Preclinical and Clinical Profile of Emmelle (Dextrin-2-Sulfate)—a Potential Anti-HIV Microbicide

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ABSTRACT

Emmelle is a vaginal gel formulation of dextrin 2 sulfate (D2S) being developed by M-L Laboratories PLC, for the potential prevention of sexual transmission of HIV. D2S, a negatively charged sulfated carbohydrate molecule, prevents HIV from entering host cells by binding to positively charged groups on the surface of HIV. The safety, acceptability, and tolerability of increasing doses of D2S have been evaluated in a number of Phase I/II clinical trials in Europe and Africa. Based on favorable outcome, preparations are underway for a planned efficacy Phase III trial of Emmelle.

INTRODUCTION

Heterosexual transmission of human immunodeficiency virus, type 1 (HIV-1), the causative agent of acquired immunodeficiency syndrome (AIDS), continues to be the predominant mode of the pandemic spread of HIV/AIDS.[1] Worldwide, it accounts for 90% of all HIV infections in women.[2] In the absence of an effective prophylactic anti-HIV therapy or vaccine, current efforts are aimed at developing safe and effective intravaginal/intrarectal topical formulations of antiviral microbicides to prevent the sexual HIV-1 transmission.[3,4] Microbicides could provide protection by directly inactivating HIV or preventing HIV from attaching, entering or replicating in susceptible target cells, as well as dissemination from target cells present in semen or the host cells that line the vaginal or rectal wall. A safe and effective microbicide is not yet available despite the fact that more than 60 candidate agents have been identified to have in vitro activity against HIV, 18 of which are in clinical development and 6 have advanced to Phase III efficacy trials.[5]

PROPERTIES REQUIRED OF TOPICAL MICROBICIDES

The desirable properties of a vaginally or rectally inserted anti-HIV microbicide are acceptability and feasibility. They must be easy to use, non-irritating, and non-toxic. They should have broad-spectrum activity, since drug-resistant HIV is common in recently infected patients.[6,7] Also, their efficacy must be governed by knowledge about the nature of sexual transmission of the virus, in that they should be fast-acting in the presence of vaginal/rectal fluids and semen. This will require careful

consideration of several variables involving the active agent, vaginal and rectal physiology, and the delivery system. Physiological differences between the vagina and rectum would necessitate different formulations for each site. In addition, vaginal drug availability can be affected by multiple factors such as the pH, menstrual cycle, and the presence of co-pathogens. Therefore, the ultimate success of a microbicide will also depend on its formulation in addition to the active agent(s).

MECHANISM OF HIV TRANSMISSION

The mechanisms involved in sexual transmission of HIV-1 appear to be complex and are not yet fully defined. Potential sources of transmissible HIV are free virus particles and infected lymphoid cells in semen, in cervicovaginal secretions, and in blood or other fluids present because of physical trauma or genital infections.[8-10] The target cells for HIV-1 infection in the female genital tract include mucosal dendritic cells and lymphocyte/macrophages in the lamina propria of the cervicovaginal mucosa, Langerhans cells in the vaginal epithelium, and genital tract epithelial cells.[11-13]

Monocytes, macrophages, and dendritic cells play an important role in the initial infection and contribute to its pathogenesis throughout the course of infection. HIV particles can also attach to the surface of dendritic cells, without infecting them, through an interaction between mannose-rich residues in gp120 and a C-type specific lectin (DC-SIGN) in the cell membrane.[14] Captured virions either infect target cells or are efficiently transmitted to lymphocytes.

MECHANISM OF MICROBICIDE ACTION

To prevent HIV transmission a microbicide must either inactivate the virus (both free and cell-associated) while it is still in the vaginal/rectal lumen, prevent the virus from attaching or fusing with its host cells, or prevent the virus from replicating in target cells. The microbicides under development work in a variety of ways including: (1) disrupting the organism's cell membrane or envelope; (2) blocking the receptor-ligand interactions or postfusion events essential for infectivity; (3) inhibiting the intracellular replication of the virus; and (4) altering the vaginal microenvironment, reducing the susceptibility to infection and enhancing the local immune response.

HIV FUSION AS A TARGET FOR MICROBICIDES

HIV-1 entry process is one of the earliest mechanisms examined for therapeutic intervention. The process of HIV-1 entry can be grouped into three sequential steps: (1) attachment of the virus to host cells; (2) interaction of the virus with coreceptors; and (3) fusion of the virus and host cell membranes.[15-20] HIV fusion is mediated by CD4 and a co-receptor (CXCR4 or CCR5, the receptors for X4 and R5 viruses) on the target cell membrane, and by the non-covalently bound glycoproteins gp120 and gp41 on the virus surface. The fusion event is initiated by attachment of viral particle on a target cell, and the binding of gp120 with CD4 and the co-receptor. This results in a conformation change in gp120 and gp41, the insertion of the gp41 N-terminus into the target cell membrane, and the formation of a 6 helical hairpin structure by the heptad repeat regions of gp41. The hairpin structure formation brings together the cell and viral membrane and eventually leads to fusion.[21,22]

Several anionic polymers including dextrin 2 sulfate (D2S) are thought to exert their anti-HIV activity by shielding off the positively charged sites in the V3 loop of the viral gp120 which is required for virus attachment to the cell surface

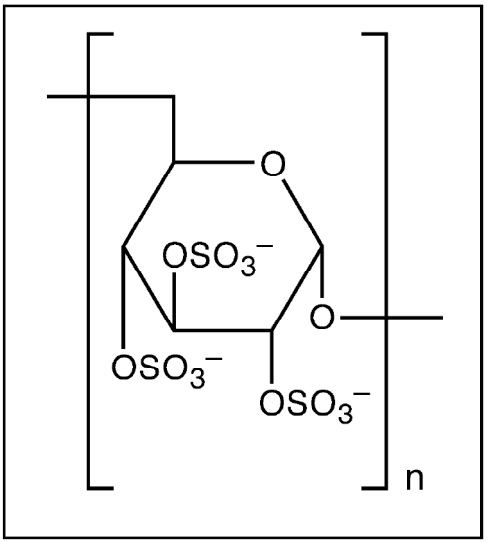

Figure 1. Chemical structure of dextrin sulfate.

heparin sulfate, before more specific binding occurs to the CD4 receptor of the CD+ cells. Therefore, both synthetic and natural anionic polymers are being evaluated as potential vaginal topical microbicides for prevention of sexual transmission of HIV. Emmelle is a vaginal gel formulation of dextrin 2 sulfate (D2S) being developed by M-L Laboratories PLC (Warrington, UK), for the potential prevention of sexual transmission of HIV.

PRECLINICAL DEVELOPMENT OF EMMELLE/D2S

D2S is a sulfonated polysaccharide with in vitro activity against HIV-1. It is a synthetic derivative of an enzymatic hydrolysis product of starch possessing a sulfate moiety on the 2-position of the glucan ring (Figure 1). Dextran sulfates are effective in vitro inhibitors of various enveloped viruses including herpes simplex, cytomegalovirus (CMV), and HIV.[23-26] The median inhibitory concentration of D2S (MW, 10,000) for CMV and HIV-1 is 0.5 µg/mL with selectivity index of greater than 800 against the host cell (MT-4). A comparative antiviral activity of D2S with MWs ranging from 1,000 to 500,000 revealed that the optimal antiviral activity was observed at a MW of 10,000. D2S is believed to block virus binding and penetration into target cells, and specifically, to block the binding of the HIV envelope protein to the CD4 receptor.[27,28] D2S also exerts its activity at a second step in infectivity, such as membrane fusion.[29] D2S fails to neutralize virions directly, but interacts with target cells to inhibit virus entry.[30] D2S inhibits the growth of diverse laboratory strains of HIV-1 in a variety of human cell lines, lymphocytes, and macrophages.[31,32] D2S has been shown to elicit gp41 six-helix bundle formation in HIV-1 IIIB. However it was less effective against HIV-1 BaL, a macrophage (M)-tropic or R5 isolate. Recent studies suggest that sexually transmitted R5 HIV-1 viruses have less positive charge on their gp120 surface compared with the R4 HIV-1 viruses.[33,34]

CLINICAL DEVELOPMENT OF EMMELLE/D2S

In gel form, D2S could be used to coat the outside of epithelial cells lining the vagina and so may prevent sexual transmission of HIV. Vaginal safety studies have evaluated the effects of D2S gel on genital signs and symptoms in sexually abstinent healthy women following 7 to 28 day product use. Safety end-points have included signs of genital irritation, vaginal leakage, systemic safety, absorption, and changes in vaginal microflora, and changes in vaginal and cervical epithelia by means of colposcopy and/or microscopic evaluation of biopsy specimen for signs of inflammation, in addition to product acceptability.

A number of Phase I studies have evaluated the safety, tolerability, and acceptability of intravaginal D2S gel as a potential vaginal microbicide. The first randomized, double blind, placebo-controlled trial to evaluate the safety and

tolerability of D2S gel was conducted by Stafford et al[35] of Chelsea and Westminster Hospital (London, UK). This Phase I study included 36 healthy sexually abstinent women who received 5 mL doses containing either 0.003% or 0.01% D2S or a placebo gel.[35] Gel tolerability was based on questionnaire and patient interview. Abstinence from coitus was a prerequisite for trial entry because it was considered an important confounding variable. Macroscopic and microscopic evidence of local inflammation was assessed by colposcopy and vaginal biopsies. The impact of D2S gel on normal vaginal flora was assessed by quantitative lactobacilli culture as well as the ratio of peroxide to nonperoxide-producing organisms. Colposcopy revealed mild erythema in 5 of 24 subjects receiving D2S gel and in none of the 12 placebo recipients. Histology in all subjects revealed no evidence of inflammation. No adverse impact on vaginal lactobacilli was found. Using the same methodology, a second safety study involving 18 subjects (10 D2S gel recipients and 8 placebo gel recipients) produced a similar favorable toxicity profile was demonstrated for 0.05% D2S gel.[36] These studies indicated that D2S gel is safe and well tolerated intravaginally at the dosing schedule used in this study.

A second randomized, placebo-controlled trial carried out at St. Mary's School of Medicine (London, UK), included 73 sexually active women and their male partners.[37] Female participants received a 2 mL dose of 0.125% D2S gel (n = 36) or a placebo gel (n = 37). A single 2-mL dose of D2S gel was self-administered every night over two 14-day periods separated by a 7-day interval, during which menses was expected to occur. Up to two supplementary doses per 24-hour period were allowed for use before sexual intercourse. Assessments of adverse events were based on semi structured interview, colposcopy, and laboratory safety studies. Male partners in a sub study were exposed to gel through sexual intercourse during the second 14-day exposure period. Seventy-three women (36 D2S recipients and 37 placebo recipients) used at least one application of gel, of whom 66 (33 D2S recipients and 33 placebo recipients) completed follow-up. Genital irritation was apparent in 26 (14 D2S recipients and 12 placebo recipients) of 67 women. Forty-five (23 D2S recipients and 22 placebo recipients) of 67 women reported unusual vaginal discharge. Twenty-two of the 45 women with discharge had mild or moderate genital irritation. Eleven women (5 D2S recipients and 6 placebo recipients) of 73 women reported intermenstrual bleeding during gel use. Use of D2S gel was not associated with an increased incidence of bacterial vaginosis or vaginal candidiasis in this study population. Overall, this study suggested that daily intravaginal administration of a 2 mL of 0.125% D2S gel had an acceptable toxicity profile compared with placebo gel. Despite the known anticoagulant activity of D2S,[38] coagulation studies, and platelet counts in both treatment groups did not suggest systemic absorption. Only transient increase in activated partial thromoboplastin time and a decrease in platelet count was noted in three women following exposure to the gel.

Ten male partners (4 with D2S exposure and 6 with placebo exposure) were enrolled in the penile safety study and all completed follow-up. There was no evidence of systemic toxicity or genital epithelial disruption attributable to D2S gel. However, at follow-up 4 men had transient genital burning or soreness during gel exposure, which was attributed to, localized erythema.

A third Phase I randomized double-blind placebo-controlled, safety and

acceptability study of D2S gel has been carried out at St. Mary's School of Medicine (London, UK), and The Institute of Tropical Medicine (Antwerp, Belgium), included 80 sexually active HIV-negative and 20 HIV-positive women.[39] This dose-ranging Phase I study assessed local toxic effects of D2S gel for vaginal use on vulvular and cervicovaginal mucosa in HIV-infected women. Fifty HIV-negative women received 0%, 1%, or 4% D2S gel and 20 HIV-positive and HIV-negative women received 4% D2S gel. An additional 20 HIV-negative women were included as no treatment controls. The gel was applied twice daily for 14 days. Local toxicity was assessed by colposcopy or histology of vaginal biopsies. Systemic toxicity was determined by coagulation studies. Cervico-vaginal lavages were assayed for cytokines, chemokines, and HIV RNA. No increased evidence of genital epithelial disruption or inflammation or evidence of systemic toxicity associated with gel use was apparent. None of the women receiving D2S gel in this Phase I study reported difficulties in applying the gel. The acceptability rates were 76% for the D2S gel and 70% for the placebo gel. However, an unexpectedly high frequency of intermenstrual bleeding occurred in the study population.

A fourth Phase I/II randomized, placebo controlled safety and acceptability study of 4% intravaginal D2S gel has been carried out at St. Francis Hospital (Nsamby, Kampala, Uganda).[40] In this study, sexually active females of reproductive age were allocated to active gel or its matched vehicle placebo, pre sex active gel and observation only arm. Total follow up period was 8 weeks during which women were interviewed, examined by colposcopy and laboratory tests (HIV test, STD screening, hematology, coagulation, and clinical chemistry). A total of 109 females comprising 71-HIV negative and 38 HIV-positive were enrolled; 80 females (65 active gel recipients, 15 placebo gel recipients) applied the gel twice daily, 9 with active gel before coitus and 20 observation only arm. In 7/322 (2.2%) colposcopy exams, abnormalities were documented among females using active gel, and in 11/74 (14.9%) within the placebo group. Only 2 of the 18 abnormalities were thought to be gel related. Six of 65 (9%) participants on active gel twice daily reported mild intermenstrual spotting, compared to 2/15 (13%) using placebo, and 3/20 (15%) using no gel. Eight of 65(12%) participants on active gel reported excessive thirst during the first week of gel use. No excess genital irritation, no evidence of change in vaginal flora, no evidence of systemic toxicity were observed as a result of gel use. These results indicated a satisfactory safety profile of 4% D2S gel in a sexually active African population.

Microbicidal gels should cover the vaginal and cervical epithelium in order to maximally protect against viral transmission. Quantifying the intravaginal distribution of topical gels is important in formulating optimal microbicidal gels. Spreadability is dependent on such factors as volume, time, ambulation, and sexual activity. A Phase I study being conducted at London, UK (St. Mary's Hospital and MRC Clinical Trials Unit) compared the intravaginal distribution of two concentrations (2% and 4%) of D2S gel by magnetic resonance imaging (MRI).[41] MRI can be used to safely and acutely visualize and quantitate spread of a potential microbicide formulation. MRI assessments are made prior to, and immediately after gel application and 12 hours and 24 hours after gel administration. Study end points include signal intensity readings for gel at predetermined sites within the vagina, assessment of the degree of cervico-vaginal mucosa covered by gel and visual assess-

ment of the uniformity and distribution of gel within the genital tract. Women will act as their own controls to enable us to estimate the effect of intercourse on the retention and distribution of gel over 24 hours. This study will determine whether sexual intercourse affects the distribution and retention of the gel over 24 hours.

Van Damme et al[42] have evaluated the safety and acceptability of penile application of a 4% D2S gel/Emmelle in a randomized, double-blind, placebo-controlled trial among 16 HIV-positive and 12 HIV-negative men which was conducted at the Institute of Tropical Medicine (Antwerp, Belgium). This study assessed whether 4% D2S gel, currently used for clinical testing in women, is also acceptable for sexually active men. In this Phase I study, men were asked to apply the gel to the penis once per day for 14 consecutive days and to leave it on for a minimum of 6 hours. The median period where the gel was in contact with the penis was 8.2 hours. Safety assessments included genital examination, coagulation studies, and sexually transmitted infection screening. Results from laboratory evaluations, genital examinations, and adverse events reports showed that 4% D2S gel administered topically to the penis for 14 days was well tolerated when compared with placebo, despite the fact that D2S is a skin irritant. However, penile erythema was evident during the study medication. No clinically significant differences were observed in hematology, clinical chemistry, coagulation parameters, or adverse events between treatment groups. Thus, penile application of 4% D2S gel for 14 consecutive days had an acceptable safety profile.

Currently, UK's Department for International Development (DfID) and the Medical Research Council's (MRC) clinical trials unit are planning to launch Phase III effectiveness trials of D2S.[43,44] The DfID/MRC human trials are planned to take place in 8 sites in 5 African countries, including South Africa, Tanzania, and Cameroon. This trial would require over 6,000 women in order to give clinically meaningful results. This anticipated trial should give results by early 2008, so that a microbicide could be available to women by 2010.

EXPERT OPINION

As a first-generation microbicide, D2S may not be highly effective as intravaginal or intrarectal microbicide, but it would still fulfill the current void in meeting the urgent need for some kind of intervention that would bring some health relief. AIDS experts estimate that even a partially effective microbicide could prevent 2.5 million deaths from AIDS over three years.[45] Independent market research studies have concluded that in developed countries the potential revenues from a product such as Emmelle could reach in excess of $1 billion per year.[45]

To be effective in preventing HIV infection, anti-HIV-1 microbicides must be virucidal against both X4 and R5 viruses, utilizing CXCR4 and CCR5 as coreceptors,[3] respectively. The macrophage tropic R5 HIV-1 is the most frequently sexually transmitted.[33,46] Persons who harbor R5 viruses are more infectious than those that have X4 variants. D2S binds to basic regions of HIV env and block viral interactions with some attachment and entry receptors. These electrostatic interactions of D2S are dependent on pH and the charge of the V3 loop. D2S has a lower binding affinity for R5 HIV-1 viruses than R4 HIV-1 viruses and fails to prevent infection of cultured target cells by some R5 isolates.[34,47,48] Since patient isolates are composed of populations of genetically and biologically distinct variants,[49] the use of D2S as a prophylactic microbicide

might select for the predominant growth of resistant strains. Furthermore, D2S does not interfere significantly with HIV-1 transmission to mucosal dendritic cells via DC-SIGN mediated lectin pathway.[50] This could limit the effectiveness of D2S in preventing sexual transmission of M-tropic HIV-1 in the clinical setting. Furthermore, among the sulfated polysaccharides, D2S exhibits stronger anticoagulant properties. Thus, the increase in blood clotting time might be potentially undesirable for repeated genital applications under conditions of local preexisting injury or bleeding.

Potential advantages of D2S as microbicide include its broad-spectrum activity and simple chemical structure, which allows production costs to be low compared with those of the available viral replication inhibitors. However, in vivo protection by D2S against sexual transmission of HIV could only be accomplished, if the compound at sufficient concentrations, and the virus both reach susceptible cells within genital mucosa before productive infection could occur. Considering the complex events involved in sexual transmission of HIV-1, this might not be easily accomplished. Interaction between D2S and HSV or HIV-1, is reversible and non-virucidal,[24,51] a release of D2S from the viral glycoprotein could occur once the virus-D2S complex has reached the surrounding vaginal tissues. Therefore, inactivation of HIV-1 infectivity preceding virus contact with susceptible target cells should be considered a preferred mechanism of protection against infection. Recently, it has become apparent that combination of microbicides with different modes of preventing infection will be needed. Combination products incorporating Emmelle/D2S could in principle provide a greater degree of protection than D2S alone, a broader spectrum of activity against various pathogens and, by permitting a lower dose of each component, a lower risk of potential adverse reactions.

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