Evaluation of Buccal Methyl-β-Cyclodextrin Toxicity on Human Oral Epithelial Cell Culture Model

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ABSTRACT: Cyclodextrins, especially methylated β-cyclodextrins offer several advantages for drug delivery which include improved drug solubilization, protection against physicochemical and enzymatic degradation, as well as a potential for absorption improvement. However, little or no data are available for their use as drug penetration enhancer via the buccal route. This study focuses on the toxicity of randomly methylated β-cyclodextrin (RAMEB) on buccal mucosa using a reconstituted human oral epithelium model composed of TR 146 cells. Toxicity of RAMEB on TR 146 cells was evaluated by measuring cell viability (MTT assay) and membrane damages followed by LDH release after single and repeated exposures to RAMEB solutions. Inflammatory effects of RAMEB are also considered by measuring expression of interleukin-1α and are supported by histological examination. The present results indicate that 10% RAMEB results in cytotoxic and inflammatory effects depending on time exposure, whereas 2% and 5% RAMEB do not induce tissue damages even after 5 days of repeated exposures. Therefore, the highly water-soluble RAMEB is thought to be a safe candidate as an excipient for buccal mucosal drug delivery. © 2005 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 94:1300–1309, 2005

Keywords: cyclodextrins; buccal mucosa; cell culture; toxicity; mucosal delivery; reconstituted human oral epithelium; TR 146 cells; RAMEB

INTRODUCTION

Buccal mucosa is a potential site for drug absorption. Active molecules administered through buccal mucosa pass directly into systemic circulation, thereby minimizing the first intestinal and hepatic pass and adverse gastro-intestinal effect.[1,2] However, permeability of buccal mucosa to large molecules is not enough to allow plasma concentration reaching therapeutic levels. Buccal permeation can be enhanced by using various penetration enhancers such as bile salts, surfactants, fatty acids and derivatives and chelators.[3–5] Cyclodextrins are considered as a new class of penetration enhancers.[5,6] These molecules are cyclic oligosaccharides of six, seven, or eight D-glucopyranose units, denoted as α-, β-, and γ-cyclodextrins, respectively. The three-dimensional ring structure of these compounds resembles a truncated cone, whose internal cavity has slight hydrophobic properties and whose surface is hydrophilic. Because of these structural features, cyclodextrins can form inclusion complexes with lipophilic molecules, thereby increasing their solubility in aqueous solutions.[7] Furthermore, cyclodextrins, especially methylated β-cyclodextrins (pure dimethyl-β-cyclodextrin (DMeβCD), randomly methylated β-cyclodextrin (RAMEB), trimethyl-β-cyclodextrin (TMeβCD)) were shown to enhance drug penetration through skin[8] and nasal mucosa.[6,9,10] The mechanism of action of methylated β-cyclodextrins as absorption enhancers for hydrophilic drugs occurs probably by transiently changing membrane permeability,[11] overcoming the aqueous diffusion barrier[12] and opening tight junctions.[13]

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During cell differentiation process in buccal mucosa, small organelles called "membrane-coating granules" (MCGs) composed of non-lamellar lipid sacks are formed in intercellular spaces of the non keratinized regions.[14,15] These MCGs, first observed in epidermis, are not different from those observed in the skin and are believed to provide an intercellular permeability barrier to buccal mucosa.[16] Since methylated β-cyclodextrins interact strongly with lipids, they could modify buccal mucosa permeability and could act as penetration enhancers for the buccal route. However, interactions of RAMEB with buccal mucosa have not been yet investigated and its toxicity toward this tissue needs to be considered.

Studies using excised tissues have provided considerable insights into the mucosal absorption process. Although being very reliable, use of human or animal buccal mucosa faces a serious and growing problem of availability. For this reason, *in vitro* cell culture systems have been developed as a model to study drug transport and toxicity. Among the different cell lines considered, the TR 146 cell line when cultured at the air-interface on an inert supporting membrane and in a chemically defined medium forms an epithelial tissue, devoided of stratum corneum and having the same histological properties than the human buccal epithelium.[17–20]

In the present study, toxicity of RAMEB on TR 146 cells is evaluated by measuring cell viability and membrane damages after single and repeated exposures to RAMEB solutions. Inflammatory effects of RAMEB are also considered and supported by histological observations.

MATERIALS AND METHODS

Materials

Reconstituted human oral epithelium (RHOE) model, growth, and maintenance medium were supplied by SkinEthic® (Nice, France). Randomly methylated β-cyclodextrin (RAMEB) with a substitution degree of 1.8 was generously given by Wacker Chemie (Burghausen, Germany). RAMEB was dissolved in phosphate-buffered saline (PBS) devoided of calcium and magnesium (Gibco™, Invitrogen, Cergy-Pontoise, France) at pH 7.0. 3,[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) and Formalin (Acustan®) were purchased from Sigma Chemical Co. (St. Louis, MO). Sodium dodecylsulphate (SDS) at 20% (w/v) in ultra pure water was obtained from Laboratoires Eurobio (Courtaboeuf, France) and diluted in distilled water (Gibco™, Invitrogen) to a concentration of 1% (w/v).

RHOE

Reconstituted epithelium is a three-dimensional tissue model consisting of transformed human keratinocytes derived from the TR 146 cell line which was originally established from a biopsy specimen of a squamous cell carcinoma of the buccal mucosa that has infiltrated a lymph node.[17,20,21] TR 146 cells were cultured at the air-interface on a 0.5 cm² polycarbonate filters in a chemically defined medium MCDB-153 (Clonetics, San Diego, CA) supplemented with 1.5 mM calcium chloride, 25 μg/mL gentamicin, 0.4 μg/mL hydrocortisone, and 5 μg/mL insulin (growth medium). After 12 days of culture, TR 146 cells formed an epithelial tissue without stratum corneum and exhibited histological properties of human buccal epithelium.

Exposure of RHOE Tissue Culture Model to Tested Compounds

Single Exposure

Tissue cultures were transferred into a 24-well plate containing 300 μL of a chemically defined medium MCDB-153 containing 1.5 mM calcium chloride, 25 μg/mL gentamicin, and 5 μg/mL insulin (maintenance medium). Thirty microliters of RAMEB solution in PBS at concentration of 2%, 5%, or 10% (w/v) were added to the inserts. Cell cultures were incubated at 37°C with 5% CO_2 at saturated humidity for 1, 4, and 24 h. Cells exposed to PBS and SDS 1% (w/v) were treated in the same conditions and used as negative and positive controls, respectively. The conditioned medium was collected after exposure and was kept frozen until running the LDH and IL-1α assays. Each experiment was carried out in triplicate on three different and independent inserts.

Repeated Exposures

RHOE were incubated 4 h per day with the tested compounds during 5 days and treated according to the following protocol. Briefly, tissue cultures were transferred into 6-well plates containing 1000 μL of growth medium and 100 μL of RAMEB solution in PBS at 2%, 5%, or 10% (w/v) were added to the inserts. Cell cultures were incubated