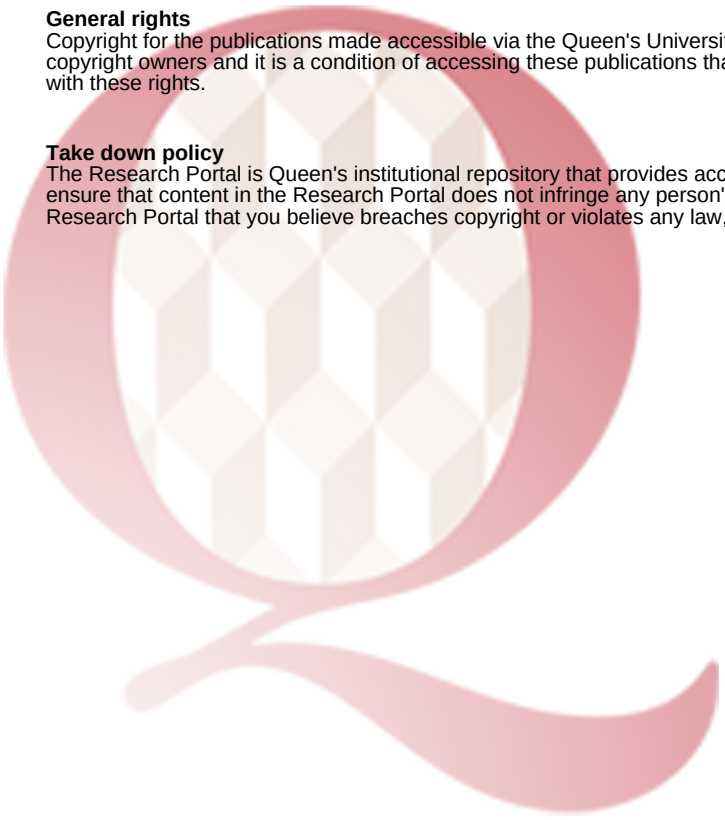
Size-fitting of Intravaginal Rings for Macaques and in vitro Release Kinetics of Zinc Finger Inhibitors



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Size-fitting of Intravaginal Rings for Macaques and *in vitro* Release Kinetics of Zinc Finger Inhibitors

James M. Smith[1], Karl Malcolm[2], Ettore Appella[3], Chris McConville[2], Marco Schito[4], David Woolfson[2], Ryo Hayashi[3], Nattawan Lanier[1], Ronald Otten[1], Salvatore Butera[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA USA; [2]School of Pharmacy, Queen's Univ. of Belfast, Northern Ireland, UK; [3]National Cancer Institute, NIH, Bethesda, MD, USA; [4]NIAID, NIH, Bethesda, MD, USA

James Smith
Centers for Disease Control and Prevention
Laboratory Branch, Div. of HIV/AIDS Prevention
1600 Clifton Rd. Mailstop A-25
Atlanta, GA 30333
tel: 404-639-1312
fax: 404-639-1174
email:ajo9@cdc.gov

Abstract

Background: Small molecule inhibitors of the zinc finger domain (ZFI) in the nucleocapsid protein (NCp7) of HIV-1 are potent inhibitors of HIV and SIV replication and may have utility as topical products to prevent infection. Furthermore, intravaginal rings (IVRs) were developed as coitally-independent, sustained release devices which could be used for administration of HIV microbicides. The aims of these studies were to demonstrate that IVRs sized for macaques are practical and compatible with the current generation of thioester-based NCp7 inhibitors.

Methods: Non-medicated silicone elastomer vaginal rings of various sizes thought to be applicable for macaques were prepared and tested for vaginal fit in Pigtailed and Chinese Rhesus macaques. Macaques were monitored for 8 weeks for mucosal disruption by colposcopy and proinflammatory cytokine markers in cervical vaginal lavages (CVL) using Luminex bead-based technology. Three different ZFIs (compounds 52, 89 and 122, each derived from an N-substituted S-acyl-2-mercaptobenzamide thioester scaffold) were loaded at 50 mg into an optimal matrix-type ring design. In vitro continuous release studies were then conducted over 28 days and analyzed by HPLC. Rate of release was determined by linear regression analysis.

Results: Qualitative evaluation at the time of ring insertion suggested that the 25 mm ring provided optimal fit in both macaque species. All rings remained in place during the study period (2 to 4 weeks), and the animals did not attempt to remove the rings. No tissue irritation was observed, and no signs of physical discomfort were noted. Also, no significant induction of cervicovaginal proinflammatory markers was observed during the 8-week period during and following ring insertion. One Pigtailed macaque showed elevated IL-8 levels in the CVL during the period when the ring was in place; however, these levels were comparable to those observed in two control macaques. In vitro release of the ZFIs peaked at day 1 and then continually declined to near steady-state rates between 20-30 mcg/day. The percent release after 14 days was 2.9, 2.0 and 0.9 for ZFI 89, 52 and 122, respectively.

Conclusions: IVRs of 25mm diameter, determined to be the optimal size for macaques, were well tolerated and did not induce inflammation. Release of all ZFI compounds followed t 0.5 kinetics. These findings suggest that efficacy testing in primate models is warranted to fully evaluate the potential to prevent transmission.

Introduction

The HIV pandemic can arguably be best slowed and eventually stopped by an effective vaccine. Although great strides have been made towards that end, an effective vaccine is realistically still many years away, providing a compelling argument for the exploration of other avenues of HIV prevention. Currently, there are a handful of effective microbicides that are designed to prevent HIV transmission by targeting specific viral proteins and hindering viral replication. Many of these compounds can potentially inhibit replication of HIV at the site of exposure and are, therefore, especially important to female sex workers and women in heterosexual relationships.

NCp7 is involved in both the early phase (facilitating reverse transcription and mediating undefined effects on integration) and the late phase (incorporation of the viral RNA genome into newly budding particles) of virus replication. HIV NCp7 contains two highly conserved zinc finger motifs that form an ordered and unique structure. Single amino acid mutations introduced within chelating (CCHC) or specific non-chelating residues of the zinc fingers result in the production of noninfectious virus particles. The potential virucidal nature of ZFI and the ability to inhibit cell-cell transmission together with the large number of conserved residues within the structure among HIV strains makes the NCp7 target highly desirable for microbicide applications.

The characteristics of an ideal vaginal microbicide are summarized in Table 1. Although many of the available antiviral drugs and compounds are highly effective, delivery and retention of the microbicide at the mucosal surface is challenging. Currently, the major challenges include the need to constantly reapply the microbicide gels and that gels are not universally received by all cultures. Therefore, there is a great need for an effective microbicide delivery device that will afford protection, is easy to use and socially and culturally acceptable.

Intravaginal rings (IVRs) are widely accepted and currently being used as contraceptive devices for the long-term, controlled release of hormones (Table 2). Adapting IVRs for sustained delivery of microbicides against HIV seems a logical progression of this technology since they possess many of the desirable attributes listed in Table 1. We describe the adaptation of this technology to the non-human primate model for studying HIV transmission, and report the initial in vitro release of a class of investigative drugs, zinc finger inhibitors.

Table 1 Characteristics of an ideal vaginal HIV microbicide product [Woolfson et. al. *Potential Use of Vaginal Rings for Prevention of Heterosexual Transmission of HIV* Am J Drug Deliv 2006; 4 (1)]

Inexpensive to manufacture	Retain activity over broad pH range
Ease of application	Maintain normal vaginal ecology
Cause no physical discomfort	Activity against other sexually transmitted pathogens
Immediate protection after application	Compatible with condoms
Long shelf-life	Negligible systemic absorption
Cause no local irritancy	High user acceptability
Maintain the integrity of the vaginal tissue	Availability of contraceptive and noncontraceptive forms
Non-messy	Tasteless
Good vaginal retention	Odorless
Good vaginal distribution	Nonteratogenic
Take account of physiological changes that occur during intercourse	Long duration of activity
Retain activity in presence of semen	Ability to be used without knowledge of male partner

Table 2 Vaginal rings either marketed or in development [Adapted from Woolfson et. al. *Potential Use of Vaginal Rings for Prevention of Heterosexual Transmission of HIV* Am J Drug Deliv 2006; 4 (1)]

Product name	Company	Indication	Elastomer description	Active ingredients (loading)	Release rate (period of use)
Estring	Pharmacia & Upjohn	HRT [1]	Silicone	Estradiol (2mg)	7.5 µg/day (3months)
Nuvaring	Organon	Contraception	Poly(ethylene-co-vinylacetate)	Etonogestrel + ethinylestradiol (11.7/2.7mg)	120 + 15 µg/day (3 weeks)
Femring	Warner Chilcott	HRT	Silicone	Estradiol acetate (12.4 or 24.8mg)	50 or 100 µg/day estradiol (3 months)
Progering	Population Council/CONRAD	Contraceptive	Silicone	Progesterone	10 mg/day (1 year)
NA	International Partnership for Microbicides	HIV	Silicone	TMC120	NA

[1] HRT - Hormone replacement therapy

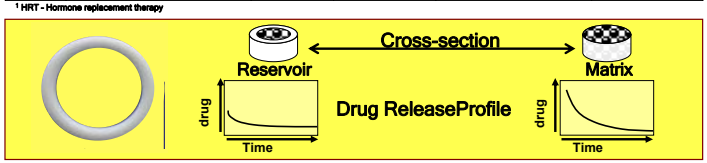

Figure 1 Types of silicone-based intravaginal rings IVRs can be manufactured to release drug at constant levels (reservoir) or at a high initial level with a decreasing release rate over time (matrix).

Size-fitting IVR for Macaques

Table 3 Study design for intravaginal ring size-fitting in pig-tailed and Chinese rhesus macaques

		Wk 0	Wk 1	Wk 2	Wk 3	Wk 4	Wk 5	Wk 6	Wk 7	Wk 8
Pig-tailed macaque Ring size	96P025/ 30 x 5mm	CVL P	IVR IN PLACE	CVL	CVL	CVL	CVL P	CVL P	CVL P	CVL P
	96P058/ 25 x 5mm	CVL P		CVL	CVL	CVL	CVL P	CVL P	CVL P	CVL P
Chinese Rhesus Ring size	RO5232/ 25 x 5mm	CVL P		CVL	CVL	CVL	CVL P	CVL P	CVL P	CVL P
	RQ5155/ 20 x 5mm	CVL P		CVL	CVL	CVL	CVL P	CVL P	CVL P	CVL P

ring observed to be in place (colposcopy) and no adverse effects or behavioral issues/problems noted, based on daily monitoring CVL - cervicovaginal levage P-Blood collected for plasma

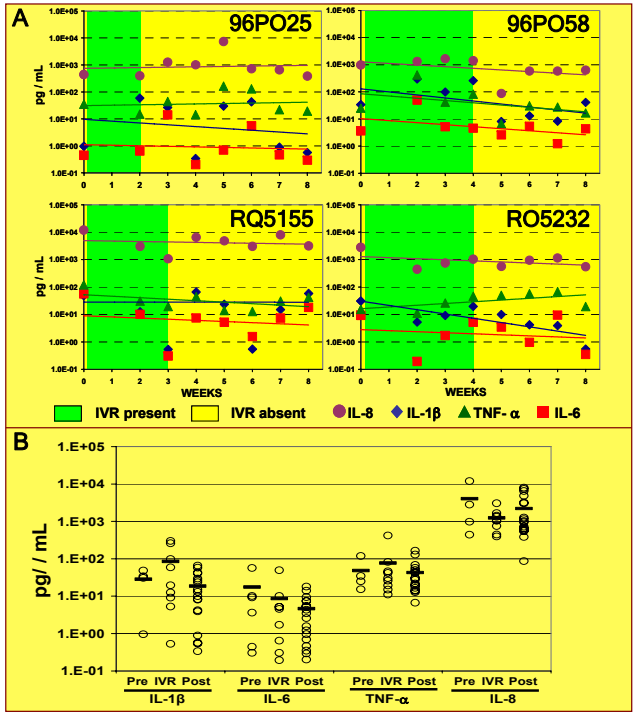

Figure 2 IVRs do not induce expression of pro-inflammatory markers A) Pro-inflammatory cytokines are plotted for each individual animal during the course of the study B) Scatter plots for each cytokine were plotted for all 4 macaques before IVR was inserted (pre); in the presence of the IVR (IVR); and after removal of the IVR (post).

Results

- As assessed by physical examination, colposcopy and behavioral observation, the 25mm rings were well tolerated by both Chinese rhesus and pig-tailed macaques.
- Levels of pro-inflammatory cytokine markers from cervicovaginal lavages remained stable throughout the study period of 8 weeks

Conclusions

- Silicone-based rings of 25mm diameter are easily inserted into the vaginal vault of both Chinese Rhesus and pig-tailed macaques and their presence does not alter the behavior of the animals or induce inflammation. These results warrant further studies utilizing IVRs loaded with antiviral drugs in the non-human primate model.

In Vitro Release of Zinc Finger Inhibitors

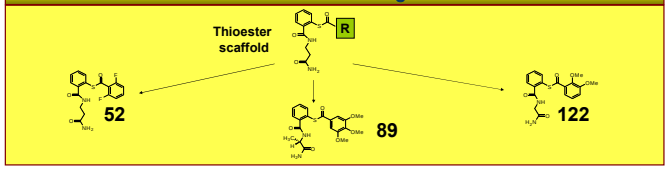

Figure 3 Zinc finger inhibitors are thioester-based compounds that interfere with the interaction of the nucleocapsid protein (NCp7) of SIV and HIV with zinc molecules.

Table 4 In vitro inhibition of replication against SIV and HIV-1 with ZFI compounds (Adapted from: Srivastava, P. et. al. Optimization of unique, uncharged thioesters as inhibitors of HIV replication. *Bioorg Med Chem* 2004, 12, (24), 6437-50)

ZFI		52	89	122
PBMC [1] SIVMac 251	EC_{50}	1	4.7	0.8
	TC_{50}	56.3	40.4	56.3
	TI	56.3	8.6	70.4
PBMC [1] HIV-1, B subtype	EC_{50}	0.5	0.02	1
	IC_{50}	>100	>100	>100
	TI	>192	>5000	>105

[1] Viral replication in PBMC and monocytes/macrophage cultures were determined on day 7 post-infection by measuring supernatant reverse transcriptase (RT) activity or p24 antigen expression by ELISA. Units are in µM. EC_{50} - high antiviral activity (low EC_{50}), IC_{50} - low cellular toxicity (high IC_{50}), TC_{50} - low cellular toxicity (high TC_{50}), TI - Therapeutic Index = IC_{50} or TC_{50} / EC_{50}

Table 5 Comparison of ZFI release rates and physicochemical properties; compounds presented in order of decreasing release rate.

ZFI Compound	MW (g/mol)	log P	DSC Melting Point (oC)	HPLC retention time (min)	Cumulative and % release after 14 days	Release Rate* (*2ASDC*)0.5
89	418.5	1.31	166.5	5.7	1.45mg / 2.9%	0.427
52	364.4	2.27	197.8	4.7	0.99 mg / 2.0%	0.253
122	374.4	1.02	167.1	5.1	0.46 mg / 0.9%	0.132

* equal to the gradient of cumulative release versus root time profile, where A is the drug loading per unit volume, S is the surface area of the ring, D is the apparent diffusion coefficient, and C is the silicone elastomer solubility per unit volume of the active agent.

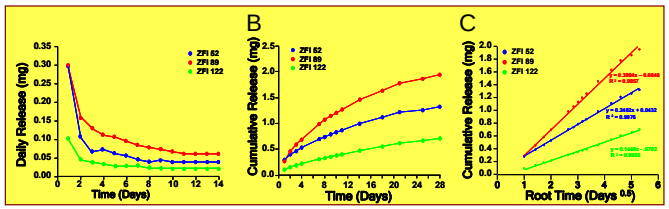

Figure 4 Matrix-type IVRs were loaded with 50mg of ZFI 52, 89 or 122. Release was determined by HPLC analysis of drug levels in media under sink conditions. A) daily release-versus-time profiles B) mean cumulative release-versus-time profiles C) mean cumulative release-versus-root time

Results

- The daily and cumulative release profiles (Fig 4) are typical of diffusion-controlled matrix-type devices, showing an initial Day 1 burst followed by a declining daily release rate on subsequent days. Daily release rates range from 20-300 mcg/day.
- Compound 89 released at the fastest rate, followed by Compound 52, and Compound 122 the slowest
- The almost perfect straight-line plots of the cumulative release-versus-root time are indicative of $t^{0.5}$ kinetics, confirming diffusion-controlled release from a matrix delivery system

Conclusions

In several independent studies, the utility and acceptance of intravaginal rings as a delivery device for hormones and contraception has been well documented. The controlled sustained release of inhibitors from IVRs for pre-exposure prophylaxis has many of the desired characteristics of an ideal vaginal microbicide. This study was launched to adapt and expand this versatile delivery device to the non-human primate model of HIV prevention. The fact that IVR did not cause inflammation and the zinc finger inhibitor was successfully released makes this combination, as well as other classes of HIV inhibitors, an excellent candidate for preclinical evaluation in the non-human primate repeat challenge model.