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Safety and tolerance of cidofovir as a 2% gel for local application in high-grade cervical intraepithelial neoplasia: A phase 1 investigation

Michel Bossens[1], Maud Jost[2], Catherine Van Pachterbeke[1], Viviane De Maertelaer[1], Philippe Simon[1], Francis Frankenne[2], Philippe Hubert[3], Brigitte Evrard[3], and Robert Snoeck[4]

[1]Université Libre de Bruxelles, ULB, Brussels, [2]Mithra Pharmaceutical, [3]Université ULG, CIRM, and [4]Rega Instituut, Leuven, Belgium

Key words
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Abstract. Objectives: The primary objective was to evaluate the safety and local tolerance of a topical 2% (w/w) cidofovir gel, applied directly to the cervices of women with high-grade cervical intraepithelial neoplasia (CIN 2+). The secondary objective was to evaluate the pharmacokinetics of cidofovir during the treatment. Materials and methods: Nine women with CIN 2+, were treated with a course of 3 g of cidofovir gel, applied locally once per week for 3 weeks in total (9 g). The treatment was administered in a cervical cap, applied to the cervix for 5 or 10 hours (n = 6 and 3 patients, respectively). Follow-up included a structured questionnaire, a gynecological examination, blood analysis for hematology, C-reactive protein (CRP), and renal function assessment plus pharmacokinetic analyses of cidofovir after each treatment and at the end of the full course. Results: No clinically significant hematological/biochemical abnormalities or serious adverse events (SAE) were reported, although 6 mild to moderate adverse events (AE) occurred in relation to the study drug: 1 flu-like syndrome and 5 local AEs. Plasma concentrations of cidofovir were very low (mean C_{max} of 103.0 and 99.2 ng/mL after 5 and 10 hours of exposure, respectively). Conclusion: Cidofovir, directly applied on CIN 2+, is reasonably well tolerated and the systemic exposure following topical application is much lower than that seen with intravenous administration, at the approved dose.

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Correspondence to
Catherine
Van Pachterbeke, PhD
CHU Brugmann, Place
Arthur Van Gehuchten, 4,
1020 Bruxelles, Belgium
Catherine.vanpachter-
beke@chu-brugmann.be

Introduction

High-risk human papilloma viruses (HPV) are implicated in the development of several epithelial cancers. HPV infections can induce cervical intraepithelial neoplasia (CIN), which can progress to invasive cancer [1]. The gold standard treatment of CIN grade 2 and 3 lesions is cervical conization. Although this procedure is well accepted and is associated with few short-term complications, the procedure is associated with increased risks of infertility, caesarean section, and preterm births in subsequent pregnancies [2]. Therefore, there is a need to develop a non-surgical alternative for cervical intraepithelial lesions grade 2 and 3 (CIN 2+) that could preserve the cervices of young women and reduce the morbidity associated with the conization.

Cidofovir, an acyclic phosphonate nucleoside with a broad-spectrum activity against DNA viruses, is approved as an intravenous treatment for cytomegalovirus (CMV) retinitis in subjects with acquired immune deficiency syndrome (AIDS) [3]. Cidofovir was initially approved under the trade name of Vistide® in 1996 by the FDA (U.S. Food and Drug Administration) for this indication. The recommended intravenous dose is 5 mg/kg given weekly for 2 weeks, followed by 5 mg/kg once every other week.

Cidofovir is also largely recognized as an effective treatment for several benign and premalignant vulvar and extra-genital HPV lesions [4, 5, 6]. Cidofovir toxicity against HPV transformed cells has been confirmed in a previous publication, both in vitro and in vivo [7].

Cidofovir is widely used intralesionally, in the treatment of recurrent laryngeal papillomatosis, with a complete response rate of ~ 60% [8]. It is also used topically, in the treatment of HPV cutaneous lesions, especially in immunocompromised hosts [9]. A

double-blind placebo-controlled study demonstrated that topical 2% cidofovir can cure up to 60% of CIN 2/3, without significant side-effects [10] and another trial showed that cidofovir is as safe and active as imiquimod for the topical treatment of vulvar intraepithelial neoplasia [11].

In such studies, transient local inflammatory reactions and/or erosions have been noticed in ~ 30 – 40% of cases after vulvar or vaginal application, but no systemic toxicity has been recorded [9, 11]. Such erosions invariably heal and may actually reflect an effective response of the lesions to cidofovir treatment. Furthermore, it has been demonstrated that systemic side effects from topical or intralesional cidofovir administration are extremely rare, and in addition, some pharmacokinetic data suggest that systemic absorption of cidofovir in rabbits is minimal and related to lesion size [12]. Of greater concern is that renal failure has been described, even after topical cidofovir application [13, 14] prompting our research team to perform a more detailed investigation into the pharmacokinetics of cidofovir, applied directly to CIN 2+ lesions, via a cervical cap.

As the primary objective of this research was to evaluate the potential systemic toxicity and local tolerance of a 2% (w/w) cidofovir aqueous gel, applied directly onto cervices with high-grade squamous intraepithelial lesions, an investigational product was developed and named "2% (w/w) cidofovir gel".

This type of topical administration has been previously tested in a local tolerance study using New Zealand white female rabbits where intravaginal treatment with cidofovir at 1 mg/kg, induced reversible local swelling and erythema with the potential to worsen with repeated exposures. However, the level of vaginal absorption of cidofovir was low and no systemic toxicity was detected (unpublished data).

Primary objective

To evaluate the safety and local tolerance of 2% (w/w) cidofovir aqueous gel, directly applied to cervices with high-grade cervical squamous intraepithelial neoplasia (CIN 2 and 3).

Secondary objective

To evaluate the pharmacokinetic profile of 2% (w/w) cidofovir gel applied in the same circumstances.

Materials and methods

This is an open-label, single-arm, phase 1 clinical study, conducted between May 31, 2010, and September 28, 2010, in a University Hospital (Erasme, Brussels, Belgium). The study was approved by the local ethics committee.

The study included 6 patients in the 5-hour treatment group and 3 patients in the 10-hour treatment group. All patients were volunteers and gave written informed consent. The treatment consisted of 3 applications of 3 g of 2% (w/w) cidofovir gel onto the cervix for either 5 or 10 hours at days 0, 7, and 14. The gel formulation was developed to be applied directly onto the cervix by vaginal route, using a cap (CCD Laboratory, Paris, France). This method was chosen to optimize the contact of the drug with CIN lesions.

Gynecological examinations of the vagina and cervix were performed at day 0 to observe the cervix before treatment, and at each subsequent visit (days 7, 14, and 21) to observe potential reactions (erythema, leucorrhea, and erosion). Subjects were systematically questioned about potential adverse events at each visit following the first treatment application (days 1, 7, 8, 14, 15, and 21). Patients were asked to keep a daily diary of any observations or symptoms. The diary was checked and transcribed into the case report form (CRF) at each visit. Adverse events were systematically recorded on a dedicated page of the CRF. Renal function (creatinine clearance and albuminuria), C-reactive protein (CRP), and differential leukocyte count were measured at the initial screening, before each application and after completion of the treatment.

Blood samples for pharmacokinetic analyses of cidofovir were collected with each application (cidofovir concentrations in plasma at 0, 1.5, 3, 5, 6, 7, 8, 9, 10, and 24 hours after treatment for patients who were treated for 5 hours, and at 0, 2.5, 5, 10, 11, 12, 13, and 24 hours after treatment for patients who

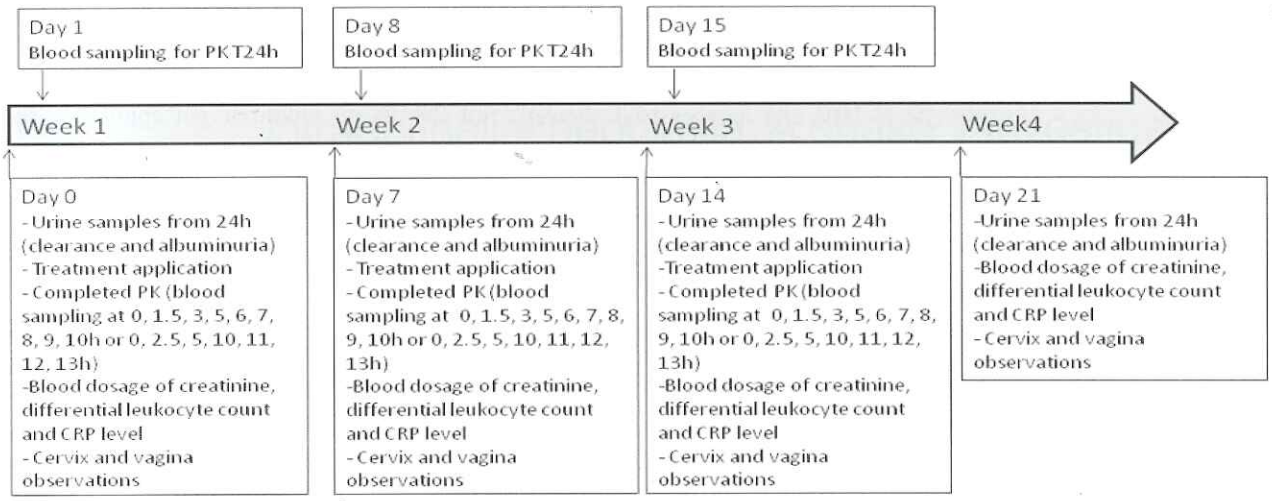

Figure 1. Study flow chart. PK = pharmacokinetic; CRP = C-reactive protein.

were treated for 10 hours) (Figure 1). The time points were chosen according to the plasma half-life of 2.5 hours and its rapid excretion in urine within 24 hours after injection (90% of administered dose) [15].The determination of cidofovir in human plasma utilized the hydrophilic interaction chromatography method as described by Lecomte et al. [16]; with a limit of detection (LOD) of 28.1 ng/mL and a lower limit of quantification (LLOQ) of 92.7 ng/mL.

After the study, all patients were referred back to their own gynecologist, for follow-up and standard treatment if required.

Women aged between 18 and 50 years with cervical lesions classified as CIN 2 or 3, on biopsies made during the preceding 6 weeks, were eligible for inclusion. A prerequisite was the current use of an effective mechanical, hormonal, or intrauterine contraception (except Nuvaring®). Exclusion criteria included current pregnancy or active breast feeding, subtotal hysterectomy, current or past history of renal impairment, an active immune disorder, concurrent treatment for any cancer, participation in another experimental study during the last 3 months before the screening visit, current, local, or general condition incompatible with the experimental treatment in the opinion of the investigator, or current use of the following: drugs that interfere with renal function, oral or parenteral anti-viral treatment, Nuvaring® contraception, vaginal application of drugs or cosmetics, treatments interfering with immunity.

The treatment consisted of three 5- or 10-hour applications at days 0, 7, and 14. The investigator loaded the cervical cap best suited to the cervical size with the 2% (w/w) cidofovir gel (3 g) immediately before placing it under direct vision. The investigator or the subject removed the cap 5 or 10 hours later, by pulling the nylon thread at the bottom of the cap. The aqueous gel contained 2% of active principle cidofovir (chemical name: (S)-1-[3-hydroxy-2-(phosphonylmethoxy) propyl]cytosine (HPMPC)) and specific excipients for this formulation (carbomer 974P, EDTA, NaOH, and methylparabens and propyl-parabens). From 7 days before the first treatment to 7 days after the third treatment, the treatments listed in exclusion criteria were not permitted, however vulvo-vaginal application of anesthetic gel (xylocaïne gel) was allowed in cases of vaginal pain.

The primary endpoints of safety and local tolerance were evaluated using adverse events reporting, blood testing, urinalysis and gynecological examinations. The potential toxicity of cidofovir was evaluated as follows: differential leukocyte count and CRP used as markers of inflammation and serum creatinine, creatinine clearance and albuminuria for the assessment of renal function. Participants were given a diary to record any specific side effects during the treatment cycles.

All statistical analyses were performed using IBM-SPSS statistical software (Version 19). Missing data were not replaced, nor extrapolated. Continuous variables

Table 1. Renal function: creatinine clearance: descriptive statistics.

			Creatinine clearance (mL/min)			
			Days 0 – 1	Days 7 – 8	Days 14 – 15	Day 21
Application group 5 hours	N	Valid	5	6	6	6
		Missing	1	0	0	0
	Mean		109.40	100.83	114.92	144.83
	Median		111.00	97.90	115.50	149.50
	SD		8.62	18.53	16.47	27.85
	Minimum		95.00	79.70	90.00	113.00
	Maximum		118.00	133.00	133.00	174.00
Application group 10 hours	N	Valid	3	3	2	3
		Missing	0	0	1	0
	Mean		104.03	97.07	93.25	92.73
	Median		113.00	103.00	93.25	112.00
	SD		17.29	30.34	42.07	49.78
	Minimum		84.10	64.20	63.50	36.20
	Maximum		115.00	124.00	123.00	130.00

were described by: number of observations (n), mean (Mean), standard deviation (SD), minimum (Min) and maximum (Max). Categorical and ordinal variables were presented using frequencies (N) and percentages (%). The time course of each biological variable according to the successive blood samplings was analyzed with the non-parametric test of Friedman. The pharmacokinetic profile ($AUC0\rightarrow\infty$, $AUC0\rightarrow t$, C_{max}, t_{max}, and $T_{1/2}$) was compared between the three blood samplings (treatment 1, treatment 2, and treatment 3), with the non-parametric test of Friedman followed when significant by Wilcoxon's tests for paired data. The pharmacokinetic profiles were compared between application groups (5 hours and 10 hours) with Mann-Whitney tests. The statistical tests were considered significant as soon as the p-value was lower than 0.05.

Results

Overall, 11 patients (6 in the 5-hour and 5 in the 10-hour application groups) were screened. Two screened patients with CIN 1 lesion were not included in the study. The 9 enrolled patients were 28.2 ± 4.2 years of age (mean ± SD) (Min = 19 years; Max = 36 years). Four patients were current smokers, and 3 patients were past smokers. At baseline, 6 patients (66.7%) were diagnosed with CIN 2 and 3 patients (33.3%) with CIN 3 (3 × CIN 2 and 3 × CIN 3 in the 5-hour group, 3 × CIN 2 in the 10-hour group). Initial gynecological examinations revealed no additional abnormal findings in patients 1 – 8. Patient 9 was diagnosed with mollusca contagiosa on the right buttock. Five patients had never received any treatment for their CIN 2+ lesions before the study. Four patients had previously received topical treatment(s) including: cryotherapy (1 patient), fluorouracil (1 patient), podophyllin (4 patients) and/or cidofovir (2 patients). All pregnancy tests were negative at inclusion.

The follow-up gynecological examinations revealed that 5 of the 9 patients developed mild cervical erosions during the treatment period. Two also complained of leucorrhea; one case mild and the other moderate. All the erosions resolved spontaneously, and no patients suffered with erythema. The comparisons between the two application groups (5 hours and 10 hours) did not reveal any statistically significant differences at all time-points ($p > 0.05$).

No patients developed albuminuria, but 1 patient from the 5-hour group showed abnormal serum creatinine on day 14 (grade 1 according to the Common Terminology Criteria for Adverse Events – NIH, May 2009) despite a normal creatinine clearance, and by day 21 the creatinine had normalized. Two patients from the 10-hour group had abnormal creatinine clearance which was transient (days 7 and 14) and not clinically significant for 1 patient; the second abnormal creatinine clearance by day 21 (with normal serum creatinine) was attributed to the observed lack of compliance (Table 1). Markers of inflammation revealed no clinically significant abnormality, but there was a statistically

Table 2. Pharmacokinetics: Descriptive statistics of PK parameters as a function of the application group (all visits pooled together).

		PK profile				
		$AUC_{0\rightarrow t}$ (ng/mL×h)	$AUC_{0\rightarrow\infty}$ (ng/mL×h)	t_{max} (h)	C_{max} (ng/mL) (< LOQ = LOQ/2)	$T_{1/2}$ (h)
Treatment group 5 hours						
N	Valid	18	18	10	18	18
	Missing	0	0	8	0	0
Mean		218.6	218.6	4.5	103.0	0.2
Median		68.2	68.2	5.0	110.8	0.1
SD		281.0	281.0	1.1	57.4	0.1
Minimum		0.0	0.0	3	46.3	0.1
Maximum		849.5	849.5	6	206.5	0.3
Treatment group 10 hours						
N	Valid	9	9	4	9	4
	Missing	0	0	5	0	5
Mean		538.1	538.1	7.5	99.2	0.1
Median		0.0	0.0	7.5	46.3	0.1
SD		674.2	674.2	2.9	65.2	0.0
Minimum		0.0	0.0	5	46.3	0.1
Maximum		1,519.4	1519.4	10	193.7	0.1

$AUC_{0\rightarrow t}$ = plasma concentration curve from time 0 to time 24; $AUC_{0\rightarrow\infty}$ = plasma concentration curve from time 0 extrapolated to infinity; t_{max} = time to maximum concentration; C_{max} = maximum plasma concentration; $T_{1/2}$ = plasma elimination half time; LOQ = limit of quantification.

significant difference between time points for eosinophils ($p = 0.003$). An increase in eosinophil count was observed between baseline and days 14 – 15 ($p = 0.008$) and between baseline and day 21 ($p = 0.016$), but all values remained within the normal range.

Overall, there were 21 adverse events (AEs) reported in 5 patients (55%). 13 AEs were reported in the 5-hour group, and 8 AEs were reported in the 10-hour group. Six AEs resulted in the prescription of additional medications or treatments. Of all the AEs reported, 6 were considered either possibly or probably related to the study drug: 2 cases of pruritus, 1 vaginal discharge, 1 flu syndrome, and 1 vulvar erosion in the 5-hour application group; and 1 case of vaginal bleeding in the 10-hour group. None of the AEs were severe, and all resolved rapidly.

Serum levels of cidofovir were monitored in both treatment groups (5 hours versus 10 hours). Following applications of the gel on the cervix, cidofovir was detected in the plasma for 10 of 18 cidofovir treatments of 5 hours and for 4 of 9 cidofovir treatments of 10 hours. For the remaining 13 treatments, serum cidofovir remained below the level of detection (LOD). If we assume a full resorption of the 60 mg of cidofovir applied on the cervix (3 g of 2% gel), as the mean weight of the patients was 61, 5 ± 8 kg (mean ± SD), the approximate dose of cidofovir received was 1 mg/kg at each treatment visit. Due to the very low cidofovir plasma concentrations detected during the study, inferential statistical analyses were not performed, and AUCs were not reliably estimated.

The descriptive statistics of the pharmacokinetic parameters as a function of the application group for all visits, pooled together, can be found in Table 2. In the 5-hour application group, the following mean parameters were measured: C_{max} = 103.0 ng/mL, t_{max} = 4.5 hours. In the 10-hour application group, the following mean parameters were measured: C_{max} = 99.2 ng/mL, t_{max} = 7.5 hours. The pharmacokinetic profiles at each visit and for all visits pooled together are found in Table 3.

Discussion

In this small phase 1 clinical study, we have demonstrated the safety and tolerance of multiple topical doses (5- and 10-hour applications) of cidofovir, in patients affected by CIN 2 or CIN 3, with minimal systemic

Table 3. Pharmacokinetic profile at each visit and for all visits pooled together: descriptive statistics.

Visits	Descriptive statistics		PK profile				
			$AUC_{0\rightarrow t}$ (ng/mL×h)	$AUC_{0\rightarrow\infty}$ (ng/mL×h)	t_{max} (h)	C_{max} (ng/mL) (< LOQ = LOQ/2)	$T_{1/2}$ (h)
Visit 1 (day 0)	N	Valid	9	9	3	9	3
		Missing	0	0	6	0	6
	Mean		120.8	120.8	7.0	75.3	0.1
	Median		0.0	0.0	6.0	46.3	0.1
	SD		244.4	244.4	2.6	43.8	0.0
	Minimum		0.0	0.0	5.0	46.3	0.1
	Maximum		711.9	711.9	10.0	145.1	0.1
Visit 2 (day 7)	N	Valid	9	9	4	9	4
		Missing	0	0	5	0	5
	Mean		237.3	237.3	4.5	87.0	0.2
	Median		0.0	0.0	5.0	46.3	0.1
	SD		498.5	498.5	1.0	53.0	0.1
	Minimum		0.0	0.0	3.0	46.3	0.1
	Maximum		1,519.4	1,519.4	5.0	184.9	0.3
Visit 3 (day 14)	N	Valid	9	9	7	9	7
		Missing	0	0	2	0	2
	Mean		617.2	617.2	5.1	143.0	0.1
	Median		543.6	543.6	5.0	168.7	0.1
	SD		488.9	488.9	2.3	59.9	0.0
	Minimum		0.0	0.0	3.0	46.3	0.1
	Maximum		1,396.4	1,396.4	10.0	206.5	0.2

$AUC_{0\rightarrow t}$ = plasma concentration curve from time 0 to time 24; $AUC_{0\rightarrow\infty}$ = plasma concentration curve from time 0 extrapolated to infinity; t_{max} = time to maximum concentration; C_{max} = maximum plasma concentration; $T_{1/2}$ = plasma elimination half time; LOQ = limit of quantification.

effects. Clinical evaluations of the patients did not significantly change during and after the treatments, regardless of treatment duration (5 or 10 hours).

However, the number of participants and applications were probably too small to fully evaluate potential deleterious effects that may be seen during the longer treatment cycles associated with clinical studies [9, 11]. The type of adverse events that were reported in relation to cidofovir administration were similar to those seen in others studies [9, 11, 17] including flu-like syndromes, pruritus, moderate vaginal discharge, mild vaginal bleeding, and moderate vulvar erosions. No SAEs were reported, consistent with the majority of clinical studies where topical cidofovir has been used over short time frames [4, 5, 6, 10, 11, 17, 18, 19]. In HIV-infected patients with high grades of anal intraepithelial neoplasia (AIN) and vulvar intraepithelial neoplasia (VIN) lesions treated with up to 6 cycles of 5 days of topical 1% cidofovir, Stier et al. [9] reported 3 SAEs in their open-label uncontrolled study: 1 patient had an invasive perianal squamous cell carcinoma diagnosed at study completion (after 18 weeks), which may either represent undiagnosed occult disease at inclusion or a true progression to cancer, and 2 other SAEs that were unrelated to the treatment.

The resulting drug plasma concentrations following topical cervical treatment with 3 g of a 2% (w/w) cidofovir gel per week for 3 weeks were very low, thereby minimizing systemic exposure and this was supported by the pharmacokinetic data, clinical examinations, and patient diaries of side effects. The mean C_{max} has been measured at 3.12 µg/mL after an intravenous (IV) infusion of 1 mg/kg of cidofovir, whilst at the approved IV dosage of 5 mg/kg with probenecid protection the C_{max} was 11.5 µg/mL [20] in comparison to only 103.0 ng/mL for the 5-hour group and 99.2 ng/mL for the 10-hour group (mean values) in our study, after ~ 1 mg/kg of topical cidofovir.

Due to the very low cidofovir plasma concentrations, we could not reliably estimate the AUC, however the mean AUCs increased between the first and the third application. One explanation for this observa-

tion could have been the presence of cervical erosions, which tended to increase with repeated treatments. This theory is supported by a study which showed that direct application of the gel onto the abraded, damaged skin of rabbits, increased the bioavailability of topical cidofovir treatment by up to 41% in comparison to a 2% bioavailability, when applied to intact skin [12].

These concerns are further reinforced by a publication from 2014, which reported two cidofovir-induced acute kidney injuries after an estimated dose of 16 and 26.8 mg/kg of cidofovir applied directly onto abraded skin [14]. These findings highlight the need for caution before applying this treatment to damaged skin and for extended periods.

Conclusion

This study suggests that 2% (w/w) cidofovir gel application, as a treatment for moderate to severe cervical dysplasia, at the dosage of 3 g a week for 3 weeks, is well tolerated both locally and systemically. The serum cidofovir level was far lower after weekly local application of 3 g of 2% cidofovir gel for a duration of 3 weeks, compared to the published results of intravenous injections at the approved dosage.

However, more research is needed to confirm the safety and efficacy of this treatment in larger-scale, randomized, controlled clinical trials.

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Conflict of interest

All authors: no conflict.

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