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Vaginal gene therapy☆

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ABSTRACT

In the last years, vaginal gene therapy has gained increasing attention mainly for the treatment and control of sexually transmitted infections. DNA delivery has been also suggested to improve reproductive outcomes for women with deficiencies in the female reproductive tract. Although no product has reached clinical phase, pre-clinical investigations reveal the potential of the vaginal tract as an effective administration route for gene delivery. This review focuses on the main advantages and challenges of vaginal gene therapy, and on the most used nucleic acid delivery systems, including viral and non-viral vectors. Additionally, the advances in the application of vaginal gene therapy for the treatment and/or prevention of infectious diseases such as the human immunodeficiency virus (HIV), the human papillomavirus (HPV) or the herpes simplex virus (HSV) are presented.

Contents

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1. Introduction

The vagina is a site for both local and systemic drug delivery [1,2]. The most common intravaginally delivered agents for local application include antibacterials, antifungal, antiprotozoal, antivirals, labor-inducing agents, spermicides, prostaglandins and, steroids. Specifically for the prevention of sexually transmitted infections, local vaginal delivery also facilitates interception of the virus at the point of entry and deposition of agents in close proximity to infectible cells. In addition, the vagina presents several features that favor the delivery of small molecules, proteins, peptides, oligonucleotides, and plasmid DNA [3], such as, large surface area, vascular tissue and mucosal permeability and, abundant vasculature. By offering an alternative to non-localized systemic or parenteral administration, intravaginal administration avoids first-pass hepatic clearance associated with most other delivery routes. Due to these advantages over other drug delivery routes, intravaginal administration is an area of substantial research interest.

An area of great interest of vaginal delivery is for the treatment and control of several viral infections in humans. Intravaginal delivery of nucleic acids offers a number of advantages relative to delivery of other active agents [3]. Nucleic acids have high specificity for their given cell or virus target, resulting in low toxicity and side effects, and low systemic absorption. Additionally, as genes have the ability to target virus or to protect the host prior to virus entry, this offers a potent strategy to block infection before the virus has adhered to or bound to target cells. Actually, the multistage processes of virus binding, glycoprotein conformation rearrangement, virus fusion, and/or endocytosis provide opportunities for interception during infection. By using specifically tailored agents against the virus or host cell, a variety of specific interactions between virus glycoproteins and cellular receptors or surface molecules can be inhibited.

In spite of the advantages of vaginal delivery in general, and vaginal nucleic acid delivery in particular, up to now there is no any product based of vaginal gene therapy under clinical investigation. One of the reasons is the low cost-effectiveness ratio of the formulations. The design of a drug product in which the active is a nucleic acid is highly costly and takes longtime, much more than that of a conventional drug product; moreover, it implies a low production capacity. In fact, the FDA identified manufacturing as the rate limiting step for new technology development due to specific challenges including physical design, characterization, scale-up, packaging, and quality control [4]. Another problem associated with vaginal delivery that limits its use is the adherence of the prescribed prophylactic/treatment regimen, required for efficacy. User compliance and acceptability of the formulations, which varies within geographical areas, are still a challenge.

To optimally design methods for vaginal gene therapy, it is important to understand the main advantages and challenges of vaginal gene therapy, and the most used nucleic acid delivery systems, including viral and non-viral vectors. In this review we also include the main advances in the application of vaginal gene therapy for the treatment and/or prevention of infectious diseases.

2. Concept of gene therapy and nucleic acid vaccines

In the last years, great advances in biotechnology have lead gene therapy to be in forefront of medical research. According to the European Medicines Agency (EMA), gene therapy medicinal product means a biological medicinal product which fulfills the following two characteristics: (a) it contains an active substance which contains or consists of a recombinant nucleic acid used in or administered to human beings with a view to regulating, repairing, replacing, adding or deleting a genetic sequence; (b) its therapeutic, prophylactic or diagnostic effect relates directly to the recombinant nucleic acid sequence it contains, or to the product of genetic expression of this sequence [5]. As an example of gene therapy medicinal product, Glybera was authorized by the EMA in 2012. This product contains the human lipoprotein lipase (LPL) gene variant in an adeno-associated viral vector, and is indicated for adult patients diagnosed with familial LPL deficiency and suffering from severe or multiple pancreatitis attacks despite dietary fat restrictions [6].

The aim of gene therapy is to deliver plasmid DNA (pDNA) or interference RNA (RNAi) into cells, where they interact with specific mediators which lead to expression of a gene of interest or inhibition of mRNA translation, respectively [7]. Therefore, this therapy enables direct intervention on the cause of the disease, rather than treating symptoms, and it is theoretically the closest approach to a cure. Fig. 1 features a scheme with the concept of gene therapy with DNA or RNAi.

Typically, the two most used RNAi molecules are short-interfering RNA (siRNA) and short-hairpin RNA (shRNA) [8]. siRNAs are double-stranded RNA molecules of about 19–23 base pair nucleotides in length, able to mediate site-specific split and destruction of the targeted messenger RNA (mRNA). The main advantage of synthesized siRNAs is that they do not need to reach the nucleus to result effective. shRNAs consist of two complementary RNA sequences of 19–22 base pair in length, linked by a short loop of 4–11. These shRNAs are synthesized within the cell by DNA vector-mediated production. Therefore, as occurs in the case of gene therapy based in pDNA delivery, there is a need to be delivered into the cell nucleus, which implies a complex and often unknown intracellular trafficking.

Gene therapy has been applied to many genetic and nongenetic disorders. Table 1 shows the indications of the approved, ongoing or completed clinical trials with gene therapy and the number of events related to each worldwide [9]. Indications addressed by gene therapy clinical trials include cancer diseases, monogenic diseases, infectious diseases, neurological diseases, and inflammatory diseases, among others [8]. However, most products under investigation do not reach clinical phases. In fact, clinical application of gene therapy is currently limited to serious diseases that have no cure. In spite of the promising strategy that gene therapy supposes for several diseases, its potential risk still makes necessary studies to extend safeness and effectiveness concerns [10].

One of the applications of gene therapy is in the field of vaccination, thanks to the development of new technologies to identify protective antigens and to present them to the immune system. Genetic immunization consists in the administration of a nucleic acid sequence directly into a host target tissue. The synthesis of specific foreign protein occurs in the host as in natural infection, with either wild type or attenuated pathogen. These host-synthesized proteins then become the subject of immune surveillance via both humoral and cellular pathways [11]. Fig. 2 shows the difference between conventional vaccines and nucleic acid-based vaccines. One of the main advantages of nucleic acid-based vaccines over conventional vaccines is the safety since living organisms and potent adjuvants are not necessary. Other advantages are effectiveness (after immunization antigens are expressed in situ, mimicking a true infection, and inducing both B and T-cell response), specificity (the immune response is only induced against the antigen of interest), and relatively low production cost, high stability, ease of manipulation, and the possibility of expressing complex antigens such as transmembrane proteins [12]. Target genes for vaccination include reporter genes, tumor antigens, allergens and viral antigens [12]. Gene-based vaccines are under development for a broad variety of indications, including immunotherapies for cancer, autoimmune diseases, infectious diseases, and allergy. For instance, HIV-1 DNA-based vaccines have been assayed in chimpanzees after intravaginal administration; these vaccines resulted to be safe and well tolerated, and induced both humor and cellular immunity [13]. Beside DNA, RNAi is seen as a possibly safer and more potent alternative to DNA for gene vaccination. As an example, Palliser et al. [14] showed that vaginal instillation of small interfering siRNA targeting herpes simplex virus 2 (HSV-2) protects mice from letal infection.

3. Advantages and challenges in vaginal gene therapy

Vaginal administration presents some advantages over conventional administration routes, mainly over oral route, since it avoids the adverse

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