VivaGel™ (SPL7013 Gel): A candidate dendrimer – microbicide for the prevention of HIV and HSV infection

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Abstract: Microbicides are compounds that applied vaginally or rectally, protect the user from sexually transmitted infections. Although no commercial product is yet available, many candidates are under development. A leading candidate, VivaGel™ (SPL7013 Gel) is the product of nanotechnology. The active ingredient is SPL7013, a dendrimer that was designed specifically with HIV and HSV antiviral activity and human safety in mind. SPL7013 has demonstrated efficacy against human immunodeficiency virus and herpes simplex virus in in vitro and animal models. VivaGel™ appears to be well tolerated in both animals and humans. This review summarizes the studies of VivaGel™ and its active ingredient, SPL7013.

Keywords: microbicide, dendrimer, SPL7013, VivaGel™

Introduction

In 2005, an estimated 4.1 million people worldwide were newly infected with human immunodeficiency virus (HIV) (UNAIDS 2006). The mainstay for controlling the AIDS epidemic remains prevention. Unfortunately, the development of a vaccine has proven elusive; therefore prevention has relied primarily on behavioral changes including condom use. Many males are unwilling to use condoms thus placing their sex partner(s) at risk. Microbicides are compounds that when applied vaginally or rectally would protect the user from sexually transmitted infections (STIs). Microbicides could offer additional protection when combined with condoms or be used alone as a means of protection for women unable to negotiate condom use. Although the emphasis has been on preventing HIV, protection from other STIs is desirable in terms of avoiding illness and associated sequelae. Importantly, many STIs may increase vulnerability to HIV infection (eg, genital herpes infection facilitates the transmission of HIV).

Microbicides, like vaccines, will be used by healthy individuals to protect against infections. Like vaccines, microbicides will need to be both highly efficacious and display an excellent safety profile. These products should have little or no effect on host defenses including the normal vaginal or rectal microflora, the mechanical barrier provided by intact epithelium and a variety of innate and adaptive immune responses (Turpin 2002). Studies using in vitro systems and animal models are important tools in the preclinical development of microbicides.

Dendrimers are a relatively new class of compounds that hold the potential to be safe and effective microbicides (McCarthy 2005). Many biologic targets are large complex macromolecules (eg, membrane receptors) on the scale of 1–100 nm. These macromolecules rely on polyvalent interactions for binding and initiating biologic processes. To date, most pharmaceutical development has focused on low molecular weight drugs that lack the capacity to interact with such targets in a multivalent manner. Dendrimers are "nanoscale" macromolecules that can be built with various

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properties including the ability to interact polyvalently with a target. Dendrimer production is a highly controlled staged process that allows for the synthesis of precisely defined macromolecular structures. The result is a highly branched three-dimensional molecule that may possess various surface features. SPL7013 is a dendrimer that was developed by Starpharma (Melbourne, Victoria, Australia) and contains a specifically designed polyanionic surface. This highly charged surface allows SPL7013 to attach to targets on viruses, blocking viral attachment and/or adsorption to cells thereby preventing infection. In the case of HIV, SPL7013 is thought to bind gp120 proteins on the surface of the virus, through which the virus normally attaches to CD4 receptors on human cells.

SPL7013 is the active product in the candidate microbicide, VivaGel™ (SPL7013 Gel). Recognizing its potential, the United States Food and Drug Administration (FDA) granted VivaGel™ Fast Track status in January 2006 for the prevention of HIV indication (Starpharma 2006a). The Fast Track program facilitates and expedites development of products that serve unmet medical needs of serious or life-threatening conditions. Recently, in collaboration with Starpharma, the United States National Institutes of Health (NIH) submitted a second Investigational New Drug application (IND) to FDA for the prevention of HSV-2 indication. This paper reviews the data on the safety and efficacy of VivaGel™.

VivaGel™ chemistry

VivaGel™ is a water-based vaginal product of 3% weight/weight (w/w) SPL7013 mixed in Carbopol® gel buffered to a pH physiologically compatible with the normal human vagina.

SPL7013 is built from a divalent core, the benzhydrylamine amide of L-lysine (McCarthy 2005). To this core, four successive layers of the branching units, L-lysine are added creating a dendrimer with 32 amine groups on the surface. A sodium 1-(carboxymethoxy) naphthalene-3,6-disulfonate group is attached to each of the 32 amine surface groups via amide linkers. The chemical product is BHA.lys15lys16(NHCOCH2O)1-(3,6-naphth$(SO_3Na)_{32}$ (BHA = benzhydrylamine) with a molecular weight of 16,581 daltons. High performance liquid chromatography (HPLC) and liquid chromatography/mass spectrometry are employed in manufacturing to ensure a single molecular entity is synthesized. Purity is determined by HPLC, capillary electrophoresis, and electrospray mass spectral analysis.

Carbopol® gel was chosen because of its mucoadhesive properties and its use in other vaginal products and microbicide formulations. Carbopol® 971P NF is a cross-linked acrylic acid listed in the USP Monograph as Carbomer 941 and is categorized by the FDA as a generally regarded as safe (GRAS) excipient. It is a weak acid with high buffering capacity and has been shown to be partially effective at inhibiting herpes simplex virus in mice and partially effective against HIV-1 in vitro.

The results of in vitro and in vivo studies of SPL7013 and the formulated product Vivagel™ are discussed in the text below. Summaries of the safety and efficacy are included in Tables 1 and 2.

In vitro studies

Gong et al (2005) evaluated SPL7013 for efficacy against herpes simplex virus (HSV) and for cell cytotoxicity. Efficacy against infection was tested by incubating Vero cells (African green monkey kidney cells) with SPL7013 prior to the addition of the viral inoculum (pre-infection prophylaxis). Plaque reduction assays demonstrated the 50% effective concentration (EC_{50}) against infection as 2.0 μg/ml for HSV type-1 (HSV-1) and 0.5 μg/ml for HSV type-2 (HSV-2). Viral absorption to the Vero cells was completely inhibited at SPL7013 concentrations >3 μg/ml.

Antiviral activity in cells already infected with HSV-1 or HSV-2 was demonstrated as well (post-infection treatment). EC_{50} for treating infections were 6.1 μg/ml for HSV-1 and 3.8 μg/ml for HSV-2. Time-of-addition studies indicated that SPL7013 inhibited both cell entry and HSV replication suggesting that it may have potential as a therapeutic agent for HSV infections. SPL7013 was shown to provide comparable protection and antiviral activity against a HSV-2 strain resistant to penciclovir/acyclovir and a HSV-1 strain resistant to foscarnet.

SPL7013 was not toxic to Vero cells up to the highest concentration tested, 10,000 μg/ml. Effects on cell proliferation were tested on two epithelial cell lines (Hela-229 and Hep-2) in both stationary and dividing phases. The 50% cytotoxic concentrations (CC_{50}) on both cell lines were greater than the highest concentration tested (10,000 μg/ml).

By serial passage in the presence of increasing concentrations of SPL7013, Gong et al (2005) generated an HSV-1 strain that was resistant to post-infection dendrimer treatment but not pre-infection dendrimer prophylaxis. Using this approach the investigators were unable to create a dendrimer-resistant HSV-2 strain. This suggested it was possible to develop resistance to the effects of SPL7013 on viral replication but not on the mechanism(s) responsible for preventing infection.

Table 1 Brief summary of safety studies

In vitro studies

Gong et al (2005)

SPL7013 found nontoxic to Vero cells in highest concentration tested (10,000 μg/ml)

Dezzutti et al (2004)

SPL7013 was relatively nontoxic to peripheral blood mononuclear cells, macrophages, urogenital and colorectal cell lines. Its toxicity was similar to that of the base carbopol gel. SPL7013 had very little effect on the ability of mucosal epithelia cells to maintain an intact, polarized monolayer when measured by transepithelial resistance.

Abner et al (2005)

The 5% formulation in colorectal explants caused epithelial sloughing while the lamina propria remained intact whereas nonoxynol-9 caused sloughing and necrosis of the lamina propria.

Bernstein et al (2003)

In Vero cells, the cytotoxic concentrations (CC_{50}) was higher than the maximum concentration tested (1000 μg/ml).

In vivo studies

Bernstein et al (2003)

In a 5-day repeat dose rabbit vagina model, the 5, 1 and 0% formulations all elicited the same minimal level of vaginal irritation.

Patton et al (2006)

In a 4-day repeat dose macaques vagina model, the 5% but not the 3% or 1% formulation caused irritation visible by colposcopic examination. The 3% did not cause histologic changes on biopsy. Rectal application of the 3% formulation yielded rectal lavage specimens no different than those of untreated animals.

Jaing et al (2005)

In vitro studies yielded a cytotoxic dose (CD50) of 1500 μM for macaques peripheral blood mononuclear cells and >2250 μM in two different human cell lines. Clinical observation did not find any signs of macaques vaginal irritation with a single dose of the 1%, 3% and 5% formulations.

Human study

McCarthy et al (2005)

A phase I human clinical trial demonstrated that 0.5%–3.0% SPL7013 formulations were safe and well tolerated following once daily vaginal application for 7 days. In addition, the SPL7013 was not absorbed systemically.

Dezzutti et al (2004) evaluated the cytotoxicity and anti-HIV activity of serial dilutions of 5% w/w SPL7013 in the VivaGel™ formulation. Cellular toxicity was appraised in peripheral blood mononuclear cells (PMBCs), macrophages (mΦ), urogenital and colorectal cell lines. SPL7013 was found to be relatively nontoxic and its toxicity was similar to that of the base Carbopol® gel. The ability of mucosal epithelial cells to maintain an intact, polarized monolayer in the presence of the various candidates was evaluated by measuring transepithelial resistance (TER). TER was affected very little by SPL7013. Anti-HIV activity was evaluated in assays for prevention of infection of PMBCs and mΦ as well as for prevention of the transfer of infectious HIV-1 from epithelial cells to activated PMBCs. SPL7013 blocked infection of mΦ by >2 $\log_{10}$ ($\geq$99% inhibition).

The safety and efficacy of SPL7013 for rectal use was evaluated using colorectal explants obtained from patients undergoing bowel surgical resection for non-inflammatory disease. (Abner et al 2005). Explants exposed to 5% SPL7013 underwent epithelial sloughing leaving the lamina propria intact whereas the control explants treated with the nonoxynol-9 suffered sloughing and necrosis of the lamina propria. As is described below, any epithelial sloughing attributed to SPL7013 in these explant studies is in contrast to the safety profile of VivaGel™ following repeated rectal administration in nonhuman primate studies. A decrease in explant viability was detected after application of both SPL7013 and the placebo Carbopol® gel by a MTT (1-(4,5-dimethylthiazol-2yl)-3,5-diphenylformazan) assay. Anti-HIV efficacy was evaluated utilizing HIV-1 CXCR4-using (R4) and CCR5-using (R5) strains. Transmission of HIV-1 infection to the rectal epithelium was reduced by >85% with SPL7013 and by 50% with the base Carbopol® formulation alone.

Nonprimate animal studies

Bernstein et al (2003) evaluated SPL7013 in vitro and in animal models. The in vitro studies utilized Vero cell cultures incubated with different concentrations of unformulated SPL7013 to evaluate cell cytotoxicity and effectiveness against HSV-2 infection. The group found an EC_{50} of 0.6 μg/ml while the CC_{50} was greater than the maximum 1000 μg/ml tested.

The effect of SPL7013 drug concentration and duration of protection was evaluated. Mice were treated vaginally with 100 mg/ml, 10 mg/ml, 1 mg/ml SPL7013 or phosphate

Table 2 Brief summary of efficacy studies

In vitro
Gong et al (2005)
SPL7013 was effective protecting Vero cells from HSV infection with an effective concentration (EC_{50}) of 2.0 μg/ml for HSV-1 and 0.5 μg/ml for HSV-2. Inhibitory effects were observed in HSV infected cells indicating a therapeutic potential for SPL7013
Dezzutti et al (2004)
The 5% SPL7013 formulation inhibited by >99% HIV infection of human macrophages in vitro
Abner et al (2005)
Transmission of HIV-1 (R4/R5 strains) infection to the rectal epithelium in explants was reduced by >85% with the 5% SPL7013 formulation
Bernstein et al (2003)
Unformulated SPL7013 protected Vero cells from HSV-2 infection
Jaing et al (2005)
In vitro SPL7013 inhibits infections by HIV and SHIV in human cell lines and macaque PBMCs
In vitro
Bernstein et al (2003)
Formulated product improves duration of protection for at least one hour in the mouse vaginal model. Guinea Pig vaginal model suggested formulation should be 3%–5% SPL7013 for optimal protection
Patton et al (2006)
The 3% formulation was not effective against cervical challenge with *Chlamydia trachomatis* in macaques
Jaing et al (2005)
Formulations of 3% and 5% SPL7013 effectively blocked vaginal transmission of SHIV in macaques

buffered saline (PBS) and challenged 20 seconds later with HSV-2. A dose related response was noted with 12/12, 10/12, 6/12 and 0/12 mice being protected from infection, respectively. The 10 mg/ml concentration was tested with HSV-2 challenges at 5, 30, or 60 minutes following SPL7013 prophylaxis. Protection against infection was seen at each respective time in 14/16, 12/16 and 4/15 mice. All PBS treated controls became infected.

Vaginal gel prototypes were prepared with various concentrations of SPL7013 in three different Carbopol® formulations differing in the amount of propylene glycol and glycerin. Gels containing 5, 1 and 0% w/w SPL7013 were evaluated in a 5-day repeat dose rabbit vaginal irritation model and all elicited the minimal vaginal irritation. The 5, 1 and 0% w/w SPL7013 concentrations in the different Carbopol® formulations were tested in mice against a HSV-2 challenge at 5 and 30 minutes following prophylaxis. All three formulations with either the 5 or 1% w/w SPL7013 provided significant protection at both time points (63%–100% efficacy).

Based on these animal studies the Carbopol® formulation containing 1% w/w glycerin and propylene glycol was selected for further vaginal testing in the guinea pig model of genital herpes disease and is now referred to as VivaGel™. The guinea pig model is felt to closer approximate human genital herpes disease than the mouse model. Concentrations of 1%–5% w/w SPL7013 were evaluated with a HSV-2 challenge at 5 minutes following prophylaxis. The results showed high levels of protection were provided by 3% and 5% w/w SPL7013 formulations. This same Carbopol® formulation was selected for evaluation in subsequent primate model studies.

In June 2006, Starpharma (2006b) announced that preliminary studies suggest that SPL7013 exhibits contraceptive activity in a rabbit model. The mechanism of contraceptive action is postulated to be interference with attachment/fertilization. The study, performed at Johns Hopkins University (Baltimore, Maryland, USA), used two formulations of 3% SPL7013. A 75% reduction in embryos was seen with the SPL7013 Carbopol® gel formulation and a 95% reduction with the SPL7013 HEC (hydroxyethyl cellulose) gel formulation. The reduction in the number of embryos in rabbits was similar to that produced by contraceptive products containing nonoxynol-9.

Nonhuman primate safety and efficacy studies

Investigators at the University of Washington at Seattle (Patton et al 2006) studied the safety and efficacy of SPL7013 vaginal gels in pigtailed macaques. In a comparative study, three concentrations (1%, 3% and 5% w/w SPL7013) and the base Carbopol® gel alone (placebo) were administered intravaginally once daily for four consecutive days. Cervicovaginal irritation was seen on colposcopy in

four of the six animals administered the 5% w/w SPL7013 formulation but in none of the animals receiving the 3% w/w, 1% w/w SPL7013 or placebo gel. Vaginal and cervical biopsies collected 24 hours after the fourth application of the 3% gel and placebo appeared similar to those obtained at baseline. A transient impact on vaginal microflora was seen 30 minutes after application in all groups but no pattern of a sustained shift in organisms was noted. Vaginal pH was significantly lower 30 minutes after application of the SPL7013 formulations but had returned to baseline by the fourth day after the final application. The pH change is due to the difference between the pH of the formulations (pH 5) and the higher pH of the normal macaque vagina. VivaGel™ has been formulated at pH 5 to be physiologically compatible with the normal acidic nature of the human vagina.

Based on the results of the vaginal tests, the 3% w/w SPL7013 formulation was selected for study in the rectal model. The macaques received either the 3% w/w SPL7013 formulation or the base Carbopol® gel alone (placebo) intrarectally on three consecutive days or received no treatment at all. The safety of VivaGel™ in this model was demonstrated following collection of rectal lavages and their evaluation for evidence of epithelial damage (ie, cellular debris, epithelial sheets and blood and/or stroma associated with the epithelial sheets). This analysis showed there were no significant differences between the animals in the nontreated control group and those animals treated with the 3% w/w SPL7013 and placebo gels. There were no significant differences in microflora between all the groups. Rectal pH was significantly lower in the placebo and 3% w/w SPL7013 gel groups, which reflects the pHs of these formulations relative to the pH of the macaque rectum, and recovered by 24 hours after the last application.

The efficacy of the 3% w/w SPL7013 gel for the prevention of cervical *Chlamydia trachomatis* infection was assessed. Four of the six animals that received the 3% w/w SPL7013 gel and five of the six animals that received no product developed infection following intravaginal inoculation. SPL7013 did not prevent cervical chlamydial infection in this model.

Jaing et al (2005) demonstrated anti-retroviral activity in vitro against both CXCR4-using (R4) and CCR5-using (R5) HIV and SHIV (chimeric simian-human immunodeficiency-1 virus) strains in human and simian cell lines. In addition, SPL7013 showed very low cytotoxicity in cell culture. Based on these findings for unformulated SPL7013, the ability of a single intravaginal application of VivaGel™ to block vaginal SHIV transmission was tested. Following challenge, all untreated pigtailed macaques (8/8) and seven of the eight animals given the base Carbopol® formulation without SPL7013 (placebo gel) developed SHIV infection as indicated by plasma viremia and $CD4^+$ cell decline. A dose-dependent response was observed among the SPL7013 treated macaques; 6/6, 5/6, and 2/6 animals treated with the 5%, 3% and 1% w/w SPL7013 gels, respectively, were protected against infection. The uninfected animals were followed for over a year and remained SHIV negative by serology and lymph node biopsy. Clinical observation did not reveal any signs of toxicity or vaginal irritation due to the single application of the SPL7013 formulations.

Human trials

A phase I clinical trial involving 36 healthy women was completed in 2004 (McCarthy 2005). VivaGel™ preparations containing 0.5%–3.0% w/w SPL7013 were evaluated for safety in comparison to the base Carbopol® formulation without SPL7013 (placebo gel). Women received either VivaGel™ or placebo once daily intravaginally for 7 days. All SPL7013 concentrations of VivaGel™ were found to be safe and as well tolerated as placebo. SPL7013 was not absorbed into the systemic circulation simplifying the toxicological aspects about this drug.

Summary

SPL7013 is a dendrimer developed specifically to work as a microbicide against HIV and HSV-2. In vitro and animal model studies have demonstrated the efficacy of the unformulated dendrimer in protecting against both HSV and HIV infections. VivaGel™, at SPL7013 concentrations up to and including 3% w/w, was shown to be safe following vaginal or rectal application in macaques and protected macaques against vaginal SHIV infection. A Phase I clinical trial in humans demonstrated VivaGel™ was well tolerated. Further trials are planned to expand on the initial Phase I clinical trial and to evaluate VivaGel™ for the prevention of HIV and HSV-2 genital infections in women. (Starpharma 2006c).

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