Permeability of Tritiated Water through Human Cervical and Vaginal Tissue

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ABSTRACT: The increased incidence of human immunodeficiency virus infection in women has identified an urgent need to develop a female-controlled method to prevent acquisition of human immunodeficiency virus and other sexually transmitted diseases. Women would apply the product intravaginally before intercourse. Development of such a product requires a better understanding of the permeability characteristics of the tissues with which such products would come into contact. However, limited studies have been performed in this area. In the present study, water permeability of fresh human cervical and vaginal tissue was evaluated. The average apparent permeability coefficient was found to be 8×10^{-5} cm/s for fresh human cervical tissue and 7×10^{-5} cm/s for fresh human vaginal tissue. Considering the lack of regularity in obtaining cervical and vaginal tissue from surgical specimens, additional tests were performed to evaluate the effect of freezing on tritiated water permeability. No statistically significant differences were observed in the permeability values obtained when comparing fresh versus frozen tissues. © 2004 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 93:2009–2016, 2004

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INTRODUCTION

Drug administration across moist epithelial surfaces, including nasal, rectal, and vaginal surfaces, offers the advantage of elimination of first pass drug metabolism, which may potentially minimize the risk of systemic toxicity. In developing any drug delivery system, it is essential to understand the transport kinetics of compounds through tissues to which the drug will be applied. For systemic delivery by this route, the drug must be transported through the tissue and be available to the systemic circulation. For local delivery, the drug and the inactive agents of the product should remain at the external surface.[1]

The vaginal route has considerable potential for delivering drugs systemically. The vagina has a reasonably large surface area, good blood supply, and acceptable permeability to a wide range of drugs.[1] However, most of the vaginal preparations on the market are intended to exert effects on microorganisms causing superficial infections, to kill sperm cells, to treat vaginal atrophy, or to provide lubrication. Particular interest to this research is the recent efforts toward the development of a vaginal topical microbicide. A vaginal

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microbicide is intended to inactivate pathogens deposited into the vagina during sexual intercourse without significant systemic absorption of the active agent.

In recent years, sexually transmitted diseases (STDs), including human immunodeficiency virus (HIV), have become a growing medical problem and concern throughout the world. According to recent statistics, >90% of new infections are spread through unprotected sex.[2] The development of an intravaginal topical microbicide to prevent acquisition of STDs, including HIV, has become a widely recognized strategy.[2] Studies have shown that the cervix is an important infection site for STDs and HIV. Because of its fragility and the presence of HIV receptor sites, the cervical mucosa is considered more susceptible to HIV than the vaginal tissue.[3]

Characterizing the permeability of vaginal and cervical epithelial tissue is one of the tools available to better understand the mechanism of transport through these tissues. Permeability studies on vaginal tissue obtained from animal models, such as rodent, rabbit, monkey, and sheep have been conducted. However, few studies comparing species have been performed.[4–7] Permeability studies using cultured human vaginal-cervical epithelial cells have been described in the literature,[8–10] but there is a lack of information available on the permeability of human vaginal and cervical tissue. Vaginal absorption may be significantly different when comparing the animal and the human models. Limited studies have been performed to establish an alternate tissue model for transport through buccal tissue using human vaginal tissue.[11–14]

Some of the difficulties in developing an *ex vivo* transport model include the storage of excised tissue specimens and the lack of regularity in obtaining cervical and vaginal tissue from surgical specimens. The best alternative is to establish a "tissue bank" where samples can be frozen and stored for use at a later date. Samples could then be thawed immediately before permeability studies. Several methods have been reported for storing human specimens.[13–19] However, it is challenging to define the best freezing technique that will preserve the permeability pathways in the tissue. In studies conducted by Van der Bijl et al.[11–14,18] evaluating the permeability of tritiated water through buccal and vaginal mucosa, it was shown that freezing at −85°C did not alter water permeability. No studies have been conducted with human cervical tissue.

To better understand the transport kinetics of vaginal and cervical tissue, the permeability coefficient of water through these tissues was determined. Studies were also conducted using two different freezing techniques. Permeability of fresh tissue was compared with frozen tissue to evaluate the possibility of storing vaginal and cervical tissue.

EXPERIMENTAL

Vaginal and Cervical Tissue

Freshly excised human ectocervical and vaginal tissue was obtained from the Tissue Procurement Facility at Magee-Womens Hospital. Tissue was obtained from 19 women undergoing hysterectomy for benign conditions. No specimens were used when there was evidence of tissue abnormality that may influence the state of the mucosa. Tissue was obtained from premenopausal women with an age range of 31–49 years (mean 39 ± 6). All tissue specimens were obtained within 1 h of surgical excision. Tissues were held at 5°C in either phosphate-buffered saline or Dulbecco's modified Eagle medium (Mediatech Inc., Herndon, VA) during transfer from surgery to the laboratory. In those studies in which freezing techniques were evaluated, tissue was stored at −80°C until use. Tissue samples were retained for histological evaluation for comparison before and after the experiment. In studies using cervical tissues, the epithelial layer was isolated using a Thomas-Stadie-Riggs tissue slicer (Thomas Scientific, Swedesboro, NJ) before permeability studies.

Freezing Process

Two techniques were evaluated with respect to tissue freezing. 1. Methanol/dry ice (frozen MeOH): crushed dry ice was added in methanol (1:1 w/v) to form a slurry. The tissue was placed in a resealable zipper closer bag (Bitran specimen storage bag; Fisher Scientific) and immersed in the slurry for a few seconds to freeze. Tissue was then stored at −80°C. 2. Liquid nitrogen (frozen N_2): tissue was placed in a resealable zipper closer bag (Bitran specimen storage bag; Fisher Scientific) and immersed in the liquid nitrogen for a few seconds to freeze. Tissue was then stored at −80°C. Frozen specimens were thawed before use using a 37°C water bath.