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A silicone elastomer vaginal ring for HIV prevention containing two microbicides with different mechanisms of action

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ABSTRACT

Vaginal rings are currently being developed for the long-term (at least 30 days) continuous delivery of microbicides against human immunodeficiency virus (HIV). Research to date has mostly focused on devices containing a single antiretroviral compound, exemplified by the 25 mg dapivirine ring currently being evaluated in a Phase III clinical study. However, there is a strong clinical rationale for combining antiretrovirals with different mechanisms of action in a bid to increase breadth of protection and limit the emergence of resistant strains. Here we report the development of a combination antiretroviral silicone elastomer matrix-type vaginal ring for simultaneous controlled release of dapivirine, a non-nucleoside reverse transcriptase inhibitor, and maraviroc, a CCR5-targeted HIV-1 entry inhibitor. Vaginal rings loaded with 25 mg dapivirine and various quantities of maraviroc (50–400 mg) were manufactured and *in vitro* release assessed. The 25 mg dapivirine and 100 mg maraviroc formulation was selected for further study. A 24-month pharmaceutical stability evaluation was conducted, indicating good product stability in terms of *in vitro* release, content assay, mechanical properties and related substances. This combination ring product has now progressed to Phase I clinical testing.

1. Introduction

In the last decade, increased access to highly active antiretroviral therapy has led to a decline in the number of new HIV infections occurring annually (3.1 million in 2001, 2.7 million in 2010) and significantly increased life expectancy for infected individuals (UNAIDS, 2010; WHO, UNAIDS and UNICEF, 2011). The positive impact of antiretroviral drugs on the treatment of HIV infection has spurred interest in their potential for preventing sexual transmission of human immunodeficiency virus (HIV), with promising results obtained following both oral (Cohen et al., 2011; Grant et al., 2010) and vaginal (Abdool Karim et al., 2010) administration.

In the context of HIV prophylaxis, a topical microbicide is a vaginally or rectally administered product designed to prevent HIV transmission across the relevant mucosal tissue (Fetherston et al., 2010; Garg et al., 2009; Kelly et al., 2011; Nuttall et al., 2010; Romano et al., 2008; Shattock et al., 2004; Stone and Harrison, 2010; Woolfson et al., 2006a). Antiretroviral-based microbicide products are now being developed and various animal studies have demonstrated some degree of efficacy (Caron et al., 2010; Fetherston et al., in press; Lederman et al., 2004; Malcolm et al., in press; Neff et al., 2011; Singer et al., 2012; Stolte-Leeb et al., 2011; Veazey et al., 2005a, 2010). Results from the CAPRISA 004 study, the first human clinical trial to assess efficacy of a vaginally administered ARV microbicide product (Abdool Karim et al., 2010), demonstrated that coitally-related twice daily application of 1.0% tenofovir vaginal gel reduced HIV transmission to women by 39% and by up to 54% in high adherence users. Unfortunately, recent data from the VOICE (Vaginal and Oral Interventions to Control the Epidemic) trial indicated that once daily administration of tenofovir, either orally (tablet) or vaginally (gel), was not effective in protecting women from sexually acquired HIV infection. Although the formulations were found to be safe in use, both arms of the trial were discontinued due to futility. Evaluation of once daily Truvada (a combination of emtricitabine and tenofovir disoproxil fumarate for oral administration) is continuing. Other lead candidate antiretroviral compounds being developed as HIV microbicides include dapivirine (an experimental, small molecule non-nucleoside reverse transcriptase inhibitor, NNRTI), and maraviroc (a small molecule CCR5-targeted HIV-1 entry inhibitor) (Dorr et al., 2005; MacArthur and Novak, 2008). Vaginally administered gels (Nel et al., 2009b, 2010a,b), rings (Gupta et al., 2008; Johnson et al., 2010; Malcolm et al., 2005; Nel et al., 2009a; Woolfson et al., 2006b) and film formulations (Akil et al., 2011) containing dapivirine have been described previously. For maraviroc, which

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is currently used as part of oral antiretroviral therapy to reduce viral load in infected people, various vaginal gel and ring formulations have been evaluated as potential microbicide strategies (Forbes et al., 2011; Malcolm et al., 2012a, in press; Neff et al., 2011; Veazey et al., 2010).

Given the ongoing concerns with adherence to prescribed use regimes (Abdool Karim et al., 2010), a single application microbicide product that provides continuous microbicide delivery over a long time period is likely to be more effective than one administered pericoitally (e.g. gels). Vaginal rings are being developed for the controlled delivery of one or more HIV microbicides to the vagina (Fetherston et al., 2010, in press; Han et al., 2007; Johnson et al., 2010; Kaur et al., 2011; Malcolm et al., 2005, 2010, 2012a; Moss et al., 2012; Nel et al., 2009a; Romano et al., 2009; Saxena et al., 2009; Singer et al., 2012; Woolfson et al., 2006a, 2006b). Use of such devices is female-initiated, which may prove useful in reducing sexual HIV transmission in women. Vaginal rings offer a number of advantages to users over conventional gel formulations, such as a longer duration of action, reduced dosing intervals, continuous placement and the possibility of covert use (Fetherston et al., 2010; Malcolm et al., 2010; Woolfson et al., 2006a).

The most clinically advanced microbicide-releasing vaginal ring is a matrix-type silicone elastomer product containing 25 mg dapivirine, a non-nucleoside reverse transcriptase inhibitor (NNRTI) with potent activity against HIV-1 *in vitro* (Fletcher et al., 2009; Van Herrewege et al., 2004a,b). Based on *in vitro* studies, reservoir-type vaginal rings loaded with 25 mg dapivirine have been reported to release sufficient quantities of dapivirine to provide *in vitro* concentrations capable of inhibiting vaginal HIV transmission (Malcolm et al., 2005; Woolfson et al., 2006a,b). Several Phase I dapivirine studies have been completed, including the testing of various vaginal gel formulations (Nel et al., 2009b, 2010a,b) and a matrix-type and reservoir-type vaginal ring (Nel et al., 2009a; Romano et al., 2009). The matrix ring entered Phase III testing in 2012.

Standard treatment for HIV/AIDS involves the use of combination ARVs as part of highly active antiretroviral therapy (HAART). By using drugs from different therapeutic classes and having different mechanisms of action, the virus is targeted at multiple stages of the infection/replication cycle, which can increase the breadth of activity and reduce the propensity for emergence of resistant viral strains (Garg et al., 2009, 2010; Herrera et al., 2009; Martinez et al., 2006; Pirrone et al., 2011; Ramjee et al., 2011). It is rational to extend this combination strategy to ARV-based vaginal microbicides (Herrera et al., 2009; Schader et al., 2011). A combination of emtricitabine (nucleoside reverse transcriptase inhibitor) and tenofovir disoproxil fumarate (nucleotide analogue reverse transcriptase inhibitor) has already been shown to confer protection (Grant et al., 2010) and the same combination is currently being investigated as part of VOICE. Other combination microbicide ring products are in the early stages of preclinical development (Johnson et al., 2010; Moss et al., 2012; Saxena et al., 2009).

Here, we report the pharmaceutical development and testing of a silicone elastomer vaginal ring formulation that provides controlled release of dapivirine and maraviroc.

2. Materials and methods

2.1. Materials

Dapivirine (micronized) and maraviroc (non-micronized) were supplied by the International Partnership for Microbicides (Silver Spring, MD, USA). Medical grade silicone elastomers LSR9-9508-30 and MED-4870 were purchased from NuSil Technology (Carpinteria, CA, USA). Both materials are addition-cure, translucent, rapid-cure, liquid silicone rubber systems supplied as two-part kits. The parts (Parts A and B) must be mixed to initiate the curing reaction. LSR9-9508-30 has a lower pre-cure viscosity and a lower post-cure Shore hardness compared with MED-4870, and was used during early stage development for ease of injection molding. MED-4870 is presently used in the manufacture of the dapivirine-only ring. HPLC-grade water was obtained using a Millipore Direct-Q 3 UV Ultrapure Water System (Millipore, Watford, UK). Dichloromethane, HPLC-grade methanol, HPLC-grade acetonitrile, potassium dihydrogen orthophosphate (AnalaR analytical reagent) and HPLC-grade isopropanol were purchased from VWR International Ltd. (Dublin, Ireland). Phosphoric acid (85% w/w in water) and 19-norethindrone (internal standard for HPLC) were purchased from Sigma–Aldrich (Gillingham, UK) and ammonium acetate was purchased from Fisher Scientific (Loughborough, UK).

2.2. Thermal analysis of microbicide-loaded cured silicone elastomer samples

Samples of cured LSR9-9508-30 silicone elastomer loaded with either maraviroc (10% w/w), dapivirine (10% w/w) or both maraviroc and dapivirine (10% w/w each) were prepared by mixing the appropriate quantities of each component in a SpeedMixer DAC 150 FVZ-K (Synergy Devices, High Wycombe, UK) (1:1 w/w mixture of Parts A and B, 3500 rpm, 20 s) before curing in an oven (50 °C, 30 min). Differential scanning calorimetry (DSC) analysis of each silicone sample (~35 mg) and the supplied microbicides (~5 mg) was conducted using a calibrated TA Instruments Q100 Differential Scanning Calorimeter (Elstree, UK) in standard heating ramp mode. Baseline calibration was carried out using an empty cell and temperature calibration was conducted using cyclopentane and indium standards. Test samples were heated under an atmosphere of nitrogen (50–300 °C, 10 °C/min) in closed aluminium pans (TA Instruments, Elstree, UK) (part numbers 900779.901 and 900786.901), alongside an empty reference pan.

2.3. Solubility of dapivirine and maraviroc in cured silicone elastomer

The saturation solubilities of dapivirine and maraviroc in LSR9-9508-30 and MED-4870 silicone elastomers were determined using a method described previously (van Laarhoven et al., 2002). Briefly, silicone elastomer films (1.5 mm thick) were prepared from each elastomer system by combining Parts A and B (1:1 w/w) and curing (LSR9-9508-30, 24 h at 25 °C; MED-4870, 1 h at 150 °C) between glass plates separated by spacers. The cured films were cut into strips (50 mm × 50 mm × 1.5 mm) and each strip ($n = 4$ per elastomer per microbicide) was weighed and placed in a glass flask containing 100 mL HPLC-grade water. Excess (~200 mg) of either dapivirine or maraviroc was added to each sample and the flasks were stored in a rotating orbital incubator (Infors HT Unitron, Infors AG, Bottmingen, Switzerland) for 8 weeks (37 °C, 60 rpm) to allow drug to saturate the silicone elastomer. Strips were removed from the flasks, rinsed with HPLC-grade water and blotted dry. Each strip was cut into small pieces (approximately 5 mm × 5 mm × 1.5 mm) and refluxed in a mixture comprising 95 mL dichloromethane and 5 mL internal standard solution (5 mg/mL 19-norethindrone in methanol) for 2 h. A 10 mL aliquot of the reflux solutions were removed and evaporated to dryness using a BUCHI® Syncore Polyvap system (BUCHI UK Ltd., Oldham, UK), reconstituted in 2 mL acetonitrile, vortexed (30 s), sonicated (20 min) and then centrifuged (10 min, 2000 rpm). This extraction method has previously been validated and used to provide complete extraction of the dapivirine and/or maraviroc content from silicone strips/rings (Nel et al., 2009a; other unpublished data). The concentration of dapivirine or maraviroc in the final solutions was quantified by reverse-phase HPLC (Section 2.4) and the data

used to calculate the solubility of each drug in the silicone elastomers.

2.4. HPLC analytical method for quantification of silicone elastomer solubility, in vitro release and drug content

HPLC analysis was performed using a Waters HPLC system (Waters, Dublin, Ireland) consisting of a Waters 1525 Binary HPLC pump, a Waters 717 Plus Autosampler, a Waters In-line Degasser AF Unit and a Waters 2487 Dual λ Absorbance Detector, along with Breeze software (Version 3.30). Each sample was injected (25 μL aliquot) onto a Thermo Scientific BDS Hypersil C18 column (150 × 4.6 mm, 3 μm particle size) (Fisher Scientific UK Ltd., Loughborough, UK) held at 30 °C. Gradient elution was performed using a mobile phase of 10 mM potassium dihydrogen orthophosphate (adjusted to pH 3.0 using phosphoric acid) (solvent A) and acetonitrile (solvent B) with a flow rate of 1.0 mL/min and run time of 7 min (0.0–4.0 min: 70–20% A; 4–4.5 min: 20–70% A; 4.5–7.0 min: 70% A). Maraviroc, 19-norethindrone and dapivirine were detected by UV absorption at 210 nm with retention times of 4.8, 6.3 and 6.6 min, respectively.

2.5. Manufacture of rings for formulation development

Matrix-type, silicone elastomer, human-sized vaginal rings loaded with 25 mg dapivirine and 25, 50, 100, 200 or 400 mg maraviroc were manufactured by high temperature reaction injection moulding. Dapivirine and maraviroc were mixed at the appropriate concentrations into both Parts A and B of the platinum-catalysed silicone elastomer LSR9-9508-30 using a SpeedMixer (1 min, 3000 rpm). The active mixes were combined (1:1 w/w), speed-mixed (1 min, 3000 rpm), injected into stainless steel molds fitted to a laboratory-scale ring-making machine, and cured for 3 min at 80 °C. The vaginal rings measured 56.0 and 7.6 mm in external and cross-sectional diameter, respectively (Fig. 1).

2.6. In vitro release testing of vaginal rings

In vitro release testing over 28 or 29 days was performed for both LSR9–9508-30 (n = 4, 28 days) and MED-4870 (n = 12 for each stability storage condition, 29 days) vaginal rings using a sink condition model. Individual rings were placed into 250 mL DURAN® laboratory glass bottles containing 200 mL 1:1 mixture of isopropanol/water and stored in an orbital shaking incubator (37 °C, 60 rpm, 25 mm orbital throw). The release medium was sampled and replaced (100 mL) daily with the exception of weekends (200 mL used to maintain sink conditions). Dapivirine and maraviroc concentrations were quantified by reverse-phase HPLC with UV detection, as described previously (Section 2.4).

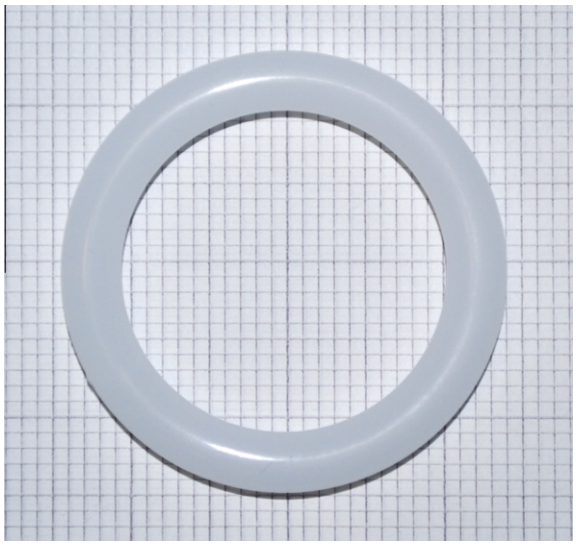

Fig. 1. Silicone elastomer vaginal ring containing 25 mg dapivirine and 100 mg maraviroc. Ring dimensions: 56 mm external diameter, 7.6 mm cross-sectional diameter.

2.7. Manufacture of rings for stability testing

Matrix-type silicone elastomer vaginal rings loaded with 100 mg maraviroc and 25 mg dapivirine were manufactured using a liquid silicone rubber injection molding machine (Arburg 125V, Loesburg, Germany). Batches of premix containing appropriate quantities of dapivirine and maraviroc in Part A of the platinum-catalysed silicone elastomer MED-4870 were produced using a paddle blade mixer (60 rpm for 5 min then 100 rpm for 35 min). Six separate batches of the Part A premix were transferred into a 20 L pail and degassed for 30 min. The same process was repeated to produce premix in Part B MED-4870. Parts A and B premixes were separately pumped via a dosing meter (E4-5M, Fluid Automation, Wixom, MI, USA) through a static mixer (1:1 mix ratio) into the injection molding machine. The mix was then injected into a heated stainless steel mold assembly comprising a single ring shaped cavity (outer diameter 57.6 mm, cross sectional diameter 7.7 mm). Each ring was cured at 140 °C for 25 s.

2.8. Testing protocols for ring stability study

Vaginal rings were packaged in semi-permeable, paper-plastic pouches and stored in stability cabinets (Binder KBF 115 Constant Climate Chamber, Binder GmbH, Tuttlingen, Germany) maintained at 30 °C/65% relative humidity (RH) or 40 °C/75% RH (ICH guidelines for accelerated stability studies). At defined monthly time-points (T_0, T_{1M}, T_{2M}, T_{3M}, T_{6M}, etc.), a sample set of 35 active vaginal rings and three placebo vaginal rings was removed for analysis (Table 1). At each time-point, vaginal rings were tested for *in vitro* release of maraviroc and dapivirine, mechanical properties, content assay and the presence of related substances.

2.9. Mechanical testing of rings

Mechanical properties of vaginal rings were assessed at each time-point (n = 10) by compression testing and measurement of tensile strength. Compression and tensile testing were performed using a TA.XTplus Texture Analyser (Stable Micro Systems Ltd., Godalming, UK). For compression testing, each vaginal ring was placed vertically in a specially designed holder fixed to the base-plate of the Texture Analyser. A probe attached to the movable

Table 1
Vaginal ring sample set codes according to storage conditions and time-points for analysis.

Storage time (months)	Storage conditions	
	30 °C/65% RH	40 °C/75% RH
0	T0	–
1	T1 (30/65)	T1 (40/75)
2	T2 (30/65)	T2 (40/75)
3	T3 (30/65)	T3 (40/75)
6	T6 (30/65)	T6 (40/75)
9	T9 (30/65)	–
12	T12 (30/65)	–
18	T18 (30/65)	–
24	T24 (30/65)	–

arm was used to compress the vaginal ring five times through a distance of 5.0 mm (speed 2.0 mm/s) and the maximum compressive force was recorded (in Newtons). For tensile testing, the Texture Analyser was fitted with a 30 kg load cell and two ring grips (attached to the base and movable arm of the analyser). Vaginal rings were stretched at a rate of 10.0 mm/s until rupture. The pass/fail criterion was set at 5 kg.

2.10. Microbicide content assay of rings

Microbicide content assay was measured for each set of vaginal rings ($n = 10$). Vaginal rings were cut into discs (~2 mm thickness), and the dapivirine and maraviroc content extracted using the method described previously (Section 2.3), with the following points of difference: 2 mL aliquot of extraction solution was evaporated to dryness, reconstituted in 10 mL acetonitrile and diluted (1 in 2) with HPLC-grade water prior to HPLC analysis (Section 2.4).

2.11. Method for extracting drugs and related substances from rings

Dapivirine and maraviroc related substances in vaginal rings at each time-point ($n = 3$) were quantified by solvent extraction, as described previously (Section 2.10), with the following points of difference: refluxed in 100 mL dichloromethane; 25 mL aliquot of extraction solution was evaporated to dryness, reconstituted in 5 mL acetonitrile and diluted (1 in 5) with acetonitrile and HPLC-grade water to give a final solution with 1:1 composition of each solvent. Dilutions of each sample (1%) were also prepared for the purpose of quantitation of unidentified peaks or identified impurities. Placebo vaginal rings were analysed in the same way ($n = 3$).

2.12. HPLC analytical method for related substances

Reverse-phase HPLC analysis was conducted using a Waters HPLC system (described previously, Section 2.4) and a Halo C18 column (150 × 3.0 mm, 2.7 μm particle size) (Wilmington, DE, USA) held at 40 °C. Gradient elution was performed using a mobile phase of 0.01 M ammonium acetate (pH 5.7) (solvent A) and acetonitrile (solvent B) with a flow rate of 0.38 mL/min and run time of 33 min (0–12 min: 92–60% A; 12–15 min: 60% A; 15–22 min: 60–35% A; 22–24 min: 35–10% A; 24–25 min: 10% A; 25–26 min: 10–92% A; 26–33 min: 92% A) (30 μL injection volume). Maraviroc and dapivirine were detected after 17.8 and 28.2 min at 210 nm and 288 nm, respectively. Standard solutions of known impurities were prepared and analysed to allow identification and quantitation of related substances where necessary. Samples obtained from placebo vaginal rings were used to identify peaks attributable to MED-4870.

2.13. Statistical analysis

Where appropriate, results were statistically analysed using a one-way ANOVA, followed by Post hoc analysis using the Tukey–Kramer multiple comparisons test. In all cases, a p value of less than 0.05 was considered significant. Analysis was conducted using GraphPad Prism.

3. Results

3.1. Thermal analysis of microbicide-loaded cured silicone elastomer samples

Representative DSC traces for cured silicone elastomer samples loaded with dapivirine and/or maraviroc, are presented in Fig. 2. Each trace is annotated with the onset temperature (the lower temperature), peak temperature (the higher temperature) and enthalpy values for all observed melting transitions. The transitions for dapivirine and maraviroc in the combination ring sample (Fig. 2A) were similar to those observed in the single active ring samples (Fig. 2B and C) and also those of the pure actives (Fig. 2D and E), suggesting no interactions between the two compounds and no evidence of eutectic formation within the cured silicone elastomer system. Eutectic formation has been observed previously between the active components in the combination contraceptive NuvaRing® (Van Laarhoven et al., 2002).

3.2. Solubility of dapivirine and maraviroc in cured silicone elastomer

Experimentally determined values for the solubility of dapivirine and maraviroc in the cured silicone elastomers fell within the range 0.21–0.34 mg/g (Table 2), values at least ten-fold lower than reported previously for etonogestrel and ethinyl estradiol in ethylene vinyl acetate copolymer (NuvaRing®) (van Laarhoven et al., 2002). The solubility of dapivirine was slightly higher in both silicone elastomer systems compared with maraviroc, while solubility was significantly greater in MED-4870 compared with LSR9-9508-30. Drug solubility in the silicone elastomer matrix is a necessary prerequisite for drug release, with the extent of solubility directly influencing the release rate.

3.3. In vitro release from LSR9-9508-30 vaginal rings

The cumulative *in vitro* release versus time profiles for LSR9-9508-30 rings are presented in Fig. 3. The release of both maraviroc and dapivirine increased with increasing maraviroc loading (Fig. 3A and B, respectively). After 28 days, cumulative maraviroc release was 5.7, 11.4 and 18.5 mg for rings initially loaded with 25, 100 and 400 mg maraviroc, respectively, clearly illustrating the effect of drug loading upon release. Dapivirine release (each ring contained 25 mg dapivirine) was also influenced by the initial maraviroc loading, with cumulative release values after 28 days of 9.4, 10.6 and 11.4 mg for rings loaded with 25, 100 and 400 mg maraviroc, respectively. Linear relationships between cumulative release and root time were observed for both drugs in all formulations tested ($R^2 > 0.990$, graphs not shown) indicating that release obeyed $t^{½}$ kinetics, typical of permeation-controlled polymeric devices containing a dispersion of solid drug (Higuchi, 1961, 1963; Malcolm et al., 2003a, 2012a; Roseman and Higuchi, 1970).

The increased maraviroc release (Fig. 3A), an effect observed previously for other drug loaded matrix-type silicone elastomer devices (Maeda et al., 2002; Malcolm et al., 2003a, 2003b, 2004; McBride et al., 2009), is entirely expected based on an understanding of the mathematics governing permeation-controlled polymeric devices containing a dispersion of solid drug (Malcolm et al., 2003a, 2012a). The smaller increases in dapivirine release (Fig. 3B) are directly attributed to the influence of increasing maraviroc content in the rings, which modifies the diffusional characteristics of the silicone elastomer network.

Dapivirine was released in greater quantities than maraviroc when the loadings of both drugs were equivalent (i.e. 25 mg maraviroc and 25 mg dapivirine, Table 3), reflecting the differing physicochemical properties of the microbicides (Table 2). For example, dapivirine has a higher log P value, lower molecular weight, and higher silicone elastomer solubility compared with maraviroc, all of which enhance drug permeation (Russell et al., 2000; Woolfson et al., 1999). The maraviroc release characteristics were most similar to those of the 25 mg dapivirine ring with 100 mg maraviroc loading (Table 3). This formulation was selected for further study (and, ultimately, progress to the clinic).

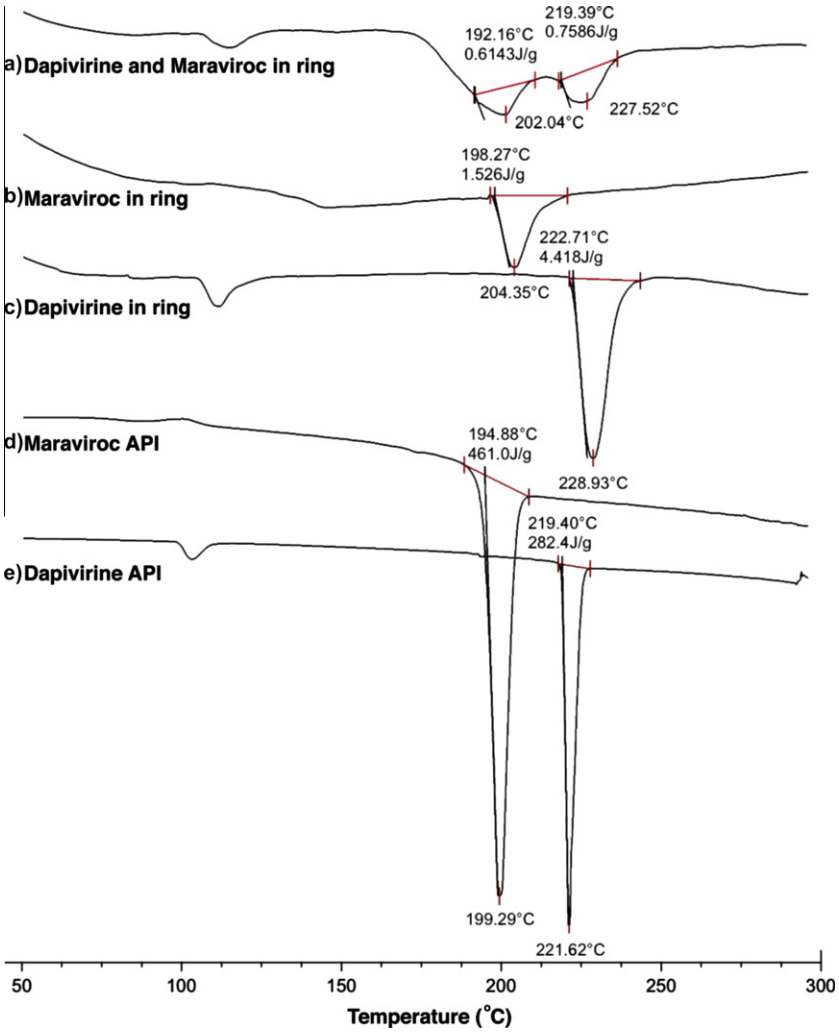

Fig. 2. DSC traces for ring samples containing dapivirine and maraviroc (A); ring sample containing maraviroc (B); ring sample containing dapivirine (C); maraviroc API (D); dapivirine API (E). The drug loadings in the DSC samples (10% w/w per active) were significantly higher than those in the rings, in order to provide suitably sized thermal transitions.

Table 2
Physicochemical properties and experimental silicone elastomer solubility data for dapivirine and maraviroc.

Property	Dapivirine	Maraviroc
Drug class	NNRTI	CCR5 entry inhibitor
MW (g/mol)	329.4	513.7
Experimental unbuffered water solubility	0.02 μg/mL[a]	1.03 mg/mL[b]
Experimental IPA*/water solubility	1.54 mg/mL[c]	>50 mg/mL[d]
Log *P*	+4.6[e]	+2.55[b]
LSR9-9508-30 solubility (mg/g)	0.26 ± 0.003	0.21 ± 0.031
MED-4870 solubility (mg/g)	0.34 ± 0.061	0.22 ± 0.032

* IPA – isopropanol.
[a] Fetherston et al. (2012).
[b] Malcolm et al., 2012a (experimentally determined log *P* value).
[c] Woolfson et al., 2006b.
[d] R.K. Malcolm, unpublished data.
[e] Woolfson et al., 2006a (calculated log *P* value).

3.4. Ring stability testing data

Daily and cumulative *in vitro* release profiles at various stability time points are presented in Fig. 4 for MED-4870 vaginal rings containing 25 mg dapivirine and 100 mg maraviroc. Although the maraviroc and dapivirine cumulative release profiles (Fig. 4B and D) show entirely similar trends to those observed previously with LSR9-9508-30 rings (Fig. 3), some variability is apparent such that the profiles at different stability time points do not overlap each other. However, as evidenced by the daily release vs. time profiles (Fig. 4A and C), this variation is almost entirely associated with variability in the first day release values, which range from 3.44 to 5.77 mg for maraviroc and 1.19–2.34 mg for dapivirine. Daily release values on subsequent days were entirely similar across the stability time points (Fig. 4A and B). For example, there was no significant difference in the total mass of maraviroc released at T_{1M} and T_{24M} (13.4 mg versus 12.3 mg; $p > 0.05$). The greater variability associated with maraviroc is most likely attributed to its non-micronised nature. The broader distribution in particle size of the solid maraviroc particles present at the ring surface (compared with micronised dapivirine) will significantly influence initial release behaviour, as observed previously (our unpublished data). However, drug release from the vaginal rings again followed $t^{½}$ kinetics ($R^2 > 0.989$ and 0.997 for maraviroc and dapivirine respectively).

The compression test data for MED-4870 vaginal rings as a function of storage time are presented in Fig. 5, where mean compression forces of ~2 N are reported. While some variability in the

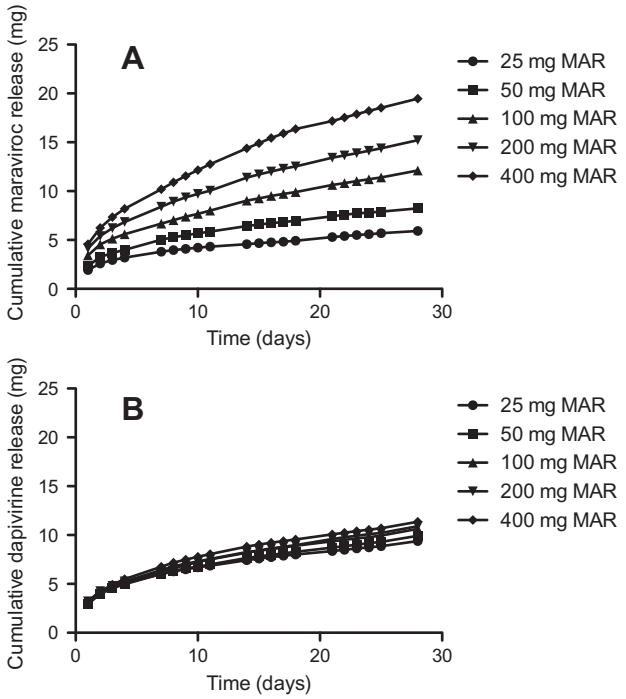

Fig. 3. Cumulative release of (A) maraviroc and (B) dapivirine from LSR9-9508-30 vaginal rings containing various loadings of maraviroc. Both axes have the same y-axis scale, for ease of comparison. The percentage relative standard deviation associated with the corresponding daily release data was less than 10% ($n = 4$).

Table 3
Summary of *in vitro* release data for LSR9-9508-30 vaginal rings containing various loadings of maraviroc and 25 mg dapivirine.

Maraviroc loading (mg)	Day 1 release (mg)	Day 25 release (mg)	Cumulative release after 28 days (mg)	% Release after 28 days	Daily release rate (mg/day$^{0.5}$)
Mean maraviroc in vitro release (various loadings)					
25	1.92	0.087	5.69	22.8	0.86
50	2.35	0.093	7.90	15.8	1.33
100	3.44	0.18	11.40	11.4	1.97
200	4.15	0.24	14.38	7.2	2.55
400	4.57	0.33	18.54	4.6	3.50
Mean dapivirine in vitro release (25 mg loading)					
25	3.17	0.12	9.38	37.5	1.34
50	2.95	0.12	9.92	39.7	1.51
100	3.08	0.16	10.64	42.6	1.65
200	3.22	0.13	10.91	43.7	1.71
400	3.18	0.14	11.36	45.4	1.82

compression force at the various stability time-points was observed (a general trend of increased compression force with storage time; 48 out of 78 comparisons measured as significantly different, $p < 0.05$), the differences were relatively small and almost certainly insignificant in a clinical setting. Storage time did not affect tensile properties, with all rings withstanding (no rupture) a 5 kg force during tensile testing (data not shown). There is no clinical/regulatory specification for the mechanical properties of vaginal rings. Similar compression testing of the commercial vaginal rings Estring®, Femring® and Nuvaring® have been reported previously, with measured compression force values ranging between 3 and 9 N (Promadej-Lanier et al., 2009). The general impact of drug loading on the mechanical properties of vaginal rings has also been assessed previously, with increasing solid drug loading producing increased ring compression force (unpublished data). The 25 mg dapivirine-only ring, currently in Phase III testing and upon which this dapivirine + maraviroc combination ring is based, has already demonstrated safety in clinical use (Nel et al., 2009a and other unpublished data). The incorporation of 100 mg maraviroc into the ring does not significantly increase the compression force compared with the dapivirine-only rings, with values maintained around 2 N.

The mean percentage contents of maraviroc and dapivirine in MED-4870 vaginal rings relative to the theoretical content (based on the active loading in premix and the final ring weight) at each time point in the stability study are presented in Fig. 6. Maraviroc content ranged from 88.1% to 100.0% and dapivirine content from 92.5% to 104.0%. No overall trend in drug content was observed as a function of storage temperature and humidity.

At each stability time-point, the related substances assay showed no degradation products or process impurities at levels high enough to be specified (i.e. >0.1%), confirming long term stability of maraviroc and dapivirine in the MED-4870 silicone elastomer rings.

4. Discussion

With a number of single-agent antiretroviral microbicide products having completed or currently undergoing human clinical testing (Abdool Karim et al., 2010; Nel et al., 2009a,b, 2010a,b; Romano et al., 2009), attention is now turning to more advanced strategies, including combination microbicides and multi-purpose prevention technologies (Johnson et al., 2010; Han et al., 2007; Moss et al., 2012; Pirrone et al., 2011; Saxena et al., 2009; Schader et al., 2011; Thurman et al., 2011). The most promising combination microbicide strategies are those that target multiple steps in the viral replication cycle, mimicking drug regimes used in highly active antiretroviral therapy. The low molecular weight and hydrophobic characteristics of non-nucleoside reverse transcriptase inhibitors and CCR5 receptor antagonist entry inhibitors make them particularly suitable for incorporation into and prolonged delivery from silicone elastomer vaginal ring devices. All commercial vaginal ring products (Estring®, NuvaRing®, Femring®, Progering®/Fertiring®) provide release of one or more steroid molecules, having similar physicochemical characteristics. The dapivirine silicone elastomer vaginal ring is already well advanced in the clinic, while the licensed drug maraviroc is effective at reducing viral loads in infected people and shows promise as a single agent microbicide (Forbes et al., 2011; Malcolm et al., 2012a, in press; Neff et al., 2011; Tsibris et al., 2011; Veazey et al., 2005a,b, 2010).

Here, we report for the first time a combination microbicide formulation, in the form of a silicone elastomer vaginal ring, providing simultaneous release of dapivirine and maraviroc. In order to facilitate development and regulatory progress, the combination ring was based on the same design, elastomer material (MED-4870) and manufacturing method as the current dapivirine-only ring, although initial formulation development was conducted using silicone elastomer LSR9-9508-30 due to its similarity to MED-4870 and its relative ease of handling and injection molding. Both dapivirine and maraviroc were released from the vaginal rings according to a permeation-controlled mechanism, as confirmed by release profiles that followed $t^{1/2}$ kinetics over the 28- and 29-day study periods. Consistent with $t^{1/2}$ kinetics, and widely reported and discussed in the literature for other matrix-type vaginal rings containing dispersed solid drug particles (Fetherston et al., in press; Malcolm et al., 2005, 2012a; Nel et al., 2009a; Woolfson et al., 2003, 2006b), both dapivirine and maraviroc show a relatively high release on day 1 followed by steadily declining release quantities on subsequent days. Initially, drug concentrations are highest at the surface of the device, leading to high drug release rates. As time progresses, drug is depleted from the surface layers,

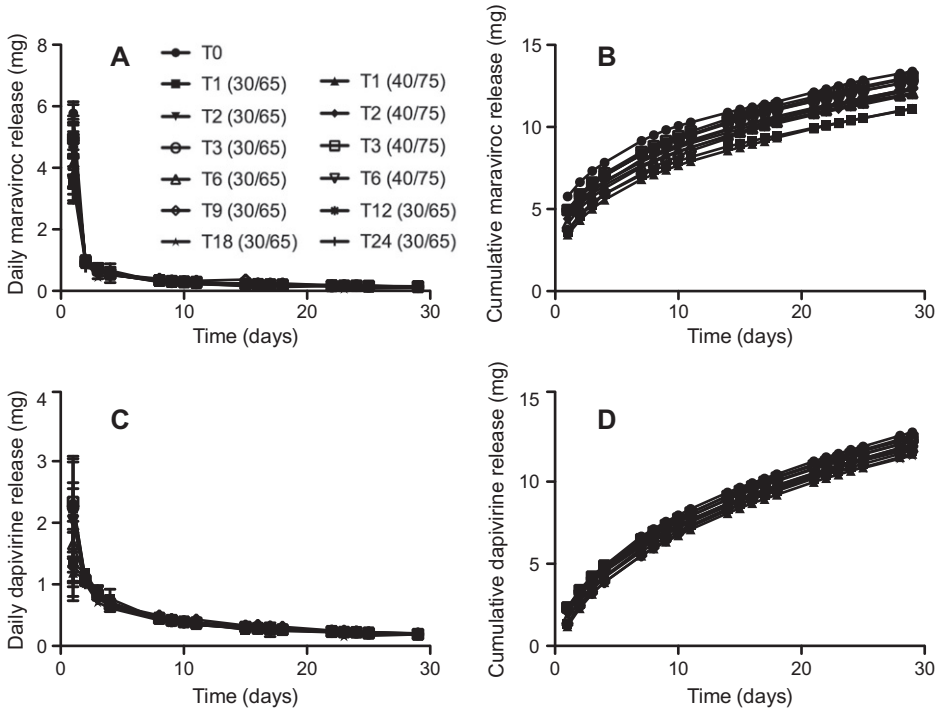

Fig. 4. Mean daily (±sd) and cumulative *in vitro* release ($n = 12$) for maraviroc (A and B, respectively) and dapivirine (C and D, respectively) from MED-4870 vaginal rings containing 100 mg maraviroc and 25 mg dapivirine following different stability storage times and conditions. The supplied legend applies to all graphs.

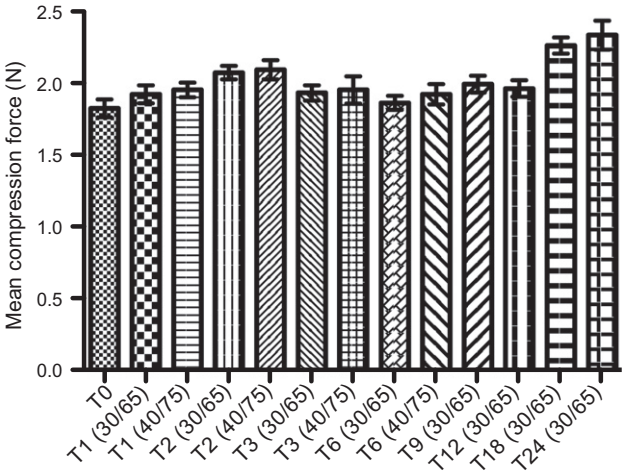

Fig. 5. Mean compression force (±sd, $n = 10$) of MED-4870 vaginal rings containing 100 mg maraviroc and 25 mg dapivirine analysed during stability study. *T* values refer to months.

and subsequent release requires the underlying drug to permeate the depletion zone in the silicone elastomer matrix. The thickness of the drug depletion zone increases as more and more drug is released, resulting in declining drug release rates with time. The cumulative quantity of drug released versus time is effectively modelled by the Higuchi equation (Higuchi, 1961, 1963) to confirm permeation controlled, $t^{1/2}$ release kinetics.

Previous pharmacokinetic studies in women with the 25 mg matrix-type dapivirine ring provided vaginal tissue and fluid concentrations that are considered sufficiently high, based on *in vitro* activity assays, to potentially provide protection *in vivo* during a 28-day continuous use regime (Nel et al., 2009a; Romano et al., 2009). Efficacy data for the 25 mg dapivirine ring will not likely be reported until 2014, as part of the recently started Phase III study. The final maraviroc loading in the combination ring was selected on the basis of its low nanomolar *in vitro* activity (similar to dapivirine) and closely matching the *in vitro* release profile of the 25 mg dapivirine-only ring. Based on the data presented in Fig. 3, a 100 mg maraviroc loading was chosen for progress.

In addition to silicone elastomer vaginal rings, various other vaginal microbicide formulation strategies for dapivirine have been described previously, including gels (Nel et al., 2009b, 2010a, 2010b; Nuttall et al., 2008), polyurethane vaginal rings (Gupta et al., 2008; Johnson et al., 2010), and films (Akil et al., 2011). Aqueous hydroxyethylcellulose (HEC) gel formulations of dapivirine have been shown to be safe and acceptable to women (Nel et al., 2009b, 2010a, 2010b). Dapivirine is distributed throughout the vaginal tract when administered in these gels, and studies in both humans and animals have suggested that once daily administration of a gel may be sufficient to provide efficacy against HIV infection (Nel et al., 2010a; Nuttall et al., 2008). Other dapivirine formulations currently in development include quick-dissolving vaginal films for rapid drug release (Akil et al., 2011) and polyurethane vaginal rings for delivery over a sustained period of time (Gupta et al., 2008; Johnson et al., 2010). Data from these various studies support the continued development of dapivirine as a microbicide.

Maraviroc formulations in development for mucosal administration include aqueous HEC gels (Malcolm et al., in press; Neff et al., 2011; Veazey et al., 2010), silicone elastomer gels (Forbes et al., 2011) and silicone elastomer vaginal rings (Malcolm et al., 2012a). Pharmacokinetic studies on maraviroc-loaded HEC gels in macaques indicate that drug levels measured in vaginal fluid, vaginal tissue and plasma increase as the administered dose is increased (Malcolm et al., in press). This formulation has also been tested for efficacy against a single high-dose vaginal challenge with SHIV-162P3, with complete protection achieved with a gel containing 3.3% w/w maraviroc (Malcolm et al., in press; Veazey et al., 2010). However, an increase in the delay between gel

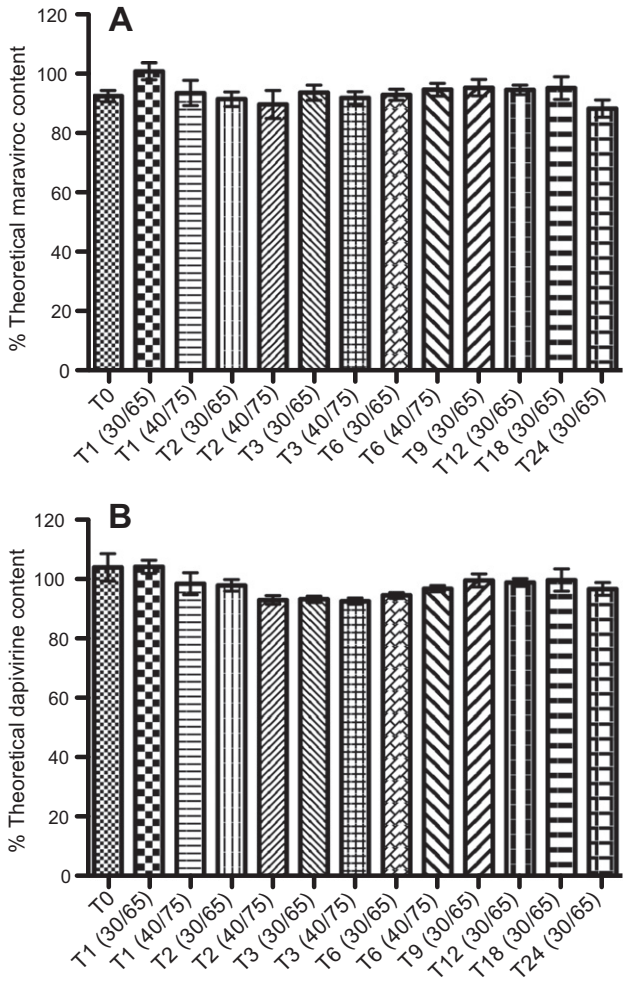

Fig. 6. Percentage of the theoretical content of (A) maraviroc and (B) dapivirine (±sd, $n = 10$) in MED-4870 vaginal rings containing 100 mg maraviroc and 25 mg dapivirine analysed during stability study. *T* values refer to months.

application and virus challenge was shown to correlate with a reduction in protection (Veazey et al., 2010). This timing issue may be reduced through the use of a silicone elastomer gel formulation, which provides higher levels of maraviroc in vaginal fluid, vaginal tissue and plasma in macaques than when the equivalent dose is delivered in a HEC gel, and for a longer period of time (up to 24 h) (Forbes et al., 2011). Sustained delivery from a vaginal ring may further increase the duration of protection afforded from the single application of a microbicide product. A pharmacokinetic study of maraviroc-loaded silicone elastomer vaginal rings in macaques has demonstrated that this compound is delivered continuously over a 28-day period (Malcolm et al., 2012a). At steady state, maraviroc concentrations in both vaginal fluid and vaginal tissue were approximately 10^6 times greater than the 50% inhibitory concentrations required for *in vitro* inhibition of SHIV-162P3 in macaque lymphocytes.

Data generated to date on both dapivirine and maraviroc indicate that a silicone elastomer vaginal ring containing both of these compounds is likely to be safe in humans and may be an effective formulation for the prevention of HIV infection in women. Although a range of other formulations are being considered, which is important from the perspective of providing women with a range of options according to their own preferences and requirements, it is likely that a vaginal ring would be well tolerated by women and may have improved user-adherence compared to a gel formulation. Here, the results of the stability study conducted with the MED-4870 vaginal rings indicated that this formulation is stable in terms of *in vitro* release, mechanical properties, assay content and related substances for 24 months when stored at conditions of elevated temperature and humidity. Given these encouraging data, a Phase I trial to assess safety and pharmacokinetics of this combination ring formulation has recently been completed and data analysis is in progress.

5. Conclusions

A silicone elastomer matrix-type vaginal ring formulation containing 25 mg dapivirine and 100 mg maraviroc has been developed. Both microbicides are continuously released *in vitro* over 29 days and the ring formulation is stable for at least 2 years under conditions of elevated temperature and humidity. This vaginal ring may be valuable in preventing sexual HIV transmission to women during heterosexual intercourse with an infected partner. Although data are not yet available, the ring was evaluated in a Phase I clinical trial in 2012.

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