the middle vocal fold areas and stained with hematoxylin-eosin (H&E) for histologic analysis using routine methods used almost daily at the University of California Los Angeles Clinical Laboratories.

TABLE I.
Experimental Groups and Cidofovir Doses.

Animal/Weight (kg)	Vocal Cord Side	Intralesional Dose (mg)	Systemic Dose (mg/kg)
Group 1			
1/14.5	L	0	0.18
	R	2.5	
2/16.0	L	5	0.94
	R	10	
3/14.0	L	20	4.11
	R	37.5	
Group 2			
1/18.0	L	0	0.14
	R	2.5	
2/13.5	L	5	1.11
	R	10	
3/13.5	L	20	4.26
	R	37.5	

Group 1 was killed at the end of study period.
Group 2 was killed after an additional 6-month observation period.

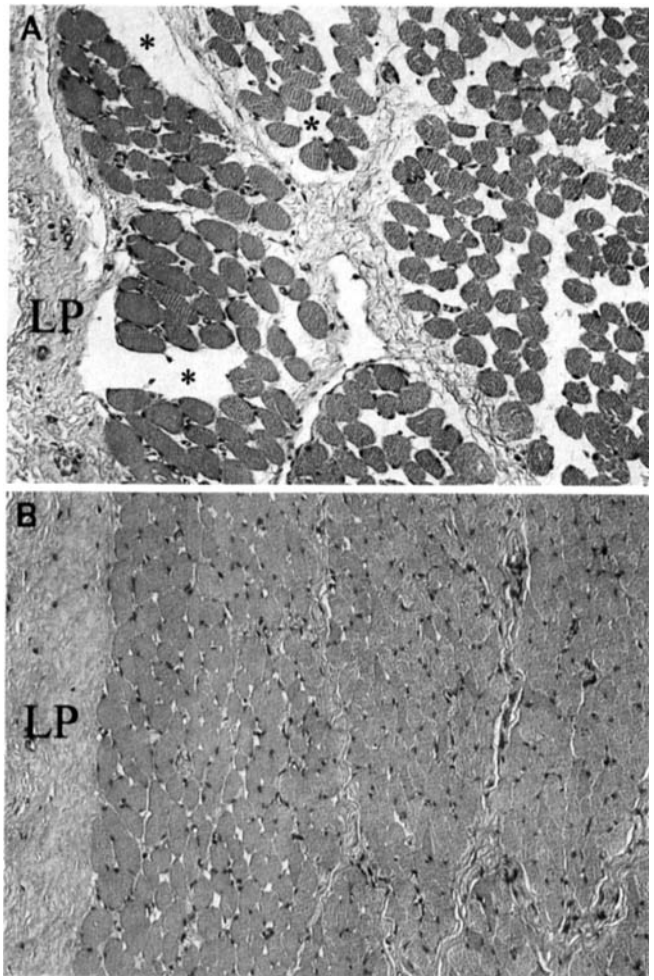

Fig. 1. Vocal folds injected with 2.5 mg (5 mg/mL) cidofovir every 2 weeks for 6 months (hematoxylin-eosin, magnification ×200). (A) At the end of the 6-month injection period, moderate endomysial edema is seen as expanded spaces between individual muscle fibers (*). (B) Complete recovery is seen after the 6-month observation period. LP = lamina propria.

RESULTS

All animals tolerated vocal fold injections. All animals were injected every 2 weeks for 24 weeks. Direct laryngoscopy was performed just before the next scheduled injection. The effective systemic dose of cidofovir ranged between 0.14 and 4.26 mg/kg of body weight (Table I).

Direct Laryngoscopy

After three injections, punctate nodular thickening was observed in several animals in a dose-independent manner at the needle insertion site on the vocal fold surface. These tended to resolve with time and were attributed to needle trauma. However, vocal folds receiving 37.5 mg cidofovir injections (75 mg/mL) appeared somewhat pale and thicker throughout the length of the vocal fold. After seven injections, these folds not only continued to

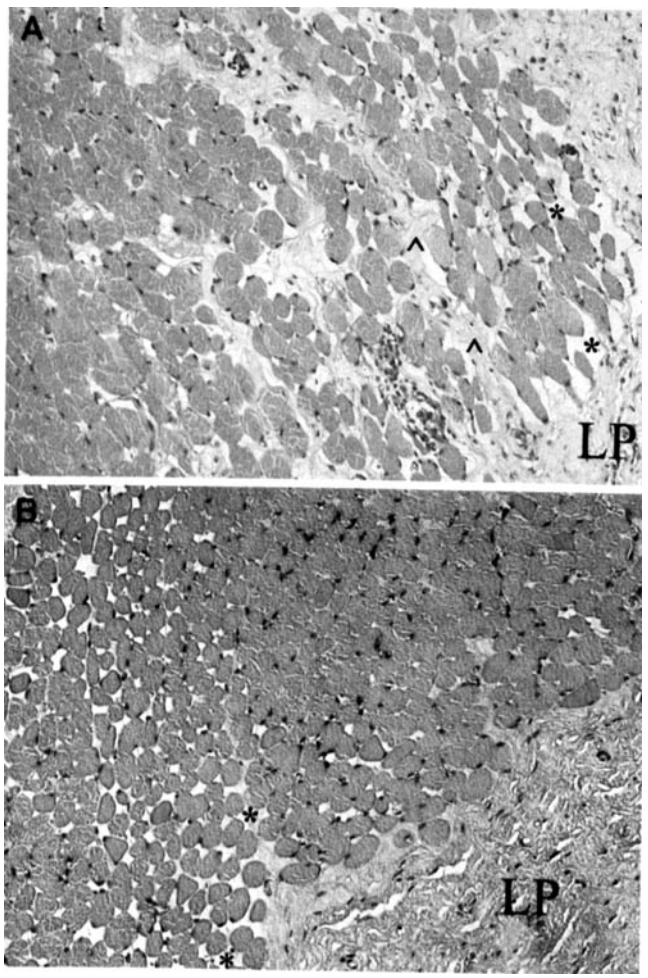

Fig. 2. Vocal folds injected with 5 mg (10 mg/mL) and 10 mg (20 mg/mL) cidofovir every 2 weeks for 6 months (hematoxylin-eosin, magnification ×200). (A) At the end of the 6-month injection period, some endomysial fibrosis is apparent as an increased amount of connective tissue between muscle fibers (^). A moderate amount of endomysial edema is also present (*). (B) Near complete recovery is seen after 6-month observation period, and only mild endomysial edema is present (*). LP = lamina propria.

appear paler but also thinner, suggesting vocal fold atrophy. At this time, vocal folds receiving 20 mg cidofovir injections (40 mg/mL) also appeared pale. After 10 injections, vocal folds injected with 20 mg and 37.5 mg cidofovir were grossly atrophic. In addition, increased resistance to vocal fold injection was encountered, suggesting vocal fold scarring. Vocals folds injected with control saline and 2.5 mg (5 mg/mL) and 5 mg (10 mg/mL) cidofovir appeared normal throughout the experimental period. Vocals folds injected with a 10 mg (20 mg/mL) dose also appeared pale after the 11th injection; however, the study was ended after 12 injections per protocol.

Histology

The epithelium of the vocal fold surface was normal in all animals without evidence of dysplasia, metaplasia, or carcinoma. No distinctive changes in the lamina propria layer were noted using the H&E stain. The muscle layer of the vocal fold was the most dramatically affected. The histologic changes were similar between the vocal folds injected with 0 and 2.5 mg cidofovir, 5 and 10 mg cidofovir, and 20 and 37.5 mg cidofovir. Therefore, for the purposes of histologic description, they are grouped in into low-, intermediate-, and high-dose groups, respectively.

A moderate amount of endomysial edema was present in the thyroarytenoid muscle at the low doses (0 and 2.5 mg cidofovir per dose). The edema was limited to approximately the superficial 20% of the muscle, and deeper muscle fascicles were intact without endomysial edema. After the 6-month observation period, this edema completely resolved, and the muscle appeared normal (Fig. 1).

Moderate amounts of endomysial edema were also found in the thyroarytenoid muscle at the intermediate doses (5 and 10 mg cidofovir per dose). The edema was limited to the superficial one third of the muscle, and

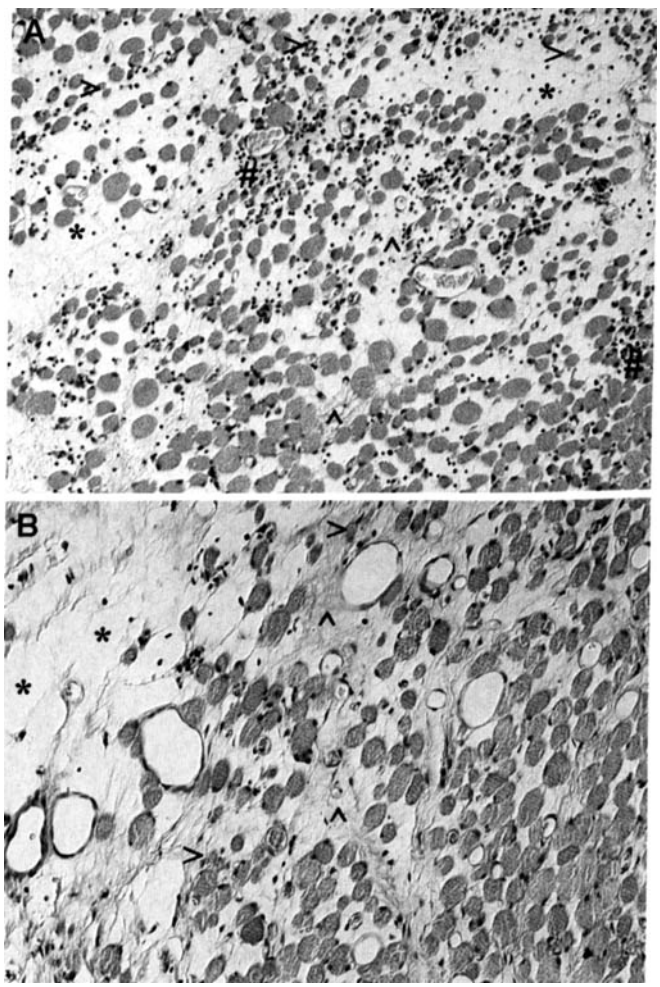

Fig. 3. Vocal folds injected with 20 mg (40 mg/mL) and 37.5 mg (75 mg/mL) cidofovir (hematoxylin-eosin, magnification ×200). (A) After the 6-month injection period, muscle damage is seen as atrophied, necrotic, and regenerative muscle fibers (>). Severe endomysial edema (*), fibrosis (^), and an associated perimysial mononuclear inflammatory infiltrate is also present (#). (B) No recovery is seen after the 6-month observation period, although there is a decrease in the inflammatory infiltrate.

TABLE II.
Hematologic (white blood cell count) and Renal Parameters (BUN/creatinine) at Baseline and at the End of the 6-Month Cidofovir Injection Period (normal parameters).

Animal/ Weight (kg)	Systemic Drug Dose (mg/kg)	WBC Count (6.0–17.0 × $10^3/\mu L$)		BUN (7.0–27.0 mg/dL)		Creatinine (0.4–1.8 mg/dL)	
		Baseline	6-Months	Baseline	6-Months	Baseline	6-Months
Group 1							
1/14.5	0.18	9.4	7.0	12.0	14.0	1.0	0.7
2/16.0	0.94	10.7	9.5	11.0	12.0	1.0	1.0
3/14.0	4.11	9.1	9.1	8.0	12.0	0.8	0.8
Group 2							
1/18.0	0.14	9.6	9.0	10.0	14.0	1.0	0.6
2/13.5	1.11	5.7	8.0	13.0	14.0	1.0	0.8
3/13.5	4.26	11.7	10.4	11.0	10.0	1.0	0.8

Group 1 was killed at the end of study period.
Group 2 was killed after an additional 6-month observation period.
WBC = white cell blood count; BUN = blood urea nitrogen.

deeper muscles appeared intact. In contrast with the low-dose group, some endomysial fibrosis was present as well. After the 6-month observation period, near complete resolution of endomysial changes were seen. Several areas of mild residual endomysial edema without fibrosis could still be appreciated in the superficial layers (Fig. 2).

The most dramatic changes of the thyroarytenoid muscle were seen at the high doses (20 and 37.5 mg cidofovir per dose). Full thickness atrophy and necrosis of the thyroarytenoid muscle was present. Severe edema and fibrosis of the endomysium and an associated perimysial inflammatory infiltrate composed of plasma cells, lymphocytes, and macrophages were present. No recovery was seen after the 6-month observation period (Fig. 3).

Laboratory

There were no clinically significant changes in leukocyte count, renal parameters (BUN/creatinine), electrolytes, or liver function tests (Table II).

DISCUSSION

Recurrent respiratory papillomatosis (RRP) is a recalcitrant disease that is often frustrating to treat. The multitude of recently published case reports reporting better control of RRP with addition of intralesional cidofovir provide a new armamentarium with which to treat this disease. However, a better understanding of the local and systemic toxicity of intralaryngeal injection of cidofovir must be obtained before embracing this therapeutic modality. Pain, pruritus, and rash at the application site after topical cidofovir (1% gel) and development of subcutaneous nodules at the injection site (presumably fibrosis) after subcutaneous injection (3 mg/kg) have been reported.[8,9] Acute renal failure in a patient with chronic renal failure after topical use of 1% cidofovir gel has also been reported.[10]

This study finds that intralaryngeal injection of cidofovir leads to dose-dependent toxicity to laryngeal muscles. Published reports on intralesional injection of cidofovir for RRP have typically used the drug at a concentration between 1.25 mg to 5 mg/mL and a maximum dose of 1 mg/kg body weight. In this study, although we see complete resolution of endomysial edema in the low-dose (2.5 mg/dose) group, residual endomysial edema was still present in the intermediate-dose (5 and 10 mg/dose) group even after 6 months of observation. Residual edema in the intermediate-dose group was limited to the most superficial thyroarytenoid muscle layer. At higher doses (20, and 37.5 mg/dose), cidofovir injections caused full thickness damage of the thyroarytenoid muscle. These changes appear irreversible because no evidence of recovery was seen after the 6-month observation period. Lower concentrations of cidofovir should therefore be used in intralesional intralaryngeal use for RRP, and injection into the true vocal folds must be limited to prevent scarring.

One may question whether the changes observed in this study were effected by the dose or the concentration of cidofovir. Would similar results be obtained if the same amount of drug were injected at different concentrations? At the small volume used to inject the vocal cords for this study, the dose and concentration are tightly related, the concentration being just twice the dose, and the results can be viewed in terms of either the dose or the concentration. However, because histologic abnormalities of muscle were localized to the injection site, and muscle further away from the injection site appear relatively normal, the concentration of the drug is likely more important. The volume injected into the vocal folds must necessarily be restricted to avoid airway compromise. In the treatment of children with laryngeal papillomatosis, we use 1 mg cidofovir/kg body weight at 5 mg/mL concentration. A 30 kg child would thus receive 6 mL of cidofovir injection at multiple sites in the larynx.[11] The vocal cords typically receive only 0.5 mL (2.5 mg) on each side. Using these parameters, we have never seen any laryngoscopic evidence of atrophy or scarring.

The pharmacokinetic properties of intralaryngeal injection of cidofovir have not been studied. Wachsman et al.[9] conducted a phase I and II trial to assess the bioavail-

ability and pharmacokinetics of cidofovir administered orally and subcutaneously. They reported equivalent bioavailability of the subcutaneous injection to intravenous route. Given anatomic similarities of the subcutaneous layer to the subepithelial vocal fold layer, the bioavailability of intralaryngeal injection is expected to be equivalent to the intravenous route. Thus, the effective systemic dose in our study animals ranged from 0.14 to 4.26 mg/kg body weight (Table I). There was no evidence of renal or hematologic toxicity at these doses (Table II). In addition, complete electrolyte panels and liver function tests were also obtained in the animals, and no noticeable changes were observed. Therefore, from the systemic standpoint, cidofovir appears to be safe at the maximum dose of 1 mg/kg typically used for intralaryngeal intralesional use.

CONCLUSION

Intralaryngeal injection of cidofovir induced dose-dependent localized toxic injury to muscle, and induced vocal fold atrophy and scarring. These effects appear irreversible at the higher doses. The epithelial and lamina propria layers appeared histologically unaffected. Renal and hematologic toxicity was not observed up to a systemic dose of 4.26 mg/kg of body weight. Lower concentrations of cidofovir should be used in intralesional intralaryngeal injections.

BIBLIOGRAPHY

1. Van Cutsem E, Snoeck R, Van Ranst M, et al. Successful treatment of a squamous papilloma of the hypopharynx-esophagus by local injections of (S)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine. *J Med Virol* 1995;45: 230–235.
2. Safrin S, Cherrington J, Jaffe HS. Clinical uses of cidofovir. *Rev Med Virol* 1997;7:145–156.
3. Snoeck R, Wellens W, Desloovere C, et al. Treatment of severe laryngeal papillomatosis with intralesional injections of cidofovir [(S)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine]. *J Med Virol* 1998;54:219–225.
4. Wilson WR, Hashemiyoon R, Hawrych A. Intralesional cidofovir for recurrent laryngeal papillomas: preliminary report. *Ear Nose Throat J* 2000;79:236–238, 240.
5. Pransky SM, Magit AE, Kearns DB, et al. Intralesional cidofovir for recurrent respiratory papillomatosis in children. *Arch Otolaryngol Head Neck Surg* 1999;125:1143–1148.
6. Pransky SM, Brewster DF, Magit AE, Kearns DB. Clinical update on 10 children treated with intralesional cidofovir injections for severe recurrent respiratory papillomatosis. *Arch Otolaryngol Head Neck Surg* 2000;126:1239–1243.
7. Chhetri DK, Blumin JH, Shapiro NL, Berke GS. Office-based treatment of laryngeal papillomatosis with percutaneous injection of cidofovir. *Otolaryngol Head Neck Surg* 2002; 126:642–648.
8. Snoeck R, Bossens M, Parent D, et al. Phase II double-blind, placebo-controlled study of the safety and efficacy of cidofovir topical gel for the treatment of patients with human papillomavirus infection. *Clin Infect Dis* 2001;33:597–602.
9. Wachsman M, Petty BG, Cundy KC, et al. Pharmacokinetics, safety and bioavailability of HPMPC (cidofovir) in human immunodeficiency virus-infected subjects. *Antiviral Res* 1996;29:153–161.
10. Bienvenu B, Martinez F, Devergie A, et al. Topical use of cidofovir induced acute renal failure. *Transplantation* 2002;73:661–662.
11. Chhetri DK, Shapiro NL. A scheduled treatment protocol for the treatment of juvenile recurrent respiratory papillomatosis with intralesional cidofovir. *Arch Otolaryngol Head Neck Surg*. 2003;129:1081–1085.