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Pharmacokinetics and Safety Assessment of Anti-HIV Dapivirine Vaginal Microbicide Rings with Multiple Dosing

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Abstract

Objectives: Self-administered vaginal rings are a promising method for delivery of topical anti-HIV microbicides and might offer an adherence advantage over daily or coitally-dependent dosage forms such as gels. This trial assessed the safety and pharmacokinetic aspects of the Dapivirine Vaginal Ring-004 when worn as multiple rings over sequential periods of ring use by healthy, sexually-active, HIV-negative women.

Methods: This double-blind trial was conducted among 48 women (18-40 years). Participants were randomly assigned to two groups (A or B) and received (3:1) either the dapivirine or a placebo vaginal ring. Group A used two rings over a 56-day period and Group B used three rings over a 57-day period. Safety evaluations were conducted throughout the trial. Dapivirine concentrations were measured in plasma, vaginal fluid and cervical tissue samples collected during and after the 56 days (Group A) or 57 days (Group B) of vaginal ring use.

Results: Ring-004 was safe and well tolerated in all participants. The pharmacokinetic profile demonstrated a rapid increase in plasma and vaginal fluid concentrations and achieved concentrations in vaginal fluids and cervical tissue well above the *in vitro* IC_{99} in cervical tissue (3.3 ng/mL) that were sustained for a 28 to 35-day ring use period (approximately 3000 times higher in vaginal fluids and 14 -1000 times higher in cervical tissue). Drug levels were associated with significant inhibitory activity of genital secretions against HIV *ex vivo*, a biomarker of pharmacodynamics. Individual plasma dapivirine concentrations did not exceed 553 pg/mL and were well below plasma concentrations at the maximum tolerated dose for oral treatment (mean C_{max} 2286 ng/mL).

Conclusions: The consecutive use of several rings over a period of up to 57 days was safe and well tolerated, and PK data indicate that a single Ring-004 is likely to be protective for at least 35 days.

Keywords: Dapivirine; Vaginal ring; HIV prevention; Microbicide; Pharmacokinetics; Women-initiated; Safety

Introduction

Women are disproportionately affected by the HIV epidemic, accounting for nearly 60% of HIV positive adults in sub-Saharan Africa where two thirds of people living with HIV reside [1,2]. Most new HIV infections in women are from heterosexual contact (84%) [3]. While male condoms are highly effective against HIV transmission, they are often inadequate prevention options for women as male partners may refuse their use [4-6]. The development of women-initiated HIV prevention options therefore remains a critical public health objective. Microbicides represent a promising prevention technology that has the potential to fulfil this need. However, in order to be effective, vaginal microbicides need to be used correctly and consistently [7]. This was clearly demonstrated in the VOICE trial, in which both oral and vaginal tenofovir-based products failed to protect against HIV infection, despite having previously been shown to be efficacious [8-10]. This was subsequently shown to be due to poor user adherence to product use. Thus, there are likely to be benefits for products that place as low a burden as possible on the user. Self-administered vaginal rings are a promising method for delivery of topical anti-HIV microbicides that require infrequent insertion and removal by the user, and therefore might offer an adherence advantage over daily or coitally-dependent dosage forms such as gels.

Dapivirine is a non-nucleoside reverse transcriptase inhibitor (NNRTI) [11] microbicide with potent activity against HIV-1 [12]. Prototypes of silicone vaginal rings that release dapivirine over a 4-week use period were found to be safe and acceptable in Phase I clinical trials [12-15]. The *in vivo* pharmacokinetic profiles of these rings, however, were quite different, depending upon the ring design and the type of silicone used. The current version of the dapivirine vaginal ring, Ring-004, which is currently being evaluated for safety and efficacy in Phase III clinical trials [16,17], has a matrix configuration in which 25 mg dapivirine is dispersed in a platinum-catalyzed silicone design to address issues observed with the previous ring prototypes. The *in vitro* dissolution profile of Ring-004 demonstrated a more consistent and sustained vaginal release of the drug over 28 days [18,19]. The trial described here was designed to assess the safety, local and systemic pharmacokinetics (PK) (measured in plasma, vaginal fluids and cervical tissue) of the Dapivirine Vaginal Ring-004 in healthy, sexually-active, HIV-negative women when multiple rings were used sequentially over various periods of use. Exploratory objectives evaluated the capacity of

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Ring-004 to protect vaginal fluids against HIV-1 infection in an *in vitro* model, as well as the local and systemic pharmacokinetics of dapivirine measured in plasma and vaginal fluids during menses among women where tampon use was allowed during the trial period.

Methods

Trial design

This double-blind, randomized, placebo-controlled trial was conducted in 2010 at SGS Life Science Services in Antwerp, Belgium among 48 women between the ages of 18 and 40 years. Participants were assigned to one of two groups, namely Group A (n=24) or Group B (n=24), in an unblinded fashion. Within Groups A and B, participants were randomized in a blinded manner, in a 3:1 ratio, to either the dapivirine or placebo ring. Within each group, 18 participants received the dapivirine ring and 6 participants the placebo ring. Of the 48 participants enrolled in the trial, the total number of participants enrolled overall to the dapivirine ring and placebo ring was 36 and 12, respectively (Figure 1).

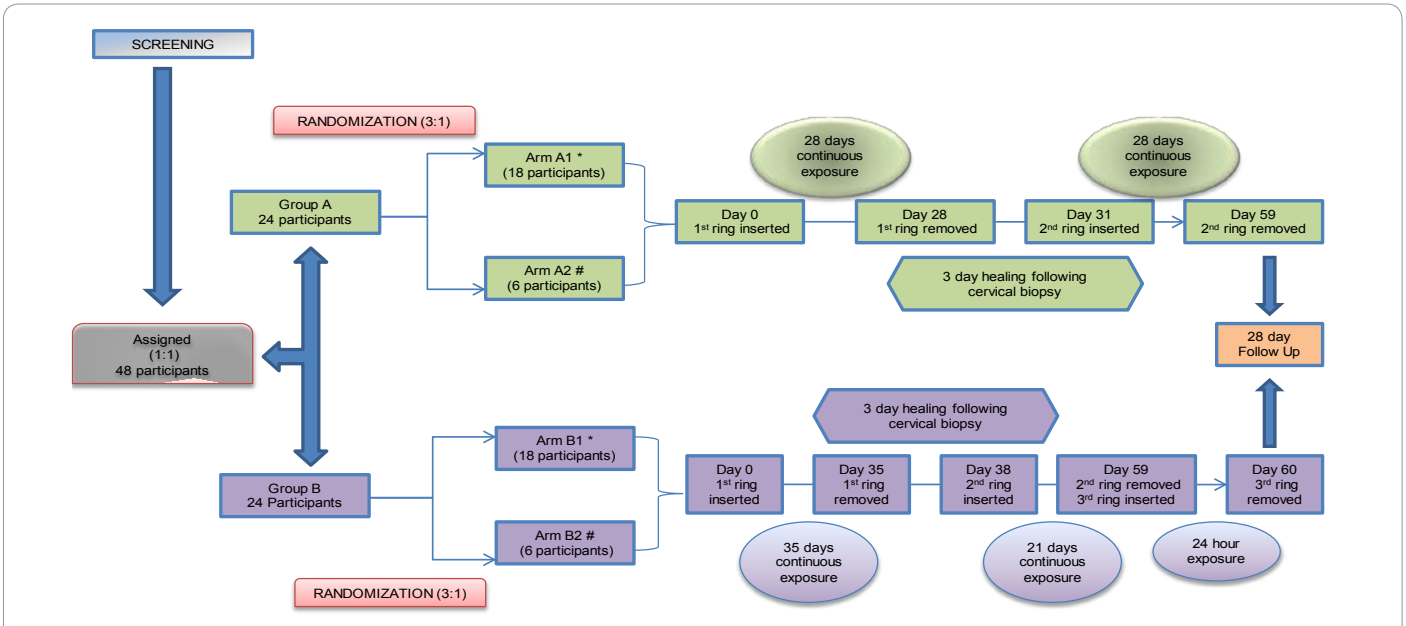
Group A used two rings over a 56-day period as follows: the first ring was inserted and worn continuously for 28 consecutive days whereafter it was removed. After a 3-day period without a ring, a second ring was inserted and worn for another 28 days whereafter it was removed. Group B used three rings but over a 57-day period as follows: the first ring was inserted and worn continuously for 35 consecutive days after which it was removed. Following a 3-day period without a ring, a second ring was inserted and worn for 21 consecutive days. The second ring was then removed and a third ring inserted immediately after removal of the second ring and worn for one day (Figure 1). The rings were inserted and removed by the trial Investigator. After removal of the last ring, all participants were monitored further for safety over a 28-day period.

Participants were eligible to participate if they were healthy, sexually active, using a stable form of contraception, had a normal appearing cervix and vagina, tested negative for HIV, hepatitis B and C, and agreed not to use vaginal products, except tampons, during the trial. "Sexually active" was defined as an average of one penetrative penile-vaginal coital act per month for the three months prior to enrollment. A "stable form of contraception" was defined as either i) a stable oral contraceptive regimen used for at least two months prior to enrollment, ii) a transdermal contraceptive patch used for at least three months prior to enrollment, iii) long-acting progestins used for at least six months prior to enrollment, iv) an intrauterine device (IUD) inserted (with no vaginal or gynecological complaints associated with its use) at least three months prior to enrollment, or v) surgical sterilization at least three months prior to enrollment. Participants were ineligible if they were pregnant, breastfeeding, had untreated symptomatic urogenital infections or other gynecological conditions, had a hysterectomy or recent gynecological surgery, or had laboratory abnormalities of Grade 2 or higher, according to the National Institutes of Health (NIH) Division of Acquired Immunodeficiency Syndrome (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events [20]. Participants were provided with HIV/STI risk reduction counseling at each trial visit, including the dispensing of male condoms for use during vaginal intercourse, but had to agree to abstain from vaginal intercourse and oral contact with the female participant's genitalia for 48 hours prior to each trial visit, as well as for 72 hours after the biopsy procedure.

Due to the exploratory nature of the trial, a sample size of 48 participants was deemed sufficient. Eligible participants were assigned an identification number in sequential order upon enrollment. Vaginal rings were packaged and numbered serially by the Sponsor according to a pre-determined randomization code, thus allocating the participant in a double-blind manner to either an active or placebo ring.

Ring adherence

Participants were instructed on how to insert the ring and were requested to refrain from removing the ring (except as directed during clinic visits). In the event of a ring being expelled, participants were told to wash the ring in water, re-insert it as soon as possible and record the



* Arm A1 and B1=25 mg dapivirine Ring-004. # Arm A2 and B2=placebo ring.

Figure 1: Randomization scheme.

event in the participant ring/symptom diary. Adherence was assessed by inspection of the diary cards and by asking the participants if the vaginal ring remained inserted the whole time.

Pharmacokinetic assessment of dapivirine in plasma and vaginal fluids

Blood and vaginal fluid samples for PK analyses were collected prior to insertion of the first ring and 4, 8 and 24 hours and 7, 14 and 21 days post-ring insertion and again just prior to first ring removal. Samples were also collected just prior to insertion and removal of the second ring, and for Group B, immediately and 4, 8 and 24 hours after insertion of the third ring. Further samples were collected from both groups at the final trial visit that occurred 28 days after the final ring removal. Each participant remained at the research center for a 24-hour period following enrollment and insertion of the first and third rings for blood and vaginal fluid collection and returned to the research center for all other specimen collections. Participants willing to provide blood and vaginal fluid samples during menstruation had samples collected on the third and fifth days of menses.

Vaginal fluid samples were collected from the surface of the cervix, introïtus and near the site of ring placement using tear test strips (Alpha Med™, Richmond, Texas, USA) at each scheduled clinic visit (excluding screening). Vaginal fluid was collected by applying the strip to the epithelial surface. Each tear test strip was weighed before and after sampling. At each blood sampling time point, 5 mL of blood was collected into a labeled 6 mL EDTA vacutainer tube, using an indwelling venous catheter, or by direct venipuncture. Blood samples were centrifuged at approximately 3000 rpm (1500 G) for 10 minutes. The centrifugation was completed within 2 hours of blood collection. The samples were stored at -20°C until shipment to the bioanalytical laboratory responsible for the quantitative analysis of dapivirine.

Plasma and vaginal fluid samples were analyzed for dapivirine content using a validated high-pressure liquid chromatography tandem mass spectroscopy (HPLC-MS/MS) method as previously described [15]. The dapivirine assay in plasma had a lower limit of quantification (LLOQ) of 3 pg/mL, and the tear test strip method had an LLOQ of 0.4 ng/tear test strip, corresponding to approximately 20 ng/g vaginal fluid.

Pharmacokinetic assessment of dapivirine in cervical tissue

Cervical tissue biopsy samples (2 mm by 4 mm) were collected using Baby Tischler biopsy forceps, under topical anesthesia if necessary, following removal of the first ring and after collection of cervicovaginal lavage (CVL) samples for pharmacodynamic assessments (see below) on Day 28 (Group A) or Day 35 (Group B) [21]. The tissue samples were placed in labeled 1.5 mL eppendorf cups, and stored at either -70°C or -80°C within one hour after collection. Dapivirine content was measured by an HPLC-MS/MS method with a calibration range of 0.500 - 250 ng (corresponding to approximately 33.3 - 16667 ng/g vaginal tissue) [21].

Pharmacodynamic assessment of anti-HIV activity of dapivirine in vaginal fluid

Additional vaginal fluid samples for pharmacodynamic assessments were collected by cervicovaginal lavage (CVL) on Day 28 (Group A) and Day 35 (Group B), following sampling with the tear test strip prior to removal of the first ring. A CVL was performed by rinsing the cervical os and posterior vaginal wall with 10 mL of sterile saline [22,23]. The fluid was collected in a labeled 15 mL conical polypropylene tube. The tube was vortexed for 30 seconds to ensure adequate mixing of the sample. A volume of 4 mL of the fluid was stored for pharmacodynamic (PD) analysis at -80°C. The remainder of the fluid was stored at -20°C within one hour after collection, for PK analysis. Shipped CVL samples were logged in upon arrival at the bioanalytical laboratory and stored at -80°C. Prior to PD analysis, samples were thawed on ice, clarified by centrifugation at 4°C for 10 min at 2000 rpm, divided into aliquots (300 µL per tube) and stored at -80°C until used. Jurkat-Tat-CCR5 T cells (1×10^5) in RPMI 1640 culture medium supplemented with 10% fetal bovine serum, 2 mM L-Glutamine, 100 U/mL penicillin and 100 µg/mL streptomycin (complete RPMI) were placed in round-bottomed 96-well tissue culture plates, and the plates centrifuged at 1500 rpm for 5 minutes. The supernatant was removed and 50 µL of neat or diluted (1:10 in normal saline) CVL sample was added to the cell pellets. Either a suspension (50 µL) of HIV-1_{BaL} in complete medium containing 10 ng/mL HIV-1 p24, or complete medium for cell control was added and the culture incubated for 2 hours at 37°C with 5% CO_2. The plates were then centrifuged and the supernatant removed. The cells were washed with serum-free medium and centrifuged as before to completely remove all unbound inoculum virus. Complete medium (200 µL) was then added to all wells and the plates were incubated again at 37°C. At various times (4-6 days) post-infection, samples (50 µL) of culture supernatant were collected and stored at -20°C pending analysis. Viral replication was assessed by determining HIV p24 core protein levels in culture supernatants by antigen capture ELISA [23,24]. All samples were tested in triplicate.

Safety assessments

Safety assessments included pelvic examinations accompanied by colposcopy; pregnancy and HIV ELISA testing; safety laboratory testing; evaluation of sexually transmitted infections (STIs); vaginal flora and vaginal pH assessments; and general physical examinations including vital signs. Concomitant medication and adverse event (AE) information was collected at every trial visit.

Pre-specified post-baseline safety endpoints included the proportion of participants with mucosal abnormalities, positive diagnostic STI tests, at least one treatment-emergent adverse event (TEAE), any abnormalities on hematology or biochemistry, and abnormal vaginal pH and/or vaginal flora. Severity of AEs and laboratory abnormalities were graded by the Investigator according to the DAIDS Table for Grading the Severity of Adult and Pediatric Adverse Events [20]. Gynecological complaints were graded according to the most recent version of the Female Genital Grading Table for use in Microbicide Studies [25].

Statistical analyses

The statistical analysis of safety data was conducted by the International Partnership for Microbicides (IPM), using SAS® for Windows XP (version 9.2; SAS Institute, Inc., Cary, North Carolina, USA) on the intent-to-treat population which included all 48 enrolled participants. The null hypothesis was that the safety profile was similar for the dapivirine and placebo treatment arms, and this was tested by comparing the incidence of the pre-specified safety endpoints. AEs were coded using the Medical Dictionary for Regulatory Activities (MedDRA) (version 10.0). Only TEAEs are presented. The PK analyses included all participants who received at least one dapivirine vaginal ring and for whom at least one blood and/or vaginal fluid sample was obtained. Pharmacokinetic variables were calculated using the validated computer program WinNonlin Professional™ (version 4.1; Pharsight Corporation, Mountain View, California, USA). Non-compartmental

analysis model 200 (extravascular input, plasma data) was applied for the PK analysis. Correlations between dapivirine concentrations (PK) and inhibition of HIV-1 infection by vaginal fluids (neat and diluted 1:10) were performed using GraphPad Prism® (version 6.0; GraphPad Software Inc., San Diego, California, USA).

Investigational products

The active vaginal ring used in this trial (Ring-004) contained 25 mg of dapivirine dispersed in a platinum-catalyzed silicone elastomer and had an outer diameter of 56 mm and a cross-sectional diameter of 7.7 mm. The placebo vaginal ring was identical in composition except it contained titanium dioxide colorant to match the active ring in appearance and did not contain active ingredient.

Ethics

Written informed consent was obtained from each participant prior to the initiation of screening procedures. The trial was approved by the Commissie Voor Medische Ethiek, Ziekenhuisnetwerk in Antwerp and was conducted in full compliance with International Conference on Harmonisation (ICH) guidelines for Good Clinical Practice (GCP). The trial was registered in the ClinicalTrials.gov database (NCT01144676).

Results

Participant disposition and demographics

Forty-five of the 48 (93.8%) randomized participants completed the trial (33 used dapivirine rings and 12 used placebo rings). Three participants discontinued early from the trial: one participant inadvertently received the wrong investigational product (IP); one participant used a prohibited concomitant medication (pantoprazole used for esophagitis); and one participant experienced an AE (generalized pruritus) that led to trial discontinuation (see safety results).

The four treatment regimens were well matched with respect to demography (Table 1). In Group A, the mean age of the participants was 30.0 years for dapivirine ring users and 28.5 years for placebo ring users compared to a mean age of 26.8 years for dapivirine ring users and 29.8 years for placebo ring users in Group B.

Ring adherence

All participants in the placebo and dapivirine arms reported adherence with the trial requirements. In addition, data from the analysis of plasma samples from the 33 participants in the active treatment arm who completed the trial were consistent with correct use of the ring, although the limited sampling during the trial and the variability in plasma dapivirine concentrations associated with use of the ring meant that it could not be concluded with certainty that these participants used the ring consistently over the course of the trial. Two participants in the dapivirine ring group reported accidental expulsion of the ring in their ring diaries. In both cases, the participants reportedly self-inserted the expelled ring within 5 minutes of expulsion.

Pharmacokinetics

Plasma concentrations of dapivirine: Quantifiable plasma concentrations of dapivirine were observed at the earliest scheduled time point (4 hours after insertion of the first ring) for all participants, indicating that dapivirine was rapidly released from the ring and systemically absorbed. In general, plasma concentrations of dapivirine were low, with individual concentrations not exceeding 553 pg/mL (observed in Group B on Day 60), which was well below the plasma dapivirine concentration observed at the maximum tolerated dose

Characteristic	Group A		Group B	
	Dapivirine Vaginal Ring (N=18)	Placebo Vaginal Ring (N=6)	Dapivirine Vaginal Ring (N=18)	Placebo Vaginal Ring (N=6)
	n (%)	n (%)	n (%)	n (%)
Gender:				
Female	18 (100%)	6 (100%)	18 (100%)	6 (100%)
Race:				
White	18 (100%)	6 (100%)	17 (94.4%)	5 (83.3%)
Black	0	0	1 (5.6%)	0
Hispanic	0	0	0	1 (16.7%)
Other	0	0	0	0
Marital Status:				
Married	**2 (11.1%)**	**1 (16.7%)**	**1 (5.6%)**	**3 (50.0%)**
Relationship with multiple partners	0	0	0	0
Not Married	**16 (88.9%)**	**5 (83.3%)**	**17 (94.4%)**	**3 (50.0%)**
One partner, living with that partner	7 (43.8%)	1 (20.0%)	4 (23.5%)	0
One partner, not living with that partner	7 (43.8%)	2 (40.0%)	7 (41.2%)	0
Not in a relationship	2 (12.5%)	2 (40.0%)	6 (35.3%)	3 (100%)
Relationship with multiple partners	0	0	0	0
Mean age in years (range):	30.0 (20.0-40.0)	28.5 (21.0-39.0)	26.8 (18.0-39.0)	29.8 (20.0-40.0)
Mean height in cm (range):	167.2 (159.0-176.0)	171.2 (168.0-178.0)	165.7 (159.0-175.0)	166.3 (162.0-174.0)
Mean weight in kg (range):	64.1 (56.0-84.0)	71.7 (56.0-95.0)	66.4 (50.0-82.0)	62.7 (53.0-78.0)
Mean body mass index in kg/m^2 (range):	22.9 (19.4-29.8)	24.4 (19.6-30.0)	24.2 (19.3-30.9)	22.6 (19.7-28.3)

Table 1: Demographic and Baseline Characteristics.

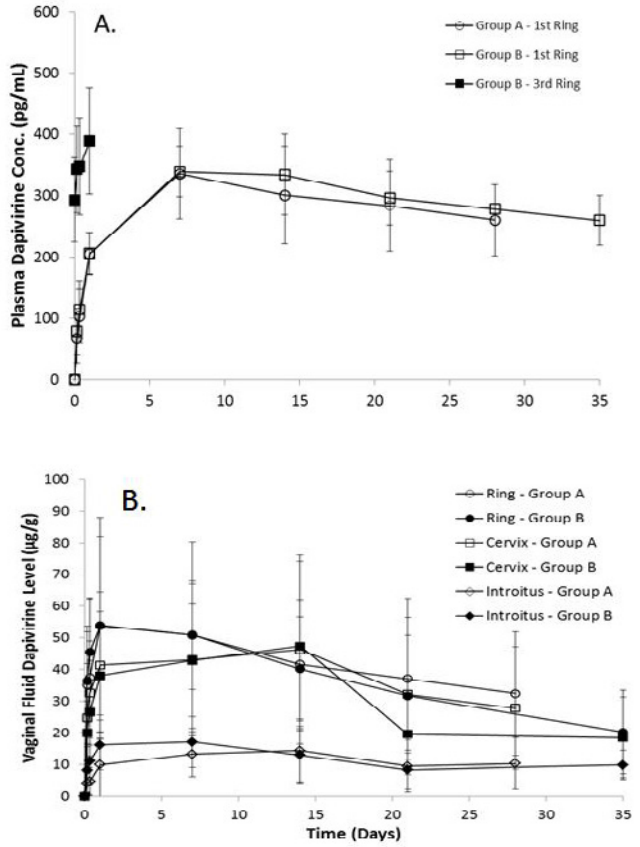


Figure 2: Pharmacokinetics of Dapivirine in Plasma and Vaginal Fluids
Mean (SD) dapivirine concentrations in (A) plasma during the first and third ring use periods, and in (B) vaginal fluid collected from near the ring, at the cervix and near the introïtus during the first ring use periods in Groups A and B. Group A participants (n=17) used the first vaginal ring for 28 days, and Group B participants (n = 16) used the first vaginal ring for 35 days and the third vaginal ring for 24 hours.

for oral treatment (mean C_{max} 2286 ng/mL) (Investigator's Brochure TMC120 Vaginal Microbicide, Tibotec 2003). The mean plasma dapivirine concentration-time curves following insertion of the first vaginal ring were similar for Groups A and B. After maximum plasma concentrations had been reached approximately 7 days post-ring insertion, concentrations showed a gradual decrease until Day 28 or Day 35, when the ring was removed (Figure 2A).

Mean dapivirine plasma concentrations at the end of the first ring use period of 28 days (Group A) or 35 days (Group B) were similar, at approximately 260 pg/mL. Plasma dapivirine concentrations declined by approximately 60% during the 3-day washout period between the first and second ring use periods (Table 2). Vaginal fluid samples were obtained through CVL immediately prior to the removal of the first ring, which could have had an indirect effect on plasma levels during the washout period. The mean plasma concentration (270.4 ± 84.58 pg/mL) at the end of the second 28-day ring use period (Group A) was similar to that observed with the first ring use period (Table 2). With no washout period between the second and third rings in Group B, and following use of the second ring for only 21 days in this group, slightly higher mean plasma dapivirine concentrations were observed over the first 24 hours after insertion of the third ring compared to the first ring (Figure 2A), as reflected in the corresponding plasma $AUC_{0\text{-}24\ h}$ values (Table 2).

Twenty-eight days after removal of the final ring in each group (second ring in Group A or the third ring in Group B), dapivirine plasma concentrations were below LLOQ for the majority of participants. Detectable concentrations on Day 87/88 ranged between 3.38 pg/mL and 8.89 pg/mL for three participants in Group A and two in Group B (data not shown).

Vaginal fluid concentrations of dapivirine collected by tear test strips: Quantifiable levels of dapivirine were also observed in vaginal fluid at the earliest time point (4 hours after insertion of the first ring) at all three collection sites. The lowest mean fluid levels at this time point (observed near the introïtus in both groups; 4222 ng/g in Group A and 8133 ng/g in Group B were at least 1200-fold above the dapivirine concentration required *in vitro* for 99% inhibition (IC_{99}) of provirus integration into cervical tissue following challenge with HIV-1_{BaL} (3.3 ng/mL) (assuming a vaginal fluid density of 1 g/mL) [12]. In vaginal fluid samples from all three collection sites (near the ring and at the cervix and introïtus), dapivirine concentrations increased rapidly following ring insertion and maximum or near maximum concentrations were reached within 24 hours. The fluid concentration-time profile established during the period of use for the first ring demonstrated that vaginal fluid concentrations tended to gradually decrease throughout the period in which the ring was worn (Figure 2B). The highest dapivirine concentrations were found near the ring, followed by the cervix and introïtus (Table 2). Based on ratios of mean values of $C_{prior\ to\ ring\ removal}$ on Day 28 in Group A to those on Day 35 in Group B, mean vaginal fluid concentrations in the area of the ring (Group A: 32.4 ± 19.5 µg/g and Group B: 20.3 ± 13.2 µg/g) and at the cervix (Group A: 27.8 ± 19.3 µg/g and Group B: 18.5 ± 12.8 µg/g) were respectively 38% and 33% lower when the ring was worn for 35 days compared to 28 days. At the introïtus, the mean $C_{prior\ to\ ring\ removal}$ value was only 4% lower in Group B (9.9 ± 4.7 µg/g) relative to Group A (10.3 ± 8.2 µg/g) . These values remained at least 3000 times higher than the *in vitro* IC_{99} (3.3 ng/mL) in cervical tissue [12].

Mean $C_{prior\ to\ ring\ removal}$ values were comparable between the first and second rings in both groups (Table 2). Dapivirine concentrations in vaginal fluid prior to insertion of the second ring (3 days after the removal of the first ring) were near or below the LLOQ. A CVL was performed prior to removal of the first ring, which likely removed much of the dapivirine from the vaginal area. No lavage was performed prior to removal of the second ring on Day 21 in Group B participants, and the third ring was inserted immediately after removal of the second ring. As a result, the vaginal fluid levels at the end of the 24-hour period that the third ring was worn ($C_{prior\ to\ ring\ removal}$) were slightly higher compared to mean values observed 24 hours after the insertion of the first ring in this group. Mean (SD) $C_{prior\ to\ ring\ removal}$ values for the third ring were 55.4 (± 42.91) µg/g (near the ring), 40.1 (± 22.40) µg/g (cervix) and 33.5 (± 48.42) µg/g (introïtus), while mean $C_{24\ h}$ values after insertion of the first ring were 53.9 (± 28.13) µg/g, 38.1 (± 20.14) µg/g and 16.2 (± 7.91) µg/g, respectively (Table 2). The inter-participant variability with respect to the vaginal fluid PK parameters was high, but generally comparable between Group B (ring worn for 35 days) and Group A (ring worn for 28 days) (Table 2).

Considering the known elimination half-life of dapivirine in vaginal fluids (mean values of approximately 12 – 14 hours [26]), total 28-day exposure is not considered to differ much between a single ring insertion or repeated ring insertions.

Vaginal fluid concentrations of dapivirine during menses: Seven participants using dapivirine rings provided plasma and vaginal fluid samples during menses. Four of these women used tampons. Menses

Dapivirine ([Mean [SD])			
Ring	**Pharmacokinetic Parameter**	**Group A (N=17)**	**Group B (N=16)**
Plasma:			
Ring 1	C_{max} (pg/mL) (± SD) (%CV)	347.6 (± 72.16) (20.76)	358.5 (± 52.32) (14.60)
	t_{max} (h)*	170.00 (168.00-674.28)	336.00 (168.00-338.00)
	$AUC_{0\text{-}24\ h}$ (pg.h/mL) (± SD) (%CV)	2939 (± 760.1) (25.86)	3103 (± 833.2) (26.85)
	$AUC_{0\text{-}last}$ (pg.h/mL) (± SD) (%CV)	190600 (± 38490) (20.20)	245500 (± 33200) (13.52)
	$C_{prior\ to\ ring\ removal}$ (pg/mL) (± SD) (%CV)	260.2 (± 58.28) (22.40)	259.8 (± 40.56) (15.61)
Ring 2	$C_{0\ h}$ (pg/mL) (± SD) (%CV)	95.06 (± 49.86) (52.46)	100.2 (± 43.18) (43.11)
	$C_{prior\ to\ ring\ removal}$ (pg/mL) (±SD) (%CV)	270.4 (± 84.58) (31.28)	293.3 (± 68.47) (23.35)
Ring 3	$C_{prior\ to\ ring\ removal}$ (pg/mL) (±SD) (%CV)	–	389.5 (± 86.44) (22.19)
	$AUC_{0\text{-}24\ h}$ (pg.h/mL) (±SD) (%CV)	–	8379 (± 1836) (21.91)
Vaginal fluid (collected by tear test strip) – Cervix:			
Ring 1	C_{max} (µg /g) (± SD) (%CV)	61.22 (± 26.09) (42.63)	55.54 (± 24.06) (43.32)
	t_{max} (h)*	24.80 (4.17 – 506.65)	168.29 (24.17-338.37)
	$AUC_{0\text{-}24\ h}$ (µg.h/g) (± SD) (%CV)	759.9 (± 332.6) (43.77)	653.9 (± 312.4) (47.77)
	$AUC_{0\text{-}last}$ (µg.h/g) (± SD) (%CV)	26070 (± 12850) (49.31)	25440 (± 11750) (46.18)
	$C_{24\ h}$ (µg /g) (± SD) (%CV)	41.39 (± 23.05) (55.68)	38.13 (± 20.14) (52.83)
	$C_{prior\ to\ ring\ removal}$ (µg /g) (± SD) (%CV)	27.84 (± 19.28) (69.23)	18.53 (± 12.80) (69.08)
Ring 2	$C_{0\ h}$ (µg /g) (± SD) (%CV)	0.1856 (± 0.3102) (167.1)	BLQ
	$C_{prior\ to\ ring\ removal}$ (µg /g) (± SD) (%CV)	27.03 (± 12.40) (45.89)	22.42 (± 12.79) (57.05)
Ring 3	$C_{prior\ to\ ring\ removal}$ (µg /g) (±SD) (%CV)	–	40.06 (± 22.40) (55.90)
	$AUC_{0\text{-}24\ h}$ (µg.h/g) (±SD) (%CV)	–	857.8 (± 418.5) (48.79)
Vaginal Fluid (collected by tear test strip) – Introïtus:			
Ring 1	C_{max} (µg /g) (± SD) (%CV)	21.38 (± 10.54) (49.29)	58.83 (± 145.8) (247.8)
	t_{max} (h)*	336.97 (24.12 – 674.98)	24.17 (4.08 - 506.27)
	$AUC_{0\text{-}24\ h}$ (µg.h/g) (± SD) (%CV)	145.1 (± 128.7) (88.73)	273.3 (± 124.5) (45.56)
	$AUC_{0\text{-}last}$ (µg.h/g) (± SD) (%CV)	7676 (± 3636) (47.37)	21420 (± 40180) (187.6)
	$C_{24\ h}$ (µg /g) (± SD) (%CV)	9.98 (± 10.36) (103.8)	16.19 (± 7.91) (48.86)
	$C_{prior\ to\ ring\ removal}$ (µg /g) (± SD) (%CV)	10.33 (± 8.235) (79.90)	9.881 (± 4.669) (47.26)
Ring 2	$C_{0\ h}$ (µg /g) (± SD) (%CV)	0.2810 (± 0.4891) (252.3)	BLQ
	$C_{prior\ to\ ring\ removal}$ (µg /g) (± SD) (%CV)	9.168 (± 6.993) (76.28)	8.327 (± 5.501) (66.07)
Ring 3	$C_{prior\ to\ ring\ removal}$ (µg /g) (± SD) (%CV)	–	33.46 (± 48.42) (144.7)
	$AUC_{0\text{-}24\ h}$ (µg.h/g) (± SD) (%CV)	–	503.5 (± 431.1) (85.62)
Vaginal fluid (collected by tear test strip) – Area near ring:			
Ring 1	C_{max} (µg /g) (± SD) (%CV)	65.14 (± 29.83) (45.80)	67.14 (± 26.84) (39.98)
	t_{max} (h)*	24.32 (4.22 - 671.77)	168.12 (4.13 - 506.87)
	$AUC_{0\text{-}24\ h}$ (µg.h/g) (± SD) (%CV)	951.7 (± 545.6) (57.33)	1035 (± 378.7) (36.58)
	$AUC_{0\text{-}last}$ (µg.h/g) (± SD) (%CV)	28770 (± 14310) (49.72)	31550 (± 12660) (40.12)
	$C_{24\ h}$ (µg /g) (± SD) (%CV)	53.92 (± 33.96) (62.98)	53.86 (± 28.13) (52.23)
	$C_{prior\ to\ ring\ removal}$ (µg /g) (± SD) (%CV)	32.41 (± 19.54) (60.30)	20.25 (± 13.17) (65.04)
Ring 2	$C_{0\ h}$ (µg /g) (± SD) (%CV)	0.8336 (± 2.103) (252.3)	BLQ
	$C_{prior\ to\ ring\ removal}$ (µg /g) (± SD) (%CV)	29.32 (± 16.65) (56.80)	31.11 (± 19.62) (63.05)
Ring 3	$C_{prior\ to\ ring\ removal}$ (µg /g) (± SD) (%CV)	–	55.41 (± 42.91) (77.44)
	$AUC_{0\text{-}24\ h}$ (µg.h/g) (± SD) (%CV)	–	1307 (± 691.3) (52.90)

*Median (range). SD = standard deviation; BLQ = below the limit of quantification; $AUC_{0\text{-}last}$ = $AUC_{0\text{-}28\ days}$ for Group A and $AUC_{0\text{-}35\ days}$ for Group B.

Table 2: Pharmacokinetic Parameters of Dapivirine.

appeared to result in temporarily lower vaginal fluid concentrations of dapivirine, with or without tampon use, but no apparent difference was observed in plasma concentrations (data not shown). This may suggest some lowering of drug levels in the vaginal fluid either due to adsorption to the tampon or increased blood in the vagina or both. However, due to the low number of participants for whom plasma and vaginal fluid samples were obtained during menses (n=7), it cannot be concluded with certainty that tampon use was indeed the reason for the temporarily lower vaginal fluid concentrations observed during menses. The lowest observed vaginal fluid concentration of dapivirine during menses was 490 ng/g, which was still more than 100-fold higher than the *in vitro* IC_{99} for provirus integration into cervical tissue (3.3 ng/mL) following challenge with HIV-1_{BaL}.

Dapivirine levels in cervical tissue: Dapivirine concentrations were quantifiable in the cervical tissue samples of all dapivirine ring users. The mean dapivirine tissue level in Group A was 1913 ± 3107 ng/g compared to 802.7 ± 839.5 ng/g in Group B. This difference was largely due to one relatively high concentration in a Group A participant (12900 ng/g). Other than this value, individual concentrations ranged

from 46.0 ng/g to 4770 ng/g in Group A and from 118 ng/g to 3090 ng/g in Group B, which are approximately 14 to 1000 times higher than the *in vitro* IC_{99} (3.3 ng/mL). In general, the inter-participant variability of dapivirine concentrations in cervical tissue was high: Group A: %CV = 162%; and Group B: %CV = 105%.

Pharmacodynamics

Anti-HIV activity of dapivirine in vaginal fluid: Antiviral activity of CVL samples collected at the end of the first ring-use period was determined *ex vivo* by challenging JT-CCR5 T cells with HIV-1_{BaL} and measuring HIV p24 core protein in culture supernatants. CVL from participants exposed to placebo vaginal rings inhibited HIV-infection by a mean of 18% when used neat, but the activity was lost when samples were diluted 1:10 (Figure 3). This low level of activity was attributed to some innate inhibition in vaginal lavage fluid, an effect that has been documented previously [22,27,28]. In contrast, samples from participants treated with rings containing dapivirine inhibited HIV-infection by a mean of 89% when used neat, and retained 71% inhibitory activity when diluted 10-fold (Figure 3). Given an approximately 50 to 170-fold dilution of cervicovaginal fluid due to the lavage procedure and expected loss of dapivirine during sample processing, these are likely underestimations of the *in vivo* biological activity.

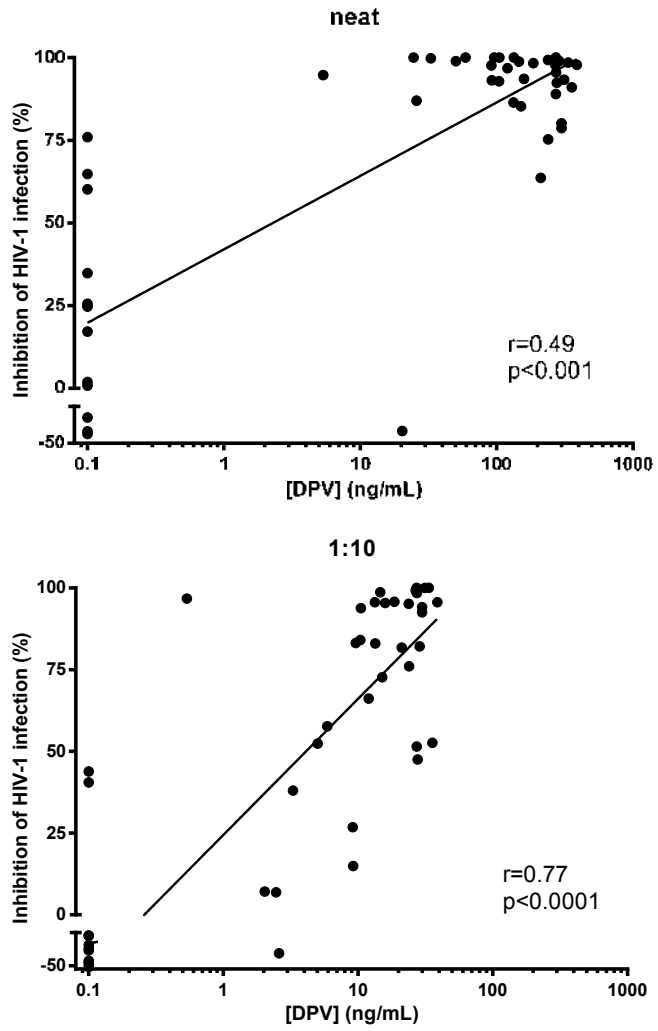


Figure 3: Dapivirine PK/PD correlation in vaginal fluid
Correlation between CVL antiviral activity against HIV-1_{BaL} infection in JT-CCR5 cells and dapivirine concentrations in vaginal fluid at the end of the first ring-use period for Group A (ring worn for 28 days) and Group B (ring worn for 35 days) participants (Spearman PK/PD correlation; neat, r = 0.49 and 1:10, r = 0.77). Samples with dapivirine levels below the LLOQ were attributed the value of 0.1 ng/ml so that values could be plotted on a log scale.

Dapivirine concentrations in the CVL samples were quantifiable in all participants using dapivirine rings and were similar in both groups: 197.3 ± 137.5 ng/mL in Group A, and 189.2 ± 113.6 ng/mL in Group B, with individual values ranging between 20.3 ng/mL and 535 ng/mL. Therefore, even a 10-fold dilution would result in a mean concentration at least 6-fold higher than the *in vitro* IC_{99} against HIV-1_{BaL} in cervical tissue (3.3 ng/mL), and this would account for the high levels of inhibition observed. Inter-participant variability in dapivirine concentrations was high, but similar between Group A (%CV = 70%) and Group B (%CV = 60%). As could be expected, these concentrations were well below the concentrations obtained by tear test strips, due to dilution in the lavage fluid. It is also likely, based on previous investigations (data not shown), that substantial amounts of dapivirine were lost during the sample collection procedure due to precipitation of the drug in the lavage fluid and adsorption of dapivirine to the collection equipment.

Safety

There were no relevant differences observed between the treatment groups with respect to pelvic examination and colposcopic findings. Ten participants, six (6/36; 16.7%) using dapivirine rings (four Group A; two Group B) and four (4/12; 33.3%) using placebo rings (three Group A; one Group B) presented with post-enrollment colposcopic abnormalities: for participants using dapivirine rings, erythema of the cervix was observed for three participants (two Group A; one Group B); ecchymosis of the cervix for one participant (Group A); petechiae (fornix) for one participant (Group A); and a superficial abrasion of the fornix for one participant (Group B). For placebo ring users, erythema of the cervix was observed for two participants (one Group A; one Group B); petechiae (fornix) for one participant (Group A); hypervascularization (cervix) for one participant (Group A); and peeling and a grossly white finding (cervix) for one participant.

Based on a definition of abnormal vaginal pH of values below 3.5 or greater than 4.8, 20 participants, 14 (15/36; 38.9%) using dapivirine rings (6 Group A; 8 Group B) and six (6/12; 50.0%) using placebo rings (3 Group A; 3 Group B) showed abnormal vaginal pH values post-enrollment. The corresponding baseline (enrollment) number was 15 participants, 11 (11/36; 30.6%) assigned to dapivirine rings (6 Group A; 5 Group B) and four (4/12; 33.3%) assigned to placebo rings (1 Group A; 3 Group B). None of these abnormal pH values were considered to be clinically significant.

At screening, none of the randomized participants had a Nugent score ≥ 7 (abnormal vaginal flora). One participant, assigned to dapivirine rings in Group A, presented with abnormal vaginal flora at the enrollment visit; this was regarded by the Investigator as not clinically significant. Three participants, all (3/36; 8.3%) using dapivirine rings (2 Group A; 1 Group B), showed abnormal vaginal flora post-enrollment. None of these instances were considered by the Investigator as clinically significant.

The majority of TEAEs reported were mild in severity. The most commonly observed TEAE was metrorrhagia (21 [58.3%] dapivirine participants and nine [75.0%] placebo participants) (Table 3). All cases were assessed by the Investigator as mild (Grade 1) in severity. Other frequently reported TEAEs were headache (14 [38.9%] dapivirine ring

MedDRA System Organ Class/Preferred Term	Overall		Group A		Group B	
	Dapivirine Vaginal Ring (N=36)	Placebo Vaginal Ring (N=12)	Dapivirine Vaginal Ring (N=18)	Placebo Vaginal Ring (N=6)	Dapivirine Vaginal Ring (N=18)	Placebo Vaginal Ring (N=6)
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Participants with one or more events	32 (88.9)	11 (91.7)	17 (94.4)	5 (83.3)	15 (83.3)	6 (100)
All Local TEAEs:						
Reproductive system and breast disorders						
Dysmenorrhoea	0	1 (8.3)	0	0	0	1 (16.7)
Metrorrhagia	21 (58.3)	9 (75.0)	11 (61.1)	5 (83.3)	10 (55.6)	4 (66.7)
Vaginal discharge	3 (8.3)	2 (16.7)	1 (5.6)	1 (16.7)	2 (11.1)	1 (16.7)
Vaginal pain	1 (2.8)	0	1 (5.6)	0	0	0
Vulvovaginal discomfort	4 (11.1)	1 (8.3)	2 (11.1)	0	2 (11.1)	1 (16.7)
Vulvovaginal dryness	1 (2.8)	0	1 (5.6)	0	0	0
Vulvovaginal pruritus	2 (5.6)	0	1 (5.6)	0	1 (5.6)	0
Infections and Infestations						
Chlamydial infection	0	1 (8.3)	0	0	0	1 (16.7)
Vaginal candidiasis	1 (2.8)	0	1 (5.6)	0	0	0
Other TEAEs Occurring in 10% or More of the Participants in any for the Dapivirine Arms:						
Gastrointestinal disorders						
Abdominal pain	4 (11.1)	5 (41.7)	2 (11.1)	2 (33.3)	2 (11.1)	3 (50.0)
Abdominal pain lower	4 (11.1)	4 (33.3)	3 (16.7)	1 (16.7)	1 (5.6)	3 (50.0)
Dyspepsia	4 (11.1)	1 (8.3)	4 (22.2)	0	0	1 (16.7)
Nausea	5 (13.9)	1 (8.3)	3 (16.7)	0	2 (11.1)	1 (16.7)
General disorders and administration site conditions						
Influenza like illness	3 (8.3)	0	2 (11.1)	0	1 (5.6)	0
Musculoskeletal and connective tissue disorders						
Back pain	2 (5.6)	0	2 (11.1)	0	0	0
Neck pain	3 (8.3)	0	3 (16.7)	0	0	0
Nervous system disorders						
Headache	14 (38.9)	6 (50.0)	7 (38.9)	3 (50.0)	7 (38.9)	3 (50.0)
Presyncope	2 (5.6)	0	0	0	2 (11.1)	0
Somnolence	2 (5.6)	0	2 (11.1)	0	0	0
Renal and urinary disorders						
Dysuria	2 (5.6)	1 (8.3)	2 (11.1)	1 (16.7)	0	0
Respiratory, thoracic and mediastinal disorders						
Pharyngolaryngeal pain	3 (8.3)	3 (25.0)	1 (5.6)	1 (16.7)	2 (11.1)	2 (33.3)
Skin and subcutaneous tissue disorders						
Rash	2 (5.6)	0	0	0	2 (11.1)	0

MedDRA=Medical Dictionary for Regulatory Activities; TEAE=Treatment-emergent Adverse Event

Table 3: Incidence of Treatment Emergent Adverse Events.

users and six [50.0%] placebo ring users) and abdominal pain (four [11.1%] dapivirine participants and five [41.7%] placebo participants). TEAEs reported by at least 10% of participants using dapivirine rings were nausea, vulvovaginal discomfort and dyspepsia, and in the placebo group, pharyngolaryngeal pain, vaginal discharge, fatigue, diarrhea and abdominal distension.

One participant was discontinued from the dapivirine ring and the trial due to Grade 2 (moderate) generalized pruritus which was assessed by the Investigator as possibly related to ring use. The event resolved completely following treatment.

Safety laboratory test results showed one Grade 4 biochemistry abnormality for a participant in the dapivirine ring group. The participant had normal calcium values of 9.1 mg/dL at baseline and 9.0 mg/dL on Day 28. Test results at Day 59 showed a low calcium value of 3.8 mg/dL (reported as Grade 4). A repeat test at the final trial visit on Day 87 showed that the parameter had returned to within the limits of the laboratory reference range (value of 9.1 mg/dL).

All participants tested negative for HIV throughout the trial and no pregnancies were reported.

The relative risk ratios of experiencing a primary safety outcome for the dapivirine compared to the placebo ring groups were 0.9 for both Groups A and B (95% CI of 0.84; 1.06 and 0.75; 1.05, respectively), suggesting no clinical safety difference between the arms.

Discussion

The Dapivirine Vaginal Ring-004 is designed to provide sustained delivery of the NNRTI dapivirine in order to afford protection against HIV-1 infection in women over extended periods without requiring

frequent interventions by the user. This reduced burden of adherence on the user is likely to improve correct use of the product compared with products intended for daily use, or those for use around the time of sexual intercourse. This, in turn, is expected to result in a product with increased efficacy.

The trial described here was the first trial in which multiple vaginal rings containing dapivirine were used consecutively, and was intended to evaluate any alterations in the safety and pharmacokinetics of dapivirine associated with use of more than one ring. In addition, the trial examined the influence of truncated or prolonged patterns of use of a single ring, along with a preliminary assessment of the effects of menses on the PK of dapivirine when delivered from the vaginal ring.

The Dapivirine Vaginal Ring-004 was safe and well tolerated with no safety concerns identified during the trial. Post-enrollment pelvic and colposcopic examination findings were minimal with none of the findings reported as deep or associated with bleeding. Most findings were transient and had resolved by the next scheduled visit and were not reported as AEs. All findings that were reportedly ongoing at the end of the trial (petechiae in one dapivirine ring user; and erythema, hypervascularization, and peeling and grossly white finding in four placebo ring users) were considered by the Investigator as not clinically significant and none required further follow-up. None of the pelvic examination or colposcopy findings were regarded by the Investigator as related to ring use.

Results from physical examinations, vital signs and clinical laboratory evaluations showed no clinically relevant changes from baseline. No differences in the incidence of TEAEs, genital symptoms or Nugent scores were observed between the dapivirine ring and the placebo ring group. These data are consistent with those from previous trials in which the use of a single Dapivirine Vaginal Ring-004 was investigated, and demonstrate a good safety profile for the use of multiple rings over approximately 56 days [15,26]. In addition, these data also correspond to the safety profile observed during a 3-month observational safety arm of an earlier IPM trial (IPM 011, unpublished data), when no device or investigational product was administered (ClinicalTrials.gov No NCT00469170). IPM 011 was an open-label, multi-center, crossover trial investigating the safety and acceptability of a silicone elastomer vaginal ring containing no active ingredient (only a placebo ring was used in the trial) at multiple research centers in South Africa and Tanzania - the placebo vaginal ring was found to be safe and well tolerated with no evidence of a difference in safety between the vaginal ring and the observational safety regimens of the trial.

Dapivirine was detectable in vaginal fluid and plasma within 4 hours after ring insertion, indicating that it was readily released from the ring and absorbed into the surrounding tissue and into the bloodstream. Compared to the vaginal fluids, systemic exposure to dapivirine in plasma was low with concentrations of not more than 553 pg/mL, whereas concentrations of up to 171 μg/g were measured in vaginal fluids. Maximum or near maximum concentrations in vaginal fluids tended to be reached within the first day after ring insertion, but in plasma it took approximately 7 days to reach peak concentrations.

Plasma concentrations at the end of the period of ring use were similar for both 28-day and 35-days use periods. Vaginal fluid concentrations after 28 days of ring use were approximately one third higher in the area of the ring and at the cervix than those in the same locations after 35 days of use, but at the introïtus the difference between the two use periods had little effect on concentration. However, inter-participant variability in vaginal fluid concentrations was generally high, so differences between Groups A and B should be interpreted with caution. Nevertheless, the data suggest that extending the period the ring is worn from 28 to 35 days does not result in a major drop in vaginal fluid concentrations.

When the second ring in Group B was removed after 21 days and immediately replaced with a third ring, dapivirine concentrations over the following 24 hours were higher in both the plasma and the vaginal fluids when compared to values over the initial 24 hours after insertion of the first ring.

The potential for the ring to be effective for a period of at least 35 days was also supported by *ex vivo* experiments of viral replication in susceptible cells challenged with HIV-1 in the presence of fluids collected by cervicovaginal lavage from women who had used the Dapivirine Vaginal Ring-004 for 28 or 35 days. The lavage procedure meant that the fluids were substantially diluted during collection, but high levels of inhibition were seen even when the fluids were diluted by a further 10-fold. Although the primary site of action for dapivirine is likely to be within target CD4 cells in the tissues of the lower female reproductive tract rather than in the vaginal lumen [29], the data demonstrate dapivirine remains biologically active in the presence of vaginal fluid which may suggest that delivery of dapivirine from the ring is sufficient to be protective.

The influence of menses on drug concentrations was of interest because of the potential impact on the efficacy of the ring. Menses did appear to temporarily result in lower vaginal fluid concentrations of dapivirine both with and without tampon use, although there was no apparent effect on plasma concentrations. However, the limited amount of data available means that no definite conclusions could be made, and this will be further investigated in future trials.

The PK assessments of dapivirine ring use demonstrated rapid achievement of drug concentrations consistent with HIV-1 infection inhibition in vaginal fluids obtained from key anatomic locations and in cervical tissue (obtained on Day 28 for Group A and Day 35 for Group B) during the entire time period each ring was worn. This suggests that the dapivirine ring may provide protection against HIV-1 infection for up to 35 days before needing replacement.

Plasma dapivirine levels for the 33 participants in the active treatment arm who completed the trial indicate that the Dapivirine Vaginal Ring-004 was used correctly by these participants. However, due to the limited number of plasma samples collected during the trial, and the variability in plasma dapivirine concentrations associated with use of the ring, it cannot be concluded with certainty that all participants were adherent with the use of the vaginal ring throughout the trial.

The HIV epidemic continues to exact a critical toll in sub-Saharan Africa where the predominant route of transmission is heterosexual intercourse, and where women represent the majority of both prevalent and incident infections. Any effective public health approach to preventing HIV transmission to women in this region of the world must not only be clinically effective and safe but also acceptable to both women and their male partners and allow women at high risk of HIV infection to be able to use it with or without their partner's consent. Ideally, it would also provide protection over relatively long periods of time to minimize issues of adherence to product use. Based on the results from this trial the Dapivirine Vaginal Ring-004 has the potential to meet these criteria.

Conclusions

Dapivirine Vaginal Ring-004 is a promising new approach to HIV prevention in women. The consecutive use of several rings over a

period of up to 57 days was safe and well tolerated, and PK data indicate that a single ring is likely to be protective for at least 35 days. These data support the continued development of Dapivirine Vaginal Ring-004 and evaluation of the safety and efficacy of this ring in a Phase III clinical program.

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