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Review

Buccal penetration enhancers—How do they really work?

Joseph A. Nicolazzo, Barry L. Reed, Barrie C. Finnin*

Department of Pharmaceutics, Monash University, 381 Royal Parade, Parkville, Victoria 3052, Australia

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Abstract

Certain agents that increase drug delivery through the skin, including surfactants, bile salts, and fatty acids, have been shown to exert a similar effect on the buccal mucosa. These agents enhance skin permeability by interacting with and disrupting the ordered intercellular lipid lamellae within the keratinized stratum corneum, and it has been assumed that a similar mechanism of action occurs in the nonkeratinized buccal mucosa. However, the chemical and structural nature of the lipids present within the intercellular regions of the buccal mucosa is quite different to that found within the stratum corneum, and so extrapolation of results between these two tissues may be misleading. To assume that the mechanism of action of buccal penetration enhancers is based on the disruption of intercellular lipids may be erroneous, and may result in the inappropriate prediction that certain skin penetration enhancers will similarly enhance drug delivery through the buccal mucosa. The data available in the literature suggest that agents that enhance buccal penetration exert their effect by a mechanism other than by disruption of intercellular lipids. Rather, buccal penetration enhancement appears to result from agents being able to (a) increase the partitioning of drugs into the buccal epithelium, (b) extract (and not disrupt) intercellular lipids, (c) interact with epithelial protein domains, and/or (d) increase the retention of drugs at the buccal mucosal surface. The purpose of this review is to identify the major differences in the structural and chemical nature of the permeability barriers between the buccal mucosa and skin, to clarify the mechanisms of action of buccal penetration enhancers, and to identify the limitations of certain models that are used to assess the effect of buccal penetration enhancers.

Keywords: Buccal mucosa; Drug delivery; Permeability; Chemical penetration enhancers; Intercellular lipids

Contents

* Corresponding author. Tel.: +61 3 9903 9520; fax: +61 3 9903 9583.
E-mail address: barrie.finnin@vcp.monash.edu.au (B.C. Finnin).

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1. Introduction

The potential of the buccal mucosa as an alternative site for the delivery of drugs into the systemic circulation has recently received much attention. There are many reasons why the buccal mucosa might be an attractive site for the delivery of therapeutic agents into the systemic circulation. Due to the direct drainage of blood from the buccal epithelium into the internal jugular vein [1,2], first-pass metabolism in the liver and intestine may be avoided. This first-pass effect is a major reason for the poor bioavailability of some compounds when administered orally [3]. Additionally, the mucosa lining the oral cavity is easily accessible, which ensures that a dosage form can be applied to the required site and can be removed easily in the case of an emergency [4–6]. However, like the skin, the buccal mucosa acts as a barrier to the absorption of xenobiotics, which can hinder the permeation of compounds across this tissue. Consequently, the identification of safe and effective penetration enhancers has become a major goal in the quest to improve oral mucosal drug delivery. Chemical penetration enhancers are substances that increase the permeation rate of a coadministered drug through a biological membrane [7]. While extensive research has focussed on obtaining an improved understanding of how penetration enhancers might alter intestinal and transdermal permeability, far less is known about the mechanisms involved in buccal penetration enhancement.

The purpose of this review is to identify the major issues relating to buccal penetration enhancement, and to review the literature relevant to the potential mechanism(s) of action of buccal penetration enhancers. While the methods used to assess buccal permeation will not be detailed in full, the limitations associated with some of the experimental models used to assess buccal penetration enhancers will be discussed. More importantly, the characteristics required for an agent to act as a buccal penetration enhancer will be outlined, and the belief that penetration enhancers increase buccal permeability by disrupting lipid organization will be challenged.

2. Structure and environment of the buccal mucosa

The primary role of the buccal mucosa, like the skin, is to protect underlying structures from foreign agents. The surface of the buccal mucosa consists of a stratified squamous epithelium which is separated from the underlying connective tissue (lamina propria and submucosa) by an undulating basement membrane (a continuous layer of extracellular material approximately 1–2 μm in thickness) [8]. This stratified squamous epithelium consists of differentiating layers of cells (keratinocytes) which change in size, shape, and content as they travel from the basal region to the superficial region, where the cells are shed [9]. There are approximately 40–50 cell layers, resulting in a buccal mucosa which is 500–600 μm thick [10–12]. The permeability of the buccal mucosa is greater than that of the skin, but less than that of the intestine [13–15]. This does not only result from the greater surface area provided by the small intestine, but also from the

structural differences between each of the tissues, as demonstrated in Fig. 1. Based on epithelial structure alone, it is not surprising that the simple columnar epithelium covering the small intestine provides less resistance to drug transfer than the stratified squamous epithelium covering the skin and buccal mucosa.

Unlike the skin, and other keratinized regions of the oral cavity (such as the gingiva and palate), the epithelium lining the buccal mucosa lacks a cornified surface. The superficial cells of the nonkeratinized buccal mucosa retain their nuclei and some cytoplasmic function, and are surrounded by a cross-linked protein envelope [16]. The differentiation processes that occur in keratinized and nonkeratinized epithelia differ significantly, and this results in either the presence or absence of a cornified surface layer. In nonkeratinized oral mucosa, cells leave the basal area and differentiate to become larger and flatter as they begin to accumulate lipids and cytokeratins; however, the cytokeratins do not aggregate and form bundles of filaments, as seen in keratinized epithelia [16]. As cells reach the upper third of the epithelium, membrane-coating granules become evident at the superficial aspect of the cells.

Membrane-coating granules are found in almost all stratified squamous epithelia, regardless of whether the epithelium is keratinized or not [17]. The appearance of membrane-coating granules in the epidermis has been well characterized [18–20], but less is known about their nature in nonkeratinized epithelia, albeit their existence has been demonstrated [21,22]. These small cytoplasmic granules, approximately 2 μm in diameter, appear in the Golgi region of the prickle cell layer, migrate to the superficial region of cells at the midlevel of the epithelium, and apparently fuse with the cell membrane in the upper regions of the epithelium [21]. It is upon fusion with the cell membrane, that the contents of the membrane-coating granules are extruded into the intercellular spaces of the epithelium [23].

The membrane-coating granules in keratinized epithelia contain electron-dense lipid lamellae [23,24], and therefore the intercellular spaces of the stratum corneum are filled with short stacks of lipid lamellae which fuse at the edges to produce multiple broad lipid bilayer sheets [25,26]. Most of the membrane-coating granules in nonkeratinized epithelia consist of amorphous material [21]. Recent studies, however, have shown that a small number of these granules in nonkeratinized epithelia contain lamellae [27]. Therefore, the intercellular spaces of the superficial layer of nonkeratinized epithelia contain electron

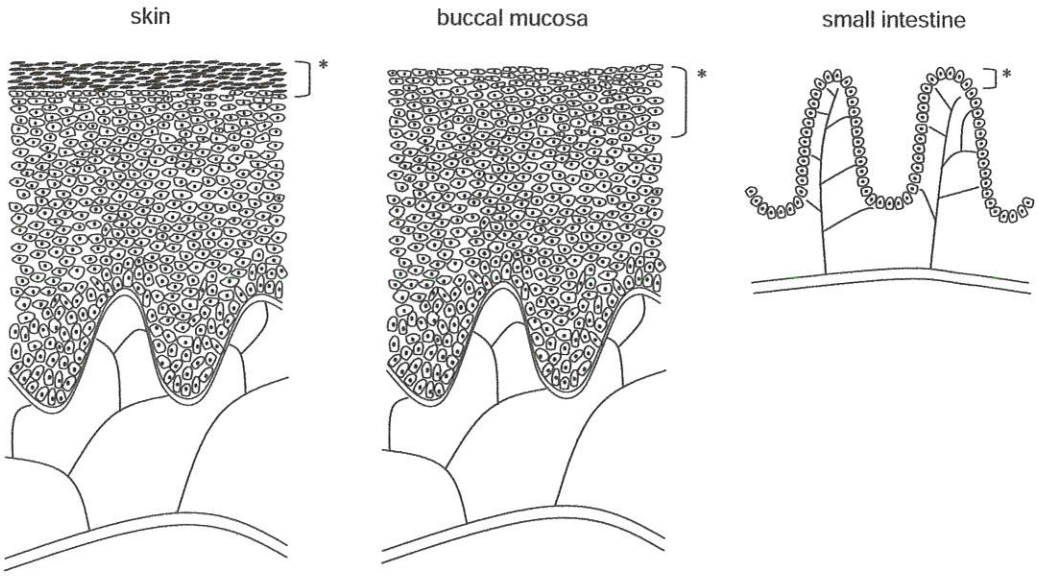

Fig. 1. A structural comparison of the skin, buccal mucosa, and small intestine. The skin and buccal mucosa are covered by a stratified squamous epithelium, whereas the surface of the small intestine consists of a simple columnar epithelium. The region associated with the barrier properties of each tissue is highlighted by the asterisk. This diagram is not drawn to scale.

lucent material, which may represent nonlamellar liquid phase lipid, with occasional short stacks of lipid lamellae [28]. The absence of organized lipid lamellae in the intercellular spaces of the buccal mucosa results in a greater permeability to exogenous compounds, compared to keratinized epithelia [29].

3. The barrier nature of the buccal mucosa

3.1. Location of the permeability barrier

The barrier properties of the buccal mucosa have been attributed to the upper one-third to one-quarter of the buccal epithelium. This was first demonstrated with the topical application of horseradish peroxidase to the oral mucosa of monkeys, rabbits and rats, where the protein was unable to penetrate deeper than the top 1–3 cell layers [30]. When injected subepithelially, horseradish peroxidase was found within connective tissue and extended through the intercellular spaces of the epithelium, up as far as the region where the membrane-coating granules first appear [30]. This suggested that the permeability barrier of the buccal mucosa may be attributed to the materials extruded from the membrane-coating granules. To ensure that this region was also the barrier to the permeation of smaller molecules, the experiments were repeated using lanthanum salts, and identical results were obtained [31].

Further evidence to suggest that the barrier properties of the buccal mucosa were due to the extruded materials of membrane-coating granules came from studies assessing the permeability of tissues lacking these granules. An example of such tissue is the junctional epithelium which attaches the gingival stratified squamous epithelium to the tooth surface [10]. When horseradish peroxidase and microperoxidase were applied to the epithelial surface of this tissue or injected subepithelially, both proteins were found to have distributed through the intercellular spaces of the entire epithelium [32,33]. A similar observation was made when horseradish peroxidase and lanthanum were applied topically to keratinized oral epithelium in tissue culture [34]. Both tracer substances penetrated to deeper layers of the epithelium; lanthanum nitrate reaching the basal cell layer and horseradish peroxidase penetrating to within 3–8 cells of the basal cell layer. Since these tissue cultures lacked membrane-coating granules [35], it became evident that the permeability barrier of nonkeratinized oral mucosa could be attributed to contents extruded from the membrane-coating granules into the epithelial intercellular spaces.

3.2. Chemical nature of the permeability barrier

It is well established that the permeability barrier of the epidermis is attributed to the neutral lipids (mainly ceramides and acylceramides) extruded from the membrane-coating granules into the intercellular spaces [36,37]. It is believed that the barrier of the nonkeratinized oral epithelium is also composed of lipid material, since treatment of oral mucosa with chloroform/methanol mixtures has resulted in a reduced barrier function [38]. To verify the chemical nature of these lipids, various regions of porcine oral cavity have been separated, and the lipids present in each region extracted and identified by thin-layer chromatography [28,38–41]. In common with porcine epidermis, keratinized palatal and gingival mucosae contained high quantities of ceramides and cholesterol, and a low proportion of cholesterol esters and glycosylceramides. In contrast, the buccal and sublingual mucosae, both of which are nonkeratinized, contained higher quantities of the more polar phospholipids, cholesterol esters, and glycosylceramides, and minimal amounts of ceramides. Histochemical staining suggested that the polar lipids were localized in the intercellular spaces of the nonkeratinized oral epithelium [39]. Therefore, the intercellular lipids of the nonkeratinized regions of the oral cavity are of a more polar nature than the lipids of the epidermis, palate, and gingiva, and this difference in the chemical nature of the lipids may contribute to the differences in permeability observed between these tissues [38]. Consequently, it appears that it is not only the greater degree of intercellular lipid packing in the stratum corneum of keratinized epithelia that creates a more effective barrier, but also the chemical nature of the lipids present within that barrier.

3.3. Routes of drug transport

The cellular organization of epithelia lining the buccal mucosa is typical of a stratified squamous

epithelium, where the epithelial cells are surrounded by a hydrophilic intercellular matrix. Due to extrusion of contents from the membrane-coating granules [21], the intercellular spaces of the epithelia are filled with polar lipids, which appear to be in an amorphous state; however, there are occasional short stacks of lipid lamellae [28]. The lipophilic cell membranes of the epithelial cells are thus surrounded by relatively polar intercellular lipids on the cell exterior and a hydrophilic aqueous cytoplasm on the cell interior. This is somewhat analogous to the situation in the intestine, where the epithelial cells are separated by a hydrophilic intercellular compartment, albeit, the intercellular spaces of the intestinal mucosa lack the polar lipids seen in the intercellular spaces of the buccal mucosa. Consequently, the existence of hydrophilic and lipophilic regions in the oral mucosa has lead the majority of researchers to postulate the existence of two routes of drug transport through the buccal mucosa—paracellular (between the cells) and transcellular (across the cells) [42]. This is analogous to the two routes of transport through intestinal epithelium, as is shown in Fig. 2.

The epithelial cell membranes are rather lipophilic and may pose a barrier to polar hydrophilic permeants, and therefore, hydrophilic molecules probably permeate the buccal mucosa via the paracellular route [15]. The presence of tight junctions between intestinal epithelial cells is the primary barrier to paracellular drug transport through the intestine [43]; however, tight junctions are rare in oral mucosa [11,44]. Consequently, passage of drugs through the intercellular domain of the buccal epithelium is more favourable than that observed in the intestine. In the intestine, the transcellular route may be more favourable for lipophilic penetrants since the polar nature of the intercellular domain may offer greater resistance to lipophilic molecules. Such lipophilic molecules may be transported through the aqueous cytoplasm of intestinal epithelial cells by various carrier proteins and/or lipoproteins [45]. If such mechanisms were present in the epithelial cells of the buccal mucosa, then it would be possible for lipophilic molecules to be transported across the aqueous cytoplasm of buccal epithelial cells. There is evidence for carrier-mediated transport of hydrophilic molecules within the buccal mucosa [46–50]; however, similar transport mechanisms for lipophilic molecules have not been identified in the buccal mucosa, which suggests that intracellular transport of lipophilic compounds is limited.

In fact, evidence in the literature suggests that most compounds actually traverse the buccal mucosa via the intercellular lipid domain. Glycosylceramides, which stain positively to periodic-acid Schiff reagent, have been shown histochemically to be located in the intercellular spaces of oral mucosa [39]. Following lipid extraction, the presence of intercellular glycosylceramides is reduced [39], and this is associated with an increase in the permeability of tritiated water [38]. This suggests that lipids within the intercellular domain act as a major hindrance to the permeability of compounds across the oral mucosa. More direct evidence demonstrating the significance of the paracellular route in buccal permeation was provided by direct visualization of certain tracer compounds (horseradish peroxidase and lanthanum salts). When

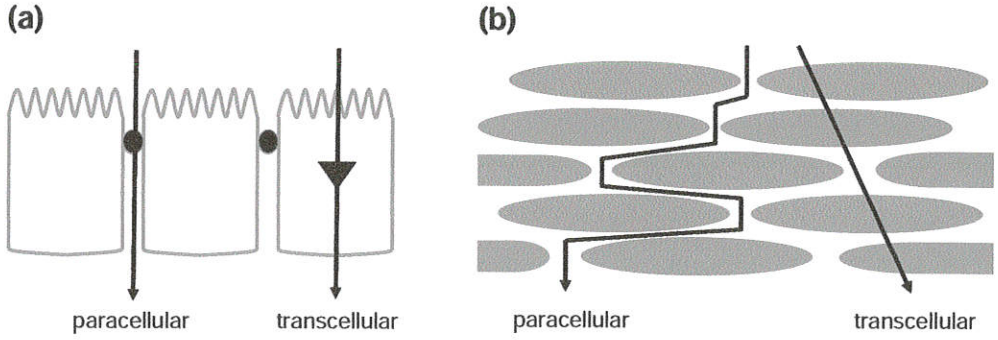

Fig. 2. (a) A schematic representation of the intestinal epithelium with the two routes of drug transport (paracellular and transcellular). The paracellular route of transport is often limited by the presence of tight junctions (●) and the transcellular route of transport can be improved by the presence of various carrier mechanisms (▾). (b) In a similar manner, the paracellular and transcellular routes of transport have been designated to the buccal mucosa, however, the validity of the transcellular route is questionable.

applied to the oral mucosa of rabbits, rats, and monkeys, these tracers reacted only with electron-dense stains in the intercellular spaces of the mucosa, as determined by electron microscopy, and so the paracellular route was considered to be their route of permeation [30,31]. The paracellular route was also found to be the major route of permeation for water, ethanol, cholesterol, and thyrotropin releasing hormone, as determined by light microscopic autoradiography [51,52]. More recently, confocal laser scanning microscopy was used to determine the route of transport of fluorescently labelled dextrans, and it was shown that these hydrophilic macromolecules also penetrated the oral mucosa via the paracellular route [53,54].

Therefore, the existence of a transcellular route is questionable, and it may be that all drugs permeate the buccal mucosa via a paracellular route. However, highly lipophilic compounds may become associated with the cellular membrane lipids or other lipidic components as they permeate through the intercellular spaces. Such thinking has been incorporated into the assignment of drug transport routes as being polar and nonpolar. The nonpolar route involves lipid elements of the mucosa by the partitioning of the drug into the lipid bilayer of the plasma membrane or into the lipid of the intercellular matrix, whereas the polar route involves the passage of hydrophilic compounds through ion channels in the intercellular spaces of the epithelium [11,12]. This classification appears to be more appropriate as it does not limit drug transport to only intercellular or intracellular domains, but rather suggests that drugs move through the tissue through lipidic or nonlipidic regions, depending on the physicochemical properties of the drug. It is therefore possible, that all compounds traverse through the intercellular lipids; however, highly lipophilic compounds may become associated with cellular membrane components, as they traverse through the intercellular space.

3.4. Importance of determining the route of drug transport

It is important to be aware of the route of drug transport through the buccal mucosa, or any biological membrane, especially when attempting to enhance drug transport. Chemical penetration enhancers may have specific effects on either the paracellular (polar) or transcellular (nonpolar) route, and may only alter the permeability of compounds being transported via that particular pathway. Consequently, knowledge of the route taken by a permeant may allow the investigator to use chemical penetration enhancers specific to a particular pathway.

4. Methods employed to improve permeability through the buccal mucosa

Since drug delivery through the buccal mucosa is limited by the barrier nature of the epithelium and the area available for absorption, various enhancement strategies are required in order to deliver therapeutically relevant amounts of drug to the systemic circulation. Various methods, including the use of chemical penetration enhancers, prodrugs, and physical methods may be employed to overcome the barrier properties of the buccal mucosa. However, this review focuses on the potential of chemical penetration enhancers to improve drug delivery through the buccal mucosa.

4.1. Chemical penetration enhancers

A chemical penetration enhancer, or absorption promoter, is a substance added to a pharmaceutical formulation in order to increase the membrane permeation or absorption rate of a coadministered drug, without damaging the membrane and causing toxicity [7]. There have been many studies investigating the effect of chemical penetration enhancers on the delivery of compounds across the skin [55], nasal mucosa [56], and intestine [57], and in recent years, more attention has been given to the effect of these agents on the permeability of the buccal mucosa.

Since permeability across the buccal mucosa is considered to be a passive diffusion process [58–71], the steady state flux (J_{ss}) should increase with increasing donor chamber concentration (C_D) according to Fick's first law of diffusion (Eq. (1)):

$$J_{ss} = \frac{DK}{h} C_D \quad (1)$$

where D is the diffusion coefficient of the drug within the buccal mucosa, K is the partition coefficient

between the buccal mucosa and the donor chamber buffer solution, and h is the length of the pathway through which the drug must traverse (paracellular or transcellular). According to Fick's first law of diffusion, the permeation of compounds across the buccal mucosa may be increased by increasing the diffusivity through the tissue (D), the partitioning into the tissue (K), or the concentration (or thermodynamic activity) of the permeant at the mucosal surface (C). By increasing any one, or all, of these factors, a chemical penetration enhancer may improve the overall flux of a compound across a biological membrane. While it has been established that many skin penetration enhancers increase the diffusivity of permeants by perturbing the ordered intercellular lipid lamellae [55], this may not be the case in the buccal mucosa, since these lipids are already in a less-organized state. In theory, if agents could reduce the viscosity of the intercellular matrix of the buccal mucosa, an improvement in permeability would be expected. However, no evidence for this exists, and so it seems possible that penetration enhancers may exert their effect through alternative mechanisms. In the following sections of this review, the mechanism of action of known buccal penetration enhancers will be discussed, and the limitations and/or strengths of each of the proposed mechanisms of action will be highlighted.

4.1.1. Surfactants and bile salts

Surfactants and bile salts have been shown to enhance the permeability of various compounds across the buccal mucosa, both in vitro and in vivo [54,72–81], and data obtained from these studies strongly suggest that the enhancement in permeability is due to an effect of the surfactants on the mucosal intercellular lipids. For example, the in vitro permeability of 2′,3′-dideoxycytidine through porcine buccal mucosa was only enhanced with concentrations of sodium glycodeoxycholate above its critical micelle concentration (CMC) [81]. A similar result has been observed with sodium dodecyl sulfate (SDS), where the in vitro penetration of caffeine through porcine buccal mucosa was only enhanced at concentrations above the CMC of SDS [82]. This suggests that mucosal lipids may become extracted in the presence of micelles, as has been shown for skin lipids (cholesterol and free fatty acids) in the presence of supramicellar concentrations of SDS [83]. Therefore, by extracting the mucosal lipids at concentrations exceeding the CMC, the barrier properties of the buccal mucosa would be reduced, resulting in enhanced drug permeability.

However, it appears that surfactants only enhance the permeability of compounds which traverse the buccal mucosa via the polar (paracellular) route. This was demonstrated by the absence of enhanced estradiol permeability through SDS-pretreated buccal mucosa [82]. Since estradiol is a poorly water soluble, lipophilic molecule, it would be expected to traverse the buccal mucosa via the nonpolar route. A similar effect has been observed with permeability experiments using rat skin, where SDS enhanced the permeability of compounds with a log $P<3$ but had no effect on compounds with a log $P>3$ [84]. This was attributed to SDS affecting the lipid bilayers in the epidermal intercellular spaces, which act as a barrier for hydrophilic, but not for lipophilic compounds. This may also be the case for the buccal mucosa, where SDS may extract the intercellular lipids, which act as a rate-limiting barrier for caffeine and other hydrophilic molecules. However, being very lipophilic, estradiol may bind to other lipidic components, such as cell membrane lipids, and so extraction of intercellular lipids may have little effect on estradiol permeability. In other in vitro experiments, sodium glycocholate was shown to enhance the buccal transport of flecainide acetate and not the more lipophilic flecainide base, which was attributed to the different pathways for each permeant and the ability of the bile salt to affect only the paracellular route [72]. This strongly suggests that the ability of surfactants to enhance the buccal permeability of a compound depends on the lipophilicity, and ultimately the permeation pathway of that compound.

However, at very high concentrations of surfactant or bile salt, it appears that both the polar and nonpolar routes are affected. Such an observation was made using confocal laser scanning microscopy to visualize various fluorescently labelled dextrans in porcine buccal mucosa in the presence and absence of bile salts [54]. At low concentrations of bile salt, the amount of dextran present in the intercellular spaces was increased, suggesting that the bile salts possibly solubilized intercellular lipids, and thus enhanced dextran diffusivity via the paracellular or polar route. At higher concentrations of bile salt, dextrans began to

appear in the epithelial cells, indicating that these concentrations of bile salt were able to increase permeability across cell membranes, possibly due to disruption of cell membrane lipids. The ability of surfactants to extract both intercellular and cell membrane lipids was verified in another study, where the application of sodium glycodeoxycholate to the mucosal surface of porcine buccal epithelium resulted in a significant reduction in the tissue levels of polar lipids (intercellular) and cholesterol (cell membrane) [85]. Additionally, electron microscopic techniques have demonstrated that surfactants induce changes within the cytoplasm of epithelial cells and produce abnormal deposits within the cells [85,86]. Therefore, solubilization of both intercellular and epithelial cell membrane lipids may be responsible for the enhanced permeability induced by very high concentrations of surfactants and bile salts.

Recently, Fourier transform infrared spectroscopy (FTIR) has been utilized to correlate the effect of sodium glycodeoxycholate on bovine buccal mucosal lipids with the permeability of the buccal mucosa. Using this spectroscopic method, it was found that, in addition to improving the permeation of morphine sulfate through the buccal mucosa, sodium glycodeoxycholate reduced the areas under the symmetric and asymmetric carbon–hydrogen stretching peaks, which are thought to be due to the buccal mucosal lipids [73]. This indicated that extraction of epithelial lipids by the bile salt was responsible for the increased permeability of morphine sulfate. Interestingly, sodium glycodeoxycholate did not induce significant shifts in these stretching peaks—an event which occurs when an agent alters the degree of order of membrane lipids [87]. This demonstrates that the effect of bile salts and other surfactants can be attributed mainly to lipid extraction, and not to the perturbation of intercellular lipid organization.

Extraction of mucosal lipids (intercellular or cellular) is not the only mechanism by which surfactants can increase drug permeability through the buccal mucosa. Using differential scanning calorimetry (DSC), it was found that treatment of excised rabbit buccal mucosa with sodium deoxycholate and sodium lauryl sulfate affected thermal transitions associated with tissue proteins and lipoproteins [88]. This was associated with an increase in salicylic acid permeability and a reduction in electrical resistance (barrier function) of the tissue. In this report, it was suggested that sodium deoxycholate and sodium lauryl sulfate caused uncoiling and extension of protein helices, thereby opening up the polar pathway for diffusion [88]. Other reports have also suggested that surfactants increase oral permeability by reacting with epithelial proteins, albeit, no mechanistic studies have been provided [76,78]. Therefore, agents which alter protein domains within the epithelium of the buccal mucosa may also increase drug permeability.

While surfactants have been shown to cause removal of the superficial cell layers [82,86,88], which are responsible for the barrier properties of the buccal mucosa, a greater body of evidence suggests that lipid extraction is the main mechanism by which these agents improve buccal permeability. This lipid-solubilizing effect generally alters paracellular or polar transport through the buccal mucosa; however, at higher concentrations of surfactant and bile salt, cellular membrane lipids may be extracted, resulting in enhanced transcellular transport. There is no evidence in the literature to suggest that lipid packing and/or organization is altered; however, some results suggest that an interaction with the proteinaceous domains of the tissue can result in reduced barrier properties of the tissue.

4.1.2. Fatty acids

Fatty acids have been shown to enhance the permeation of a number of drugs through the skin, and this has been shown by DSC and FTIR to be related to an increase in the fluidity of intercellular lipids [89–91]. There have been a number of studies demonstrating the enhancing effect of fatty acids on drug delivery through the buccal mucosa; however, either an inappropriate model was utilized or no mechanism of action was investigated. The permeability of insulin from Pluronic F-127 gels was assessed through rat buccal mucosa, and the presence of oleic acid within the gel appeared to produce an increased hypoglycaemic effect [92]. However, the reader should use caution in extrapolating such results to human buccal absorption, since rat buccal mucosa is keratinized [16], in contrast to the buccal mucosa of humans. Two other studies which demonstrate the enhancing effect of fatty acids are (1) the improvement in ergotamine tartrate permeation through keratinized epithelial-free hamster cheek pouch by cod-liver oil extract

(which contains 16 types of fatty acids) [93], and (2) the enhanced permeation of [D-Ala^2, D-Leu^5]enkephalin through porcine buccal mucosa from a cubic phase of glyceryl monooleate, containing oleic acid [94]. In these two studies, no mechanism of action of the fatty acids was investigated or provided by the authors. In a report on the enhancing effect of oleic acid on the in vitro permeability of propranolol through porcine buccal epithelium, it was assumed, and not proven, that the enhancing effect was due to the lipid-fluidizing effects of the fatty acid [95]. However, the mechanisms of action have never been demonstrated for these fatty acids in the buccal mucosa, by means of DSC and/or FTIR.

It has been shown by fluorescence anisotropy, that oleic acid reduces the lipid packing order in buccal epithelial cell membranes [96]. Although the epithelial cell membrane lipids are different from those found in the intercellular spaces of the buccal mucosa, these results suggest that enhancers may interact with the phospholipids of cell membranes, resulting in increased drug diffusion via the nonpolar route. However, these buccal epithelial cells were present in suspension form, and it is unclear whether a similar mechanism would operate with intact buccal mucosa. Some authors have suggested that oleic acid may improve the permeability of compounds by an increase in partitioning [67]; however, direct evidence for this is lacking in the literature. Therefore, the actual mechanism whereby fatty acids enhance buccal permeation is unknown, and it should not be assumed to be an effect on intercellular lipid order, since no direct evidence for this exists.

4.1.3. Ethanol

In addition to smoking, ingestion of alcohol is a major risk factor for the development of oral cancer [97]. This may be due to the ability of ethanol to enhance the absorption of potential carcinogens, including nitrosonornicotine, across the mucosa of the oral cavity [98–100]. Additionally, pretreatment with ethanol has been shown to enhance the permeability of tritiated water and albumin across ventral tongue mucosa [100], and to enhance caffeine permeability across porcine buccal mucosa [101]. Being hydrophilic, such molecules are expected to traverse the oral epithelium via the polar route, and since their permeation was enhanced with ethanol, this solvent must be affecting the intercellular domains of the epithelium. In fact, it has been suggested that the enhancing effect of ethanol on the permeability of tritiated water across the oral mucosa was attributed to the ability of ethanol to disrupt the lipid molecules from their normal orderly arrangement [100]. Ethanol has been suggested to induce modifications at the polar head group region of lipid bilayers in skin [102]—whether this occurs in buccal mucosa is questionable, since the intercellular lipid domains are less ordered than in skin.

At higher concentrations, however, ethanol has been shown to extract stratum corneum intercellular lipids, using FTIR [103,104]. Extraction of buccal intercellular lipids would seem an appropriate mechanism of action for ethanol, since ethanol is a lipid solvent. However, there have been no FTIR studies demonstrating the ability of ethanol to extract buccal mucosal lipids. Such an investigation is needed to help verify that the mechanism of action of ethanol is in fact due to extraction of intercellular lipid components.

4.1.4. Azone®

The skin penetration enhancing effects of Azone® have been extensively studied, using a range of permeants [105,106]. The enhancing effect of Azone® on skin permeability has been attributed to a disruption of the organized lipid structure in the intercellular region of the stratum corneum, resulting in increased lipid fluidity and enhanced drug diffusivity [55,107]. There are several reports of the enhancing effect of Azone® on the permeability of compounds through oral mucosa. Pretreatment with Azone® has been shown to increase the in vitro and in vivo permeability of salicylic acid through hamster cheek pouch buccal mucosa [108,109]. In addition, Azone® has been shown to increase the fluidity of lipids extracted from the hamster cheek pouch [108]. Therefore, it is possible that Azone® enhances the permeability of the buccal mucosa in a manner similar to its action in skin.

However, one should take particular note of the model membrane employed in these studies. In most of these reports, hamster cheek pouch is used, which has a keratinized surface closely resembling the stratum corneum of skin [110]. Whether Azone® or other chemical penetration enhancers, which affect the

ordered intercellular lipid lamellae in stratum corneum, have the same enhancing effect in nonkeratinized buccal mucosa is not known. Since the intercellular spaces of the buccal epithelium contain lipids of a more polar nature and these lipids are in a less organized state [28,38–41], results obtained using keratinized buccal mucosa may not be directly representative of results to be expected from nonkeratinized tissues. This was demonstrated recently with in vitro permeability experiments using nonkeratinized porcine buccal mucosa. Following a 2 h pretreatment with ethanolic solutions of Azone®, the permeability of caffeine and estradiol were not significantly enhanced [101]. This demonstrates that skin penetration enhancers do not always improve buccal drug delivery, since the barrier nature of these two tissues is quite different, and it is possible that these agents have no direct effect on the intercellular lipids of the buccal mucosa.

While there have been no reported studies on the effect of Azone® on the intercellular lipids of nonkeratinized buccal mucosa, fluorescence anisotropy has shown that Azone® reduces the lipid packing order of buccal epithelial cell membranes [96]. These membrane lipids are not representative of the lipids in the intercellular spaces of the buccal mucosa, so caution should be used when extrapolating the effect of enhancers on these lipids to their effect on intercellular lipids. Although the epithelial cell membrane lipids are different from those found in the intercellular spaces of the buccal mucosa, these results suggest that Azone® may interact with the phospholipids of cell membranes, resulting in increased drug diffusion via the transcellular route.

There is only one report in the literature demonstrating the enhancing effect of Azone® on nonkeratinized buccal mucosa [101]. In this report, the in vitro permeability of triamcinolone acetonide was improved 3.8-fold by pretreating porcine buccal mucosa with an ethanolic solution of Azone®. Although no calorimetric or spectroscopic studies were performed to elucidate the mechanism of enhancement, mucosal-buffer partitioning experiments demonstrated that the enhancing effect of Azone® was actually due to increased uptake into the buccal mucosa. By improving the solubility of triamcinolone acetonide in the buccal mucosa, an overall improvement in buccal permeability would be expected according to Fick's first law of diffusion (Eq. (1)). Further evidence to demonstrate the improved uptake of triamcinolone acetonide into Azone®-pretreated buccal mucosa was provided by simultaneously assessing triamcinolone acetonide disappearance from the donor chamber and appearance in the receptor chamber in an in vitro model [111]. Pretreatment of the buccal mucosa enhanced the disappearance permeability coefficient of triamcinolone acetonide 1.5-fold, and increased the tissue concentration of the corticosteroid 4.4-fold. There have been no other reports on the effect of Azone® on the permeability of nonkeratinized buccal mucosa, and so from the data available in the literature, it appears that this skin penetration enhancer may enhance the permeability of certain compounds by improving the partitioning of such compounds into the buccal mucosa.

4.1.5. Sunscreen skin penetration enhancers

Recent investigations have demonstrated that the sunscreen agents octisalate and padimate O improve the transdermal permeability of various compounds, both in vitro and in vivo [112–114]. Since it was assumed that most skin penetration enhancers alter buccal mucosal permeability, an investigation on the effects of these sunscreens on the permeability of the buccal mucosa was undertaken. It was shown that neither octisalate nor padimate O significantly improved the permeability of caffeine, estradiol, or triamcinolone acetonide through porcine buccal mucosa [101]. This supports the theory that skin penetration enhancers do not always have a similar effect on buccal mucosal permeability.

While the exact mechanism of transdermal enhancement for these sunscreen agents has not been fully elucidated, it is probable that they have an effect on the skin similar to that of Azone®. Preliminary data obtained using DSC suggests that Azone®, octisalate, and padimate O all reduce intercellular lipid order, since all enhancers reduced the transition temperature of a model mixture of stratum corneum lipids [115]. Although these experiments were conducted on dry model stratum corneum lipids, the results suggested that octisalate and padimate O interacted with intact stratum corneum lipids in a similar manner to that of Azone®. Further evidence to suggest that these sunscreen agents disrupt the packing of stratum corneum intercellular lipids was

provided by the use of attenuated total reflectance FTIR [116]. In these spectroscopic studies, treatment of human stratum corneum with octisalate resulted in a shift in the vibrational frequencies associated with stratum corneum lipids—an effect which has also been observed with Azone® pretreatment [117]. This suggests that octisalate may fluidize the stratum corneum surface lipids, resulting in a lower resistance to drug transport. However, since the intercellular lipids in the buccal mucosa are less ordered, it is not surprising that pretreatment with octisalate and padimate O had no effect on buccal mucosal permeability. This clearly demonstrates that the structural characteristics of the permeability barrier in buccal mucosa and skin are different, and that agents that enhance permeability through one biological membrane do not necessarily have the same effect on other biological membranes.

4.1.6. Chitosan

Chitosan, a biocompatible and biodegradable polymer, has been shown to enhance drug delivery through various tissues, including the intestine [118] and nasal mucosa [119]. However, chitosan has also been reported to improve the in vitro permeability of hydrocortisone and transforming growth factor-β through porcine buccal mucosa [120,121], in addition to improving the permeability of dextrans through a buccal mucosa cell culture model [122]. The authors attributed this enhancing effect to the bioadhesive nature of chitosan, resulting in increased retention of the drug at the buccal mucosal surface. Although not confirmed, such a hypothesis seems plausible since hydrogels containing chitosan have been shown to have prolonged retention times on oral mucosa [123].

Within the intestine, the enhancing effect of chitosan has been attributed to binding of the polymer to the epithelial membrane through a charge-dependent effect, followed by opening of the tight junctions [118]. While this would be beneficial for the enhancement of compounds traversing the intestine via the paracellular route, it is anticipated that such a mechanism is not responsible for the enhancing effect of chitosan seen in the buccal mucosa, since tight junctions are rare in the buccal epithelium and do not contribute to its barrier properties [11,44]. It has also been suggested that the enhancing effect of chitosan is due to an interference with the intercellular lipid organization in the buccal epithelium [121,122], however, such a mechanism has not been proven. Therefore, from the data available in the literature, it appears that the enhancing effect of chitosan on buccal drug delivery may be due to increasing the retention of the drug at the mucosal surface. This could be beneficial in the clinical setting, since clearance of the drug by salivary flow, would be reduced.

5. Summary and conclusions

From an extensive review of the literature regarding buccal penetration enhancement, it appears that chemical penetration enhancers improve buccal drug delivery by one or more of the following mechanisms:

(a) increasing the partitioning of drugs into the tissue,
(b) extracting (and not disrupting) intercellular lipids,
(c) interacting with epithelial protein domains, and/or
(d) increasing the retention of drugs at the buccal mucosal surface.

While chemical penetration enhancers may have other additional mechanisms of action, limited research has been performed in the area to elucidate the exact mechanisms involved. In order to have greater predictive power, more focus should be given to various calorimetric and/or spectroscopic methods (such as DSC and FTIR)—methods that have been successful in elucidating the mechanism of action of penetration enhancers in the skin. In addition, one should be mindful of the models used when assessing the potential of novel chemical penetration enhancers on the buccal mucosa. Use of keratinized mucosae, such as the buccal mucosa of rats or the cheek pouch of hamsters, may provide data that cannot be extrapolated to human buccal mucosa, due to the significant differences in the nature and organization of intercellular lipids between these species. For this reason, nonkeratinized buccal mucosa, such as porcine buccal mucosa, appears to be a more appropriate model.

From the published data available, and an understanding of the chemical and structural organization of

the buccal epithelium, it is very unlikely that enhanced buccal drug penetration will result from the use of skin penetration enhancers which act solely by disrupting stratum corneum intercellular lipid organization. This misconception may lead to the inappropriate assumption that all skin penetration enhancers will affect buccal mucosal permeability. More importantly, agents which do not improve transdermal permeability may be erroneously ignored as candidates to enhance buccal permeation, if this concept is not understood.

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