ABSTRACT SUMMARY

Simple, safe and effective permeability enhancers are crucial for successful non-invasive drug delivery methods. We seek local permeability augmentation mechanisms for integration into passive or active architectures in order to enable therapeutic delivery routes of the target drug while minimizing drug formulation challenges. This study demonstrates the efficacy of hydrogen peroxide, H$_2$O$_2$, as a permeability enhancer for transvaginal delivery of macromolecules in an animal model.

H$_2$O$_2$ at low concentrations is an effective permeability enhancer that is locally metabolized. H$_2$O$_2$ improves drug permeation through mucosa by altering tight junctions (TJ) between cells and oxidizing enzymes that function to degrade the foreign species. Our previous work using trans-epithelial electrical resistance measurements and an MTT cell viability assay show reversible disassembly of TJ with minimal cell damage demonstrating the feasibility of H$_2$O$_2$ as a safe permeability enhancer for drug delivery. Permeation studies show that H$_2$O$_2$ treatment of cell cultured vaginal mucosa significantly enhances the permeability to insulin and follicle-stimulating hormone (FSH) by more than an order of magnitude. All such in vitro work established safe experimental H2O2 dosage limits for subsequent animal experiments.

EXPERIMENTAL METHODS

Transepithelial electrical resistance applies a low frequent AC current source and measures the voltage using Ag/AgCl electrodes. Intact TJs provide primary resistance to ionic transport. Changes in the resistance can indicate either a breakdown of the transport barrier or the recovery of this barrier.

H$_2$O$_2$ dosages ranging from 2.4 to 24 μmol/cm$^2$ were administered and TEER values were measured over the course of 24 hours.

An animal study was carried out on nulliparous rabbits at ISIS Services, LLC (San Carlos, CA). A positive control rabbit was directly given a 1 mL subcutaneous injection of urine-derived human FSH, while a negative control rabbit was administered u-hFSH intravaginally without an H2O2 tissue pre-treatment. The delivery success of administering u-hFSH across the rabbit vaginal epithelium after selected concentrations of H2O2 tissue pre-treatment was compared to both controls.

Blood samples were collected every 20 minutes over the course of 7 hours and administered proteins and peptides thereby reducing the amount of potent drug that can reach the underlying vasculature.

The histology of the vaginal wall physical blocks the uptake of foreign substances. The most superficial layer of the mucosa consists of nonkeratinized, nonsecretory stratified squamous epithelium ~20 to 45 cell layers thick depending on the phase of menstrual cycle, with the peak thickness occurring at ovulation.

Cell-to-cell junctional molecules are localized in the basal 2/3 of the vaginal epithelium. Disassembly of crucial components of these junctional complexes such as TJs enables improved paracellular transport.

H$_2$O$_2$ can cleave these tight junctions and provide a path for drug delivery.

INTRODUCTION

The vaginal tract is naturally protected by both a physiochemical barrier and a physical barrier.

Vaginal transudate and mucus secretions from the upper reproductive tract such as the cervix and endometrium impede the permeation of macromolecular drugs such as polypeptides. Proteases in particular, with aminopeptidases being a major constituent, directly degrade
Processed into serum. The serum samples were assayed using a commercial hFSH ELISA.

RESULTS AND DISCUSSION

Figure 1: H_{2}O_{2} dosages ranging from 2.4 to 16 μmol/cm^2 showed reversible changed in TEER measurements over the course of 24 hours. Dosages of 20 to 24 μmol/cm² showed reducing TEER values but with no observed reversibility.

Figure 2: u-hFSH concentration in blood samples every 20 minutes from transvaginal administration.

CONCLUSION

H_{2}O_{2} can reversibly disassemble TJ barrier functions between epithelial cells over a range of dose concentrations.

An H_{2}O_{2} mediated permeability enhancement step followed by drug administration onto the treated tissue was studied using in vitro and in vivo models. In both cases dosages of H_{2}O_{2} successfully increased the permeation across the vaginal epithelial tissue.

This study provides a preliminary assessment of the safety and efficacy of using HP treatments to enhance the permeability of vaginal epithelium to macromolecular drugs. Results provide reference concentrations upon which future studies on HP treatment of the vaginal mucosa can be based and demonstrate potential permeability enhancement with implications for applications in drug delivery and women’s health.

This platform offers a promising new drug delivery route that may be employed for treatment of a variety of diseases within women’s health including osteoporosis, infertility, diabetes, cancer and pain management.

REFERENCES


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