Sustained Delivery of Hydroxychloroquine from a Polyether Urethane Intravaginal Ring

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ABSTRACT SUMMARY

The aim of the current study is to develop and characterize a novel intravaginal ring for the sustained delivery (>14 days) of hydroxychloroquine (HCQ) as a strategy for preventing male-to-female transmission of HIV. This intravaginal ring (IVR) system is non-cytotoxic and may be a suitable platform for the prevention of HIV transmission and other sexually transmitted diseases.

INTRODUCTION

In 2010, it was estimated that 2.7 million people became newly infected with human immunodeficiency virus (HIV) with Sub-Saharan Africa being the region most affected. Young women aged 15-24 years old are as much as eight times more likely to be HIV positive. Thus, there is a desperate need to develop innovative strategies that provide female-controlled prevention of HIV infection. IVR is a preferable female-initiated microbicide with high user adherence comparing to other dosage forms such as gels. HCQ has been shown to demonstrate anti-inflammatory properties and broad anti-HIV activity by inhibiting HIV replication. Since it has been shown that inflammation of the female genital tract can increase susceptibility to HIV-1 infection, the anti-inflammatory effects of HCQ may potentially be protective.

EXPERIMENTAL METHODS

HCQ was uniformly incorporated into a polyether urethane via solvent-casting method, resulting in 2% or 4% (w/w) drug-loaded films. The IVRs were then fabricated through hot melt injection molding. 100 mg of IVR segments either non-coated or coated with 10% polyvinylpyrrolidone (PVP) or 5% poly(vinyl alcohol) (PVA) were used for in vitro release studies. End-capped segments were incubated in 5 mL 25 mM sodium acetate buffer at pH 4. HCQ release was quantitated using a RP-HPLC method. Accelerated stability studies were carried out by exposing 50 mg of segments at room temperature (R.T.) or at 40°C/75% relative humidity (RH) in a climate chamber. Samples were collected at different time intervals (0, 15, and 30 days) and remaining HCQ was extracted and quantified. In vitro cytotoxicity studies were evaluated using the human vaginal epithelial cell line VK2/E6E7 and ectocervical epithelial cell line Ect1/E6E7. The impact of drug-free IVR segments on cell viability was evaluated by incubating the cells with non-coated and PVP/PVA-coated segment elution medium up to 30 days. Cell viability was determined by incubating the cells with elution medium for 24 hr using MTS assay. Inflammatory cytokine (IL-1β, IL-6, and IL-8) production in the supernatants were quantitated using sandwich ELISAs. To examine the cytotoxic effects upon individually treated cells, colony formation assay was performed by exposing 800 cells from both cell lines with the collected elution medium for 12 days. Colony formation efficiency was calculated in comparison to the negative control. Data are presented as mean ± standard deviation (SD). The n-value refers to number of replicates performed for each study. Student’s t-tests (unpaired, two-sample, unequal variance with two-tailed distribution) was performed on all results, with P < 0.05 considered as significant.

RESULTS AND DISCUSSION

Two sizes of IVRs were fabricated. The larger IVR has dimensions of 55 x 5.5 mm (outer diameter x cross-sectional diameter) while the smaller IVR has dimensions of 25 x 5 mm. Smaller IVR was used for the following release, stability, and cytotoxicity studies.

Figure 1. The aluminum mold along with its respective fabricated IVRs: a large placebo IVR (left, 55 x 5.5 mm), small placebo IVR (middle, 25 x 5 mm), and a HCQ-loaded small IVR.

During the first 24 hr, a burst release of HCQ at 43.52 ± 2.41% and 41.08 ± 2.24% of initial loading was observed for 2% and 4% (w/w) HCQ loaded IVR segments, respectively, followed by sustained HCQ release for 18 days. During the study period, 83.83 ±
3.57% and 85.51 ± 1.81% of total loaded HCQ was released from 2% and 4% (w/w) IVR segments (100 mg), respectively. It was estimated that approximately 1533.53 mg and 3131.81 mg of HCQ could be released from a 2% and 4% (w/w) full size human IVR within 18 days. IVRs coated with PVP or PVA exhibited a reduced burst release on the first day (34.63 ± 2.39% and 25.36 ± 2.62%, respectively).

Figure 2. (A) Daily release and (B) cumulative release. Data was scaled up to represent the release from a full size small IVR. n = 6 for 2% and 4% non-coated segments, n = 4 for PVP/PVA coated segments.

Stability studies demonstrated that HCQ was stable in both coated and non-coated IVRs when stored at room temperature or at 40°C/75% relative humidity for 30 days. No significant differences in cell viability, pro-inflammatory cytokine production, or colony formation were observed when vaginal and ectocervical epithelial cells were treated with elution medium incubated with drug-free IVR segments up to 30 days comparing to negative control.

Figure 4. MTS assay using non-coated and PVP/PVA-coated elution medium to treat VK2/E6E7 and Ect1/E6E7 cells. 1M acrylamide in medium was used as positive control (P) and regular medium was used as negative control (N). n = 5.

CONCLUSIONS
This is the first study to fabricate a matrix-type IVR that can provide sustained release of HCQ over 18 days in vitro. This is also the first study to demonstrate that IVRs coated with PVA or PVP can significantly reduce the burst release of HCQ within the first 24 hrs. HCQ in the IVR was stable under accelerated stressed conditions and the IVR was non-cytotoxic when incubated in the presence of human vaginal and ectocervical epithelial cell lines. Overall, the current study indicates that our PU IVR device can potentially be used for the intravaginal delivery of HCQ. Further studies are required to determine the impact of our HCQ IVR on preventing HIV infection.

REFERENCES

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