A novel intravaginal ring to deliver a unique combination microbicide, and a contraceptive for 90 days

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ABSTRACT SUMMARY

We developed a novel core - matrix intravaginal ring (IVR) that co-delivers a unique microbicide combination composed of three active pharmaceutical ingredients (APIs), MIV-150, zinc acetate (ZA), and carrageenan (CG) to block HIV-1, HSV-2, and HPV infections and a contraceptive, levonorgestrel (LNG), for 90 days (d). The IVR components have diverse physicochemical properties and mechanisms of action. The proof-of-concept IVR was tested in vitro and in vivo in macaques.

INTRODUCTION

A multipurpose prevention technology (MPT) that could simultaneously prevent unintended pregnancy and multiple STIs like HIV-1, HSV-2, and HPV is urgently needed. Designing a MPT for sustained delivery of multiple APIs with diverse physicochemical properties is challenging within the framework of widely acceptable drug delivery systems, such as the simple donut shaped structure of the IVR. Currently, IVRs are marketed mainly for contraception and hormonal replacement therapies.

To address the above challenge, we developed a novel IVR to deliver multiple APIs for 90d. The IVR matrix, composed of ethylene vinyl acetate (EVA-28) matrix, contained two water-insoluble small molecules, MIV-150 and LNG, indicated in blocking HIV replication and conception, respectively. The IVR core contained a mixture of ZA, a water-soluble metal salt with activity against HIV and HSV-2, and CG, a polysaccharide that blocks HPV and HSV-2 infections. The compartmentalized design allowed release of MIV-150 and LNG from the IVR matrix by diffusion while the core contents diffused out from a pore drilled through the depth of the matrix after hydration. Here we present in vitro release of the four APIs for 90d; in vivo release of MIV-150, CG, and LNG for 23-28d; and the corresponding activities in cell based in vitro models.

EXPERIMENTAL METHODS

The core-matrix macaque sized IVRs (20mm x 4mm) were fabricated by a three-step extrusion-compression-extrusion technique. One half of the EVA matrix IVR (lateral cross section) with a central empty groove was produced by hot melt extrusion (120 psi, 245°F) using customized molds. A mixture of ZA and CG (3:7) ratio was filled in the empty groove and compressed, and this process was repeated until the core capacity was reached (~100 mg). The full IVRs were prepared by extruding EVA matrix over the first half such that the core was sandwiched between the two matrices. A 500 µm pore was drilled through to the core. Total loading was 3 mg MIV-150 and for MZCL also 0.6 mg LNG per IVR. Control placebo IVRs were matrix type.

In vitro release testing was performed in 10 mL 25 mM sodium acetate buffer (± 0.05 wt% Solutol) at pH 4.2, 37°C and 100 rpm. The samples were collected periodically over 94d with daily media replacement. MIV-150 and LNG were analyzed by HPLC using a gradient method at 260 nm and 245 nm, respectively. CG and ZA were analyzed using colorimetric assays at 550 nm.

The IVRs (placebo, MZC and MZCL) were inserted in non-DepoProvera (DP)-treated macaques, and the MIV-150 and LNG levels in blood were analyzed by LC-MS/MS and ELISA, respectively, while the MIV-150 levels in swabs and in acetate buffer were quantified by radioimmunoassay (RIA). The CG levels in the swabs were determined by ELISA. Methods to assay zinc in vaginal fluid (VF) are being developed. The antiviral activity of in vitro and in vivo released APIs were tested using the TZM-bl assay for anti-HIV-1ADA-M activity and the luciferase assay in HeLa cells for anti-HPV16 PsV activity, while the cytotoxicity was tested using the XTT assay. The samples were diluted to obtain dose-response curves and estimate the IC₅₀ values with the 95% confidence interval.
RESULTS AND DISCUSSION

In vitro (Figure 1) release of CG (1.2 ± 0.5 mg/d) and ZA (0.36 ± 0.15 mg/d) was steady for nearly 46d, and declined until d94 as the core exhausted: CG: 0.18 ± 0.07 mg/d, ZA: 0.12 ± 0.06 mg/d. The release rates of CG and ZA were comparable in the two media. Addition of Solutol enhanced solubilities and thereby release of MIV-150 (14.8 ± 0.9 µg/d, d75) and LNG (3.7 ± 0.3 µg/d, d75) (release being monitored until d90) relative to the corresponding release in acetate buffer alone.

In vivo (Figure 2), CG was detected within 24 hours (h) post-IVR insertion (PI). Levels increased after day 7, peaked to 85 µg/mL by d13, and were above 50 µg/mL for three weeks (protection observed against HSV-2 and HPV infections in mice at that level, unpublished2).

Figure 1: The API release from MZCL IVRs in 25 mM acetate buffer (± 0.05 wt% solutol) was measured over time. The data are shown as mean ± SD for n=3 for all but MIV-150 in the acetate buffer (n=2).

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CONCLUSION

We developed a novel IVR that can deliver four APIs to prevent multiple STIs and conception. We aim to develop the design to optimize in vivo release.

REFERENCES


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