European Journal of Pharmaceutics and Biopharmaceutics 99 (2016) 35–44

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

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

Research Paper

Characterization of cornified oral mucosa for iontophoretically enhanced delivery of chlorhexidine

Wei Ren [a,*], Arif Baig [b], Donald J. White [b], S. Kevin Li [a]

[a] Division of Pharmaceutical Sciences, James L. Winkle College of Pharmacy, University of Cincinnati, Cincinnati, OH 45267, USA
[b] Procter & Gamble Company, Mason, OH 45040, USA

ARTICLE INFO

Article history:
Received 3 August 2015
Revised 3 November 2015
Accepted in revised form 9 November 2015
Available online 17 November 2015

Keywords:
Iontophoresis
Palate
Cornified epithelium
Transmucosal drug delivery
Permeability
Chlorhexidine

ABSTRACT

Topical administration of chlorhexidine for periodontal disease can provide advantages over systemic delivery, but is limited by the permeability of the cornified oral mucosal tissue. In the present study, passive and iontophoretic transport of tetraethylammonium, salicylate, mannitol, dexamethasone, fluoride, and chlorhexidine across bovine palate was investigated to (a) determine the intrinsic barrier properties of bovine palate for its eventual use as a model of human cornified oral mucosa, (b) examine the feasibility of iontophoretically enhanced transport of chlorhexidine into and across bovine palate, and (c) identify the transport mechanisms involved in iontophoretic transport across the palate. The histology study suggests that bovine and human palates have similar cornified epithelium structures; bovine palate could be a model tissue of human hard palate for drug delivery studies. Transport study of tetraethylammonium, salicylate, and mannitol suggests that bovine palate was net negatively charged and the cornified epithelial layer was the rate-determining barrier. The direct-field effect (electrophoresis) was shown to be the dominant flux-enhancing mechanism in iontophoretic transport of ionic compounds. Electroosmosis also contributed to the iontophoretic transport of both neutral and ionic permeants. Anodal iontophoresis enhanced the delivery of chlorhexidine into and across the palate, reduced the transport lag time, and provided tissue concentration above the drug minimum inhibitory concentration, and therefore could be a promising method to assist in the treatment of periodontal disease.

1. Introduction

Periodontal disease, a chronic infection to the gum and bone tissues around the teeth, can lead to tooth loss. Periodontitis, the advanced stage of periodontal disease, shows a strong association with cardiovascular disease and type 2 diabetes mellitus [1,2]. Periodontal disease is believed to be primarily associated with bacteria (including gram-positive and gram-negative bacteria), fungi, and yeast, and can be associated with chronic medical conditions, poor dental hygiene, and smoking habits [3,4]. Chlorhexidine (CHX) or CHX gluconate is a biguanide, which was introduced into dentistry more than 40 years ago [5,6] because of its broad-spectrum activity, low toxicity, and substantivity [7,8]. The mechanism of action of CHX is the binding of the cationic drug to the negatively-charged cell walls of the bacteria, destroying the cell wall and breaking the osmotic equilibrium of the cell [9].

Systemic administration of antibacterial generally requires a high dose to reach its effective inhibition concentration for microorganisms at the target tissues. Local treatment is an alternative route of administration that can prevent adverse effects due to the high systemic drug concentration. For example, mouth-rinses are the most common form of CHX administration [10]. Numerous studies have demonstrated that CHX-containing mouthwash, varnishes, chips, and gels are efficacious in the prevention and therapy of periodontitis [11,12]. Stanley et al. have shown that on average 99% of the cultivable bacteria can be inhibited at 0.125 mg/mL CHX [6]. However, to reach the effective concentration of CHX in the infected tissues for the treatment of periodontal disease (e.g., to reduce the potential of bacteria invasion that affects systemic health), topical administration of CHX in the oral cavity has its shortcomings. High dose and dosing frequency are needed via this route because (a) the drug is easily diluted and rapidly eliminated due to the flushing action of saliva, (b) drug distribution is not easily controlled to achieve therapeutic concentration at the target site, and (c) drug permeation into and across the gingival tissue is poor. To overcome these shortcomings, bioadhesive polymers have

* Corresponding author at: 3225 Eden Avenue 136 HPB, Cincinnati, OH 45267, USA. Tel.: +1 (513) 558 0398; fax: +1 (513) 558 4372.
E-mail address: Renwi@mail.uc.edu (W. Ren).

http://dx.doi.org/10.1016/j.ejpb.2015.11.005
0939-6411/

been used in the forms of gels or films to prolong the release and residence time of CHX in the mouth; this reduces the frequency of CHX application and the amount of CHX used [13,14]. An example is PerioChip®, a commercial biodegradable matrix of CHX that releases the drug after its insertion into a periodontal pocket. Other commercial products include Atridox® and Arestin® that use a gel and microsphere, respectively, for local delivery of antibiotics in periodontal treatments. Another potential method to enhance local drug delivery is iontophoresis. Iontophoresis enhances the delivery of both charged and uncharged drugs by an externally applied electric current across a membrane. This method is generally considered to be safe and non-invasive in drug delivery in dentistry, nail treatment, and transdermal and topical skin applications.

The highly organized intercellular lipids within the epithelium of buccal mucosa provide the major physical barrier in buccal drug delivery [15,16]. For comparison, the buccal mucosa is more permeable than skin and is less permeable than the mucosal monolayer in the gastrointestinal tract. Previous iontophoresis studies have shown enhanced and controlled delivery of galantamine HBr and naltrexone HCl across buccal mucosa [17] and improved delivery of CHX dihydrochloride across skin by iontophoresis [18]. Moreover, chemical enhancers, polymeric hydrogel, and iontophoresis were also utilized to enhance the delivery of macromolecules such as peptides and proteins across buccal mucosa [14,19]. The combined use of iontophoresis and chemical enhancers was also investigated and found to provide limited synergistic effects compared to the treatment of iontophoresis or enhancers alone [20,21]. However, all these studies were carried out on buccal mucosa, which has a different anatomical structure compared to the cornified gingival tissue. Information on the barrier properties of gingiva and drug transport across this tissue such as CHX is limited.

The objectives of the present study were to (a) determine the intrinsic barrier properties of bovine palate for its eventual use as a model of human hard palate and gingival tissues, (b) examine the feasibility of iontophoretically enhanced transport of CHX into and across the palate, and (c) investigate the mechanisms involved in iontophoretic transport across the palate. Histology and electrical resistance studies were first performed on the palates. Passive and iontophoresis studies of bovine palate were conducted using tetraethylammonium, salicylate, mannitol, dexamethasone, and fluoride as the model permeants to investigate the barrier properties of the palate and the mechanisms of iontophoresis across this barrier. The delivery of CHX into and across the palate was examined under passive and iontophoresis conditions.

2. Experimental section

2.1. Materials

Phosphate buffered saline (PBS), pH 7.4, consisting of 0.01 M phosphate buffer, 0.0027 M potassium chloride, and 0.137 M sodium chloride was prepared using PBS tablets (MP Biomedicals, LLC, Solon, OH) and deionized water (DI water). CHX, sodium fluoride (NaF), and sodium salicylic acid (NaSA) were purchased from Sigma–Aldrich (St. Louis, MO). Tetraethylammonium bromide (TEABr) was purchased from Acros Organics (Morris Plains, NJ). Dexamethasone was purchased from Letco Medical (Decatur, AL). Monobasic sodium phosphate was from Professional Compounding Center of America (PCCA, Houston, TX). ^{14}C-tetraethylammonium (TEA), ^{14}C-salicylate (SA), ^{3}H-dexamethasone (DEXA), and ^{3}H-mannitol (MA) were purchased from PerkinElmer Life and Analytical Sciences (Boston, MA). The radioactive materials had purity > 97%. Triethylamine (HPLC grade) was purchased from Fisher Scientific (Fair Lawn, NJ). Acetonitrile and methanol (HPLC grade) were from Pharmaco-AAPER (Shelbyville, KY). All materials were used as received.

2.2. Bovine palate

Fresh bovine palates from healthy cows were obtained from Tri State Beef Co (Cincinnati, OH) and were stored in PBS at 4 °C until use. All experiments were conducted within 2 days after the cows had been sacrificed. This time frame was established based on the results of the barrier stability experiments in the present study (see Section 3.2). Bovine palatal tissues of different thickness with and without the superficial cornified layer were used. To prepare the palatal tissues, the palates were dermatomed to the desired thickness using a dermatome (Padgett Model B Dermatome, Kansas City, MO), cut to appropriate sizes, and carefully inspected with a stereo microscope (10× magnification) prior to use. For the palatal tissues without the superficial cornified layer, the tissues were prepared by the removal of the 0.2-mm superficial layer using the dermatome prior to the procedure described above. The thickness of the palatal tissues after tissue preparation was from 0.5 to 1.1 mm (Fig. 1), measured with a micrometer (Mitutoyo, Kawasaki, Kanagawa, Japan) when the tissue was sandwiched between two microscope glass slides. The full-thickness palate was approximately 1.1 mm thick and included the cornified epithelium and part of lamina propria.

2.3. Histological study

Full-thickness bovine palates were prepared as described in Section 2.2. Cadaver intact human hard palate was obtained from National Disease Research Interchange (NDRI; Philadelphia, PA). The tissue was stored at 4 °C and used within 2 days postmortem. The use of human tissue was approved by the Institutional Review Board (IRB) at the University of Cincinnati (Cincinnati, OH).

Both human and bovine palates were fixed in 10% formalin after careful examination under a microscope. The samples were then dehydrated with graded ethanol (50%, 70%, 75%, 90%, 95%, and 100%) and embedded in paraffin. The prepared paraffin-embedded samples were cut into 5 μm-thick sections and mounted on microscope slides, which were stained with haematoxylin and eosin (H&E) afterward. The slides were examined under a microscope at 100× magnification connected with a camera. The thickness of each tissue structure layer within the palate was measured based on their dimensions in the microscopy images.

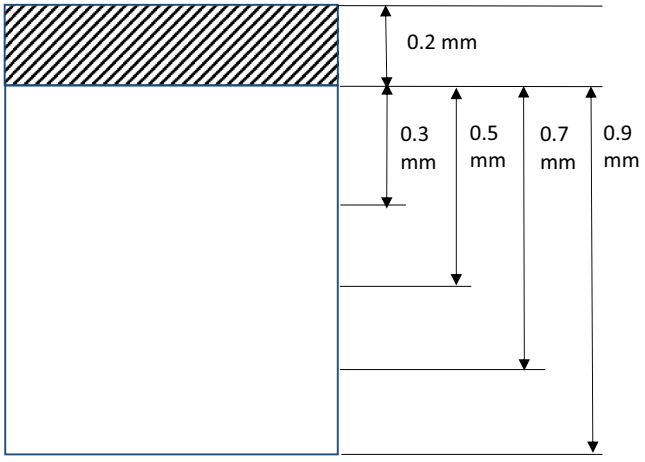

Fig. 1. A schematic diagram of the bovine palatal tissue of different thickness with or without superficial cornified layer.