Safety and pharmacokinetics of aciclovir in women following release from a silicone elastomer vaginal ring

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Received 12 January 2012; returned 7 February 2012; revised 29 March 2012; accepted 1 April 2012

Objectives: Systemic aciclovir and its prodrug valaciclovir are effective in treating and reducing recurrences of genital herpes simplex virus (HSV) and reducing transmission. Local aciclovir delivery, if it can achieve and maintain comparable intracellular genital tract levels, may be equally effective in the treatment and suppression of genital HSV. Intravaginal ring (IVR) delivery of aciclovir may provide pre-exposure prophylaxis against HSV acquisition.

Methods: Tolerability and pharmacokinetics were evaluated in six HIV-negative women with recurrent genital HSV who switched their daily oral valaciclovir suppression to an aciclovir IVR for 7 days (n = 3) or 14 days (n = 3). Blood and cervicovaginal lavage (CVL) were collected after oral and IVR dosing to measure aciclovir concentrations and genital swabs were obtained to quantify HSV shedding by PCR.

Results: The rings were well tolerated. Median plasma aciclovir concentrations were 110.2 ng/mL (IQR, 85.9–233.5) 12–18 h after oral valaciclovir. Little or no drug was detected in plasma following IVR dosing. Median (IQR) CVL aciclovir levels were 127.3 ng/mL (21–660.8) 2 h after oral valaciclovir, 154.4 ng/mL (60.7–327.5) 12–18 h after oral valaciclovir and 438 ng/mL (178.5–618.5) after 7 days and 393 ng/mL (31.6–1615) after 14 days of aciclovir ring use. Median CVL aciclovir levels 2 h after oral dosing were similar to levels observed 7 (P = 0.99) and 14 (P = 0.75) days after ring use. HSV DNA was not detected in genital swabs and there was no significant change in inflammatory mediators.

Conclusions: This first-in-human study demonstrated that an IVR could safely deliver mucosal levels of aciclovir similar to oral valaciclovir without systemic absorption. More intensive site-specific pharmacokinetic studies are needed to determine whether higher local concentrations are needed to achieve optimal drug distribution within the genital tract.

Keywords: herpes simplex virus, vaginal microbicides, genital herpes

Introduction

The total number of people living with herpes simplex virus type 2 (HSV-2) worldwide is estimated to be 536 million, with an annual incidence of 23.6 million cases among persons aged 15–49 years. HSV-2 is the most frequent cause of genital ulcer disease in all regions of the world and 38% of people who are HSV-2-infected experience six or more genital outbreaks/year. In the USA, only 10%–25% of HSV-2-seropositive persons have recognized genital herpes and most HSV-2 infections are acquired from persons without a clinical history of genital herpes. Importantly, HSV-2 is also associated with a
3.1-fold increased risk of HIV acquisition in women. These epidemiological findings prompted the evaluation of oral suppressive therapy. Specifically, daily oral valaciclovir has been shown to prevent or delay genital recurrences by 85% and to reduce the risk of transmission among HSV-2-discordant couples by 48%.

More recently, tenofovir gel applied vaginally before and after sex was shown incidentally to reduce HSV-2 acquisition in women by 51%. This surprising observation suggests that topically delivered products could protect against HSV. Aciclovir is 100-fold more potent than tenofovir in vitro (IC50 data), suggesting that a vaginal formulation of aciclovir could provide greater protection against HSV. Although topical aciclovir was shown to be safe and provided some clinical benefit for treatment of primary or recurrent lesions by shortening the duration, it was not associated with a decrease in frequency of clinical recurrences or an increase in the time to the next recurrence in patients with either first or recurrent genital herpes. However, we hypothesize that sustained local delivery from an intravaginal ring (IVR) may provide an alternative approach to oral suppressive therapy. Potential advantages of sustained aciclovir ring delivery to the genital tract include a decrease in systemic toxicity and improved adherence.

The technology platform is based on polymer-coated solid cores of drug, so-called pods, incorporated into a silicone elastomer ring, which exhibits pseudo-zero order kinetics (i.e. linear cumulative drug release over time). Drug is released through a delivery window in the silicone ring with the release rate determined by the window diameter. The amount of drug released from each ring can be adjusted by changing the amount and composition of the polymer coating of the drug core, the size of the delivery window and the number of drug pods in each ring.

The goal of the present study was to evaluate the safety and pharmacokinetics of aciclovir administered from a silicone elastomer IVR in women with recurrent genital HSV. The objectives were to assess the safety of the IVR and to compare plasma and genital tract aciclovir levels following oral valaciclovir dosing with levels obtained following aciclovir ring dosing.

Exploratory objectives were to measure immune mediators in the genital tract and to assess the rings for biofilm formation after ring use.

**Methods**

**Ethics statement**

The study was conducted according to the Declaration of Helsinki and was approved by the Albert Einstein College of Medicine Institutional Review Board. All study participants provided written informed consent. An investigational new drug application (IND) was submitted to the FDA (IND number 108536).

**Participants**

Six women between the ages of 18 and 50 years were recruited between November 2010 and March 2011. Participants were eligible if they had recurrent genital herpes, were taking 500 mg daily of oral valaciclovir and were willing to change their oral suppressive therapy to an aciclovir IVR for up to 14 days. Participants were excluded for pregnancy, breastfeeding, HIV infection, autoimmune disease, malignancy, abnormal renal function, irregular menses and active vaginal infection.

At screening, participants had a urine pregnancy test, gynaecological examination and blood collection for HIV and to assess renal function. Blood and cervicovaginal lavage (CVL) for aciclovir levels and a swab of the cervix, vagina and perineum for HSV shedding were collected 12–18 and 24 h after oral valaciclovir dosing. CVL and a swab were also collected 2 h after oral aciclovir dosing. The first three participants were an aciclovir IVR for 7 days and had blood, CVL and a swab collected 1, 3 and 7 days after IVR insertion and 1 day after ring removal; blood and CVL were also collected 4 h after ring insertion. The final three participants were an aciclovir IVR for 14 days and the study visits were extended to include blood, CVL and swab collection 10 and 14 days after ring insertion and 1 day after ring removal (Figure 1). CVL was obtained by washing the cervix and posterior fornix with 10 mL of normal saline (pH ≈ 5.0). A pelvic examination and colposcopy were performed at all study visits. A brief questionnaire was administered at visits 5–8 to assess comfort, whether the ring was removed or involuntarily expelled, and whether participants were willing to use the ring in the future if it was found to decrease HSV recurrences.

**Figure 1.** Study scheme. Participants wore an aciclovir (ACV) IVR for 7 or 14 days. The first three participants had blood and CVL for ACV levels and a swab of the cervix, vagina and perineum for HSV shedding collected at 12–18 and 24 h after oral valaciclovir (VCV) dosing as well as 1, 3 and 7 days after ACV ring insertion and 1 day after ring removal. Sample collection for the final three participants occurred 12–18 and 24 h after oral VCV dosing as well as 1, 3, 7, 10 and 14 days after ring insertion and 1 day after ring removal (day 15).
Safety and pharmacokinetics of an aciclovir vaginal ring in women

Manufacture of rings

Silicone rings were prepared in a multi-step process from Nusil MED-4840 liquid silicone elastomer (Nusil Silicone Technology) using an injection moulding system developed at Oak Crest Institute of Science (OCIS). The ring dimensions were similar to those of the commercially available Estring (outer diameter 55 mm; cross-sectional diameter 7.5 ± 0.5 mm). Each ring contained four 16 ± 0.1 mg pellets of aciclovir coated with poly-lactic acid, resulting in a total of 64 ± 0.4 mg of aciclovir. A single delivery window of 1.5 mm diameter exposed a small surface of each aciclovir pod to vaginal fluid when the ring was in use. In vitro release studies demonstrated an average release rate of 356 µg/day in acetate buffer at pH 4.2 and 37°C.

Measurement of aciclovir in plasma and CVL

Blood samples were immediately chilled on ice, centrifuged for 10 min at 2000 g within 30 min of collection and the plasma was stored at −80°C. CVL samples were placed on ice and clarified by centrifugation at 700 g for 10 min at 4°C, and the cell pellets and supernatants were stored at −80°C. Plasma and CVL supernatants were thawed and prepared by acetonitrile protein precipitation and aciclovir levels were measured by HPLC–mass spectrometry (HPLC-MS/MS) (Analytical Bio-Chemistry Laboratories, Inc., Columbia, MO, USA). The lower limit of quantification (LLOQ) was defined as the lowest concentration that provided 20% accuracy of an aciclovir sample in test matrix (i.e. CVL or plasma). The LLOQ was 25 ng/mL for CVL and 1 ng/mL for plasma.

Quantification of HSV DNA

Each genital tract swab was evaluated for HSV DNA by quantitative, real-time, fluorescence-based PCR as previously described. The detection assay, which uses primers to the type-common region of HSV glycoprotein B (gB), is reproducible, has a large linear range (<10–10^8 copies of HSV DNA) and has <3% variability.

Measurement of immune mediators

Interleukin (IL)-1α, IL-1β, IL-6, IL-8, interferon (IFN)-γ 2, IL-1 receptor antagonist (IL-1ra), macrophage inflammatory protein (MIP)-1α, MIP-1β and RANTES (regulated upon activation, normal T cell expressed and secreted) were quantified in each CVL sample using a multiplex proteome assay, which uses primers to the type-common region of HSV glycoprotein B (gB), is reproducible, has a large linear range (<10–10^8 copies of HSV DNA) and has <3% variability.

HSV plaque assays

Vero (monkey kidney epithelial) cells were challenged with HSV-2(G) in the absence or presence of increasing doses of aciclovir (0, 500, 1000, 5000 and 10000 ng/mL) or in the presence of CVL or matched control buffer (saline containing 200 µg/mL BSA to control for protein concentration). After 1 h of incubation the inoculum was removed and the cells were overlaid with 0.5% (w/v) methylcellulose in medium 199 containing the same final concentration of aciclovir or CVL or control buffer. Viral plaques were counted 48 h post-infection.

IVR processing for biofilm characterization

Aciclovir rings were removed aseptically on day 7 or 14. The rings were cut into sections and segments without pods were placed in 70% ethanol in water and transported on ice to OCIS for biofilm characterization.

Light microscopy

Individual IVR pieces were immersed in 100% ethanol in a 4.0 cm diameter Petri dish. Specimens were imaged using a Discovery.V12 stereo microscope (Carl Zeiss MicroImaging) at ×8 magnification with transmitted light.

Scanning electron microscopy (SEM)

Ethanol-fixed samples were prepared for SEM as described previously and were analysed using published methods.

Statistical analysis

Friedman tests were used to examine changes in immune mediators during the study period, accounting for repeated cytokine measures taken from the same women over time. Differences in aciclovir concentrations between oral and ring dosing were examined using Wilcoxon signed rank tests. Spearman’s correlation coefficients were calculated to determine whether CVL aciclovir levels correlated with plasma levels and anti-HSV activity.

Results

Study subjects

Eight women were assessed for eligibility and six were enrolled; one participant discontinued valaciclovir prior to enrolment and one had irregular menses. All six participants tested negative for HIV infection and had normal renal function. The mean age and standard deviation (SD) of the subjects was 37.6 ± 7.5 years. Women reported being diagnosed with genital HSV 2–8 years ago (mean duration of HSV 4.7 ± 2.4 years). Five of six participants had recurrent lesions located on the external genitalia (Table 1). The women had been on valaciclovir suppressive therapy for a mean of 5.4 months. Three subjects reported prior use of the NuvaRing.

Tolerance of aciclovir ring

There were no adverse events related to the ring, no abnormal colposcopic findings and no reports of ring expulsions. All six participants reported the ring to be very comfortable and all stated that they would use the ring in the future if it were found to reduce genital HSV recurrences. None of the participants experienced a clinical recurrence during the study period.

Table 1. Characteristics of subjects with genital HSV infection

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Ethnicity</th>
<th>Location of lesions</th>
<th>Duration of HSV (years)</th>
<th>Number of recurrences/year</th>
<th>Duration on valaciclovir (months)</th>
</tr>
</thead>
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<tr>
<td>32.7</td>
<td>non-Hispanic</td>
<td>perineum, labia</td>
<td>5</td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td>36.5</td>
<td>non-Hispanic</td>
<td>labia</td>
<td>8</td>
<td>10</td>
<td>8</td>
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<tr>
<td>43.9</td>
<td>Hispanic</td>
<td>labia</td>
<td>3</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>41</td>
<td>Hispanic</td>
<td>labia</td>
<td>7</td>
<td>3–4</td>
<td>0.8</td>
</tr>
<tr>
<td>45.5</td>
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<td>labia, vagina</td>
<td>2</td>
<td>2</td>
<td>0.5</td>
</tr>
<tr>
<td>25.8</td>
<td>Hispanic</td>
<td>vagina</td>
<td>3</td>
<td>2</td>
<td>8</td>
</tr>
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</table>
As an additional safety measure, concentrations of pro-inflammatory and anti-inflammatory cytokines and chemokines were measured in CVL following oral and ring dosing. There was no significant increase in the levels of IL-1α, IL-1β, IL-6, IL-8, IL-1ra, IFNα2, MIP-1α, MIP-1β or RANTES throughout the study period (Table 2).

SEM and light microscopy were used to examine the surfaces of the worn IVRs to determine whether any bioerosion and/or build-up of biological material had taken place during the course of the study. The results are shown in Figure 2 and suggest that the IVRs became colonized by a monolayer of epithelial cells over the course of the 14 day study. At day 7, sporadic clusters of these cells were observed, with little or no associated microbial growth. At day 14, large areas of the ring surface were covered with a mat of epithelial cells, which harboured the development of polymicrobial biofilm islands. No erosion of the ring surface was observed.

**Pharmacokinetics**

The concentration of aciclovir in plasma was significantly higher following oral compared with ring dosing ($P=0.031$). The plasma aciclovir levels ranged from 76.7 to 529 ng/mL 12–18 h after oral dosing and from 12.6 to 44.9 ng/mL 24 h after the last valaciclovir dose, whereas aciclovir concentrations were below the limit of detection (1 ng/mL) in the plasma 7 and 14 days after ring insertion (Figure 3a). Low levels were detected in the plasma shortly after ring placement (Figure 3a), which may reflect drug released from the ring or residual drug from oral aciclovir dosing.

In contrast, comparable drug levels were achieved in genital tract secretions following oral versus ring dosing (Figure 3b). The median (IQR) CVL aciclovir levels 2 h after oral valaciclovir dosing were 127.3 ng/mL (76.7–210.8), which were similar to levels observed 7 days [438 ng/mL (178.5–618.5), $P=0.99$] and 14 days [393 ng/mL (31.6–1615), $P=0.75$] after aciclovir ring use. The median CVL aciclovir levels 24 h after the last oral valaciclovir dose were 12.7 ng/mL (0–43.8) and increased 7 days ($P=0.031$) and 14 days ($P=0.25$) after aciclovir ring use.

For the timepoints in which plasma levels were above the level of detection, correlations between plasma and CVL aciclovir levels were non-significant except for 24 h after ring dosing. CVL and plasma aciclovir levels were significantly and negatively correlated in samples obtained 24 h after ring dosing ($r=-0.94$, $P=0.02$). Also, of note, the CVL aciclovir levels correlated positively with plasma concentrations in samples collected 12–18 h after oral valaciclovir dosing ($r=0.75$, $P=0.10$).

Daily release rates of aciclovir were estimated based on residual drug content of the used IVRs. The mean release rate and SD was $322\pm172$ µg/day for the rings worn for 7 days and $219\pm109$ µg/day after 14 days of ring use, which is similar to the daily release rate observed following 28 days of ring delivery in rabbits ($343\pm335$ µg/day) and greater than the daily release rate observed in sheep ($174\pm14$ µg/day).16

**Anti-HSV activity in CVL**

The anti-HSV activity of CVL collected 24 h after the last oral dose of valaciclovir (just prior to ring insertion) and 7 days after ring insertion were tested in parallel with aciclovir in plaque assays.
The concentration of aciclovir that inhibited infection by 50% (IC$_{50}$) when drug was added at the time of HSV challenge and in the overlay medium was $\approx 700$ ng/mL (Figure 4a). The concentration of aciclovir measured in the majority of CVL samples collected was below the IC$_{50}$ level (Figure 3b). However, the median anti-HSV activity of CVL increased from 31.5% (range, 9%–52%) in CVL collected just prior to ring insertion to 57.5% (range, −6% to 70%) in CVL collected 7 days after ring use, suggesting that there was enough drug present to augment the endogenous anti-HSV activity of genital tract secretions (Figure 4b).$^{18,19}$ The anti-HSV activity of the CVL correlated positively and significantly with the drug levels ($r=0.63$, $P=0.03$) (Figure 4c).

**Assessment of asymptomatic HSV shedding in the setting of oral and ring dosing**

A swab of the vagina, cervix and perineum was collected following oral dosing and ring use. HSV DNA was not detected by quantitative PCR in any of the genital tract swabs collected after oral valaciclovir and aciclovir ring dosing.

**Discussion**

This first-in-human aciclovir ring study indicates that the drug can be safely delivered to women from a silicone elastomer IVR. The ring was well tolerated with no abnormal colposcopic...
findings or significant changes in inflammatory cytokine or chemokine concentrations in CVL. We did, however, detect biofilm formation on all three rings after 14 days of continuous use.

The aciclovir ring delivered sufficient drug to achieve similar concentrations of aciclovir in the CVL as found following oral dosing of valaciclovir, with little or no systemic absorption. The low plasma levels of aciclovir detected in the first 24 h after ring application may have been due to residual oral valaciclovir rather than systemic absorption of topically delivered drug as there was no wash-out period between oral valaciclovir and aciclovir IVR dosing. Whether achieving CVL levels similar to what is detected following oral administration will translate to a comparable level of protection is not known. None of the participants had detectable HSV viral DNA in their genital tract during this short study. Possibly, higher levels may need to be delivered topically to provide adequate distribution of drug throughout the genital tract including the external genitalia (particularly the labia) and the buttocks, which are the more common sites for HSV recurrences. Higher drug levels may also be required if the rings are to be used as a pre-exposure prophylactic strategy to reduce HSV acquisition. Thus, future studies should include intensive and more frequent sampling with measurements of epithelial and keratinocyte intracellular drug levels, the sites of active HSV replication.

The dose of aciclovir needed to suppress HSV replication or reduce acquisition in humans is unknown. Surprisingly few studies have measured drug levels in the genital tract with oral suppressive therapy. One study found that women who were treated with 200 mg of aciclovir five times daily had peak vaginal levels of 0.8–3.6 nmol/g or 0.18–0.81 μg/mL 0.5–1 h after the last oral dose. Presuming that CVL samples represent ~50-fold dilutions of genital tract secretions, the CVL concentrations observed in this study following IVR use are similar to what was observed in the oral aciclovir study.

The relationship between aciclovir levels in cervicovaginal fluid and tissue is currently unknown. Aciclovir is only phosphorylated efficiently by viral thymidine kinase. It is possible that aciclovir diffuses in and out of cells until HSV infection occurs and thymidine kinase is available to phosphorylate aciclovir to its monophosphate and then its active triphosphate form. Whether the CVL aciclovir concentrations observed in this study are high enough to inhibit HSV infection will depend on the steady state of intracellular and extracellular pools of drug, which requires further study.

Future studies should include intravaginal and external genital biopsies to determine whether drug released from a ring is sufficient to inhibit HSV infection. Biopsies collected before and after ring use can be challenged with HSV ex vivo to determine whether there is sufficient drug available to protect against
infection. Animal studies could also provide important information as to whether aciclovir delivered locally from an IVR could reach the dorsal root ganglia to reduce HSV replication following reactivation from latency.

Microorganisms originating from normal flora can colonize IVRs and form biofilms. Biofilm formation may promote changes in the vaginal microbiome that could adversely impact host mucosal defences or serve as a barrier to drug release. Consequently, documentation of biofilm formation following IVR use is an integral part of evaluating safety and pharmacokinetics. To our knowledge, biofilm formation on silicone IVRs, such as Estring®, or on rings composed of ethylene vinyl acetate, such as NuvaRing®, has not been studied.

In prior research, we found that microbial biofilms formed on the surface of tenofovir and placebo silicone IVRs worn for 28 days by female pig-tailed macaques. Large areas of the ring surfaces were covered with monolayers of epithelial cells and two bacterial biofilm phenotypes were found to develop on these monolayers. Results from the present study in women support these findings and suggest that the epithelial cell monolayer develops over the first 14 days and subsequently becomes colonized by islands of polymicrobial communities embedded in extracellular material. Phenotype II, with an open architecture containing interwoven networks of uniform fibres, was the predominant structure morphology. The relatively low density of clustered microbial communities partially explains the lack of an immune response to the IVRs worn for up to 14 days. The biological significance of surface-attached bacterial biofilms and the impact of biofilm formation on the vaginal microbiome, host mucosal defence and pharmacokinetics are currently unknown and are being studied actively.

This first study in women of an aciclovir IVR demonstrates that genital tract levels of aciclovir that are similar to oral valaciclovir can be delivered without irritation, inflammation or systemic absorption. These findings support further development and study of an aciclovir IVR.

Acknowledgements

Some of the data presented in this manuscript were presented at the Forty-ninth Annual Meeting of the Infectious Diseases Society of America, Boston, MA, USA, 2011 (Oral Presentation 143).

We thank Erin Diament and Anna Lee for their assistance with data collection, Etana Kopin for her efforts in fabrication and in vitro evaluation of the IVRs, and Paul Webster for guidance with the microscopy studies.

Funding

This work was supported by Auritec Pharmaceuticals, Inc. Additional support was provided by grants from the National Institutes of Health (UL1 RR025750 and P30 AI51519).

Transparency declarations

A. M. M., R. W., C. N., M. G. and T. J. S. are employees of Auritec Pharmaceuticals, Inc. A. M. M. and T. J. S. own shares in Auritec Pharmaceuticals, Inc., and both had a role in the design, execution, analysis and reporting of the data. All other authors: none to declare.

Author contributions

M. J. K., B. C. H., T. J. S., A. M. M., M. M. B., R. W. and J. A. M. conceived and designed the study and experiments. C. A. C., M. H., C. N., S. K., M. G. and D. G. performed the experiments. M. J. K., B. C. H., T. J. S., A. M. M., M. M. B., C. A. C., M. H., L. C., C. N., S. K., M. G., D. G. and Y. L. analysed and interpreted the data. M. J. K., B. C. H. and M. M. B. drafted the manuscript. All authors critically appraised the final version of the manuscript.

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