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Preclinical assessments of vaginal microbicide candidate safety and efficacy

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ABSTRACT

Sexually transmitted infections like HIV, HPV, and HSV-2, as well as unplanned pregnancy, take a huge toll on women worldwide. Woman-initiated multipurpose prevention technologies that contain antiviral/antibacterial drugs (microbicides) and a contraceptive to simultaneously target sexually transmitted infections and unplanned pregnancy are being developed to reduce these burdens. This review will consider products that are applied topically to the vagina. Rectally administered topical microbicides in development for receptive anal intercourse are outside the scope of this review. Microbicide and microbicide/contraceptive candidates must be rigorously evaluated in preclinical models of safety and efficacy to ensure that only candidates with favorable risk benefit ratios are advanced into human clinical trials. This review describes the comprehensive set of *in vitro*, *ex vivo*, and *in vivo* models used to evaluate the preclinical safety and antiviral efficacy of microbicide and microbicide/contraceptive candidates.

Contents

1. Introduction

The origins of sexually transmitted infections (STIs), also known as venereal infections, may date back as far as the very beginning of civilization. The etymology of the name venereal is related to the goddess Venus, synonymous of love and fertility, perhaps to describe the need for

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intimate sexual contact to spread the infectious agents although the consequences of acquiring such infections are far from pleasant [1]. The etiological agents of STIs are bacteria, protozoans, viruses and chlamydias among which are: *Neisseria gonorrhoeae*, *Treponema pallidum*, *Trichomonas vaginalis*, *Chlamydia trachomatis*, human immunodeficiency virus (HIV), herpes simplex virus 2 (HSV-2), human papillomavirus (HPV) and hepatitis B virus (HBV).

According to the World Health Organization (WHO) estimates, 499 million curable STIs occur each year in the world [2]. This number excludes pathogens like HIV, HSV-2 and HPV, the three sexually transmitted viral infections with the greatest impact on human health. All three viruses cause chronic or latent infections, which cannot be eliminated through any antiviral treatment, resulting in high morbidity or mortality. Additionally, infections by HSV-2 or HPV can increase the risk of HIV infection [3,4] making novel strategies to prevent their transmission a priority area of research to improve the welfare of millions of human beings, most of them living in third world countries.

Vaccines are available for the prevention of HPV 6, 11, 16 and 18 types [5]. However, there are some limitations that may require additional prevention tools for HPV infections. These limitations include the lack of protection against other HPV types associated with anogenital infections (36 types), the need of cold chain distribution and storage and low worldwide vaccine coverage, in part due to a very high cost [6]. Changes in sexual behavior through counseling, availability of condoms for men or women, the use of antiretroviral (ARV) therapy in HIV infected persons in serodiscordant couples, male circumcision and treatment of STIs can reduce the risk of acquiring HIV or other STIs. But despite having all these tools, infections by sexually transmitted pathogens have stabilized or increased in various parts of the world and new strategies are needed to fight STIs. A more recent idea proposes the use of microbicides, which are novel topical products containing active pharmaceutical ingredients (APIs) to block the infection by these pathogens. These APIs may be delivered intravaginally or intrarectally, using different delivery systems including gels, creams, films, suppositories, probiotics, nanofibers or intravaginal rings (IVRs). The vaginal formulations may also include contraceptives, opening the field of multipurpose prevention technologies (MPTs), to not only prevent HIV and/or other STIs but also unintended pregnancy [7].

The novelty of this approach imposes a very cautious and rigorous preclinical evaluation of safety and efficacy in order to move forward the most promising microbicides and microbicide/contraceptives into clinical trials. These studies, mostly guided by prerequisites suggested or imposed by regulatory agencies that approve studies in humans, help to follow a rational and ethical approach that will finally allow the start of clinical trials. This review will focus on the preclinical assessment of safety and efficacy of vaginal microbicide and microbicide/contraceptive candidates with particular emphasis on preclinical models to evaluate efficacy against HIV, HSV-2 and HPV, as well as safety. It is important to emphasize the equal importance of development of rectal microbicides to prevent these STIs also transmitted through rectal intercourse. However, this review will focus on vaginal models as part of this special issue devoted to vaginal drug delivery.

2. The female reproductive tract as a media for establishing HIV, HSV-2 and HPV infections

The design of an effective vaginal microbicide requires understanding the steps that the viruses follow to establish infection in the female genital tract (Fig. 1). Female genital mucosa consists of stratified squamous epithelial tissue in the vagina and ectocervix, while the endocervix is composed of columnar epithelium. The vaginal mucus and intact vaginal epithelial tissues provide the first barriers that HIV must overcome before it can infect the host cells, the CD4 + T cells [8]. However, the loss of tissue integrity as a result of ulcerative genital infections (as caused by HSV-2, for example) or abrasions that occur during intercourse or possibly transcytosis [9], can allow HIV to enter epithelial tissues and establish productive infection in target cells. The main cellular targets are CD4 + T lymphocytes, CD4 + cells of the macrophage lineage and dendritic cells (DCs). DCs efficiently capture HIV and transmit captured or newly produced virus to T cells [10], where the DC–T cell communication drives robust virus replication [11]. Interestingly, HSV-2 may modulate its microenvironment after infecting DCs to drive HIV infection in the DC–T cell mixtures [12] (Goode et al., in preparation). HIV starts its replication cycle by interacting with receptors (CD4, $\alpha 4\beta 7$) [13,14] and coreceptors (CCR5, CXCR4) [15] on the surface of target cells. This process of adsorption and subsequent entry into the cells, followed by reverse transcription and integration, are all targets being exploited to develop potential microbicides that may block infection [16–38].

Epithelial cells are also the first type of cells that HSV-2 and HPV encounter, although unlike with HIV, epithelial cells constitute the primary target and site of replication of these viral infections. HSV-2 has several viral glycoproteins in the virion surface that play an important role in adsorption and entry to epithelial cells, which makes them attractive targets to develop microbicidal compounds that could block these steps [39]. The adsorption and entry are a complex process that involves interaction with heparan sulfate and other receptors like herpesvirus entry mediator (HVEM) and nectin to then induce conformational changes that result in fusion with the cell membrane [39]. The next steps are translocation of the nucleocapsid to the nucleus, viral gene expression and genome replication. Inhibition of viral enzymes that participate in these steps, like the viral DNA polymerase, could help prevent HSV-2 infection [39]. After replicating initially in epithelial cells, HSV-2 is transported retrogradely along the axon of sensory neurons to establish latency in the sensory ganglion. The latency is kept under surveillance of the immune system that also controls the virus present in the mucosa [39]. Under certain conditions, including stress, immunodeficiency or immunosuppression, the virus is reactivated and taken through anterograde transport back to the genital mucosa where once again HSV-2 replicates [39]. During this replication, HSV-2 may or may not cause lesions in epithelial tissues and be transmitted to another susceptible host [39]. HPV limits its tropism to epithelial cells and requires damaged epithelia, where the basement membrane is exposed, to start the infection. The virus first attaches to heparan sulfate on the basement membrane and then undergoes a conformational change followed by cleavage (perform by furin and/or PC5/6) of the structural protein L2. The cleavage results in a modified virion that can now attach to a secondary receptor in basal keratinocytes and enter these cells [40]. Although heparan sulfate has been suggested as the universal receptor for attachment to the basement membrane, other studies have shown that infection of human keratinocytes with tissue-derived HPV 31 does not required heparan sulfate [41]. After entering basal keratinocytes the virus continues through a complex cycle of replication that requires cellular factors that are present at different stages of the epithelium differentiation. During this process viral enzymes like DNA polymerase may constitute interesting targets to prevent infection, but compounds like cidofovir, known to inhibit this particular enzyme [42], may not have a favorable enough toxicity profile to warrant its use as a potential microbicide [43].

3. Selecting a microbicide candidate

The first step in the development of microbicides is to identify APIs that may block STIs. Candidates must have a good therapeutic index (TI), inhibit virus replication at low, non-toxic concentrations *in vitro*, have a good resistance profile, be stable, and have the potential for reasonable pricing. Fig. 2 shows a go/no-go chart that combines the preclinical assessment of safety, efficacy and quality of microbicide candidates, highlighting in green boxes the steps that will be discussed in the next sections.

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