Regional Variation in Oral Mucosal Drug Absorption: Permeability and Degree of Keratinization in Hamster Oral Cavity

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The regional permeability of oral mucosa to salicylic acid was investigated *in vivo* in hamsters along with histological variations, especially the degree of keratinization. Histological sections from six regions, i.e., sublingual mucosa, buccal mucosa, dorsum of tongue, ventral surface of tongue, labial mucosa, and cheek pouch mucosa, were prepared to assess the degree of keratinization. The area under the plasma concentration–time curve of salicylic acid following the administration of salicylic acid to the oral mucosa with a film dosage form and the thickness of stratum corneum of each site were in inverse proportion to each other, suggesting that the stratum corneum layer represents the principle barrier to drug absorption.

KEY WORDS: oral mucosa; permeability; histological variation; stratum corneum; hamster; salicylic acid.

INTRODUCTION

Drugs absorbed via the oral–mucosal route avoid both intraalimentary canal and hepatic first-pass eliminations (1–3). However, the oral mucosae were not well characterized in terms of permeability. We have investigated the absorption characteristics from keratinized oral mucosa using a hamster cheek pouch mucosa (4–8). Regionally different oral mucosae exhibit marked variations in degree of keratinization or thickness of stratum corneum layer (9). Squier and Hall reported that the keratinized porcine oral mucosa was significantly less permeable to water and horseradish peroxidase than the non-keratinized one (10). However, the role of the stratum corneum layer in barrier property has not previously been studied with simultaneous morphological assessment of each mucosa.

In this study, the relationship between the *in vivo* permeability barrier characteristics of oral mucosae from different regions to salicylic acid and the degree of keratinization was investigated in the hamster.

MATERIALS AND METHODS

Chemicals

Salicylic acid was purchased from Nacalai Tesque Co. (Kyoto, Japan). Hydroxypropylcellulose (HPC; HPC-L) was supplied from Nippon Soda Co. (Tokyo). All other chemicals used were of the finest grade available. Isotonic buffer solution used was citric acid–Na_2HPO_4 (pH 3.0).

Animals

Male golden hamsters (100–120 g) were used under urethane anesthesia (1.5 g/kg, i.p.).

Histological Assessment of Oral Mucosae

Six regions of hamster oral mucosa were surgically removed. They were the sublingual mucosa from the floor of the mouth adjacent to the lingual frenum (sublingual mucosa), the posterior part of the buccal mucosa adjacent to the molar teeth (buccal mucosa), the mucosa of the dorsal surface of corpus linguae (dorsum of tongue), the mucosa of the ventral surface of corpus linguae (ventral surface of tongue), the labial mucosa from the inside of the lower lip (labial mucosa), and the mucosa of the middle part of a cheek pouch (cheek pouch mucosa). Small blocks of each region were fixed in formalin and embedded in paraffin wax, and the sections, 6 μm thick, were stained with hematoxylin and eosin and examined with a light microscope. Two nonserial sections showing the narrow elongate connective tissue papillae were used for histological assessment. The thicknesses of both the stratum corneum layer and the epithelial layer (except the stratum corneum layer) of tops and bottoms of five papillae were determined in every section. In selecting the sites for determination, care was taken not to slant the cross sections of their basal cells. The thickness of papillae was also determined in three mucosae; ventral surface of tongue, dorsum of tongue, and buccal mucosa, where the papillae were well developed.

Preparation of Film-Dosage Form Containing Salicylic Acid

Film-dosage forms were prepared with HPC according to our previous paper (3). A mixture of 2.0 g of HPC-L and 280 mg of salicylic acid was dissolved in 25 ml of ethanol, and 0.75 ml of isotonic buffer solution (pH 3.0) and 0.2 g of polyethylene glycol 300 were added to the ethanolic solution. The pH of the resulting viscous solution was 2.9–3.1. This viscous solution of HPC containing salicylic acid was molded into a Teflon tray and dried at 60°C for 24 hr. The film-dosage form thus prepared was nearly transparent and approximately 0.3 mm thick. The apparent content of salicylic acid was 43.3 μmol/cm^2.

Absorption Experiments of Salicylic Acid *in Vivo*

Solution

A plastic cell system (4-mm i.d.), shown in Fig. 1, was newly designed. Salicylic acid dissolved in the isotonic buffer solution (pH 3.0) was applied (15 μmol/0.5 ml/kg) to the cell system, which was fixed on the surface of an oral mucosa of hamster with cyanoacrylate tissue cement (Aron Alpha, Toa Chemicals Co., Tokyo). Because of the limitation of mucosal area, the mucosae capable of investigation

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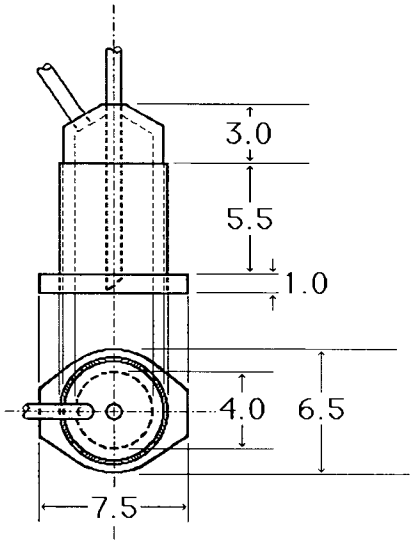

Fig. 1. Schematic representation of an absorption cell. The unit of length represented is millimeters.

were restricted to four mucosae: sublingual mucosa, dorsum of tongue, ventral surface of tongue, and cheek pouch mucosa. Blood samples were then collected periodically from the carotid artery and plasma concentrations of salicylic acid were determined by high-performance liquid chromatography (HPLC) equipped with a fluorescence detector at 300 and 430 nm for excitation and emission, respectively, as described previously (4,8).

Film-Dosage Form

Aluminum foil was used as a backing of the film-dosage form containing salicylic acid, not to contact the back surface of the preparation with other mucosae in application. The preparation was cut with a circular steel punch (5-mm i.d.), and the resulting disk preparation containing salicylic acid (8.5 μmol/piece) was administered onto an oral mucosa. The preparation adhered easily to the mucosa and swelled gradually (3). The plasma concentrations of salicylic acid were periodically determined by HPLC in a similar manner to that described above.

Evaluation of Absorption Characteristics

The area under the plasma concentration–time curve

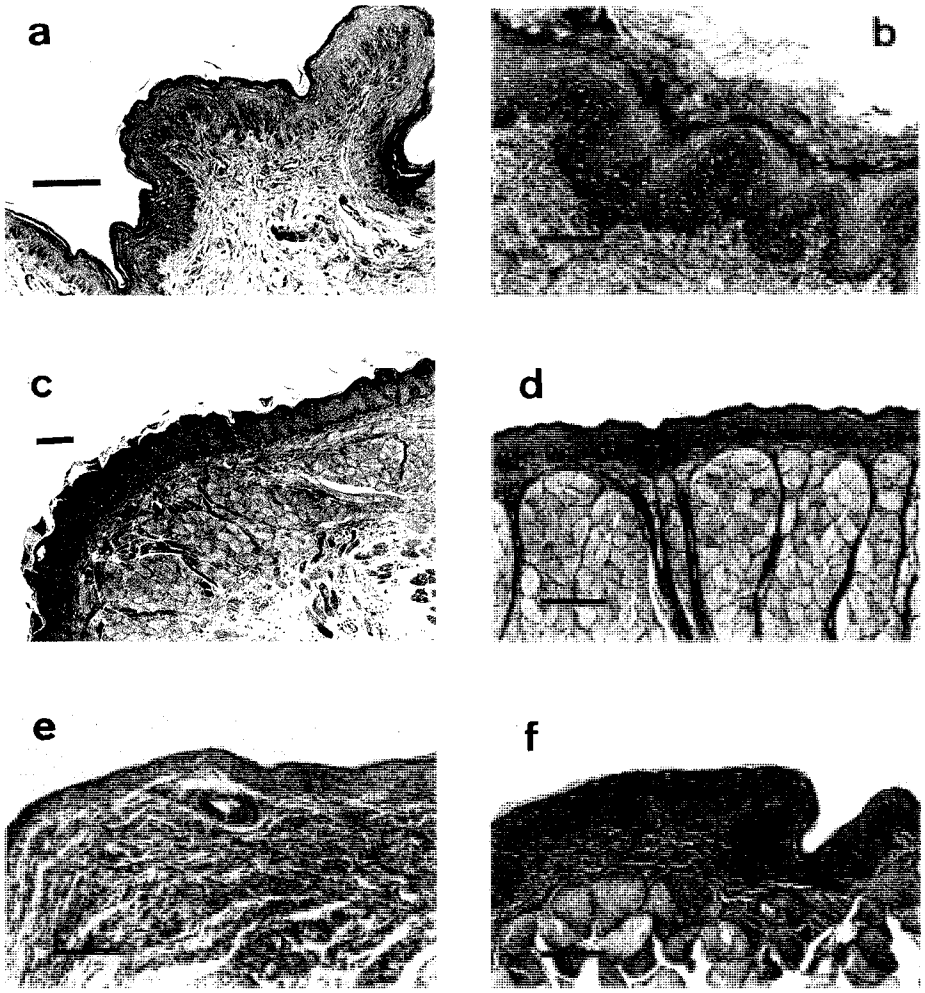

Fig. 2. Photomicrographs of histological sections of hamster oral mucosa stained with hematoxylin and eosin. Sites: (a) sublingual mucosa; (b) buccal mucosa; (c) dorsum of tongue; (d) ventral surface of tongue; (e) labial mucosa; (f) cheek pouch mucosa. Each bar represents 100 μm.

Table I. Histological Variations in Hamster Oral Mucosae

Region	Thickness (μm)[a]		
	Stratum corneum	Epithelium	Papillae
Sublingual mucosa	3.3 ± 1.4	42.4 ± 9.2	Short or absent
Buccal mucosa	73.5 ± 37.0	115.3 ± 11.5	Long (>100 μm)
Dorsum of tongue	29.0 ± 9.8	82.5 ± 12.3	Long (>50 μm)
Ventral surface of tongue	12.3 ± 3.1	50.9 ± 7.8	Short
Labial mucosa	7.8 ± 2.4	40.6 ± 9.9	Short or absent
Cheek pouch mucosa	6.6 ± 1.9	34.1 ± 8.0	Absent

[a] Results are expressed as the mean ± SE of 20 measurements.

(AUC) for salicylic acid was calculated by the trapezoidal method.

RESULTS AND DISCUSSION

Histological Assessment of Oral Mucosae

Histological sections of six regionally different oral mucosae of hamsters, i.e., sublingual mucosa, buccal mucosa, dorsum of tongue, ventral surface of tongue, labial mucosa, and cheek pouch mucosa, were stained with hematoxylin and eosin. Photomicrographs of these sections are shown in Fig. 2. Histological variations assessed from nonserial sections of these mucosae are summarized in Table I. Marked variations could be observed in the degree of keratinization and the order of the thickness of stratum corneum was sublingual mucosa < cheek pouch mucosa < labial mucosa < ventral surface of tongue < dorsum of tongue ≪ buccal mucosa. Hamster buccal mucosa is highly keratinized, though human (9) and porcine (10) buccal mucosae are nonkeratinized. However, as to the thickness of the epithelial layer, the sublingual mucosa, cheek pouch mucosa, labial mucosa, and ventral surface of tongue showed similar thicknesses. The buccal mucosa and dorsum of tongue possess relatively thicker epithelia compared with those of the other four mucosae examined. Well-developed papillae, which are found in masticatory mucosae being particularly susceptible to the stresses and strains of masticatory activity (9), were observed in buccal mucosa and dorsum of tongue. A secretory duct was observed in sublingual mucosa.

Regional Variation in Salicylic Acid Absorption

Some investigators used perfusion-cell systems for studying the *in vivo* absorption characteristics of drugs from the oral mucosa in humans (11) and in dogs (12,13). However, the size and the weight of these perfusion systems are inadequate to apply in hamsters. We designed a plastic cell (Fig. 1) to show the regional variation in salicylic acid absorption from hamster oral mucosae. Plasma concentration–time profiles of salicylic acid applied as the solution form (15 μmol/0.5 ml/kg, pH 3.0) to the cell fixed on sublingual mucosa, cheek pouch mucosa, ventral surface of tongue, and dorsum of tongue are shown in Fig. 3. A regional variation in the profiles is evident. The maximal plasma concentrations (C_{max}) were attained at 45, 60, 120, and 180 min in the sublingual mucosa, ventral surface of tongue, cheek pouch mucosa, and dorsum of tongue, respectively. The C_{max} observed in sublingual mucosa was approximately 4.5 times higher than that in the dorsum of tongue. The cell system could be applied only onto these four mucosae, whereas it could not be physically applied onto labial and buccal mucosae.

Mucoadhesive devices make it possible to study the regional differences in oral mucosal permeability more precisely. Several mucoadhesive devices have been reported for oral mucosal application (11,14–16). The application of HPC to mucoadhesive dosage forms has also been attempted to obtain suitable mucosal adhesion properties (3,17). In order to discuss the relationship between the permeability to salicylic acid and the morphological characteristics of oral mucosae, we prepared a mucoadhesive film-dosage form containing salicylic acid with HPC. A disk preparation (5 mm in diameter and approximately 0.3 mm thick) was successfully applied onto every mucosa examined. The plasma concentration–time profiles of salicylic acid after application of the film-dosage form (8.5 μmol/piece) are shown in Fig. 4. Similar to the results in Fig. 3, the absorption of salicylic acid from sublingual mucosa, where the thickness of the stratum corneum layer is the thinnest (Table I), was superior to that from other mucosae examined. The absorption from buccal mucosa or dorsum of tongue, where the stratum corneum layer is highly developed, was inferior to that from the moderately keratinized mucosae.

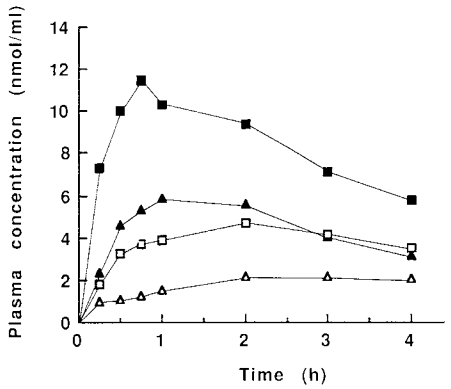

Fig. 3. Plasma concentrations of salicylic acid following the administration on various sites of hamster oral mucosa as the pH 3.0 buffer solution. Dose of salicylic acid was 15 μmol/0.5 ml/kg. Application sites: (■) sublingual mucosa; (△) dorsum of tongue; (▲) ventral surface of tongue; (□) cheek pouch mucosa. Results are expressed as the mean of at least four experiments.

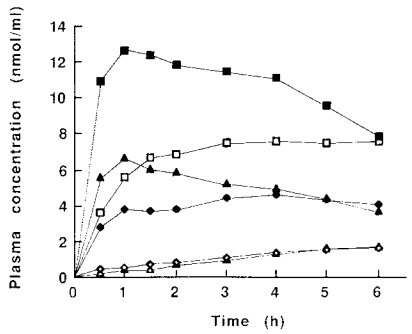

Fig. 4. Plasma concentrations of salicylic acid following the administration on various sites of hamster oral mucosa as the HPC film-dosage form. Dose of salicylic acid was 8.5 μmol/head. Application sites: (■) sublingual mucosa; (◇) buccal mucosa; (△) dorsum of tongue; (▲) ventral surface of tongue; (◆) labial mucosa; (□) cheek pouch mucosa. Results are expressed as the mean of at least three experiments.

Drug absorption through epithelia might be restricted by at least two significant barriers. One is an enzymatic barrier for drugs that are highly metabolized during the absorption process (18,19). The other is a physicochemical barrier for all drugs diffusing through the tissue. The biodegradation of salicylic acid during oral mucosal absorption is negligible in hamsters (4). Therefore we can discuss the latter barrier properties of hamster oral mucosae from the plasma concentration–time curves in relation to their characteristics determined by morphological assessment (Table I).

Squier and Hall showed that the keratinized oral mucosa was significantly less permeable to tritiated water and horseradish peroxidase than the nonkeratinized ones in the pig *in vitro* (10). Garren and Repta reported that the superficial keratinized layer of the epithelial lining of the hamster cheek pouch provided the permeability barrier in this tissue (20). Further, we have reported that the *in vitro* permeability of isolated stratum corneum sheet of hamster cheek pouch to salicylic acid is similar to that of full-thickness cheek pouch mucosa (7). Accordingly, the stratum corneum layer is generally regarded as the major permeability barrier of the keratinized oral mucosa to drug absorption. Although all the mucosae that we investigated in this study are keratinized, our present results are basically consistent with above findings (7,10,20). Recently, Yamahara *et al.* investigated the oral mucosal absorption of salicylic acid in dogs using an *in situ* perfusion system and reported that the disappearance rates of salicylic acid perfused on buccal mucosa, floor of mouth, dorsum of tongue, and ventral surface of tongue were almost the same (13). But they did not discuss their results from the morphological view point.

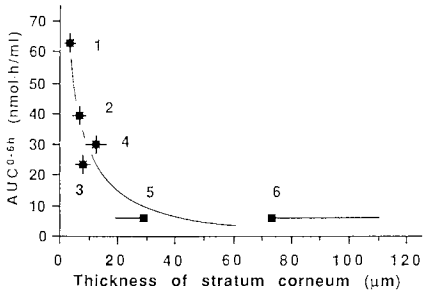

Fig. 5. Relationship between AUC of salicylic acid and thickness of stratum corneum of oral mucosa. $AUC^{0-6\ hr}$ for each curve in Fig. 4 was calculated by the trapezoidal method. Application sites: 1, sublingual mucosa; 2, cheek pouch mucosa; 3, labial mucosa; 4, ventral surface of tongue; 5, dorsum of tongue; 6, buccal mucosa. Each bar represents the standard error.

As shown in Fig. 5, the AUC up to 6 hr for salicylic acid and the thickness of stratum corneum layer, but not whole epithelium, are in inverse proportion to each other ($P < 0.01$), suggesting that the stratum corneum layer represents the principal permeation barrier to drug absorption. Since drug absorption from oral mucosa is highly dependent on the application site, it is important for designing the transoromucosal drug delivery system to clarify regional differences in the oral cavity. The barrier properties at the different sites of the human oral cavity remain to be clarified.

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