Perfusion Cells for Studying Regional Variation in Oral-Mucosal Permeability in Humans. I: Kinetic Aspects in Oral-Mucosal Absorption of Alkylparabens

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Purpose. To evaluate regional differences in permeability of human oral mucosa.

Methods. Newly designed perfusion cells were used for the investigation. The cells were applied to 5 different sites, i.e., dorsum of tongue, ventral surface of tongue, labial mucosa, floor of mouth and buccal mucosa of human volunteers. Model drugs used were methyl-, ethyl-, propyl- and butylparaben, which are passively absorbed from oral mucosa and have different lipophilicities. Biexponential disappearance profiles of the alkylparabens were analyzed kinetically using a two-compartment linear open model.

Results. Both the partitioning parameters to the oral mucosa and the absorption rate constants to the blood circulation correlated to the lipophilicities of the compounds in all mucosa. As to the former parameter, no significant difference was recognized in all mucosa. While, the latter parameter exhibited the regional difference; the absorption rate constants in buccal mucosa were approximately one-half of those estimated in other oral mucosa. A positive relation was recognized between the retention in oral-mucosal compartment and the drug lipophilicity.

Conclusions. The newly designed perfusion cells used in this study were useful to examine the regional variations of drug absorption from oral mucosa in humans. The absorption rate constant, the partition to oral mucosa and the residence time in oral mucosa increased with lipophilicity of the compound. The regional difference in the drug absorption process was demonstrated; the slow absorption and the prolonged retention were demonstrated in buccal mucosa.

KEY WORDS: oral mucosa; permeability; regional difference; perfusion cell; human; alkylparaben.

INTRODUCTION

Oral mucosal route is of great advantage to systemic drug delivery because drugs absorbed from the oral mucosa can avoid both intra-alimentary canal and hepatic first-pass elimination (1–3). Buccal absorption test of Beckett and Triggs (4) has generally been used to evaluate drug absorption from human oral cavity (5,6). This method enables to evaluate the disappearance kinetics of drugs from whole human oral cavity in a simple manner. However, the results never cover the information about the regional variations in oral mucosal absorption (7).

The oral mucosa consists of both the keratinized and the non-keratinized mucosa. We have reported the existence of a specialized absorption mechanism for amino-cephalosporins in human oral cavity, while it was not recognized in keratinized hamster cheek pouch mucosa (8). The mechanism of drug absorption across keratinized oral mucosa is a passive diffusion and the absorption phenomena obey the pH-partition hypothesis (9,10). As to regional variations in permeation of drugs in keratinized oral mucosa, we have clarified that the permeability to passively absorbed drugs is inversely proportion to the thickness of the stratum corneum layer of the mucosa in hamsters (11). In order to develop precise oral-mucosal dosage forms (3), the information about the regional difference in drug absorption properties in human oral mucosa is necessary. For this purpose, a method applicable to human and being able to evaluate the kinetic aspect of oral-mucosal absorption is required.

In this study, we will discuss regionally different aspects in oral-mucosal absorption kinetics of methyl-, ethyl-, propyl- and butylparaben characterized by using three types of newly designed perfusion cells for human oral mucosa.

MATERIALS AND METHODS

Materials

4-Hydroxybenzoic acid methyl ester (methylparaben), 4-hydroxybenzoic acid ethyl ester (ethylparaben), 4-hydroxybenzoic acid propyl ester (propylparaben) and 4-hydroxybenzoic acid butyl ester (butylparaben) were purchased from Tokyo Kasei Kogyo Co. (Tokyo, Japan). All other chemicals were reagent grade commercial products.

Preparation of Test Solution

Alkylparabens were dissolved in 5 mM phosphate buffered saline (pH 6.5) at the concentration of 10 μg/ml.

Preparation of Perfusion Cells

Three types of newly designed perfusion cells were prepared from polyvinyl chloride. The size of the perfusion cells was shown in Fig. 1 (left).

Perfusion Cell Study

Three healthy male volunteers, aged 23–25, participated in this study. Written informed consent, in accordance with the principles of the Declaration of Helsinki, was obtained from each volunteer prior to the study. A perfusion cell was clamped on either buccal mucosa, dorsum of tongue, ventral surface of tongue, labial mucosa or floor of mouth of a volunteer as shown in Fig. 1 (right). A test solution (5 ml) was put into the cell and was recirculated at a flow rate of 0.8 ml/min using a peristaltic pump (Atto, Tokyo) for 30 min. One hundred μl of the perfusion solution was sampled out at every 5 min, and the concentration of the alkylparaben was determined.

Pharmacokinetic Analysis

Pharmacokinetic analysis of oral-mucosal drug absorption process was performed from biexponential disappearance profile of drug amount in the perfusion solution. A two-compartment linear open model (12) was applied (Fig. 2). The

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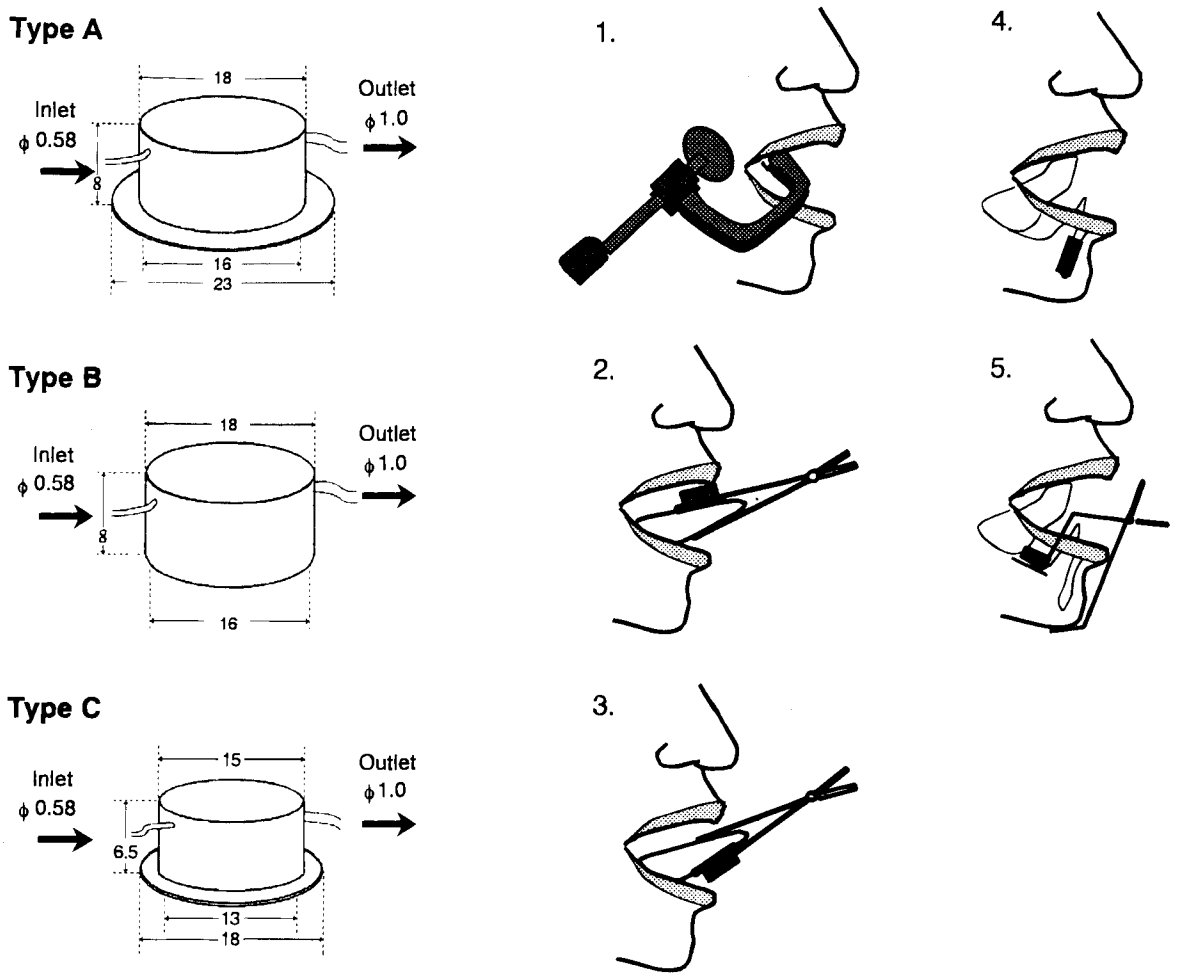

Fig. 1. Perfusion cells for studying drug absorption from human oral mucosa (left) and schematic representation of their application onto human oral mucosa (right). Type A cell (2.01 cm^2)is for buccal mucosa (1), dorsum of tongue (2) and ventral surface of tongue (3); Type B (2.01 cm^2) cell for labial mucosa (4); Type C cell (1.33 cm^2) for floor of mouth (5).

biexponential disappearance profile (X_1) was fitted to the following equation and the parameters of A, B, α and β were estimated by a nonlinear least-squares program, MULTI (13).

$$X_1 = A \exp(-\alpha t) + B \exp(-\beta t)$$

The transfer rate constants were calculated using following equations.

$$k_{12} = (A\alpha + B\beta)/(A + B)$$

$$k_{23} = \alpha\beta/k_{12}$$

$$k_{21} = \alpha + \beta - k_{12} - k_{23}$$

The amount of drug in oral-mucosal compartment (X_2) and the cumulative amount of drug cleared from the absorption site (X_3) are expressed as follows:

$$X_2 = (A + B)k_{12}\{\exp(-\alpha t) - \exp(-\beta t)\}/(\beta - \alpha)$$

$$X_3 = (A + B)[1 - \{\beta \exp(-\alpha t) - \alpha \exp(-\beta t)\}/(\beta - \alpha)]$$

The profile of the amount in oral-mucosal compartment (X_2) was simulated using these parameters and was analyzed using moment analysis. The statistical moment parameters, the area under the amount in mucosal tissue *versus* time curve (AUC) values and the mean transit time (MTT) values of alkylparabens in each oral mucosal compartment were calculated (13). The MTT in oral mucosa was estimated by subtracting the mean residence time (MRT) in donor compartment from MRT of the mucosal retention profile.

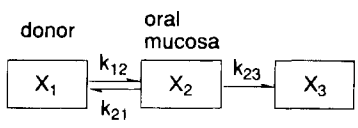

Fig. 2. Compartment model for pharmacokinetic analysis of oral-mucosal drug absorption. X_1 and X_2 are the drug amount in the donor (perfusion solution) and in oral mucosa, respectively. X_3 represents the cumulative amount of drug cleared from the absorption site. X_1, X_2 and X_3 are expressed as % of dose. k_{12} and k_{21} are the first-order transfer rate constants between the donor compartment and oral-mucosal compartment. k_{23} is a first-order transfer rate constant from oral-mucosal compartment to the successive deeper compartment, *i.e.*, systemic circulation.

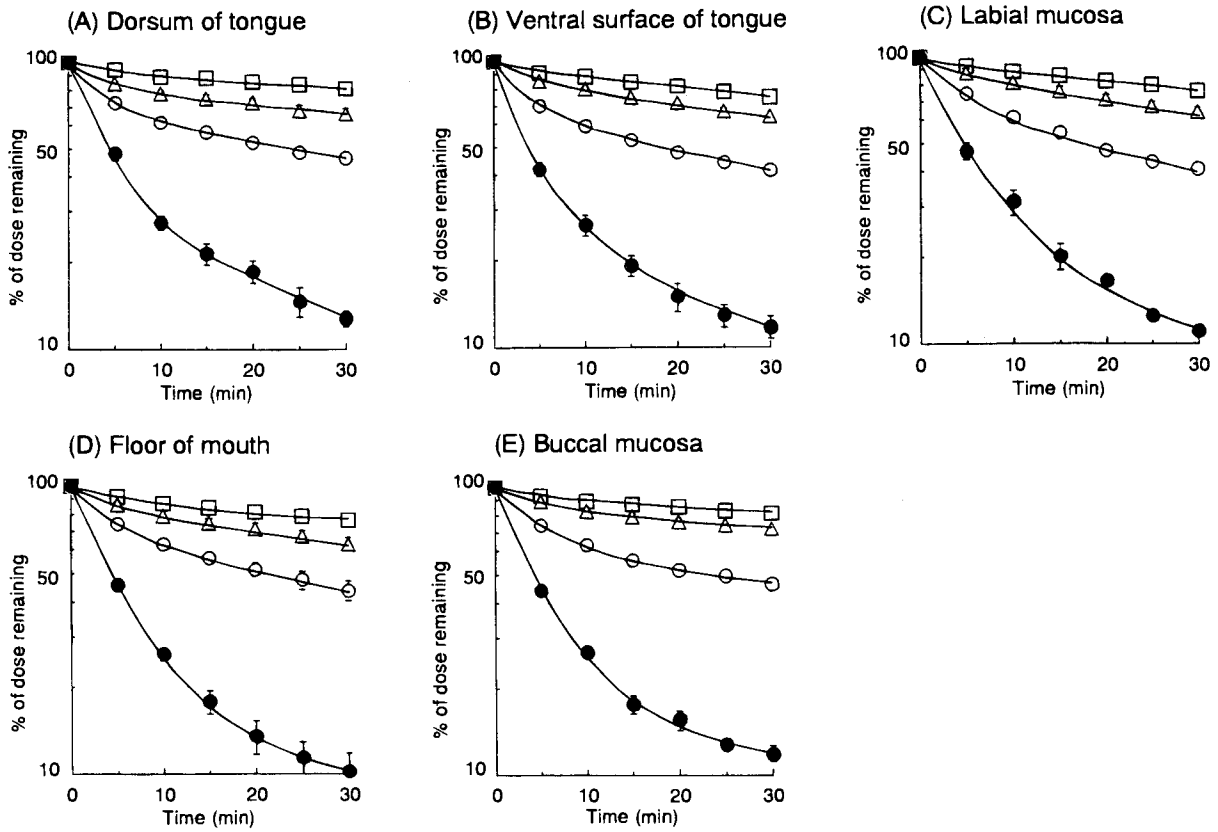

Fig. 3. Time course of the amount of alkylparabens in the donor compartment (perfusion solution). The perfusion experiment was carried out for 30 min using 5 ml of 0.001% alkylparaben solution. Results are expressed as the mean ± S.E. of 3 experiments. □, methylparaben; △, ethylparaben; ○, propylparaben; ●, butylparaben.

Determination of Lipophilic Index

The lipophilic index, log k'_0, of each alkylparaben at pH 6.5 was determined by HPLC according to the method of Yamana *et al.* (14). The column used was Inertsil ODS (4.6 mm i.d. × 150 mm, GL Sciences Inc., Tokyo) and the mobile phase of methanol-phosphate buffer solution (pH 6.5) was run at a flow rate of 1.0 ml/min. Formamide was used as an unretained substance. When log k′, which was defined as follows;

$$\log k' = \log [(t_R - t_0)/t_0]$$

where t_R and t_0 are the retention times of a retained peak and of an unretained peak, respectively, was plotted against methanol concentration (v/v%), reasonable linear relationships were obtained. The lipophilic index, log k'_0, was defined as a log k′ value extrapolated to 0% methanol.

Analytical Methods

Concentrations of alkylparabens were determined by HPLC. An aliquot of the sample solution was filtered through a 0.45 μm membrane filter (Nihon Millipore Kogyo, Yonezawa, Japan). The filtrate was used for a HPLC analysis. The chromatograph was LC-6A (Shimadzu Co., Kyoto, Japan) equipped with a Shimadzu SPD-2A ultraviolet detector. The column used was a reversed phase Inertsil ODS (4.6 mm i.d. × 150 mm). Mobile phases were 0.025% phosphoric acid-methanol (45:55, 45:55, 40:60 and 30:70 by volume for methyl-, ethyl-, propyl- and butylparaben, respectively). The flow rate was 1.0 ml/min and the column temperature was 40°C. The drugs were monitored at 254 nm. The concentrations of the parabens were calculated by the peak area measurements.

RESULTS AND DISCUSSION

There are morphologically and functionally marked regional variations in mucosa of oral cavity. In order to develop an optimal dosage form for oral mucosal drug delivery, precise information on drug absorption characteristics in each region of human oral mucosa is very important. However, since the area of morphologically uniform mucosa in oral cavity is narrow, there is no information about regional variations in oral mucosal drug absorption. Two perfusion cells have been used in human (15,16), but both of them have utilized only the buccal mucosa as the platform for perfusion. Thus, we developed new perfusion system for the study of regional variations in drug absorption from human oral mucosa. Newly designed perfusion cells (Fig. 1 (left)) could be clamped on five regions of human oral mucosa, i.e., buccal mucosa, dorsum of tongue, ventral surface of tongue, labial mucosa, or floor of mouth (Fig. 1 (right)). The size of Type C cell is smaller in comparison with the others, because the applicable area of the floor of mouth is narrow. Small margin was fixed on the surface of Type A and Type C cells to avoid the pain by pressure. Then, the cells were acceptable for volunteers for periods of at least 1 h without discomfort. The smooth perfusion without any leak of the perfu-

Table 1. Pharmacokinetic Parameters of Alkylparabens in Perfusion Cell Study

Parameter	(Unit)	Alkylparaben			
		Methyl-	Ethyl-	Propyl-	Butyl-
(a) Dorsum of tongue					
A	(% of dose)	5.3	15.9	32.7	69.9
B	(% of dose)	94.7	84.1	67.3	31.0
α	(10^{-3} min^{-1})	255	273	252	255
β	(10^{-3} min^{-1})	5.15	8.34	12.89	29.40
k_{12}	(10^{-3} min^{-1})	18.4	50.3	91.1	185.4
k_{21}	(10^{-3} min^{-1})	170.2	185.9	138.1	58.2
k_{23}	(10^{-3} min^{-1})	71.1	45.3	35.7	40.4
AUC[a]	(% of dose · min)	1406	2208	2805	2478
MTT[a]	(min)	4.1	4.3	5.7	10.0
(b) Ventral surface of tongue					
A	(% of dose)	4.5	11.4	34.1	73.2
B	(% of dose)	95.5	88.6	65.8	25.7
α	(10^{-3} min^{-1})	791	375	254	251
β	(10^{-3} min^{-1})	8.15	11.20	15.96	27.13
k_{12}	(10^{-3} min^{-1})	43.1	52.8	97.4	193.2
k_{21}	(10^{-3} min^{-1})	606.3	253.8	131.3	50.0
k_{23}	(10^{-3} min^{-1})	149.4	79.6	41.7	35.3
AUC[a]	(% of dose · min)	669	1256	2399	2833
MTT[a]	(min)	1.3	2.9	5.7	11.6
(c) Labial mucosa					
A	(% of dose)	5.1	11.4	36.9	68.4
B	(% of dose)	94.9	88.6	62.9	30.4
α	(10^{-3} min^{-1})	265	202	164	218
β	(10^{-3} min^{-1})	7.58	11.27	15.53	34.79
k_{12}	(10^{-3} min^{-1})	20.7	33.0	70.6	161.3
k_{21}	(10^{-3} min^{-1})	154.6	111.1	73.2	44.2
k_{23}	(10^{-3} min^{-1})	97.2	69.0	36.2	46.9
AUC[a]	(% of dose · min)	1029	1450	2764	2131
MTT[a]	(min)	3.9	5.5	9.1	10.9
(d) Floor of mouth[b]					
A	(% of dose)	7.7	11.7	28.6	80.6
B	(% of dose)	92.4	88.3	71.4	18.7
α	(10^{-3} min^{-1})	337	462	382	304
β	(10^{-3} min^{-1})	9.48	16.44	24.95	32.01
k_{12}	(10^{-3} min^{-1})	34.6	68.8	126.8	252.4
k_{21}	(10^{-3} min^{-1})	220.2	299.5	204.6	44.7
k_{23}	(10^{-3} min^{-1})	92.5	110.5	75.1	38.5
AUC[a]	(% of dose · min)	1082	905	1332	2598
MTT[a]	(min)	3.2	2.4	3.5	11.9
(e) Buccal mucosa					
A	(% of dose)	6.2	14.3	35.7	78.9
B	(% of dose)	93.9	85.7	64.2	20.4
α	(10^{-3} min^{-1})	242	181	198	219
β	(10^{-3} min^{-1})	4.87	6.27	11.10	18.69
k_{12}	(10^{-3} min^{-1})	19.4	31.2	77.7	177.9
k_{21}	(10^{-3} min^{-1})	166.5	119.6	102.8	36.9
k_{23}	(10^{-3} min^{-1})	60.5	36.3	28.2	23.0
AUC[a]	(% of dose · min)	1651	2752	3542	4342
MTT[a]	(min)	4.3	6.3	7.6	16.5

[a] Both AUC and MTT values were calculated for oral mucosa compartment.

sion solution could be performed by the use of wider inside diameter tubing for the outlet tube (Fig. 1).

The absorption of 4 alkylparabens from five regions of human oral mucosa was examined using the perfusion cells. Lipophilic indexes, log k'o of the 4 alkylparabens were estimated by HPLC as 1.65, 2.12, 2.64 and 3.08 for methyl-, ethyl-, propyl-, and butylparaben, respectively. There was no peak corresponding to 4-hydroxybenzoic acid, a possible metabolite of alkylparabens, in the chromatogram of each sample, suggesting that the degradation of the parabens during the perfusion

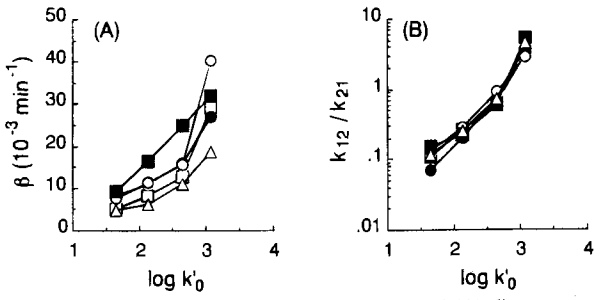

Fig. 4. Relationships between lipophilic index and (A) disappearance rate constant in the terminal phase (β) or (B) partitioning parameter (k_{12}/k_{21}) of alkylparabens. □, dorsum of tongue; ●, ventral surface of tongue; ○, labial mucosa; ■, floor of mouth; △, buccal mucosa.

experiment was negligible. Likely to the findings reported by Rathbone (16), adsorption of parabens to the apparatus for the perfusion study was negligible. In order to improve the clarification of the concentration change of the perfusate during the perfusion experiment, the volume of the perfusate was designed to 5 ml. Transmucosal water movement during the perfusion experiment, determined by using fluorescein isothiocyanate dextran (MW 35,600), varied with both the site of the mucosa and the individual, but was ranged only 1 to 4% dilution of the perfusate by the secretion in the preliminary study. The dilution of the perfusate seemed not to affect the kinetic evaluation of the disappearance profiles of the parabens. Therefore we did not take into account the transmucosal water movement during the perfusion study for these relatively well-absorbable alkylparabens. As shown in Fig. 3, the disappearance profiles of alkylparabens were biexponential, suggesting the rapid distribution to the mucosa followed by relatively slow transfer to the circulation. Pharmacokinetic parameters of the alkylparabens were estimated using a two-compartment linear open model (12) shown in Fig. 2. The results are summarized in Table 1. The rate constants in the floor of mouth listed in Table 1 were normalized by the area of the cell to 2.01 cm^2. Apparent

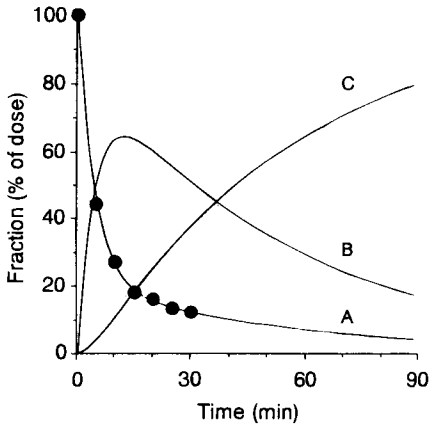

Fig. 5. Plots of the fraction of butylparaben in the donor compartment (A), the oral-mucosal compartment (B) and the cumulative amount of drug cleared from the absorption site (C) *versus* time in case of buccal mucosa. The points were obtained experimentally from the perfusion solution. Simulation curves were generated using pharmacokinetic parameters listed in Table 1.

first-order disappearance rate constants of alkylparabens from the donor in the terminal phase (β) as well as the partitioning parameters to the oral mucosa (k_{12}/k_{21}) correlated to the lipophilicities of the compounds in all mucosa (Fig. 4). As to the partitioning parameter, no significant difference was recognized in all mucosa, suggesting that properties of the mucosal surface are not so different in these five regions. On the other hand, the regional variation was observed in β. Although the parameter in buccal mucosa was slightly inferior to other oral mucosa, the regional difference was at most 2–3 fold.

The regional difference was not recognized in the partitioning parameter but in the disappearance rate constant, suggesting the regional difference in the transit process across the mucosal tissue. In order to clarify the mucosal retention characteristics of the compound, simulation analysis was carried out using the parameters listed in Table 1. Figure 5 shows the simulation curves of the distribution time courses for butylparaben in each compartment in buccal mucosa. AUC and MTT values of alkylparabens in each oral-mucosal compartment were calculated from the retention profile and were listed in Table 1. Both AUC in oral mucosa and MTT in oral mucosa show positive relation with the drug lipophilicity, indicating that the more lipophilic compounds provide the longer retention in oral mucosa. Both AUC and MTT values in buccal mucosa were larger than those in other regions, suggesting that buccal mucosa is the site of high drug retention. The thickness of the epithelium seems to be one of the factors determining the mucosal retention in addition to the lipophilicity of drugs, while the morphological data of human oral mucosa is not sufficient at present. The findings in this study suggest the usefulness of controlled release dosage forms for lipophilic drugs to improve oral-mucosal retention characteristics of the drug.

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