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The rational design and development of a dual chamber vaginal/rectal microbicide gel formulation for HIV prevention

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ABSTRACT

The DuoGel™ was developed for safe and effective dual chamber administration of antiretroviral drugs to reduce the high incidence of HIV transmission during receptive vaginal and anal intercourse. The DuoGel™s containing IQP-0528, a non-nucleoside reverse transcriptase inhibitor (NNRTI), were formulated from GRAS excipients approved for vaginal and rectal administration. The DuoGel™s were evaluated based upon quantitative physicochemical and biological evaluations defined by a Target Product Profile (TPP) acceptable for vaginal and rectal application. From the two primary TPP characteristics defined to accommodate safe rectal administration three DuoGel™ formulations (IQB3000, IQB3001, and IQB3002) were developed at pH 6.00 and osmolality ⩽400 mmol/kg. The DuoGel™s displayed no *in vitro* cellular or bacterial toxicity and no loss in viability in ectocervical and colorectal tissue. IQB3000 was removed from consideration due to reduced NNRTI delivery (~65% reduction) and IQB3001 was removed due to increase spread resulting in leakage. IQB3002 containing IQP-0528 was defined as our lead DuoGel™ formulation, possessing potent activity against HIV-1 (EC_{50} = 10 nM). Over 12 month stability evaluations, IQB3002 maintained formulation stability. This study has identified a lead DuoGel™ formulation that will safely deliver IQP-0528 to prevent sexual HIV-1 transmission in the vagina and rectum.

1. Introduction

In addition to the ongoing development of products to effectively prevent the transmission of HIV in the vagina, there is a growing appreciation of the significant need to mitigate rectally transmitted HIV (Beyrer et al., 2012, 2013; El-Sadr et al., 2010). Unprotected receptive anal intercourse (RAI) is practiced by both women and men in the developing (Kalichman et al., 2009; Lane et al., 2006) and developed (Gorbach et al., 2009) world, representing one of the highest risk sexual behaviors for HIV transmission with rates of transmission 10–20 times greater than unprotected vaginal intercourse (Vitinghoff et al., 1999). Consequently, as microbicide products advance in the clinic, it is critical that rectally-delivered microbicides also be evaluated. Microbicides are agents applied topically to the vagina or rectum prior to sexual intercourse to prophylactically inhibit transmission of STIs, including HIV, and are currently in relatively early stages of development. Gels are the leading dosage form since minimal behavior modification is needed due to common use of lubricants by men who have sex with men (MSM) during RAI (Carballo-Dieguez et al., 2000). However, many of the regularly used lubrication products are hyperosmolar and have shown toxicity to colonic epithelial cells and tissues *in vitro* and use of these products has resulted in increased risk of pathogen acquisition (Begay et al., 2011; Dezzutti et al., 2012a; Gorbach et al., 2012; Rebe et al., 2014). This osmolality issue must be considered as a critical aspect in the design of microbicide formulations such as

Abbreviations: API, active pharmaceutical ingredient; GRAS, generally regarded as safe; HIV-1, human immunodeficiency virus; HPLC, high pressure liquid chromatography; MSM, men who have sex with men; NNRTI, nonnucleoside reverse transcriptase inhibitor; PBMC, peripheral mononuclear blood cell; RAI, receptive anal intercourse; STI, sexually transmitted infection; TPP, target product profile.

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gels and liquid/semi-solid dosage forms. Since the practice of RAI is not limited to MSM, with reports of approximately 30% of women also engaging in RAI, there is additional rationale for development of a single microbicide formulation that is suitable for both rectal and vaginal applications (Gorbach et al., 2014).

Willingness to use a product is a critical issue for microbicide development in general. It has become well accepted in the microbicide field that even without a rectal microbicide product with proven efficacy, there is a need to integrate qualitative and quantitative user sensory perception and experience (USPE) data to direct the formulation characteristics most desired by the targeted users of a product (Morrow and Ruiz, 2008). Indeed, lack of product efficacy in the VOICE and FACTS 001 trials of a vaginal gel delivering tenofovir are attributed, in large part, to poor adherence by trial participants (Marrazzo et al., 2015; Rees et al., 2015). To better inform microbicide development, participants in user acceptability studies have been asked to comment upon hypothetical as well as specific products, including preferred product characteristics and their willingness to use such products. Women have expressed an interest in a rectal microbicide (Exner et al., 2008) and gels have been indicated as the preferred dosage form for rectal use, in volumes of up to 35 mL (Carballo-Diéguez et al., 2008, 2007). A user acceptability study of a thiocarboxanilide (UC781)-containing rectal gel resulted in a favorable rating and high intention to use such a product among both men and women (Ventuneac et al., 2010).

To date, the rectal microbicide field has focused primarily on rectal application of formulations which were created for vaginal use. However, the significant physiological differences between the vaginal and rectal compartments challenge a simple translation of a vaginal product to a rectal product. Structurally, the vagina is composed of a stratified squamous epithelium, while the rectum/lower gastrointestinal tract is covered by a simple columnar epithelium Additionally, the length of the colon as an "open cavity," compared to the "closed cavity" of the vagina, poses challenges for choosing dosage volume as it provides a greater surface area for infection than does the vagina. The normal vagina is acidic (pH 4–4.5) due to the presence of lactic acid producing *Lactobacilli*, whereas the rectum has neutral to slightly alkaline pH. Microbicide gels suitable for rectal delivery have been developed (Anton et al., 2012; Dezzutti et al., 2012b; McGowan, 2011; McGowan and Dezzutti, 2014). In this process, early vaginal microbicides and commercial rectal lubricants were found to be hyperosmotic, and the prevailing thought is that to minimize damage to the rectal tissue, a rectal microbicide gel should be iso-osmotic and at a neutral pH (Wang et al., 2011). There is evidence of a direct relationship between higher product osmolality and epithelial damage to vaginal and rectal epithelial tissue and structure (Fuchs et al., 2007; Rebe et al., 2014; Rohan et al., 2010).

Because of the need to protect MSM populations from rectal HIV exposure, and increasing evidence that a significant proportion of women engage in RAI (often in the same sex act involving vaginal sex), there is a need to develop a microbicide product that is specifically designed for both vaginal and rectal applications (Dezzutti et al., 2012b; Gorbach et al., 2014). For women who engage in both vaginal and anal sex in the same sexual encounter, use of a single product that is safe for both compartments would be convenient and more likely to be used. Herein, we describe the development of a gel formulation, designed for safe and efficacious use in both the vagina and rectum, which delivers the nonnucleoside reverse transcriptase inhibitor (NNRTI) IQP-0528 as a topical anti-HIV microbicide. The data presented summarize diverse, pharmacologically relevant evaluations of candidate gels (termed "DuoGel™s") including their rheological, *in vitro* and *ex vivo* safety and bioactivity properties. These performance analyses of prototype DuoGel™s yielded a lead candidate formulation indicated for further development toward joint vaginal and rectal use.

2. Materials and methods

2.1. Compound

IQP-0528 was provided by Samjin Pharmaceutical Co. (Seoul, Korea), and is licensed to ImQuest BioSciences Inc. (Frederick, MD). IQP-0528 is a nonnucleoside reverse transcriptase inhibitor with a molecular weight of 340.42 Da and an EC_{50} of 0.0005 μM (Buckheit et al., 2008, 2007). It is practically insoluble in water and has a calculated Log P of 4.1 (Mahalingam et al., 2011). ImQuest BioSciences currently has an open Investigational New Drug program for the clinical evaluation of a vaginal gel containing IQP-0528.

2.2. Cell lines, virus and bacteria

The TZM-bl-FcRI cells were a gift from Dr. David Montefiori (Duke University, Durham, NC). The Ca Ski, ME180, HEC-1A, END1, ECT1, VK2, Caco-2 and *Lactobacillus* strains (*Lactobacillus crispatus* ATCC #33820, *Lactobacillus jensenii* ATCC #25258 and *Lactobacillus acidophilus* ATCC #11975) were purchased from the American Type Culture Collection (Manassas, VA). The HIV-1_{BaL} and clinical virus isolates were obtained from the NIAID AIDS Research and Reference Reagent Program (Rockville, MD) or HIV-1_{BaL} was purchased from Advanced Biotechnologies Inc. (Eldersburg, MD). Human peripheral mononuclear blood cells (PBMCs) were derived from human blood which was purchased from Biological Specialty Corporation (Colmar, PA). The cell lines were propagated as recommended; titered stocks of HIV-1_{BaL} and clinical virus strains were stored at −80 °C prior to being used in the antiviral assays.

2.3. Vaginal and seminal fluid simulants

The vaginal and seminal fluid simulants used in the HIV-1 efficacy assays in TZM-bl-FcRI cells were prepared as previously reported (Owen and Katz, 1999, 2005).

2.4. Human tissue

Normal human ectocervical and colonic tissues were acquired from pre-menopausal women undergoing hysterectomy or persons undergoing gastrointestinal surgery for non-inflammatory conditions, respectively, under approved IRB protocols with all patient identifiers removed. Normal human ectocervical tissue was also purchased from the National Disease Registry Interchange (http://ndriresource.org/) through an approved protocol, and shipped overnight on ice. Polarized explant cultures were set-up in duplicate as previously described (Rohan et al., 2010). Briefly, an explant was placed with the luminal side up in a transwell. The edges around the explant were sealed with Matrigel™ (BD Biosciences, San Jose, CA). The explants were maintained with the luminal surface at the air–liquid interface. The lamina propria was immersed in medium for cervical explants or rested on medium-soaked gelfoam for colonic explants. Cultures were maintained at 37 °C in a 5% CO_2 atmosphere.

2.5. DuoGel™ formulation development

Working with excipients that pharmacologically were generally regarded as safe (GRAS), prototype gels were formulated in several iterations. These gels were evaluated against a defined Target Product Profile (TPP) developed by ImQuest BioSciences (Fig. 1). The physicochemical characteristics of these prototype formulations included gel appearance, pH (SI Analytics Lab850), viscosity