Journal of Pharmaceutical Sciences xxx (2018) 1-10

Contents lists available at ScienceDirect

Journal of Pharmaceutical Sciences

journal homepage: www.jpharmsci.org

Pharmaceutics, Drug Delivery and Pharmaceutical Technology

Development of a Vaginal Fast-Dissolving Insert Combining Griffithsin and Carrageenan for Potential Use Against Sexually Transmitted Infections

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ARTICLE INFO

Article history:
Received 12 February 2018
Revised 24 April 2018
Accepted 5 June 2018

Keywords:
freeze-drying/lyophilization
formulation
protein delivery
mucosal drug delivery
stabilization
solid dosage form
solid state stability

ABSTRACT

Precoital, on-demand topical microbicides to reduce a woman's risk of sexually transmitted infections have been in development for nearly 3 decades, but no product has been approved due to acceptability issues and poor adherence in clinical trials. We set out to develop a self-administered vaginal fast-dissolving insert (FDI) produced by freeze-drying that would deliver safe and effective amounts of the antiviral agents griffithsin (GRFT) and carrageenan (CG) and would have properties women and their partners find acceptable. We evaluated FDI physical criteria, attributes of the gel produced upon dissolving, and GRFT stability. The lead formulation, FDI-024, was selected from 13 candidates and contains 4 mg of GRFT, 15 mg of CG, and excipients (the cryoprotectant sucrose and bulking agents dextran 40 and mannitol). The FDI exhibits good friability and hardness and is stable for at least 6 months at up to 40°C/75% relative humidity. It disintegrates in less than 60 s in a physiologically relevant volume (~1 mL) of simulated vaginal fluid, forming a viscous semi-solid gel with favorable mucoadhesive and spreading properties. The formulation retains the antiviral activity of GRFT and CG against HIV type 1 and human papillomavirus, respectively, in cell-based assays.

Introduction

Incurable, highly prevalent sexually transmitted viral infections such as those caused by HIV type 1 (HIV-1) and high-risk types of human papillomavirus (HPV) have a devastating effect on the sexual and reproductive health of millions of women worldwide, particularly in sub-Saharan Africa and South and West Asia. In 2016, there were 36.7 million people living with HIV/AIDS, 1.1 million HIV-1 related deaths, and 2.1 million new HIV-1 infections.[1] An estimated 290 million women worldwide are infected with HPV,[2] and high-risk HPV genotypes are the leading cause of genital cancers, notably cervical cancer. This is the fourth most common cancer in women, with an estimated 270,000 deaths and 530,000 new cases in 2012, more than 85% of which occurred in less-developed regions of the world.[3] Most people clear HPV infections naturally, but women infected with HIV-1 are more likely to have persistent infections and progress to high-grade cervical dysplasia and invasive cancer if they become infected with HPV.[4] Vaccines against some of the most prevalent high-risk HPV types (16, 18, 31, 33, 45, 52, and 58) are now available and have shown high efficacy at clinical trial end points.[5] However, these vaccines require a cold chain for delivery. Also, there is reportedly poor vaccine uptake for girls aged 9 to 14 years—the WHO-recommended primary target group for vaccination[4]—due to parental concerns, lack of sufficient information, and other social and cultural reasons.[6] In addition, several low- and high-risk HPV types are not yet included in any vaccine, and a broad-spectrum prevention strategy, used in conjunction with vaccines, may be necessary to help curb HPV infections.

Abbreviations used: BP, British Pharmacopoeia; CG, carrageenan; EC_{50}, half-maximal effective concentration; FDI, fast-dissolving insert; FDT, fast-dissolving tablet; GRFT, griffithsin; HEC, hydroxyethyl cellulose; HIV-1, HIV type 1; HPV, human papillomavirus; PBS, phosphate-buffered saline; PCR, polymerase chain reaction; RH, relative humidity; SVF, simulated vaginal fluid; USP, United States Pharmacopeia.

Conflicts of interest: The authors declare no conflicts of interest.

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https://doi.org/10.1016/j.xphs.2018.06.002
0022-3549/

Microbicides are topical products applied inside the vagina or rectum to kill or inactivate pathogens, and many studies are now investigating their efficacy, safety, and acceptability for preventing HIV and HPV infections.[7] The active pharmaceutical ingredient(s) may be delivered via gels, films, rings, or suppositories. Some studies have shown that women are at significantly lower risk of acquiring HIV when such products are used consistently and correctly.[8,9] However, low adherence rates have led to unsuccessful end points in clinical trials,[10-12] and no topical microbicide has been licensed to date.[7]

Although much work is focused on developing sustained-release microbicide dosage forms such as vaginal rings,[13,14] implants, and injectables,[15] many women—for example, those who have infrequent sex and/or concerns about the side effects and health risks of long-term products—prefer on-demand, that is, coitally-dependent, products.[16] Aqueous semi-solid gels are the most common delivery systems for on-demand microbicides; others include films, inserts/tablets, and suppositories. Positive attributes of well-designed gels such as spreadability, mucoadhesion, and low cost to manufacture are countered by undesired gel attributes such as leakage and messiness,[17] as well as the need to ship and carry bulky, 4-mL gel-filled applicators. The latter characteristics have reduced interest in and adherence to on-demand gel products and thwarted phase III clinical trials. Moreover, water- and/or temperature-sensitive drugs are poor candidates for aqueous gel-based microbicides.

To overcome the disadvantages of current gel microbicide delivery systems, we adapted a technology previously developed in our laboratory for creating fast-dissolving tablets (FDTs) for oral use.[18-20] This technology produces FDTs or fast-dissolving inserts (FDIs) by freeze-drying aqueous solutions of drugs and excipients directly in blister packaging (in this article, FDTs will refer to oral dosage forms and FDIs to vaginal or rectal dosage forms). Other freeze-dried tablets already on the market deliver small-molecule drugs, allergy vaccines, and animal vaccines; however, these tablets are lightweight, fragile, and unsuitable for use as a vaginal or rectal microbicide delivery system. The inserts developed in our laboratory are robust, with low friability, which permits blister or bulk packaging as well as everyday handling and vaginal/rectal administration without crumbling.

To test the feasibility of using our FDI technology for administering microbicides vaginally, we chose the potent antiviral microbicide griffithsin (GRFT). Isolated from the red alga *Griffithsia* sp, GRFT is a carbohydrate-binding protein, or lectin, that blocks HIV replication *in vitro* at half-maximal effective concentration (EC_{50}) values in the 2 to 800 picomolar range.[21,22] GRFT binds to a component of the HIV envelope, gp120, blocking the virus from binding to CD4 receptor-expressing cells.[23] It also prevents cell fusion and cell-to-cell transmission of HIV and has an excellent safety profile in human cells *in vitro*.[24] GRFT can be mass produced via expression in *Nicotiana benthamiana* leaf tissues, using a recombinant tobacco mosaic virus vector system.[25-27] The first in-human phase I trial for GRFT to demonstrate safety has been initiated.[28]

We also evaluated 3 common gelling agents—carrageenan (CG), hydroxyethyl cellulose (HEC), and xanthan gum—for their impact on FDI properties and GRFT stability. CG is a sulfated polysaccharide from seaweed that is widely used in food and in drug delivery systems[29-35]; it also has been shown to inhibit a number of common high- and low-risk types of HPV *in vitro*, in mouse and macaque studies with HPV pseudovirions, and in some human studies.[36-41] CG gels have excellent rheological properties[42] and a proven track record of safety when administered vaginally.[11,43-45] HEC is the so-called "universal placebo" and also has a good safety record when administered vaginally.[46,47] Xanthan gum is a polysaccharide produced from simple sugars by a fermentation process.[48] It is used as a thickener in a variety of commercial products, in mucoadhesive vaginal gel formulations, and sustained-release tablets, including vaginal tablets.[49-54]

The goal of this study was to produce a thermally stable, vaginally administered solid dosage form—an FDI—containing doses of GRFT/CG capable of rapidly producing targeted drug levels against HIV/HPV in the fluid volume available in the vaginal lumen. The targeted drug levels were defined as 100 to 1000 times the EC_{50} of GRFT/CG in *in vitro* assays. We aimed to develop a compact and portable self-administered product that is easy to use and disintegrates within 60 s in minimal fluid volume to form a mucoadhesive spreading gel that can deliver GRFT/CG throughout the vagina. We also evaluated the effect of different geometries on physical properties of the FDIs by creating and testing a library of prototypes in this study.

Materials and Methods

FDI Formulations and Production

Selection of excipients and lyophilization methodology were based on our previous experience developing FDTs for vaccines[18,19] and antiretroviral medications.[20]

Excipients

Four types of methocel (Colorcon Inc., Harleysville, PA) were tested, as were xanthan gum (Spectrum Chemical, New Brunswick, NJ) and HEC (Sigma-Aldrich, St. Louis, MO), which were used as supplied. Mannitol, sucrose (J.T.Baker/Thermo Fisher Scientific, Bothell, WA), and dextran 40 (Sigma-Aldrich) were used as supplied.

Antiviral Agents

GRFT (~20 mg/mL) as bulk recombinant protein in phosphate-buffered saline (PBS) was provided by the Population Council (New York, NY). CG (>85% lambda fraction, CarraSol PGU 5563, lot 7) derived from *Gigartina skottsbergii*, sourced from Gelymar (Santiago, Chile), was purified in Callaghan Innovation's laboratories in Wellington, New Zealand, using a proprietary purification method.

Freeze-Drying Process

The compositions of the prelyophilized aqueous placebo solutions and the process to produce different types of FDIs were the same for mouse, macaque, and human inserts; however, molds and volumes differed (0.02 and 0.2 mL, respectively, for mouse and macaque; varying volumes for human). Mouse and macaque FDIs were produced in 1-mL polymerase chain reaction (PCR) tubes (Agilent Technologies, Santa Clara, CA), whereas human FDIs were formed in blister sheets or in a tooling device (Proto Labs, Inc., Maple Plains, MN). The required volume of placebo solution was added to the tubes or molds with a HandyStep® electronic repeating pipette (BrandTech Scientific, Essex, CT), and the solution was lyophilized by placing the filled forms on trays into a laboratory freeze-dryer (LD85; Millrock Technology, Kingston, NY) with a condenser temperature of −70°C and vacuum pressure of 100 mTorr. The formulations were rapidly frozen to −45°C and held for 3 h, followed by primary drying from −40°C to 30°C over a period of 22 h. Secondary drying was at 30°C for 5 h.

Sealing and Packaging

A peelable foil lid (Delta Circle Industries, Richmond, VA) was sealed onto the blister sheets containing the inserts using a B2FS thermoforming and sealing machine (Applied Engineering