An Evaluation of Polycaprolactone Matrices for Vaginal Delivery of the Antiviral, Tenofovir, in Preventing Heterosexual Transmission of HIV

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ABSTRACT: Tenofovir was incorporated in controlled-release polycaprolactone (PCL) matrices designed for production of vaginal inserts for prevention of HIV transmission. Rapid cooling of suspensions of the drug powder in PCL solution resulted in micro-porous matrices with tenofovir loadings up to 12% (w/w) and high incorporation efficiencies in excess of 90%. The release behaviour of tenofovir in simulated vaginal fluid (SVF) demonstrated high delivery efficiency of 85%–99% over 30 days and could be described effectively by a first-order kinetics model giving a mean value of 0.126 day-1 for the release constant (k_1). Tenofovir released from PCL matrices into SVF exhibited high relative activity ranging from 70 to 90%, against pseudo-typed HIV-1-infected HeLa cells. The inhibitory activity of tenofovir standard solutions in SVF provided an IC_{50} value of 2.38 μM. Besides confirming high levels of *in vitro* antiviral activity, the predicted concentrations of tenofovir, which would be released from a PCL intra-vaginal ring *in vivo*, exceeded the IC50 value for HIV-1 by a factor of 35–200 and clinically protective concentrations by a factor of 50. These findings recommend further investigations of antiviral-loaded PCL matrices for controlling heterosexual transmission of HIV. © 2013 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 102:3725–3735, 2013

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INTRODUCTION

The requirement for novel approaches to limit the spread of sexually transmitted infections is highlighted by the continuing HIV/AIDS pandemic.[1] Highly active anti-retroviral therapy suppresses virus replication in host cells and has greatly reduced the spread of AIDS in developed countries. However, 90% of infected people live in developing countries and have limited access to this treatment. Furthermore, the number of new diagnoses attributed to heterosexual contact is on the increase. In sub-Saharan Africa, for example, women are disproportionately affected with those between the ages of 15 and 24 being eight times more likely to be HIV positive than men. The scale of the problem, the lack of progress in developing a vaccine against HIV and the urgent need for improved HIV prevention options that can be controlled by women has intensified research on vaginal delivery of microbicides that kill or inactivate viruses at the mucosal barrier.[2,3]

First-generation microbicides included non-specific agents such as nonoxynol-9 surfactant and buffering agents, which functioned respectively by solubilising virus envelope proteins and maintaining the natural defensive acidic pH of the vagina (3.5–4.5). A number of anionic polymers including cellulose acetate phthalate and naphthalene disulphonate have been widely investigated as virus entry/fusion inhibitors.[4–6] These substances are considered to bind to virus envelope proteins (gp120 and gp141 in HIV) by electrostatic interaction, thereby preventing virus entry into target cells ($CD4^+$-T cells, macrophages and dendritic cells) in the genital sub-mucosal region. Vaginal formulations of galactose-linked polysaccharides (carrageenan) have also been reported to protect mice from *Herpes simplex* virus infection,[7] block HIV infection of cervical epithelial cells and trafficking of HIV-infected macrophages from the vagina to lymph nodes.[8] Although these first generation microbicides showed promise *in vitro* and in clinical trials, none met with clinical success because of safety issues or lack of efficacy.[9]

Second-generation microbicides based on HIV-specific antiretrovirals[10] function as post-entry inhibitors, preventing virus release from cells by disrupting essential steps in the replication cycle. Several compounds, widely used in systemic oral treatments of HIV, are currently being evaluated as topical vaginal microbicides. Application of the non-nucleoside reverse transcriptase inhibitor dapivirine (TMC-120) in a Carbopol 940/hydroxyethylcellulose gel formulation in (hu-SCID) mice prevented systemic infection by cell-associated HIV.[11] A limited study in HIV^- women showed that dapivirine released from a vaginal gel applied once daily over 11 days was well distributed throughout the cervico-vaginal area at concentrations several orders of magnitude above the EC_{50}.[12] Recently, the CAPRISA 004 clinical trial reported that the nucleoside reverse transcriptase inhibitor, tenofovir, applied in a hydroxyethylcellulose gel in a pre-exposure prophylactic study, reduced HIV acquisition by an estimated 39% overall and by 54% in women with high adherence to the study protocol.[13] However, the tenofovir gel was found to perform no better than a placebo in the larger-scale VOICE trial.[14]

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Most candidate microbicides undergoing clinical trials are formulated as semi-solid gels or creams which are inherently "messy" to use, prone to leakage and elicit concerns over ineffective coverage of the epithelium. In addition, they must be applied prior to intercourse (often using an applicator) to reduce the risk of virus infection. These factors make it difficult to guarantee adhesion to trial protocols and to ensure protection for women who may be at risk from HIV transmission. As a result, interest in intra-vaginal ring inserts (IVRs) for controlled, long-term, delivery of antivirals is intensifying[3,15,16] as improved user compliance and sustained concentrations of the drug in vaginal fluids at therapeutic levels are expected to reduce the risk of virus transmission. A 7-day clinical study involving HIV$^-$ women revealed that silicone elastomer IVRs releasing dapivirine elevated drug concentrations in vaginal fluid and tissues to levels three orders of magnitude above the EC_{50}.[17] However, conventional IVRs, produced from silicone elastomer or thermoplastic poly(ethylene vinyl acetate) (pEVA), display certain disadvantages for microbicide delivery. They are generally restricted to delivery of low-molecular-weight, hydrophobic drug candidates such as dapivirine and curing of silicone elastomer at 80°C or hot melt extrusion of pEVA at 110°C is required during manufacture, which could deactivate heat-labile compounds. Pod-type IVR designs have been developed to overcome these limitations and to achieve simultaneous delivery of multiple drugs. Independent control of the release rate of hydrophilic tenofovir and acyclovir has been achieved by embedding polymer-coated drug cores in silicone elastomer IVRs, which permit drug release through a delivery channel. Tenofovir release of 144 μg/day may be sustained for 28 days.[18] In addition, tenofovir-loaded pod-type IVRs were found to produce mean tenofovir levels in vaginal secretions of macaques of around 2 mg/mL over 28 days and intracellular tenofovir diphosphate concentrations in the range that may be protective against simian-human immunodeficiency virus infection in non-human primates.[19] The challenge of incorporation and delivery of hydrophilic compounds in IVRs has also been approached by application of thermoplastic polyether urethane elastomers that incorporate covalently bonded polyethylene oxide segments in the copolymer structure. Clark et al.[20] manufactured tenofovir-loaded IVRs by melt extruding drug-loaded pellets at 147°C, followed by injection moulding at 130°C–135°C. The material swells on hydration, resulting in drug release at levels greater than 2 mg/day for 90 days. Johnson et al.[21] recently described a novel reservoir-type IVR consisting of end-sealed polyether urethane tubing filled with a tenofovir/glycerol/water semi-solid paste. Tenofovir release in excess of 10 mg/day was sustained for 90 days, whereas vaginal concentrations of the drug in sheep (10^6 ng/mL) were reported to be 1000× higher than that, which provided significant protection against HIV transmission in human clinical trials using tenofovir-loaded gel. Polyether urethane has also been utilised to deliver hydrophilic tenofovir disoproxil fumarate. Drug released *in vitro* after 24 h exhibited potent anti-HIV activity in TZM-Bl cell culture and ectocervical explant models of infection.[22]

The synthetic polyester, polycaprolactone (PCL), has been investigated extensively for production of biomedical implants and drug delivery devices in the form of films, micro- and nanoparticles.[23] We have previously shown that micro- or macro-porous matrices based on PCL are effective for controlling delivery of small drug molecules (e.g., steroids and anti-bacterials) and macromolecules such as enzymes with retained activity.[24–26] More recently, the potential utility of these materials was demonstrated for vaginal delivery of anti-bacterials (ciprofloxacin) and anti-fungal agents (miconazole) in the treatment of gonorrhea and candida.[27] In this study, we describe investigations of PCL matrices loaded with the hydrophilic antiviral, tenofovir (aqueous solubility 0.6 mg/mL), as part of an ongoing evaluation of the materials for the prevention and treatment of sexually transmitted infections. The *in vitro* release kinetics of tenofovir were determined in simulated vaginal fluid (SVF) and the antiviral activity of released drug was assessed using a luciferase gene reporter assay involving HeLa cell cultures infected with pseudo-typed HIV-1.

MATERIALS AND METHODS

Materials

Polycaprolactone (Mw 115,000 Da, Capa 650) was obtained from Solvay Interox (Warrington, UK). Tenofovir was supplied by Taizhou Crene Chempharm Company (Zhejiang, China). Sodium chloride, potassium hydroxide, calcium chloride, bovine serum albumin, glucose, glycerol, urea, lactic acid and acetic acid were purchased from Sigma–Aldrich (New South Wales, Australia). All solvents (acetone, methanol, ethanol, dichloromethane) were of analytical grade and were obtained from Sigma–Aldrich. SVF (pH 4.2) was prepared following the method of Owen and Katz[28] and consisted of 3.51 g NaCl; 1.40 g KOH; 0.222 g $Ca(OH)_2$; 0.018 g bovine serum albumin; 2.00 g lactic acid; 1.00 g acetic acid; 0.16 g glycerol; 0.40 g urea and 5.00 g glucose in 1 L of distilled water.

Preparation of Tenofovir-Loaded PCL Matrices

Polycaprolactone solution (15% or 20%, w/v) was prepared by dissolving the polymer in acetone at 50°C for 30 min. Tenofovir powder was dispersed in the PCL solution to produce loadings equivalent to 5%, 10% or 15% (w/w) of the PCL content. The resulting suspensions were poured into a polypropylene syringe body (3 mL) and rapidly cooled in ethanol at −80°C for 24 h to allow crystallisation of PCL. Following the hardening process, the matrices were removed from the moulds and immersed in methanol for 24 h to extract acetone. Residual solvents (acetone and methanol) in the matrices were evaporated under ambient conditions for 24 h prior to testing.

Determination of the Tenofovir Content of PCL Matrices

Sections (approximately 100 mg) were cut from each end of the matrix sample, weighed and dissolved in dichloromethane (2 mL). Precipitation of PCL was induced by adding 5 mL of SVF, followed by vortexing (Vibrax; IKA, Werke Staufen, Germany) at 1000 rpm overnight to allow dichloromethane to evaporate and the drug content to partition into the SVF phase. The residue was washed twice with SVF (10 mL) to extract residual drug and the washings were combined. Tenofovir concentrations in SVF were assayed using UV spectrophotometry at 260 nm and obtained by comparison with a calibration curve constructed using a series dilution of tenofovir in SVF. The linearity ranged from 3 to 20 μg/mL ($R^2 = 0.9996$). The measured drug loading (w/w) of the matrices was compared with the theoretical loading and expressed as incorporation efficiency (%). The experiment was conducted using triplicate samples.

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