Advanced Drug Delivery Reviews xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Advanced Drug Delivery Reviews

journal homepage: www.elsevier.com/locate/addr

Studies and methodologies on vaginal drug permeation ☆

Rita Monteiro Machado [a], Ana Palmeira-de-Oliveira [a,b], Carlos Gaspar [a,b], José Martinez-de-Oliveira [a,c], Rita Palmeira-de-Oliveira [a,b,d,*]

[a] CICS:UBI — Health Sciences Research Center, Faculty of Health Sciences, University of Beira Interior, Covilhã, Portugal
[b] Labfit, HPRD — Health Products Research and Development, Lda, Covilhã, Portugal
[c] Child and Woman's Health Department, Centro Hospitalar Cova da Beira EPE, Covilhã, Portugal
[d] Pharmacy Department, Centro Hospitalar Cova da Beira EPE, Covilhã, Portugal

ARTICLE INFO

Available online xxxx

Keywords:
Vaginal drug delivery
Drug permeation
Vaginal permeability
Vaginal dosage forms
Franz cell systems
Ussing chambers

ABSTRACT

The vagina stands as an important alternative to the oral route for those systemic drugs that are poorly absorbed orally or are rapidly metabolized by the liver. Drug permeation through the vaginal tissue can be estimated by using *in vitro*, *ex vivo* and *in vivo* models. The latter ones, although more realistic, assume ethical and biological limitations due to animal handling. Therefore, *in vitro* and *ex vivo* models have been developed to predict drug absorption through the vagina while allowing for simultaneous toxicity and pathogenesis studies.
This review focuses on available methodologies to study vaginal drug permeation discussing their advantages and drawbacks. The technical complexity, costs and the ethical issues of an available model, along with its accuracy and reproducibility will determine if it is valid and applicable. Therefore every model shall be evaluated, validated and standardized in order to allow for extrapolations and results presumption, and so improving vaginal drug research and stressing its benefits.

Contents

1. Introduction

Researchers are now devoted to find new forms or to re-discover safer and more effective alternative routes for the administration of drugs that are poorly absorbed orally or precociously suffer metabolization [1–3]. The vaginal route has been considered of great interest for drug delivery, since it enables both local and systemic drug delivery [4,5], allowing for the absorption of peptide and other macromolecules, and even nanoparticles [6–8].

The vaginal route provides different advantages over the oral one but it is not deprived of inconveniences [9,10]. Its large surface area, rich blood supply, ability to bypass hepatic first-passage, avoidance of gastrointestinal side effects, and relatively high permeability to a wide range of molecular weight drugs are some of its physiological characteristics that

☆ This review is part of the Advanced Drug Delivery Reviews theme issue on "Vaginal Drug Delivery".

* Corresponding author at: CICS–UBI — Health Sciences Research Center, Faculty of Health Sciences, University of Beira Interior, Av. Infante D. Henrique, 6200–506 Covilhã, Portugal. Tel.: +351 275329002; fax: +351 275329099.
E-mail address: rpo@fcsaude.ubi.pt (R. Palmeira-de-Oliveira).

http://dx.doi.org/10.1016/j.addr.2015.02.003
0169-409X/

contribute to its pharmacokinetic advantages [11–14]. However, drug absorption through the vagina may be affected by variations in epithelial thickness and by changes in the vaginal milieu composition that occur as a consequence of age dependent and cyclic physiological conditions or sexual intercourse. Moreover, leakage and self-cleaning action of the vaginal tract may reduce drug bioavailability [5,15]. Furthermore, general disadvantages of vaginal drug delivery include its obvious gender specificity, cultural background limitations and personal hygienic care interference [12,14].

Vaginal drug delivery systems include solutions, semisolids (creams, ointments and gels) and solid formulations (tampons, capsules, pessaries, suppositories, films, sponges, powders and special controlled release devices like the intravaginal ring) as well as other types of formulations such as aerosols and particulate systems integrated in adequate drug delivery systems [16]. Efficacy of drug delivery systems will rely on their ability to promote adequate drug concentrations at the targeted site of action. When a systemic effect is the objective through this route, drugs must be transported across the epithelium to gain access to dermal vessels and the systemic circulation [17]. On the other hand, when a local effect is the goal, as is the case for some antimicrobials and microbicides, retention of the drug at the surface of the vagina is desirable with low grade of absorption [18–21].

Drug permeation studies are mandatory for vaginal drug administration when systemic delivery is intended, and are important for safety characterization when the objective is to limit the activity to the vaginal wall surface or its contents. The *in vitro* models developed to predict drug permeation not only provide information on absorption rates and efficacy, but also help in investigating and understanding the pathogenesis of various microbiological diseases [22–24].

So, applicable and reproducible test assays are important to provide comprehensiveness about mechanisms of permeation, absorption and mode of action of active substances for vaginal application [25] and also to characterize vaginal drug delivery systems and the formulation ability to either promote or avoid permeation through the vagina [26,27].

Previously published scientific studies on this subject are quite unclear concerning the specific meaning of permeability and permeation terms. We assume that, by definition, *permeability* is the property of membranes or barriers of an organ or structure of being permeable to substances, while *permeation* denotes the ability of substances, like drugs, to permeate through a membrane/barrier [28]. Nevertheless, it is clear that this difference is not assumed uniformly and that the terms “drug permeation” and “drug permeability” have been and still are used interchangeably in the literature.

2. Vagina: anatomy, histology and physiology

The vagina is described as an expandable, longitudinally *S*-shaped, fibromuscular, collapsed canal showing at transverse cross-section an *H* configuration, with the anterior and posterior walls contacting each other in current conditions. It extends from the cervix of the uterus to the vestibule [2,29,30], presenting approximately 7–10 cm in length, more than 4 cm in width and 150–200 μm in thickness. The posterior wall is longer than the anterior one, a consequence of the asymmetrical position of the cervix at the vaginal vault [25,31–33].

The vagina is the female sexual organ by definition, and though it normally does not harbor glands, it is usually referred as a mucosa. In fact, despite not having a secreting role, the vaginal epithelial surface is actually coated by a thin layer of fluid that includes endometrial, cervical and vestibular secretions, residues of urine and products of cellular autolysis, and variable amounts of vaginal wall transudate [34,35]. Additionally, the composition of the vaginal fluid varies according to age, menstrual cycle and health status condition [31,36]. For instance, the vaginal pH is acidic (3.5–4.5) in healthy women during the reproductive age but it fluctuates along the different stages of the menstrual cycle and it is also dependent on coitus frequency, the amount of cervical mucus present in the vagina, the amount of vaginal transudate and it also varies along the vagina being higher close to the cervix and lower at the anterior fornix [2,35]. The maintenance of the pH is accomplished by lactic acid bacteria, mainly *Lactobacillus* spp., since these microorganisms metabolize into lactic acid the mono and di-saccharides that result from the autolytic breakage of desquamated vaginal cells glycogen [31].

The vaginal wall consists of various cell layers: nonkeratinized stratified squamous epithelium, lamina propria, muscular layer and tunica adventitia (covering only their proximal segments). The lamina propria is constituted of connective tissue rich in blood and lymphatic vessels draining to the internal iliac vein, this explaining why the absorbed products do avoid the hepatic circulation as an initial passage [30]. The vaginal cell turnover is estimated to replace 10–15 layers in a week [37]. The nonkeratinized stratified squamous epithelium, settled on glycogen containing keratynocytes but also integrating other cell types (such as macrophages and Langerhans' cells), is grounded on the lamina propria consisting of fibroblasts, elastic and collagen fibers, vessels and nerves, and defense cells, mainly polymorphonuclear leukocytes and occasional lymph nodules [38]. The vaginal epithelial cells are disposed according to different stages of differentiation, identifiable through different keratins expression, such as K10 and K13, being the differential expression arrangement function of the cell location within the epithelium (Fig. 1) [39]. Numerous folds and microrridges called “*rugae*” are present in the epithelium, largely increasing the vagina's surface area and providing distensibility [7].

The vaginal innervation depends on two types of sources: a peripheral one providing a highly sensible lower quarter segment and an autonomic fiber network in the upper vagina, which is more sensitive to stretch than to touch or to painful stimuli. This explains why women do not feel discomfort when using continuous intravaginal drug delivery systems [7].

Several conditions influence vaginal physiology: hormonal balance, pregnancy, pH, microflora and age, being the last one the best biomarker for epithelium layer thickness, enzyme concentrations and vaginal fluid production [41]. These vaginal characteristic changes influence drug permeation as it depends mainly on the superficial layer characteristics, as thickness, cell tightness, and lipids composition and organization in the intercellular space [25,42,43].

3. Drug absorption from the vagina

Drug dissolution in the vaginal fluid and epithelial penetration are the two key steps for a drug to be absorbed through the vagina. As a result all factors associated with vaginal physiology and formulation profile will greatly influence the success of drug delivery to the target [6,7]. Even though the vagina is not a real mucosa, drug transport is accomplished in a multi-way mechanism similar to the other biological membranes. Drug absorption can occur passively or actively. Passive mechanisms include the transcellular route, through the cells' membrane and the paracelullar route, representing a diffusion process through intercellular fluid and tight junctions [44,45]. Tight junctions and other intracellular junctions (adherens junctions and desmossomes) are present in the vaginal and cervical epithelium having a higher expression in the endocervix. Although the uppermost layers of the ectocervical and vaginal epithelium are devoided of tight junctions, these and other intercellular junctions have been identified right beneath the most apical epithelium layers. The study of these junctions is particularly important to understand, for example, the invasion of microbes and drug permeation. Tight junctions are composed of transmembrane proteins (occludin, claudins and junctional adhesion molecules — JAMs) which contact across the intercellular space and create a seal to restrict paracellular diffusion of molecules across the epithelial sheet. Furthermore, tight junctions have a structural role in epithelial polarization by limiting the mobility of membrane-bound molecules between the apical and basolateral domains of the plasma membrane of each epithelial cell. In general, tight junctions are

Please cite this article as: R.M. Machado, et al., Studies and methodologies on vaginal drug permeation, Adv. Drug Deliv. Rev. (2015), http://dx.doi.org/10.1016/j.addr.2015.02.003