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Enhancing effect of 1-dodecylazacycloheptan-2-one (Azone) on the absorption of salicylic acid from keratinized oral mucosa and the duration of enhancement in vivo

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Summary

Enhancing effect of the pretreatment with 1-dodecylazacycloheptan-2-one (Azone) on the absorption of salicylic acid from keratinized oral mucosa was investigated in vivo using a hamster cheek pouch. The absorption was significantly increased after the 4 h pretreatment with Azone-emulsion when the pretreatment medium contained Azone above 0.2%. The enhancement was observed at all pH conditions examined irrespective of the degree of dissociation of the drug. The apparent disappearance rate constant of salicylic acid, calculated from the time course of the sum of remaining amounts of the drug in both the luminal fluid and the tissue, in Azone-pretreated cheek pouch was approximately 2.7 times larger than in the non-treated one. The pharmacokinetic analysis of the plasma concentration of salicylic acid after the intra-cheek-pouch administration revealed that Azone pretreatment enhanced the absorption, i.e., increased the absorption rate constant and shortened the mean absorption time by one-fifth, and brought the peak plasma concentration approximately twice higher. The enhanced absorption after 1 h pretreatment with 5% Azone-emulsion diminished with the lapse of time and the barrier function of the cheek pouch was completely recovered within 6 h after the removal of the Azone-emulsion.

Introduction

The oral mucosal route has advantages over systemic therapy because the drugs can avoid both the alimentary canal and the hepatic first-pass eliminations (Alkalay et al., 1973; Bogaert, 1983; Bell et al., 1985; Hussain et al., 1986, 1987). However, the barrier properties of the oral mucosa for permeation of drugs are greater, in general, than those of the gastrointestinal tract. There exist a relatively small number of studies concerning the pharmaceutical approaches to facilitate the absorption of drugs from the oral cavity, and these studies can be summarized as follows: approximately 0.5% of insulin was absorbed from the oral mucosal dosage form containing sodium glycocholate (Ishida et al., 1981) and the ionic surfactants increased the permeability of oral-mucosa (Siegel and Gordon, 1985; Kurosaki et al., 1988a).

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It is well known that there exist regional variations in the epithelial thickness and the degree of keratinization which may affect the permeability to drugs (Squier and Johnson, 1975; Squier and Hall, 1985a and b). Recently, we have developed an experimental method for studying absorption processes across the keratinized oral mucosa in vivo using a hamster cheek pouch (Kurosaki et al., 1986) which is completely covered with a few stratum corneum layers. Keratinized oral mucosa is morphologically similar to the skin. And the possible barrier layer of the cheek pouch for the permeation speculated is the stratum corneum like that of the skin (Kurosaki et al., 1989).

1-Dodecylazacycloheptan-2-one (Azone) is an agent which enhances the dermal penetration with a wide variety of compounds (Stoughton, 1982; Stoughton and McClure, 1983). In the previous report we showed the enhancing effect of Azone on the in-vitro permeability of hamster cheek pouch to salicylic acid and clarified that Azone acts directly on the stratum corneum of the cheek pouch where the major barrier function exists (Kurosaki et al., 1989).

In this study, we examined the effect of Azone pretreatment on the absorption kinetics of salicylic acid after the administration in hamster cheek pouch and clarified the duration of action of the absorption enhancement induced by Azone-pretreatment in vivo.

Materials and Methods

Chemicals

Azone was kindly supplied by Nelson Research (Irvine, CA) and was used as supplied. An emulsifier, polysorbate 20, was purchased from Tokyo Kasei Kogyo Co. (Tokyo, Japan). All other chemicals were reagent grade products obtained commercially.

Animals

Male golden hamsters (100–130 g) were used under urethane anesthesia (1.5 g/kg, i.p.).

Pretreatment mediums

The required concentration of Azone was emulsified with 0.1% polysorbate 20 in pH 7.0 isotonic buffer solution and the emulsions were used for the pretreatment of hamster cheek pouch. For the control study, 0.1% polysorbate 20 solution without Azone in the same buffer solution was also prepared.

Procedure of absorption experiment

Hamster was fastened to a platform and a cheek pouch was cleaned as described previously (Kurosaki et al., 1986). Pretreatment of the cheek pouch was carried out for a definite period (1–12 h) by the application of 1.5 ml of a pretreatment medium. Then the medium was withdrawn and the cheek pouch was rinsed 3 times with 3 ml of saline. The lumen of the cheek pouch was wiped with the cotton balls to remove excess moisture. Salicylic acid was dissolved in appropriate isotonic buffer solution (Kurosaki et al., 1988a) and 1 ml of the drug solution was administered into the cheek pouch. Procedures of the collection of the remaining drug from the lumen of the cheek pouch, the extraction of the remaining drug from the tissue of the cheek pouch, and the determination of the amounts of salicylic acid remaining in both the lumen and the tissue by a high pressure liquid chromatography (HPLC) were the same as described previously (Kurosaki et al., 1986). For the calculation of the net absorption, the amount in the tissue was subtracted from the amount disappeared from the lumen of the cheek pouch.

Estimation of plasma concentration of salicylic acid

Under urethane anesthesia, the carotid artery of the hamster was cannulated with polyethylene tubing (o.d. 0.8 mm, i.d. 0.5 mm, Dural Plastics, Australia) and then heparin (500 unit/kg) was administered i.v. In an i.v. study, salicylic acid dissolved in saline (20 μmol/ml/kg) was injected into a femoral vein. In the study of intra-cheek-pouch administration, salicylic acid dissolved in isotonic buffer solution (pH 3.0) (100 μmol/10 ml/kg) was administered into the cheek pouch in a similar manner as described in the previous chapter. In both studies, blood samples (0.2 ml each) were collected periodically from the cannula for 3 h and the plasma concentration of salicylic acid was determined by HPLC as described previously (Kurosaki et al., 1986).

Pharmacokinetic studies

Compartment analysis. Plasma concentration–time data from i.v. and intra-cheek-pouch administration studies were simultaneously fitted to a two-compartment open model (Fig. 3) with independent parameters concerning the absorption process, i.e., the lag time and the absorption rate constant (Kurosaki et al., 1986) using a non-linear least-squares program (MULTI, Yamaoka et al., 1981).

Moment analysis. The plasma concentration–time data were analyzed non-compartmentally by the statistical moment theory (Yamaoka et al., 1978). The moments were calculated by the trapezoidal method with a monoexponential extrapolation of terminal phase.

The mean absorption time (MAT) was estimated by the following equation:

$$MAT = MRT_{icp} - MRT_{iv}$$

where MRT is the mean residence time, and icp and iv are the subscripts for the intra-cheek-pouch and intravenous administrations, respectively.

The systemic availability (F) of salicylic acid was calculated by the following equation:

$$F(\%) = (AUC_{icp}/AUC_{iv}) \times (Dose_{iv}/Dose_{icp}) \times 100$$

Statistical analysis

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

Results and Discussion

Effect of Azone-pretreatment on the absorption of salicylic acid from hamster cheek pouch

It has been reported that the enhancing effect of Azone on the percutaneous drug absorption is much influenced by the composition of the vehicle (Wotton et al., 1985; Sheth et al., 1986). In the previous study, we could reveal the enhanced permeability of the cheek pouch to salicylic acid by the pretreatment with 5% Azone emulsified in 0.1% polysorbate 20 in vitro (Kurosaki et al., 1989). The pretreatment of hamster cheek pouch with Azone-emulsion was carried out to neglect the interaction between salicylic acid and Azone molecules. At first, we examined the effect of the period of pretreatment with 5% Azone emulsion on the absorption of salicylic acid from the cheek pouch at pH 3.0 in vivo. Results are summarized in Table 1. Both the disappearance from the lumen of the cheek pouch and the net absorption were significantly increased after the pretreatment with Azone emulsion in all the conditions examined. The enhancement was reduced slightly when the absorption experiment was carried out after 12 h pretreatment compared with those after 4 h pretreatment. This accounts for the long-term

TABLE 1

Effect of pretreatment with 5% Azone-emulsion on the absorption of 5 mM salicylic acid from hamster cheek pouch at pH 3.0

Pretreatment period (h)	n [a]	% of dose administered into the cheek pouch after 1 h		
		Disappeared from lumen	Remaining in tissue	Net absorption
1 (Control [b])	4	59.0 ± 3.4	15.5 ± 0.8	43.5 ± 4.0
1	8	83.1 ± 1.7 ***]#	9.8 ± 0.7 ***]#	73.4 ± 2.2 ***
4	4	89.6 ± 0.9 ***]#	6.4 ± 0.7 ***]#	83.2 ± 1.6 ***]#
12	8	77.9 ± 3.1 **]	13.0 ± 1.6]	64.9 ± 4.5 **]

Results are expressed as the mean ± S.E.M.

[a] Number of experiments.

[b] Hamster cheek pouch was pretreated with 0.1% polysorbate 20 solution for 1 h.

** $P < 0.01$; *** $P < 0.001$, compared with each control value.

# $P < 0.05$, between two groups.

TABLE 2

Enhanced absorption of 5 mM salicylic acid from hamster cheek pouch at pH 3.0 after 4 h-pretreatment with various concentrations of Azone emulsion

Azone conc. (%)	n [a]	% of dose administered into the cheek pouch after 1 h		
		Disappeared from lumen	Remaining in tissue	Net absorption
Control [b]	3	61.2 ± 1.1	10.2 ± 0.2	51.3 ± 1.3
0.05	4	68.0 ± 2.8	n.d. [c]	n.d.
0.2	3	90.1 ± 4.7 **	6.3 ± 2.0	83.8 ± 6.6 **
1.0	3	86.4 ± 1.5 ***	5.9 ± 1.2 *	80.4 ± 2.5 ***
5.0	4	89.6 ± 0.9 ***	6.4 ± 0.7 **	83.2 ± 1.6 ***

Results are expressed as the mean ± S.E.M.

[a] Number of experiments.

[b] Hamster cheek pouch was pretreated with 0.1% polysorbate 20 solution.

[c] Not determined.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, compared with each control value.

anesthesia which makes it difficult to maintain physiological conditions, such as the blood flow.

The relation between the concentration of Azone in the emulsion and the enhancing effect was examined after 4 h pretreatment and the results are summarized in Table 2. There was no significant increase in the disappearance of salicylic acid from the lumen when the cheek pouch was pretreated with 0.05% Azone emulsion. However, when Azone concentrations in the pretreatment mediums were above 0.2%, both the disappearance from the lumen and the net absorption were significantly increased compared with each control value, and the magnitude of the increments was statistically identical to each other in the concentration range of Azone from 0.2 to 5.0%.

The effects of Azone pretreatment on the pH–absorption profile of salicylic acid in the cheek pouch were examined in vivo and the results are

TABLE 3

Effect of 4 h pretreatment with 5% Azone emulsion on pH absorption profile of salicylic acid (5 mM) in hamster cheek pouch

pH	Medium	n [a]	% of dose administered into the cheek pouch after 1 h		
			Disappeared from lumen	Remaining in tissue	Net absorption
3.0	Control [b]	3	61.2 ± 1.1	10.2 ± 0.2	51.0 ± 1.3
	Azone [c]	4	89.6 ± 0.9 ***	6.4 ± 0.7 **	83.2 ± 1.6 ***
4.0	Control	4	22.7 ± 1.1	n.d. [d]	n.d.
	Azone	4	56.3 ± 1.9 ***	n.d.	n.d.
5.0	Control	4	5.8 ± 0.9	3.9 ± 0.4	1.9 ± 1.2
	Azone	4	14.5 ± 1.1 **	5.0 ± 0.2	9.5 ± 1.2 **
6.0	Control	3	1.5 ± 0.4	n.d.	n.d.
	Azone	3	9.1 ± 1.7 *	n.d.	n.d.
7.0	Control	3	0.9 ± 0.2	0.5 ± 0.1	0.4 ± 0.2
	Azone	3	2.4 ± 0.4 *	1.0 ± 0.1 *	1.4 ± 0.4 *

Results are expressed as the mean ± S.E.M.

[a] Number of experiments.

[b] Hamster cheek pouch was pretreated with 0.1% polysorbate 20 solution for 4 h.

[c] Hamster cheek pouch was pretreated with 5% Azone emulsion for 4 h.

[d] Not determined.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, compared with corresponding control values.

summarized in Table 3. The disappearance from the lumen and the net absorption were significantly increased after the pretreatment in all pH conditions. Hadgraft et al. (1985) demonstrated that Azone facilitated the transport of salicylate anion across an artificial lipid membrane over a pH range of 2.5–7.0 and proposed that the mechanism of the facilitation was the ion pair formation of Azone with anionic drug molecule. In our in vitro permeation study, the enhanced permeability of hamster cheek pouch, a keratinized oral mucosa, to salicylic acid ($pK_a = 3.0$) was also evident at pH 6.0 (Kurosaki et al., 1989). The results shown in Table 3 agree well with these in vitro findings.

Effect of Azone-pretreatment on the absorption kinetics of salicylic acid in hamster cheek pouch

To clarify the effect of 4 h-pretreatment with 5% Azone emulsion on absorption kinetics of salicylic acid after the intra-cheek pouch administration, the amounts of salicylic acid remaining in both the lumen and the tissue of the cheek pouch were determined periodically. Fig. 1(a) shows the time course of the remaining amounts of salicylic acid in the luminal fluid of the cheek pouch. Salicylic acid disappeared biexponentially from

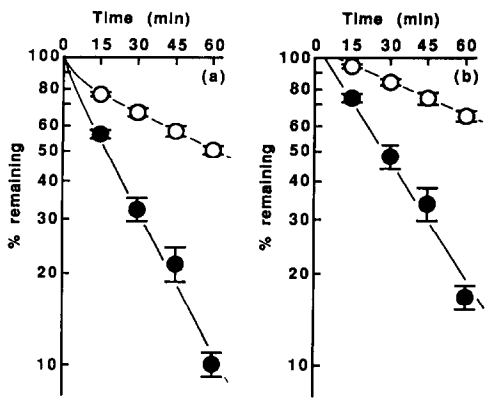

Fig. 1. Effect of Azone-pretreatment on the time course of salicylic acid absorption from hamster cheek pouch at pH 3.0. Semilogarithmic plots of (a) salicylic acid remaining in luminal fluid and (b) sum of salicylic acid remaining in luminal fluid and in tissue: absorption experiments were carried out without any treatment (open circle) and after pretreatment with 5% Azone emulsion for 4 h (closed circle). Results are expressed as the mean ± S.E.M. of 4 experiments.

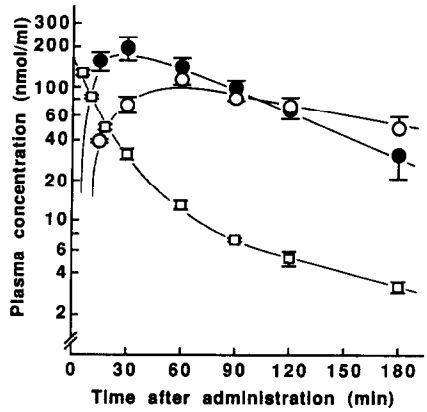

Fig. 2. Plasma concentration–time profiles of salicylic acid after i.v. (open squares) and intra-cheek-pouch administrations to non-treated (open circles) or Azone-pretreated (closed circles) cheek pouch. Doses of salicylic acid were 20 μmol/kg and 100 μmol/kg for i.v. and intra-cheek pouch administration, respectively. Results are expressed as the mean ± S.E.M. Numbers of the experiments are listed in Table 5. Each line represents the curve fitted with the two-compartment model shown in Fig. 3.

the lumen and the disappearance obviously became faster when the cheek pouch was pretreated with 5% Azone-emulsion for 4 h. We have already reported that the semilogarithmic plots of the sum of the amounts remaining in the luminal fluid and in the tissue of the cheek pouch explain well the systemic transfer process, which can be approximated as a first-order one after a lag time (Kurosaki et al., 1986). In the present study, both the data with and without Azone-pretreatment also gave semilogarithmically linear plots (Fig. 1b). The apparent disappearance rate constant in Azone-pretreated cheek pouch calculated from the slope in Fig. 1b was approximately 1.6 h^{-1} and was approximately 2.7 times larger than that in the untreated one (0.6 h^{-1}). In addition, the lag time estimated by the extrapolation was shortened from 8 to 3 min by the Azone-pretreatment.

Plasma concentrations of salicylic acid after the i.v. (20 μmol/kg) and the intra-cheek pouch administrations (100 μmol/10 ml/kg, pH 3.0) are shown in Fig. 2. The plasma concentration of i.v. administered salicylic acid declined biexponentially. In the intra-cheek pouch administration studies, the plasma concentrations of salicylic acid were significantly increased by the Azone pretreat-

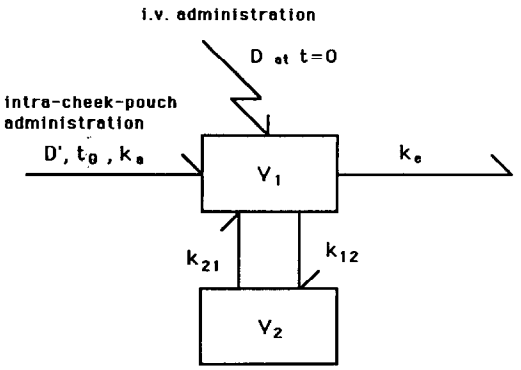

Fig. 3. Pharmacokinetic model for salicylic acid in the hamster. D or D', dose of salicylic acid; t_0, lag time; k_a, first-order absorption rate constant; k_e, first-order elimination rate constant; k_{12} and k_{21}, first-order transfer rate constants between two compartments; V_1, volume of distribution of central compartment; V_2, volume of distribution of peripheral compartment. Salicylic acid was administered by i.v. or intra-cheek pouch administration.

ment at 15 and 30 min. The peak plasma concentration in the Azone-pretreated hamsters was approximately twice that in non-treated ones, and the time of peaks plasma concentration also became earlier after the Azone pretreatment. These plasma concentration data were simultaneously fitted to the two-compartment model with a first-order absorption process shown in Fig. 3 (Kurosaki et al., 1986) using a non-linear least-squares fitting program, "MULTI" (Yamaoka et al., 1981). The pharmacokinetic parameters concerning the distribution and the elimination were close to our previous estimation (Kurosaki et al., 1986). The absorption rate constant (k_a) and the lag time (t_0) in the Azone-pretreated group were approximately 2.4 times and one-fourth compared with each parameter in the untreated one, respectively. These parameters agree well with the lag times and the net absorption rate constants estimated from Fig. 1b. The simulation curves shown in Fig. 2 calculated by using the model and the parameters were quite adequate to explain the plasma levels of salicylic acid.

Parameters of moment analysis are listed in Table 5. The systemic bioavailability of salicylic acid after the intra-cheek pouch administration was virtually complete and was not affected by the Azone pretreatment. However, the mean absorption time of the Azone pretreated group was shortened to 25.1 min, approximately one-fifth compared with that of the non-treated group. We could clarify from these results that Azone enhances the absorption of salicylic acid from the

TABLE 4

Pharmacokinetic parameters for salicylic acid after the intra-cheek pouch administration in the hamster based upon the model shown in Fig. 3

Pharmacokinetic parameter		Pretreatment	
		None [a]	Azone [b]
t_0	(min)	9.5	2.6
k_a	(h^{-1})	0.60	1.44
k_{12}	(h^{-1})	1.19	
k_{21}	(h^{-1})	1.00	
k_e	(h^{-1})	2.48	
V_1	(liter/kg)	0.120	
V_2	(liter/kg)	0.143	

The parameters were estimated by the damping Gauss–Newton method. In estimation, $(C_i)^{-2}$ was adopted as the weight, where C_i is the value of the i-th point (Kurosaki et al., 1986). Akaike's information criterion, AIC, in this estimation was -27.9.

[a] Absorption experiment was carried out without any pretreatment.

[b] Hamster cheek pouch was pretreated with 5% Azone emulsion for 4 h.

TABLE 5

Pharmacokinetic parameters for salicylic acid after the i.v. and the intra-cheek pouch administrations in the hamster estimated by the moment analysis

Parameter	Intravenous [a]	Intra-cheek-pouch [b]	
		None [c]	Azone [d]
n [e]	3	4	4
AUC (μmol·min/ml)	4.29 ± 0.08	20.6 ± 2.5	20.8 ± 2.9
MRT (min)	68.1 ± 7.4	170.8 ± 10.6	93.2 ± 18.3
MAT (min)	–	102.7	25.1
F (%)	100	96.0	97.0

[a] Dose of salicylic acid was 20 μmol/kg.

[b] Dose of salicylic acid was 100 μmol/kg.

[c] Salicylic acid was administered into the cheek pouch without any pretreatment.

[d] Salicylic acid was administered into the cheek pouch after 4 h-pretreatment with 5% Azone emulsion.

[e] Number of experiments.

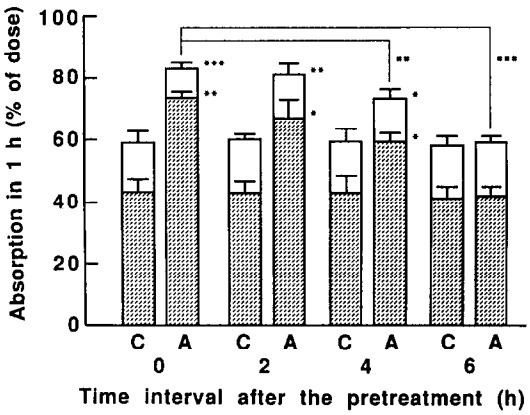

Fig. 4. Duration of the enhancing effect of Azone pretreatment on salicylic acid absorption. Absorption experiments were carried out after 1 h pretreatment with 0.1% polysorbate 20 solution (control, C) or with 5% Azone emulsion (Azone-pretreated, A). Net absorption (shaded column) was calculated by the subtraction of tissue remainings (open column) from disappearance from the lumen of the cheek pouch (full column). Results are expressed as the mean ± S.E.M. of 4–8 experiments. Significantly different from corresponding control values: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Significantly different from each 0-time interval value: ## $P < 0.01$; ### $P < 0.001$.

keratinized oral mucosa and that the enhanced absorption by the Azone-pretreatment enables to make not only the peak plasma concentration higher but also the time to reach the peak concentration earlier.

Duration of the enhancement effect after Azone-pretreatment

After the pretreatment of hamster cheek pouch with 5% Azone-emulsion for 1 h, the absorption experiments were carried out at 2-h intervals to investigate the duration of the enhancing effect of Azone in vivo. Results are shown in Fig. 4. The absorption did not change at any time-intervals in controls. On the contrary, the enhanced absorption observed at short intervals diminished with the lapse of time and there was no significant difference between control and Azone-pretreated groups when the absorption experiments were carried out 6 h after the pretreatment. This means that the barrier function of the keratinized cheek pouch, which was lowered by Azone pretreatment, was completely recovered within 6 h after the removal of Azone-emulsion in vivo. Wotton et al. (1985) reported that the enhancing effect of Azone on the in-vitro permeation remained at least over a period of 120 h after the single application in human skin. However, a recent report on the percutaneous absorption and the elimination of Azone in humans showed that the reservoir formation of Azone in the stratum corneum did not occur and that the incorporated Azone in the stratum corneum, though the amount was small, was rapidly cleared by the urinary excretion in vivo (Wiechers et al., 1987). Our in vitro study showed that Azone mainly acts on the stratum corneum layer, in which the major permeation barrier of the cheek pouch exists, and causes the permeability enhancement (Kurosaki et al., 1989). The results shown in Fig. 4 can be explained as follows. Azone incorporated in the stratum corneum of the cheek pouch during pretreatment is rapidly cleared to the deeper parts, and thus the barrier function of the stratum corneum recovered within 6 h after the pretreatment. The rapid recovery of the barrier function is desirable for the enhancers. In this respect, Azone is one of the useful candidates for the absorption enhancers acting on the keratinized oral mucosa.

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