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Effects of surfactants on the absorption of salicylic acid from hamster cheek pouch as a model of keratinized oral mucosa

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Summary

The effect of surfactants on the absorption of salicylic acid from keratinized oral mucosa was investigated by hamster cheek pouch method in vivo at pHs 3.0, 4.0 and 7.0. Four surfactants, sodium laurylsulfate (SLS), cetylpyridinium chloride (CPC), polysorbate 80 (PS-80) and sodium taurocholate (STC), were examined as adjuvants. The interaction between salicylic acid and each surfactant was determined by the molecular sieve method using Sephadex G-25. Decreased absorption of salicylic acid by the presence of PS-80 was observed in the lower pH conditions and this phenomenon was explained by the decrease in the free fraction of salicylic acid. The absorption of salicylic acid in the presence of ionic surfactants, SLS or CPC, was much larger than that predicted by the loss of activities which was caused by the interaction between the surfactant and the drug molecule. Pretreatment with SLS or CPC brought the salicylic acid absorption to increase in all pH conditions examined and the effects were dependent on the surfactant concentration. STC and no effect on the absorption. The mechanisms of the effects of ionic surfactants on the permeability of keratinized oral mucosa were discussed.

Introduction

When drugs are absorbed from the oral cavity, they can avoid both the exposure to gastrointestinal juices and the hepatic first-pass elimination. So the application of drugs to this site seems to have an advantage for the systemic administration (Bell et al., 1985; Hussain et al., 1986, 1987). The permeability of oral mucosa is superior to that of skin (Squier et al., 1985a). However, the permeability of oral mucosa, especially keratinized parts, is inferior to that of gastrointestinal mucosa in general.

It is well known that surfactants interact with not only drug molecules but also with biological membranes and that consequently they may alter the drug absorption (Gibaldi and Feldman, 1970; Attwood and Florence, 1983). However, there is little information concerning the influence of surfactants on the oral mucosal absorption. Siegel and Gordon (1985) reported that ionic surfactants such as sodium laurylsulfate (SLS) and cetylpyridinium chloride (CPC) increased the permeability of non-keratinized oral mucosa to several substances though polysorbate 80 (PS-80), a non-ionic surfactant, did not bring on any increases.

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We have developed an experimental method to investigate the permeability of keratinized oral mucosa using a hamster cheek pouch as a model membrane in vivo (Kurosaki et al., 1986). In this study, we examined the absorption of salicylic acid from keratinized oral mucosa in the presence of surfactants using the hamster cheek pouch method and analyzed the effects of surfactants on the interaction between drug molecules and surfactant micelles and on the change in the permeability of the keratinized oral mucosa.

Materials and Methods

Chemicals

Salicylic acid was purchased from Nakarai Chemical Co. (Kyoto, Japan). Polyoxyethylene sorbitan monooleate (PS-80), SLS and CPC were purchased from Tokyo Kasei Kogyo Co. (Tokyo, Japan). Blue dextran 2000 and Sephadex G-25 (Fine) were purchased from Pharmacia Fine Chemicals (Uppsala, Sweden). Sodium taurocholate (STC) was synthesized according to the method of Norman (1955) and was chromatographically pure. All other chemicals used were of the finest grade available.

Procedure of absorption experiments

Absorption from keratinized oral mucosa was determined by the hamster cheek pouch method described previously (Kurosaki et al., 1986). The swallowing during the experiment is negligible in this method. Male golden hamster (100–120 g body weight) were used under urethane anesthesia (1.5 g/kg, i.p.) and the cheek pouch was cleaned with saline. Salicylic acid solution (5 mM) was prepared with isotonic buffer solutions (citric acid–Na_2HPO_4 for pH 3.0–5.0 and NaH_2PO_4–Na_2HPO_4 for pH 6.0–7.0) containing a surfactant at an appropriate concentration. One ml of the drug solution was administered into the cheek pouch. After 1 h, the luminal contents were withdrawn and the cheek pouch was washed with the saline. The washings were combined with the luminal contents and made up to 20 ml with saline. Then the amount of salicylic acid recovered from the lumen was determined. Since the amount absorbed correlated well with that which disappeared from the lumen in general (Kurosaki et al., 1986), the latter was defined as the apparent absorption in this paper. In the case of the pretreatment experiment, 1.5 ml of the pretreatment solution made by dissolving the surfactant at an appropriate concentration in isotonic buffer solution (pH 7.0) was administered into the cheek pouch for 2 h and washed 5 times with 3 ml each time of saline. Then the absorption experiment was carried out without surfactant for 1 h in the same manner described above.

Measurement of micellar complex formation

Micellar complex formations between salicylic acid and the surfactants were determined by a molecular sieve technique of Ashworth and Heard (1966) using Sephadex G-25 as a molecular seive. Sephadex G-25 (1.000 g, dry weight) was allowed to swell in 10.0 ml of the sample solution and then incubated at 37 °C for 4 h with gentle shaking. The volume of the external phase of the molecular seive was previously determined in a similar manner by an unretained macromolecular-marker compound, Blue dextran 2000 (mean mol. wt. = 2,000,000). Free fraction (= free/(free + micellar)) of salicylic acid was calculated from the concentrations in the internal phase and the external phase of the gel network at 37 °C.

Analytical methods

(i) Salicylic acid. The sample solution was made acidic with 1.0 ml of concentrated HCl and salicylic acid was extracted with 5 ml of chloroform. From an aliquot of the organic layer, salicylic acid was re-extracted with 0.1 N NaOH solution and the resulting alkaline aqueous layer was determined spectrophotometrically at 296 nm.

(ii) Blue dextran 2000. The concentration of Blue dextran 2000 in the bulk phase was determined colorimetrically at 615 nm.

Statistical analysis

Results were expressed as the mean ± one S.E. The statistical analysis was carried out by the Student's *t*-test.

Results

Salicylic acid absorption in the presence of surfactants

Hamster cheek pouch, where the absorption obeys the pH–partition hypothesis (Kurosaki et al., 1986, 1987a), was used as a model for keratinized oral mucosa. The effects of surfactants, SLS, CPC, PS-80 and STC, on the absorption of salicylic acid from keratinized oral mucosa were examined by the hamster cheek pouch method in vivo (Kurosaki et al., 1986) at pHs 3.0, 4.0 and 7.0, and the results are shown in Fig. 1. *Control* means the absorption without surfactants. The absorption of salicylic acid ($pK_a = 3.0$) from the cheek pouch in 1 h decreased from 49.8 ± 1.1% to 0.2 ± 0.1% as the pH value of the drug solution increased from 3.0 to 7.0, due to the reduction of its unionized fraction from 50% to 0.01%. When 20 mM SLS was present, the absorption of salicylic acid was not affected in the lower pH region, but was significantly increased to approximately 8% at pH 7.0 where almost all the salicylic acid molecules exist in the ionized form. On the contrary, the absorption of salicylic acid was significantly decreased in the lower pH region when 20 mM CPC or 5% PS-80 was present. 20 mM STC showed no effect on salicylic acid absorption in any conditions examined.

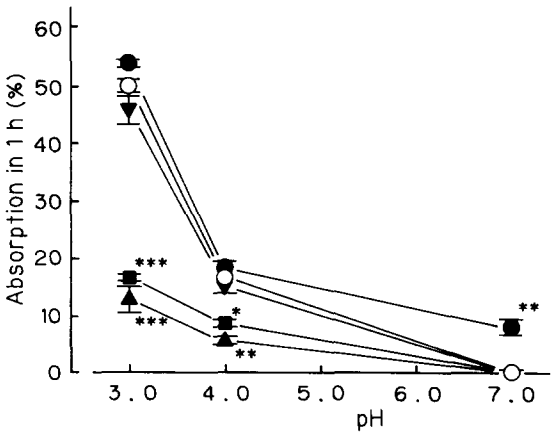

Fig. 1. Effect of surfactants on salicylic acid absorption from hamster cheek pouch. Control (○), 20 mm SLS (●), 20 mM CPC (▲), 5% PS-80 (■), and 20 mM STC (▼). Results are expressed as the mean ± S.E. of at least 4 experiments. Significantly different from corresponding control at each pH condition: * $P < 0.05$; ** $P < 0.01$; *** $p < 0.001$.

TABLE 1

Micellar complex formations between salicylic acid and surfactants

Surfactant	Free fraction (%)		
	pH 3.0	pH 4.0	pH 7.0
20 mM SLS	81.0	95.2	97.1
20 mM CPC	3.3	2.7	0.4
5% PS-80	31.4	55.2	67.9
20 mM STC	89.7	95.0	92.2

Interactions between salicylic acid and surfactants

Drug–surfactant interactions such as the micellar complex formation or the intermolecular electrostatic interaction may reduce the thermodynamic activity of the administered drug which is closely associated with the absorption (Kakemi et al., 1970a). To determine the degree of salicylic acid–surfactant interaction, the molecular sieve technique using Sephadex G-25 as the molecular sieve was employed. Free fractions of salicylic acid in the administered drug solutions were calculated and were summarized in Table 1. The interactions between salicylic acid and STC were almost negligible in all conditions tested. Likewise, SLS, an anionic surfactant, showed little interaction with salicylic acid especially in the higher pH region and the slight interactions observed at pH 3.0 were assumed to arise from the decrease in electrostatic repulsions between SLS molecules and the salicylic acid anions. On the other hand, PS-80, a non-ionic surfactant, showed more marked interactions with salicylic acid which were highly dependent on the dissociation of salicylic acid. CPC, a cationic surfactant, interacted most markedly with salicylic acid according to their strong electrostatic interactions in all the conditions examined. If the surfactants have no effect on the permeability of keratinized oral mucosa, the absorption of salicylic acid was presumed to be directly in proportion to the thermodynamic activity of salicylic acid in the presence of surfactants. Thus, the mean values of salicylic acid absorption with surfactants, shown in Fig. 1,

were normalized against corresponding controls under each pH condition. Values at pH 7.0 were omitted since the control was quite small at this pH. Then the normalized values were plotted against the percentages of the free fraction (Table 1) to evaluate the effect of surfactants on the permeability of hamster cheek pouch to the drug (Fig. 2). It is obvious from Fig. 2 that the points for PS-80 and STC were located exactly on the dotted line, showing that the absorption was merely proportional to the thermodynamic activity of salicylic acid. However, the normalized values for ionic surfactants, SLS and CPC, became rather larger than expected and the plots were located significantly upward along the line. It is suggested from these results that both SLS and CPC caused an increase in mucosal permeability of hamster cheek pouch to salicylic acid but the interactions of CPC with salicylic acid were too high to enhance the absorption of salicylic acid in vivo (Fig. 1).

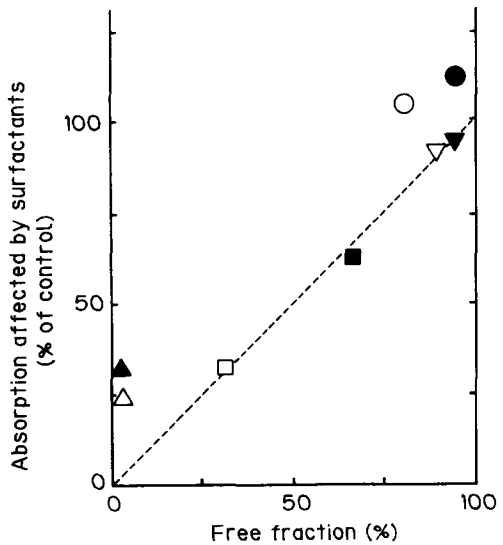

Fig. 2. Plots of the effect of surfactants on salicylic acid absorption from hamster cheek pouch against the free fraction in the surfactant solution. The absorption of salicylic acid with 20 mM SLS (○, ●), 20 mM CPC (△, ▲), 5% PS-80 (□, ■), and 20 mM STC (▽, ▼), at pH 3.0 (open symbols) and at pH 4.0 (closed symbols), were expressed as % of control at each pH condition. Each point represents the mean of at least 4 experiments. Dotted line represents the ideal case that the absorption is proportional to the free fraction of salicylic acid (see text).

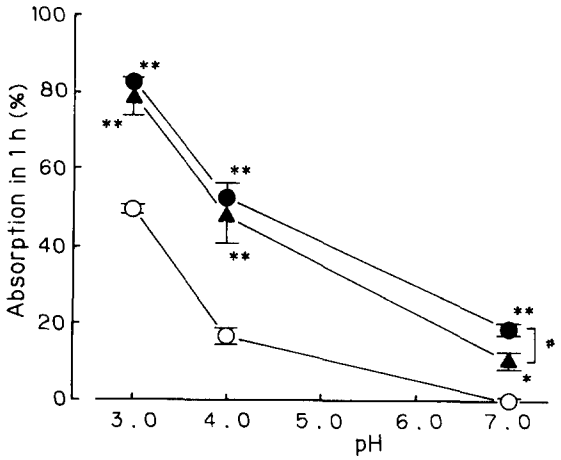

Fig. 3. Salicylic acid absorption from hamster cheek pouch after 2 h pretreatment with ionic surfactants. Control (○), 20 mM SLS (●), and 20 mM CPC (▲). Results are expressed as the mean ± S.E. of at least 4 experiments. Significantly different from corresponding control: ** $P < 0.01$; *** $P < 0.001$. Significantly different in each surfactant: $^{\#}P < 0.05$.

Effect of pretreatment with ionic surfactants on absorption of salicylic acid

To clarify the effects of ionic surfactants on the permeability of keratinized oral mucosa, the absorption was examined after the pretreatment of the cheek pouch with ionic surfactants. As shown in Fig. 3, the absorption of salicylic acid increased significantly after the pretreatment with 20 mM solution of SLS or CPC regardless of the dissociation of salicylic acid. Although the effects of SLS and CPC were nearly identical to each other at pHs 3.0 and 4.0, the enhancement caused by SLS was significantly greater ($P < 0.05$) than that by CPC at pH 7.0. Table 2 summarizes the effect of the concentration of pretreating surfactants on the permeability of cheek pouch mucosa to salicylic acid at pH 7.0. The pretreatment with 1.0 mM solution of either ionic surfactants did not bring about a significant increase in permeability in comparison with that of a simple buffer solution (expressed as *Control* in Table 2). However, when the pretreatment was carried out with the solution of concentration higher than 5.0 mM surfactant, the absorption increased significantly. The effect of SLS on lowering the barrier function of cheek pouch mucosa to the hydrophilic compound appeared more intense than that of CPC

TABLE 2

Effect of the concentration of pretreating ionic surfactant on the permeability of the cheek pouch mucosa to salicylic acid at pH 7.0

Results are expressed as the mean ± S.E. with the number of experiments in parentheses.

Concentration of surfactant (mM)	Absorption in 1 h (%)	
	SLS	CPC
Control [a]	1.4 ± 0.4 (4)	
1.0	1.9 ± 0.3 (4)	2.8 ± 0.8 (4)
5.0	8.5 ± 0.9 (3) *	6.2 ± 0.6 (4) *
20.0	19.0 ± 1.7 (8) *	10.3 ± 2.5 (4) *
		#

[a] Cheek pouch was pretreated with isotonic buffer solution (pH 7.0) for 2 h.

Significantly different from control: * $P < 0.01$. Significantly different in each surfactant: # $P < 0.05$.

when compared at 20 mM ($P < 0.05$). These findings indicate that the two ionic surfactants can lower the barrier function of the keratinized oral mucosa to polar substances.

Discussion

It is generally recognized from the human buccal absorption tests that the absorption of drugs from whole oral cavity obeys the pH–partition hypothesis which is well explained by the passive diffusion mechanism (Beckett and Moffat, 1969, 1970; Achari and Beckett, 1982). To investigate the permeability of keratinized oral mucosa, we developed an in vivo experimental method using a hamster cheek pouch as a model for the keratinized oral mucosa and clarified that the absorption from the cheek pouch also depends on both the lipophilicity and the ionization of the test substance (Kurosaki et al., 1986). In addition, we could clarify that the unstirred water layer adjacent to the mucosal surface of the cheek pouch plays a part in the barriers against drug permeation in vivo and that the effect of the unstirred water layer on the absorption of salicylic acid is negligible when the pH of the drug solution was above its pK_a (Kurosaki et al., 1987a). Tanaka et al. (1980) investigated the absorption of salicylic acid applied to hamster cheek pouch as ointments. They showed that salicylic acid was absorbed faster from ointment bases containing surfactants than from bases without surfactants, while the complexity of the formulations complicates the detailed analysis. In this study, we determined the absorption and the micellar complex formation of salicylic acid ($pK_a = 3.0$) in the presence of surfactants at pHs 3.0, 4.0 and 7.0. In addition, the pretreatment experiments were carried out to examine the direct effect of surfactants on cheek pouch mucosa.

It is demonstrated that surfactants can influence the rate and the extent of drug absorption (Attwood and Florence, 1983). As shown in Fig. 1, the enhancement of the absorption as well as the inhibition was observed depending on the surfactant and pH of the solution. Surfactants can interact not only with drug molecules but also with biological membranes, and consequently may alter the drug absorption (Gibaldi and Feldman, 1970). As to passively absorbed drugs, the absorption rate might be directly proportional to the concentration of the drug molecules as the free-form. So, when the drug molecules form the micellar complexes with surfactants, the absorption might be decreased (Kakemi et al., 1970a; Dalvi et al., 1981; Miyamoto et al., 1983). Withington and Collett (1973) showed that PS-80 in the drug solution markedly decreased the apparent transfer rate constant for salicylic acid across a cellophane membrane at low pH conditions, suggesting that the drug in the micelle did not participate in the transfer process. The present study showed that 5% PS-80 decreased the free fraction of salicylic acid to 31.4% and 55.2% at pH 3.0 and pH 4.0, respectively (Table 1). These results agree well with those reported by Collett and Withington (1973). On the other hand, Siegel and Gordon (1985) reported that PS-80 has no effect on the permeability of rat non-keratinized oral mucosa to butyric acid at pH 6.5. The results shown in Fig. 2 for PS-80 are compatible with these findings.

It has been reported that bile salts influence membrane permeability and drug absorption. For example, the enhanced absorption by STC were reported in intestinal mucosa (Kakemi et al., 1970b), in nasal mucosa (Hirai et al., 1981a) and

in cornea (Morimoto et al., 1987). However, the effect of STC on the absorption of salicylic acid from the keratinized oral mucosa was negligible (Figs. 1, and 2), suggesting the difference in the mucosal properties.

Surfactants may also be capable of modifying the properties of biological membranes. It has been reported that there are some relations between the solubilization of membrane components by surfactants and the increased absorption of solutes. Yasuhara et al. (1979) showed the relation between the protein release from the mucosal membrane and the increased intestinal absorption of *p*-acetamidobenzoic acid in the presence of surfactants. Whitmore et al. (1979) examined the release profiles of proteins and phospholipids from the intestinal tissue and concluded that the release of a comparatively small amount of protein is accompanied by a large increase in the absorption of salicylate, whereas a much larger release of phospholipids was necessary to increase the absorption. SLS shows almost 6-times higher hemolytic activity and the protein-releasing action of the nasal mucosa compared with STC (Hirai et al., 1981b). The differences in the enhancement effect observed in this study among SLS, CPC and STC may be attributable to the differences in these activities against the keratinized oral mucosa. Some attempts to characterize the permeability barrier of the oral mucosa have been made microscopically (Squier, 1984; Squier and Hall, 1985a and b) or biochemically (Wertz et al., 1986). Squier and Hall (1985a and b) showed that the functional permeability barrier of the oral mucosa is located in the epithelium and occupies the superficial layers similar to the skin. We showed that the permeability coefficients of the stratum corneum of hamster cheek pouch for salicylic acid did not differ from those obtained in the full-thickness tissue (Kurosaki et al., 1987b). Keratinized regions of the oral mucosa contain significant quantities of acylglycosylceramide, acylceramide and ceramide, all of which are characteristic lipid components of the epidermis, while non-keratinized regions possess more glycosylceramide (Wertz et al., 1986). Intercellular lipids are assumed to play an important role in maintaining the barrier function of the stratum corneum to polar substances. The ionic surfactants would probably interact with these lipids and increase the permeability of keratinized oral mucosa, though the detailed mechanisms remain unsolved. Our pretreatment experiments demonstrated that the effect of ionic surfactants on the permeability of keratinized oral mucosa is dependent on their concentrations and the enhancing effect of SLS was significantly larger than that of CPC when the pretreatment was carried out at a concentration of 20 mM (Table 2). The absorption of SLS itself through the non-keratinized oral mucosa is also concentration-dependent; that is, the percentage absorbed increases as its concentration increases (Siegel and Gordon, 1985). These results suggest that SLS causes increasing damage to the permeation barrier as its concentration increases.

In conclusion, surfactants alter the drug absorption from oral mucosa by two mechanisms; the drug–surfactant interaction and the direct action of the surfactant on the mucosal membrane. The ionic surfactants, SLS and CPC, diminish the barrier function of keratinized oral mucosa especially to polar substances. Further investigations concerning the mechanism of the permeability enhancement induced by these surfactants are a prerequisite to evaluate available promoters for drug absorption from oral mucosa.

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