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Review

Mechanisms of oral permeation enhancement

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Abstract

This paper reviews the effects of a diverse range of penetration enhancers, including fatty acids, bile salts, surfactants, Azone® and ethanol, on oral mucosa. We describe in detail the effects on the intercellular lipid domains, the changes induced in proteinaceous regions and potential interactions between the enhancer and endogenous structures along the transport pathway. Since there have been few structure-based studies of the oral mucosa, which have discussed enhancer effect as a function of specific physicochemical properties, we have drawn upon analogous studies of skin and other mucosal membranes, as well as model lipid systems, e.g. liposomes. © 1997 Elsevier Science B.V.

Keywords: Oral mucosa; Lipid organization; Permeation enhancers

1. Introduction

Penetration enhancers are substances that increase the membrane permeation rate of a coadministered drug (Aungst, 1996). Although most penetration enhancers were originally designed for purposes other than absorption enhancement, a systematic search for safe and effective penetration enhancers must be a priority in drug delivery. The goal of designing penetration enhancers, with improved efficacy and reduced toxicity profiles,

Abbreviations: ATR-IR, Attenuated Total Reflectance Infrared spectroscopy; CMC, Critical micellar concentration; DecMSO, Decyl-methyl sulphoxide; DPPC, Dipalmitoylphosphatidylcholine; DSC, Differential Scanning Calorimetry; HLB, Hydrophilic-lipophilic balance; IR, Infrared spectroscopy; MCG, Membrane coating granules; PC, Phosphatidylcholine; PG, Propylene glycol; SLS, Sodium lauryl sulphate; T_M, Transition temperature.

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will be more readily realised by an improved understanding of the relationship between enhancer structure and the effect induced in the membrane and, of course, the mechanism of action.

Enhancer efficacy depends on the physicochemical properties of the drug, administration site, nature of the vehicle and whether the enhancer is used alone or in combination. Differences in cellular morphology, membrane thickness, enzymatic activity, lipid composition and potential protein interactions, are structural and functional factors that make the effectiveness of enhancers vary from site to site (Lee et al., 1991). Even though effective absorption enhancers for nasal, rectal or intestinal drug delivery, should not be assumed a priori to have identical effects on oral delivery, it is possible to make extrapolations based upon their actions on non-oral membranes, in an attempt to delineate their effect on oral mucosa. However, it is necessary to take into consideration the structural and metabolic differences between oral mucosa and other epithelial membranes, in particular, skin. Local differences in both the structure and the composition of the different oral regions must be also considered when studying enhancer action. Variations in permeability result from the presence or absence of a keratinized layer, but also from the lipid composition.

The objective of this review is to describe the main mechanisms of action of the principal groups of permeation enhancers on oral mucosa, primarily on the intercellular lipid barrier, although their effects on protein domains and their interactions with drugs are also discussed. Emphasis is placed on the role played by surfactants, bile salts and fatty acids. Most of the compounds used as oral mucosal enhancers belong to the typical enhancer categories that are generally used to compromise barrier function. In fact, there has been little work done to develop chemical agents that are targeted specifically at the oral mucosa and, in contrast to the skin, a clearly defined structure/effect relationship has not yet been established for oral mucosal enhancers. Therefore, in some sections of this paper we have looked to the more numerous mechanistic descriptions for skin and other mucosal membranes to shed light on the effects of enhancer action on oral mucosa.

2. Differences between oral epithelium and other membranes

The oral mucosa offers a number of advantages not only as a local, but as a systemic drug delivery site. One of the most important advantages is that drugs are able to bypass gastrointestinal and hepatic drug metabolizing enzymes. However, in order to improve drug absorption, it is frequently necessary to coadminister a penetration enhancer. Although extrapolations can be made from the numerous studies explaining the actions of enhancers on non-oral mucosal membranes or skin, as well as any corresponding structure/effect relationship, it is always necessary to consider the structural similarities and differences between oral mucosa and these other membrane systems.

Oral mucosa has many similarities with skin, more than with other biological tissues. For example, the intestinal epithelium is a columnar epithelium with a single layer of cells; rectal mucosa has a surface composed of closely packed columnar cells, with some areas interrupted by crypt regions; the surface of the nasal mucosa is lined with both ciliated columnar and squamous cutaneous epithelium. The cells of these tissues are typically interconnected by tight junctions (which are almost absent in the oral mucosa). In contrast, oral mucosa is a stratified squamous epithelium, whose intercellular spaces are filled with lipids extruded from the membrane coating granules (MCG). The lipids may be organized into lamellae and they constitute the principal barrier against molecular diffusion. Some areas of the oral mucosa (e.g. the palate and gingiva) have a cornified layer, which is similar in its pattern of maturation, to the stratum corneum of the skin. These areas, even though they are among the most resistant to enhancer action, are generally more permeable than the skin because the intercellular lipids are less well structured, since they exist mainly in discrete lamellar domains and there are fewer structural contributions from lipids (ceramides) covalently bound to the corneocyte surface (Wertz and Squier, 1991; Wertz et al., 1993; Squier and Wertz, 1996). Lipids including small amounts of ceramides, monohexosylceramides, cholesterol esters, cholesterol sulphate,

fatty acids and a high proportion of phospholipids, triglycerides and cholesterol, fill the intercellular space of oral keratinized tissues. In nonkeratinized regions (e.g. cheek, floor of the mouth and lips) the chemical nature of the intercellular material is less well defined and the barrier is less efficient than that in the keratinized epithelia. The lipids, which seem to be in a nonlamellar liquid phase, with only occasional short stacks of lipid lamellae, are mainly composed of phospholipids, cholesterol and an unusual proportion of glycosylceramides (Wertz et al., 1996).

These regional variations corresponding to the local degree of keratinization and lipid organization, suggest that selective targeting of the drug delivery site may enable the use of milder enhancers, thereby reducing adverse tissue reactions and improving patient compliance. The presence of phospholipids in the epithelial oral surface, particularly in the cornified regions, is one of the major differences between these and the epidermis. Phospholipids are conspicuous by their absence in the epidermal stratum corneum. It was suggested that phospholipid catabolism is required to allow the conversion of short lamellar disks into broad lamellar sheets, as found in epidermis. This could explain the differences in permeability between epidermis and the oral tissues (Wertz et al., 1992).

It is important to consider that oral mucosa is very sensitive to the local effects of chronically administered substances, e.g. tobacco, alcohol, mouthrinses, toothpastes, etc. Furthermore, irritation may lead to tissue inflammation, in response to mechanical or chemical insult, and this may result in an altered permeability. However, on the other hand, the rate of tissue turnover is in general faster in oral mucosa than in epidermis and oral mucosa is rarely involved in hypersensitive reactions, like skin, which could confer a certain robustness and allow a rapid recovery (Squier and Wertz, 1996).

A great deal of information about membranes and their interaction with exogenous substances, has been obtained through the study of pure lipids and simple lipid mixtures. The studies of phospholipid bilayer systems may be useful indicators of oral mucosal behaviour and deductions made from these data have more relevance for the oral mucosa, considering the presence of phospholipids in the oral surface, than for the epidermal stratum corneum. However, it is important to remember that different conformational and polymorphic states may exist in the isolated components, when compared to the intact tissue samples. Much of the information about enhancer agents has also been obtained from the study of easily manipulated bacterial membranes. This knowledge can, to some extent, be used to explain the effect of enhancers on oral mucosal tissues.

3. Penetration enhancers have different mechanisms of action

Mechanisms by which penetration enhancers are thought to improve mucosal absorption include the following (Lee et al., 1991):

(i) Changing mucus rheology: Mucus covers the respiratory, gastrointestinal and genitourinary tracts, and forms a viscoelastic layer of varying thickness which affects drug absorption (Lee et al., 1991). In the mouth, it is covered by a layer of saliva. There is evidence that, for certain drugs, saliva can hinder absorption, but usually it is insignificant compared to the other barriers during passage through the oral mucosa (Rathbone and Tucker, 1993). Therefore, saliva may have only a transient contribution to the oral barrier function, for example, decreasing the initial passage of water through the buccal epithelium (Adams, 1975).

(ii) Increasing the fluidity of membrane lipid bilayers: As the intercellular pathway is the most generally accepted route for drug absorption (Squier, 1973; Squier and Rooney, 1976; Squier and Lesch, 1988; Hoogstraate and Boddé, 1993; Hoogstraate et al., 1993) the disruption of intercellular lipid packing, by interaction with either lipid or protein components, is thought to increase permeability. Biophysical techniques, e.g. differential scanning calorimetry (DSC) and infrared spectroscopy (IR), have demonstrated that,

there is indeed, a correlation between increased lipid fluidity and enhanced membrane permeability. Varying degrees of insult may occur in tissues that are in intimate contact with the enhancer (Lee et al., 1991), therefore, a transient increase in the fluidity of the intercellular lipids may be thought of as a relatively nontoxic effect, whereas extraction of the intercellular lipids or denaturation of cellular proteins may be viewed as being somewhat more drastic. Therefore, an important consideration is to ensure that the effect of the enhancer on membrane permeability is reversible (Aungst, 1996).

(iii) Affecting the components involved in the formation of intercellular junctions: This could be particularly important in the case of intestinal membranes, where the barriers to paracellular diffusion of molecules and ions are the tight junctions or 'zona occludens'. Although in oral mucosa tight junctions are almost absent, desmosomes, which are thought confer added structural integrity, occupy $\sim 47\%$ of the intercellular space of the palatal stratum corneum and a further $\sim 10\%$ is occupied by saccules (Swartzendruber et al., 1995). This suggests that disruption of these structures may provide a permeability pathway through the oral stratum corneum, but this has not been entirely confirmed (Egelrud and Lundstrum, 1991; Swartzendruber et al., 1995).

(iv) Overcoming the enzymatic barrier: Protease inhibitors for endo- and exo-peptidases are potential penetration enhancers. Although various peptidases and proteases are present within the oral mucosa, and it is possible that metabolism may act as an enzymatic barrier, the intercellular pathway is thought to be deficient in proteolytic activity (Aungst, 1996; Lee et al., 1991). However, changes in membrane fluidity induced by penetration enhancers may indirectly alter enzymatic activity (Lee et al., 1991).

(v) Increasing the thermodynamic activity of drugs: This may be affected by the vehicle composition, which will influence drug solubility (De Carvalho et al., 1993, 1996) and also by ion-pair formation between the enhancer and the drug (Coutel-Egros et al., 1992; Ganem et al., 1996).

4. Fatty acids

4.1. Introduction

Fatty acids are the most abundant lipids in biological membranes. In oral mucosa they represent $\sim 8\%$ of the total lipids in the cornified layer and $\sim 11\%$ of the lipids in the nonkeratinized buccal barrier (Wertz et al., 1992, 1996). They are present as free acids, in addition to being components of more complex lipids: ceramides, triglycerides and phospholipids. Administration of exogenous free fatty acids, mainly of the *cis*-unsaturated variety, has been reported to increase membrane permeability and although their mechanism of action as penetration enhancers has not been completely elucidated, it appears to be primarily related to the disruption of intercellular lipid packing (Muranushi et al., 1981).

4.2. Mechanism of action

4.2.1. Structure/effect relationship. Effect on the intercellular lipid domains

It has been shown that the magnitude of enhancement with fatty acids seems to follow a structure/effect relationship, being influenced by the presence and the position of double bonds, isomer type (*cis* or *trans*), ionization state, chain length and the degree of branching. Other important factors are the nature of the vehicle used, fatty acid concentration and the contact time. It is generally accepted that unsaturated fatty acids are more disruptive than saturated fatty acids with the same carbon number (Francoeur et al., 1990; Takeuchi et al., 1992; Aungst et al., 1986; Aungst, 1989; Kitagawa et al., 1995). Inclusion of fatty acids in a membrane may change the Van der Waals interactions between the hydrocarbon chains (Eliasz et al., 1976), and form hydrogen bonds between the fatty acid carboxyl groups and neighbouring moieties (Schullery et al., 1981; Marsh and Seddon, 1982).

In the homologous series of saturated straight-chain fatty acids, lauric acid (C_{12}) has been used to promote the absorption of drugs across the keratinized buccal mucosa, e.g. lauric acid was shown to effectively promote the absorption of

propranolol through hamster cheek pouch (Coutel-Egros et al., 1992); and lauric acid/propylene glycol (PG) was quite effective in enhancing the absorption of insulin through rat mucosa in vivo (Aungst and Rogers, 1989). It has been demonstrated that longer ($C > _{14}$) as well as shorter fatty chains (C_6–C_{10}) are generally less effective than the medium-chain fatty acids (Ogiso and Shintani, 1990).

It has been proposed that the presence of *cis* double bonds introduces an accentuated flexion in the hydrocarbon chain, which prevents the formation of well-ordered compact crystals (Gay et al., 1989; Horton et al., 1994). The perturbation induced by a *cis* double bond, influences the proportion of gel and liquid-crystalline lipid in a membrane (Macdonald et al., 1985). Moreover, DSC and fluorescence studies with mixtures of phospholipids and fatty acids indicated that saturated fatty acids partition preferentially into gel phases, while their *cis*-unsaturated counterparts partition primarily in liquid-crystalline domains (Ortiz and Gomez-Fernandez, 1987; Klausner et al., 1980).

In general, calorimetric studies with stratum corneum lipids, following treatment with exogenously applied fatty acids, showed a reduction of the transition temperature (T_M) associated with the intercellular lipids, at $\sim$60–65 and $\sim$70–75°C, with no change in the protein-related endotherm at 90–105°C (Golden et al., 1986, 1987a,b; Francoeur et al., 1990). The largest increases in lipid fluidity resulted from treatment with *cis* isomers; treatment with *trans* isomers had little or no effect on T_M (Golden et al., 1987b; Kitagawa et al., 1993).

Treatment of the stratum corneum with *cis*-octadecenoic acids with the site of unsaturation centrally located, e.g. oleic acid, resulted in a shift to a higher frequency and a broadening of the C–H asymmetric stretch peak near 2920 cm^{-1} and an increase in the absorption frequency of both the C–H asymmetric (2920 cm^{-1}) and C–H symmetric (2850 cm^{-1}) stretching vibrations (Golden et al., 1987b; Mak et al., 1990; Takeuchi et al., 1992). Verma et al. (1980) showed that *cis* double bonds positioned at 9, 10 or 10, 11 are more disruptive than those at other positions in C_{18} alkyl chains. Isomers with the site of unsaturation nearer to the centre of the chain decrease the lipid-T_M more than their counterparts (Macdonald et al., 1985).

Opinions are divided about the effect of the number of double bonds in the alkyl chains on membrane permeability, while some authors argue that increasing the number of double bonds may result in a greater enhancement (Aungst et al., 1986; Ortiz and Gomez-Fernandez, 1987; Aungst, 1989), others find that *cis*-polyunsaturated acids have packing properties similar to those of saturated acids, and as a consequence, propose that their fluidizing properties would be similar to those of saturated acids (Golden et al., 1987b).

Aungst (1989) showed that the effects of branched fatty acids were not significantly different from those of unbranched fatty acids with the same carbon number. This, was probably because the short- and medium-chain unbranched fatty acids used, already had a maximal disruptive effect. However, saturated fatty acids with a hydrocarbon chain length similar to normal stratum corneum lipids, are not very disruptive. Thus, in these cases, the effect of branching could increase the disruptive effect, depending on the position and the chain length of the branch, as has been reported by the same author for stearic acid and its branched isomers.

Fluorescence anisotropy studies with liposomes made of stratum corneum lipids have indicated that perturbation of lamellar lipids is induced primarily by the undissociated form of the fatty acids. Apparently the unionized form of fatty acids tend to fluidize the lipid bilayer. This effect was attributed to the interaction between fatty acids and other lipid components, which differs for ionized and unionized fatty acid forms. It seems that the absence of an electric charge, enables the distribution of the undissociated form of the fatty acid in the lipid bilayer, with a concomitant change in the local Van der Waals interactions (Kitagawa et al., 1995). This also suggests that disruption of the hydrophobic interactions between alkyl side chains is more important than breaking electrostatic linkages between adjacent polar head groups. It might be argued that pertur-

bation of the alkyl side chain packing frees up greater molecular volume, thus facilitating diffusional 'hopping' from site to site. The type of functional group present in the exogenous compound has been shown to have an influence on the extent of percutaneous absorption enhancement. For example, although the enhancing effect was demonstrated to be still present when a carboxyl group was replaced by a hydroxyl group, e.g. lauryl alcohol was as effective as lauric acid and oleyl alcohol was half as effective as oleic acid, the replacement by either a methyl ester group, or the use of the sodium salts (sodium laurate and sodium oleate) brought about a decrease in the enhancing effect (Yamada and Uda, 1987).

Other factors, that may influence the enhancing effect of fatty acids, include conformational alteration of proteins (Takeuchi et al., 1992) or the probable complexation of the drug and fatty acid (Ogiso and Shintani, 1990). Green et al. (1987, 1989) proposed an ion pairing mechanism for the penetration enhancement of β-blockers across an isopropyl myristate membrane in the presence of fatty acids; and Coutel-Egros et al. (1992) reported the formation of a lauric acid-propranolol complex as the most probable mechanism for buccal absorption, without any direct action of lauric acid on membrane permeability.

4.2.2. A special case: oleic acid

Oleic acid (C_{18} Δ9:10) is one of the most frequently used *cis*-unsaturated fatty acid enhancers and its mechanism of action on the skin has been widely studied (Macdonald et al., 1985; Aungst et al., 1986; Yamada and Uda, 1987; Aungst, 1989; Gay et al., 1989; Francoeur et al., 1990; Mak et al., 1990; Takeuchi et al., 1992; Kitagawa et al., 1993; Turunen et al., 1994; Hadgraft, 1996). However, to date, no theory has been completely accepted and penetration enhancement could result from a combination of several mechanisms.

In this respect, it has been previously noted that the alkyl chains of phospholipid liposomes as well as those of stratum corneum extracted lipids, may coexist as an equilibrium of liquid-crystalline and gel-states in the same lamellae under certain hydration and temperature conditions (Papahadjopoulos et al., 1973; White et al., 1988). The boundaries between the 'solid' and 'liquid' domains, may be considered as regions of 'disorder' or permeable interfacial 'defects', which might be related to increased permeability. The heterogeneous insertion of unsaturated fatty acids, like oleic acid, may accentuate these 'defects', thereby favouring the transport of small (polar) molecules, e.g. water. Under certain conditions, mixtures of *cis*-unsaturated fatty acids and phospholipids, show the appearance of a solid/fluid phase separation (Ortiz and Gomez-Fernandez, 1987). These results would explain the enhancement action of oleic acid at physiological temperatures (below the lipid transitions seen by DSC) (Francoeur et al., 1990). This effect appears to be selective for *cis*-unsaturated fatty acids, as revealed by DSC and Raman spectroscopy studies (Verma et al., 1980; Ortiz and Gomez-Fernandez, 1987).

Similar findings have been reported for oral mucosal membranes. The effect of oleic acid on human buccal epithelial cells labelled with different fluorophores was studied by Turunen et al. (1994). The results showed that oleic acid disrupts strongly the polar head group and the hydrophobic region of the membrane lipids, causing large decreases in anisotropy values. Clancy et al. (1994) suggested that epidermal lipid extraction plays a role in the penetration enhancing effects of oleic acid/ethanol and this is not inconceivable considering that ethanol itself, has extracting properties; however, the proposed extraction of stratum corneum lipids by oleic acid/PG systems (Yamada et al., 1987), suggests that oleic acid penetrates into the skin, dissolving some lipoidal components, and allowing the passage of the drug dissolved in PG. It has been shown that oleic acid is able to dissolve considerable amounts of cholesterol (about 175 mg/ml; Leopold and Lippold, 1995), which may act as a membrane stabilizer (Kitagawa et al., 1993).

4.3. Adverse effects

Concentration and contact time are important factors that influence not only enhancement effi-

cacy, but also the effect of enhancers on membrane integrity. Even though enhancement is frequently accompanied by barrier damage, some epidermal studies have shown that the choice of the fatty acid may influence the balance between enhancement and irritation. Some fatty acids (i.e. neodecanoic, elaidic) were found to be less irritating than others (lauric, oleic) and were reported, in certain cases, as having similar enhancing effects (Aungst, 1989). However, most of these studies have been carried out in the skin and unfortunately there is not consistent information about the effect of fatty acids on oral mucosal membrane integrity.

4.4. Importance of vehicle and concentration

The relative disruptive power of fatty acids will depend on the uptake of fatty acid by the membrane, which is related to the thermodynamic activity of the fatty acid in the vehicle (Mak et al., 1990). Francoeur et al. (1990) showed that the amount of oleic acid taken up by the stratum corneum and silastic membranes reached a maximum value when a 40% ethanolic solution was used. In addition, there was a high correlation between uptake and the changes in the lipid-associated T_M. In effect, the use of 40% ethanol enabled the greatest amount of oleic acid to be delivered into the stratum corneum and this was also the same amount of ethanol that caused the greatest lowering of T_M. The decrease in T_M reported by these authors, is a good example of the synergistic effect between oleic acid and ethanol, and emphasises the importance of the vehicle used on fatty acid action. Synergy between enhancers such as ethanol, PG or Transcutol®, that are thought to improve the solubility of the penetrants in the membrane, and lipid 'fluidisers' like oleic acid or Azone®, has been already discussed elsewhere (Hadgraft, 1996). Aungst and Rogers (1989) reported a high pharmacological effect for buccal administered insulin using 10% lauric acid/PG.

Concentration may also influence the enhancer activity, e.g. 5% lauric acid/PG was shown to be much less effective in enhancing the buccal absorption of insulin, than a 10% solution (Aungst and Rogers, 1989). Oleic acid has also demonstrated a dose-dependent effect in lowering the T_M of liposomes composed of stratum corneum lipids. The T_M occurred near 66°C with 10 mol% oleic acid; at 62°C with 20 mol% and at 73°C in its absence (Kitagawa et al., 1993). Furthermore, Turunen et al. (1994) found a decrease in the fluorescence anisotropy of labeled buccal epithelial cells, with increasing concentrations of oleic acid, until a limiting concentration value, when the maximum decrease in anisotropy was attained. The decrease in fluorescence anisotropy may be interpreted as a decrease in the structural order of lipids. These reports suggest that lipid disordering in buccal mucosa may be saturable.

5. Surfactants

5.1. Introduction

The widespread use of surfactants in pharmaceutical dosage forms and cosmetic formulations, has prompted considerable interest in the influence of these agents on drug absorption, as well as their direct effect on the tissue. Both enhancement and inhibition of drug absorption have been observed in the presence of surfactants. The difficulty in interpreting the nature of surfactant action on drug absorption, arises from the different types of effect that they can exert. These include: (i) interaction with biological membranes, thereby modifying membrane permeability; (ii) interaction with the dosage form; and (iii) interaction with the drug: surfactants enhance drug dissolution and solubilize fat-soluble compounds (Gibaldi and Feldman, 1970).

5.2. Mechanism of action

5.2.1. Structure/effect relationship

Surfactants can interact with the tissues, skin or mucosal sites (nasal, rectal, buccal) in different ways. Depending on the type of surfactant used, the concentration and the exposure time, they can cause protein denaturation or extraction, enzyme inactivation, swelling of tissue and extraction of lipid components. Surfactants are able to increase

the permeability of oral mucosa, to substances with different physicochemical properties (oil-soluble compounds as well as small and large water-soluble compounds) (Siegel and Gordon, 1985a,b, 1986).

In general, it has been shown that enhancers having C_{12} chain lengths have the greatest effect on nasal (Hirai et al., 1981), rectal (Ichikawa et al., 1980) and transdermal (Aungst et al., 1986) routes. However, with buccal mucosa, this relationship is not very evident. For example, it was shown that the absorption of calcitonin was improved when using sugar esters of myristic and palmitic acids, while the esters of capric, lauric and stearic, had no effect (Nakada et al., 1988). A consistent relationship between alkyl chain length and absorption promoting effect was not found when using alkylglycoside surfactants to promote buccal insulin absorption in rats in vivo (Aungst, 1994).

Absorption has been shown to be related to the hydrophobicity of the surfactant. Unconjugated bile salts (less hydrophobic) are more effective than their conjugated counterparts in the absorption of calcitonin through buccal rat mucosa in vivo (Nakada et al., 1989). It has been found, that non-ionic ester surfactants (e.g. PEG-8 laurate), have no effects, while ether surfactants (e.g. Laureth-9), may be effective buccal absorption enhancers (Aungst and Rogers, 1989).

5.2.2. Effect on the intercellular lipids

Theories explaining the action of surfactants as promoters focus either on their effect on the intercellular lipids or on protein domains. To explain the action of a range of polyethoxylated non-ionic surfactants on the transport of methyl nicotinate across hairless mouse skin, Walters et al. (1988) proposed two possible mechanisms: initial penetration of the surfactant into the intercellular space increasing fluidity and eventually solubilizing and extracting lipid components; and second, penetration of the surfactant into the intracellular matrix and subsequent disruption within the corneocyte.

With respect to bile salts, their known lipid-solubilizing power in the intestinal tissue, may suggest that application to the buccal epithelium would result in solubilization of lipids, via micellization, from both the intercellular domain and the cell membranes. At high levels, bile salts perturb the lipid membranes of the epithelial cells, possibly facilitating transcellular transport. Above a threshold concentration, bile salt treatment is followed by a disruption of the hemidesmosomes (Hoogstraate et al., 1996a).

The effect of surfactants on the lipid domain has been widely studied using liposomes as model systems. Perturbation of the packing state of the bilayer by the presence of surfactants has been studied using different techniques. DSC has shown that this perturbation is frequently associated with a change in T_M, or a broadening of the interval over which the transition takes place. Both parameters are a linear function of the molar ratio of surfactant to phospholipid (French et al., 1988) e.g. deoxycholate and benzalkonium chloride, when mixed with dipalmitoylphosphatidylcholine (DPPC) bilayers (Chantres et al., 1996), and also dodecyl ether ethoxylates ($C_{12}E_1 - C_{12}E_8$), when incorporated into distearoylphosphatidylcholine liposomes (French et al., 1988). This may involve the localization of polar hydroxyl groups into the hydrophobic core of the bilayer (Chantres et al., 1996). Even though the effect of sodium glycodeoxycholate on T_M was less evident, the enthalpy decreased sharply. The same results were obtained with sodium deoxycholate and sodium taurodeoxycholate (Martini et al., 1996). Alkyltrialkyl and tetraalkylammonium bromides show a variety of effects on the lipid phase transition when included in DPPC bilayers (Eliasz et al., 1976). It has been shown for example, that the pretransition normally observed with these phospholipids at 34.5°C (Janiak et al., 1976) broadens and shifts to lower temperatures as the relative amount of tetrapentyl and tetrahexyl ammonium bromides increases and eventually disappears at higher concentrations. In contrast, solutions of tetramethyl-, tetraethyl-, and tetrapropyl-ammonium bromide have no effect on the DPPC endotherms, while solutions of tetrabutylammonium bromide of the same concentration, remove the pretransition endotherm, but have no effect on the main transition at 41.5°C. Hexadecyl- and octadecyl-trimethylammonium

bromide also have only a small effect on the main lipid transition. This was attributed by the authors (Eliasz et al., 1976), to the repulsive interactions between the positive charges carried by these molecules, which are expected to favour dispersion throughout the lipid bilayer; and also to their bulky polar headgroup which is thought to be projected beyond the choline headgroup. If so, a much smaller length of alkyl chain extends into the hydrocarbon chain region of the DPPC molecules compared to alcohols or monocarboxylic acids of the same length.

A spin labelling technique in which 5-doxyl stearic acid was incorporated as a probe into DPPC liposomes, was used to study the effect of Brij 36T and *n*-decyl-methyl sulphoxide (DecMSO) on the lipid bilayer (Gay et al., 1989). The results showed that DecMSO induced the greatest degree of disorder and that a higher percentage of DecMSO than Brij 36 T is tolerated by the lipid bilayer. Both enhancers are straight chain molecules but the smaller molecular weight of DecMSO could mean that it is more easily accomodated within the bilayer. It was found that both enhancers had a pronounced effect on the fluidity of the bilayer at temperatures below the T_M, where the phospholipid molecules are tightly packed.

It was shown that fluidisation of stratum corneum lipids was greatest when surfactants of intermediate HLB (hydrophilic-lipophilic balance) were present (dodecyl ether ethoxylates: $C_{12}E_3$; $C_{12}E_4$). The partitioning of a hydrophilic surfactant between the aqueous phase and the membrane lipids appears to be highly dependent on the presence of water at the skin surface. This is an important aspect, especially when considering the moistened surface of oral mucosa (French et al., 1988).

5.2.3. Effect on protein domains

Many reports suggest that surfactants act strongly on protein domains within membranes, causing denaturation or even extraction of proteins. As is the case with lipids, evidence for surfactant action on protein domains comes from DSC by the alteration of the endotherms associated with proteins and lipoproteins. It was found, for example, that treatment of rabbit buccal mucosa with surfactants such as sodium deoxycholate or sodium lauryl sulphate (SLS), significantly affected the endotherms at 105, 154 and 166°C, and that above a threshold concentration, these protein-related endotherms disappeared (Gandhi and Robinson, 1992).

Permeability experiments with salicylic acid, carried out by the same authors (Gandhi and Robinson, 1992), showed a higher enhancement factor with sodium deoxycholate than with SLS, which may be indicative of the more profound effect of sodium deoxycholate on protein domain integrity. The decrease in electrical resistance of rabbit buccal mucosa and hamster cheek pouch, produced after treatment with certain surfactants, e.g. SLS, has been explained as a disruption caused by the surfactant in the protein matrix (Araki et al., 1992; Gandhi and Robinson, 1992).

5.3. Adverse effects

As for other chemical accelerants, the effects of surfactants on biological membranes are concentration- and time-dependent, thus, larger tissue perturbation is expected when either the concentration or the contact time is increased. The observed increases in membrane permeability have been frequently related to surfactant irritative potential (Siegel and Gordon, 1986).

Oral mucosa may undergo a sloughing of the epithelial surface, resulting from the use of synthetic detergents (sodium-*N*-lauroyl sarcosinate and SLS) contained in commercial mouthrinses or toothpaste. In addition, it has been shown that the continuous use of SLS induces the appearance of ulcerations and desquamation of the oral mucosa in hypersensitive patients (Barkvoll and Rølla, 1989).

Typically, both cationic and anionic surfactants, are more potent enhancers than non-ionic compounds but they are also considered more toxic, as they can damage the permeability barrier at relatively low concentrations (Siegel and Gordon, 1985a,b, 1986). It has been shown that ionic surfactants cause injury to the keratin of the epithelium, separating the keratin layers, with loss of surface squames (Siegel and Gordon, 1985a,b),

and they can also cause swelling of the buccal cells (Gandhi and Robinson, 1992).

Minimal irritation of dog buccal mucosa in vivo was observed after 10 min of contact with 3% sodium taurocholate solution (Zhang et al., 1991). Of the steroidal detergents, fusidates were less lytic than bile salts when applied to buccal mucosa (Aungst and Rogers, 1989). Sucrose palmitate, an effective promoter for the absorption of human calcitonin in rat buccal mucosa, was less toxic than other surfactants such as sodium deoxycholate, sodium tauroglycocholate, sodium myristate and SLS, which also have a promoting effect (Nakada et al., 1988).

Penetration of surfactants into the membrane can, under certain conditions, have profoundly negative effects. It was proposed that the entry of SLS into the bloodstream when applied on rat oral epithelium implied that the surfactant came into contact with, and disrupted, both MCG and the basal lamina (Siegel and Gordon, 1985a).

Addition of secondary substances can be used to modulate the interaction between enhancers and a membrane, in this way reducing irritative effects. The addition of lecithin or oleic acid and glyceryl monooleate protects the intestinal membrane against the toxic effects of bile salts (Gibaldi and Feldman, 1970). Cyclodextrins have also been shown to reduce the damage induced by penetration enhancers (e.g. laureth-9 and bile salts) in the nasal mucosa (Jabbal et al., 1994). In addition, the T_M of DPPC bilayers showed no change when cyclodextrins were present.

5.4. Importance of vehicle and concentration

Surfactant concentration is an important factor in drug permeation. Typically, drug penetration is improved at surfactant concentrations below the critical micellar concentration (CMC). Concentrations above the CMC, may provoke a reduction of the absorption rate, due to the 'entrapment' of drug in micelles. The data suggest that in general, membranes are impermeable to the drug-micelle species. Evidence in support of this has been found using different drugs and tissues in the presence of non-ionic surfactants, e.g. the rectal absorption of sulfonamides in rats (Kakemi et al., 1965); hydrocortisone transfer across everted rat intestine (Saski, 1968); percutaneous penetration of benzocaine (Dalvi and Zatz, 1981); and the transport of thioridazine (Florence and Gillan, 1975) and barbiturates (Levy et al., 1966), across goldfish membrane.

In general, surfactants have shown a concentration-dependence in improving the absorption of substances across the different regions of the oral mucosa (Siegel and Gordon, 1985a,b, 1986). However, a clear relation was not found between CMC and the absorption enhancing effect of some surfactants, e.g. alkylglucosides (Aungst, 1994). A sigmoidal concentration dependence was observed for the enhancement behaviour of sodium glycodeoxycholate, when studying the passage of dextrans across porcine buccal mucosa. Maximal enhancement was observed with 10 mM bile salt (above CMC, which is in the order of 2–5 mM) (Hoogstraate et al., 1996a,b,c); and a 100 mM solution of sodium glycodeoxycholate significantly enhanced the permeation of morphine sulfate across bovine buccal epithelium (Senel et al., 1997). Concentrations greater than 1% of sodium glycocholate were required to increase insulin absorption in rats in vivo (Aungst and Rogers, 1989). A clear dose-related response was also observed for sodium taurocholate in enhancing the absorption of hybrid (BDBB) α-interferon in rat buccal mucosa (Steward et al., 1994). It was also found that the effect of ionic surfactants in oral mucosa is manifested at lower concentrations than that of nonionic surfactants (Siegel and Gordon, 1985a,b).

On the other hand, the promoting effect of bile salts has been related to micelle formation, which protects peptide-drugs from oral proteolytic enzymes (Aungst et al., 1988; Nakada et al., 1989). Bile salts were suggested as increasing buccal insulin absorption by solubilization of insulin monomers in micelles and the formation of reverse micelles (Aungst and Rogers, 1989). Moreover, treatment with various concentrations of sodium deoxycholate showed the progressive disappearance of the endotherms normally found in the DSC thermogram of rabbit buccal mucosa (Gandhi and Robinson, 1992).

6. Azone®: a designer enhancer

Azone® (laurocapram) is a substance that was developed specifically as a skin penetration enhancer, however, it has also been used to promote the oral mucosal absorption of different solutes, such as methyl paraben, theophylline, 5-fluorouracil, sulfanilic acid (Araki et al., 1992); propranolol (Kurosaki et al., 1988); salicylic acid (Kurosaki et al., 1989a,b) and Sandostatin® (Wolany et al., 1990). These studies have shown that Azone® enhances primarily the transport of lipophilic drugs across the keratinized oral mucosa, without a reduction in the electrical resistance of the tissue. It was also found that Azone® exerts little effect on the permeability of low lipophilicity substances (Araki et al., 1992). However, even if Azone® does not have a significant effect on ion permeability, it could facilitate the transport of anionic drugs by formation of ion pairs (Araki et al., 1992).

It has been shown that insertion of Azone® alters the packing of DPPC molecules and disrupts electrostatic interactions. The long alkyl chain of Azone® may insert into the alkyl chain regions of the lipids and the lactam grouping (more polar) might be expected to interact with the polar regions of the lipids. The conformation adopted by the Azone® headgroup depends on the interactions with the surrounding molecules (Lewis and Hadgraft, 1990).

Since hydrogen-bonding between lipid headgroups is an important factor for membrane stability (Pascher, 1976), therefore, when Azone® inserts into the intercellular lipids, it has been proposed that it forms hydrogen bonds with one adjacent molecule, leaving the other unbound, thereby creating a region of fluidity (Hadgraft et al., 1996). It has also been proposed that, when intercalated between the hydrocarbon chains of a membrane, Azone® causes lateral swelling of the lipid matrix due to its large head group (Schuckler et al., 1993). This may explain the large expansion in the mean area/molecule of DPPC films in the presence of Azone® (Lewis and Hadgraft, 1990). Fluorescence spectroscopy studies, with human buccal cell membranes, showed that Azone® altered molecular movement on the surface of the bilayers. A decrease in anisotropy following Azone® treatment suggests again, an increase lipid fluidity (Turunen et al., 1994).

Numerous studies have also shown that Azone® interacts extensively with membrane lipids, increasing lipid fluidity and reducing T_M. Increasing the concentration of Azone® in DPPC bilayers caused a linear reduction in T_M (Beastall et al., 1988; Schuckler et al., 1993; Harrison et al., 1996).

Scanning electron microscopy studies showed loosening of cells and increased intercellular spaces in stratum corneum treated with Azone® (Ganga et al., 1996). Goodman and Barry (1985) found using DSC that treatment of stratum corneum simultaneously with 3% Azone® and 0.1% Tween 20 for 24 h caused the lipid associated peaks to disappear, in a similar way to the thermogram obtained with a delipidised stratum corneum sample. These changes in lipid fluidity are closely related to membrane permeability.

With respect to the toxicity of Azone® derivatives, it was shown that toxicity in cultured human skin cells increased with chain length, from C_2 to C_8. It remained constant between C_8 and C_{14}, and decreased with longer chains. Since a similar trend has been observed for flux enhancement with Azone®, this again suggests that a parallelism exists between skin cell toxicity and penetration enhancing effect (Ponec et al., 1990).

7. Ethanol

One of the most common agents to be introduced into the mouth is alcohol. Even though there is little information about the direct effect of ethanol on the human oral mucosa (Hillman and Kissin, 1980), long-term ethanol administration has been reported to cause severe histological and physiological changes (Martin and Pangborn, 1971; Becker, 1979; Wang et al., 1992). Furthermore, there is evidence related to the enhanced absorption of carcinogens, when tobacco and alcohol are used together; alcohol may exert a topical effect improving absorption into the underlying tissues of the oral cavity (Squier et al., 1986).

The action of ethanol on the thermotropic properties of phosphatidylcholine (PC) liposomes has been widely studied. Some of these studies (Rowe, 1983, 1985) have shown that ethanol has a biphasic effect on the gel-to-liquid crystalline phase transition of PC depending on its concentration. Ethanol has two distinct interactions with PC: at a low ethanol concentration there is a linear decrease of T_M explained by increased ethanol partitioning in the liquid-crystalline phase as compared to the gel phase. However, at a certain threshold ethanol concentration, there is a sharp change in T_M, which marks the induction of an irreversible transition from a normal gel state into an interdigitated state, in which it partitions preferentially into the gel phase, anchoring its hydroxyl group to the bilayer interface. DSC studies show that pretreatment of stratum corneum with ethanol, shifts the T_M of the endotherms related to lipid phase changes to lower values, with no apparent effect on the protein-related transitions (Golden et al., 1986, 1987a,b; Corbo et al., 1990; Francoeur et al., 1990; Gandhi and Robinson, 1992).

Several authors (Kurihara-Bergstrom et al., 1990; Manabe et al., 1996) reported increasing extraction of stratum corneum lipids with increasing ethanol concentration. The effect of ethanol on human buccal epithelial cells has been studied by fluorescence anisotropy (Turunen et al., 1994), the authors attributed the decrease in anisotropy to a reorganization of the lipid domains following lipid extraction, as the ethanol concentration was increased.

The enhancer function of ethanol may, however, involve action on protein domains, by changing conformation or by denaturing proteinaceous material, in addition to lipid extraction (Kurihara-Bergstrom et al., 1990; Manabe et al., 1996). Ethanol may also influence the physicochemical properties of the solute, by altering its solubility in the vehicle (Obata et al., 1993) and in the membrane (Coutel-Egros et al., 1992), thereby changing the partition coefficient. Increasing amounts of ethanol in the vehicle may favour the passage of ionic solutes as ion-pairs (Kurihara-Bergstrom et al., 1990; Maitani et al., 1991; Obata et al., 1993; Ganem et al., 1996). Furthermore, it has been demonstrated that the flux of ethanol itself, could enhance the passage of a solute by a cotransport mechanism (Berner et al., 1989a,b; Liu et al., 1991).

8. Concluding remarks

One of the most important advantages afforded by the absorption of drugs from the oral cavity is the possibility of bypassing gastrointestinal and hepatic enzymes. However, as the oral mucosa can be an effective barrier to the absorption of several solutes, particularly peptides, the coadministration of a penetration enhancer may be necessary to improve drug absorption. When studying the action of an enhancer on oral mucosa, three factors might be considered: the physicochemical properties of the enhancer (i.e. nature, conformation, degree of unsaturation, ionization state and chain length); the structural organization within the membrane (e.g. lipidic composition and organization), taking also into account the variations in the different regions of the oral mucosa (keratinized and nonkeratinized); and the physicochemical characteristics of the drug. Understanding the mechanism of action of an enhancer may help not only to design more effective agents, but to minimise or abolish toxic effects on oral mucosa, thereby not only improving delivery but increasing patient compliance.

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