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The Effect of Additives on the Oral Mucosal Absorption of Human Calcitonin in Rats

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The oral mucosal absorption of human calcitonin (HCT) was investigated in rats. Enhanced absorption of HCT was observed by coadministration of additives such as sodium deoxycholate, sodium tauroglycocholate, quillajasaponin (Quillayanin P-20®), sodium lauryl sulfate, sodium myristate and sugar esters. The contribution of sugar esters to oral mucosal absorption of HCT was studied in some detail. The addition of a sugar ester having a hydrophilic-lipophilic balance value between 11 to 16 was found to be effective in increasing the absorption of HCT. Furthermore, it suggested that the type of constituent fatty acid of sugar ester was one of several important factors for the promotion of the oral mucosal absorption of HCT.

Keywords — human calcitonin; oral mucosal absorption; promoter; surfactant; sugar ester; hydrophilic-lipophilic balance (HLB)

Introduction

Calcitonin (CT), which is a polypeptide composed of 32 amino acids, lowers blood calcium levels, inhibits bone resorption, and has been used for the treatment of Paget's disease, osteoporosis and hypercalcemia. However, all human, pig and salmon CTs and the CT analogue, [Asu^{1,7}]-eel CT, in clinical use, are presently administered by frequent injections. Therefore, the development of another administration route would be desirable for long term therapy with CT. It has been reported that rectal and nasal administration would be possible as a dosage route for human CT (HCT)[1] and [Asu^{1,7}]-eel CT.[2] Although oral mucosal administration is considered to be a favorable route in daily clinical use, there appears to be no studies on the oral mucosal absorption of CT. The administration of HCT to humans is more favorable than the administration of other CTs in view of immunological compatibility.

In this article we demonstrate the enhanced absorption of HCT through the rat oral mucosa and describe the effect of additives on the absorption of HCT.

Materials and Methods

Chemicals — HCT was obtained from Peptides Institute (Minou, Japan). Quillajasaponin (Quillayanin P-20®) was a generous gift from Maruzen Kasei Co., Ltd. (Onomichi, Japan). The purity of the fatty acids of sucrose monocaprate (Ryoto Sugar Ester® MO-C10), sucrose monolaurate (Ryoto Sugar Ester® MO-C12), sucrose monopalmitate (Ryoto Sugar Ester® MO-C16) and sucrose monostearate (Ryoto Sugar Ester® MO-C18) were 100% by gas chromatography according to the method of Kimura *et al.* [3] Sucrose laurate (Ryoto Sugar Ester® L-1695) and sucrose myristate (Ryoto Sugar Ester® M-1695) were composed of 81% and 82% of monoester, respectively, as shown in Table III, but the constituent fatty acid was found to be pure by the above same method. Sugar esters consisted mainly of sucrose stearate (Ryoto Sugar Ester® S-270, S-370F, S-570, S-770, S-970, S-1170, S-1570, S-1670) and were composed of mixtures of mono, di and triester, and the ratio of the constituent fatty acids, myristic acid, palmitic acid and stearic acid was 2:31:68. Sugar esters which consisted mainly of sucrose palmitate (Ryoto Sugar Ester® P-1570, P-1670) were also mixtures of mono, di and triester, and the ratio of the constituent fatty acids, myristic acid, palmitic acid and stearic acid was 2 : 80 : 18. All sugar esters were supplied by Mitsubishi-Kasei Food Corporation (Tokyo, Japan). Other chemicals used were: γ-

cyclodextrin, sodium deoxycholate and sodium hexanate (Tokyo Kasei Kogyo Co., Ltd., Tokyo, Japan); α-cyclodextrin, polyoxyethylene(20) sorbitan monooleate, saponin (tea nut), sodium lauryl sulfte (SLS) and sodium tauroglycocholate (Wako Pure Chemical Ind., Ltd.); sodium glycodeoxycholate and sodium taurodeoxycholate (Sigma Chemical Co., USA); sodium octanoate, sodium nonanoate, sodium myristate and sodium palmitate (Kanto Chemical Co., Inc., Tokyo, Japan). Other reagents used were of reagent grade obtained commerciallv.

HCT Preparations — HCT solution with or without additives was prepared by dissolving 0.51mg of HCT in 1 ml of distilled water. HCT preparation was prepared by mixing equal volumes of the HCT solution and either 0.1 M sodium acetate buffer (pH 3.6), 0.2 M tris (hydroxymethyl) aminomethane hydrochloride buffer (pH 7.4) or 0.1 M sodium bicarbonate buffer (pH 11.3).

Animals — Wistar male rats (4 weeks old) were fasted for 20 h prior to experimentation. During the experiment, rats were anesthetized with sodium pentobarbital (40 mg/kg i.p.) and kept on a warm surface at 38 °C.

Absorption Study — The oral mucosal absorption study was carried out according to the method of Aoki and Yata.[4] At first, a cotton ball (1 mg) was placed between the rat's labium inferius and gingiva. Then 50 μl of HCT preparation were administered to the cotton ball. After the administration, blood samples (about 0.2 ml) were withdrawn periodically from the jugular vein. Plasma was obtained by centrifugation at 15000 × *g* for 3 min.

Analytical Method — Plasma calcium level was determined to evaluate the oral mucosal absorption of HCT by Calcium C Test Wako®. The decrement of the plasma calcium level (ΔA, total decrease) from 0 to 4 h after dosing with HCT was calculated from the following equation according to the method of Hirai *et al.*[5]

$$\Delta A\ (\%) = \frac{AUC_C - AUC_H}{AUC_C} \times 100$$

where AUC_C is the area under the plasma calcium level *versus* time curve from 0 to 4 h after administration of additives only, and AUC_H is the area under the plasma calcium level *versus* time curve from 0 to 4 h after administration of HCT with additives.

Statisitics — All data were tested statistically for significant differences using the Bartlett and Danncan tests.

Treatment of the Glass Vessel Surface — The surface of all glass materials was treated with 3% (w/v) trimethylchlorosilane in benzen to prevent the adsorption of HCT. After treatment, the glass surface was washed 5 times with 5 ml of MeOH, and air-dried at room temperature.

Results

Effects of Various Additives

Table I shows the effects of various additives, which are known as promoters[5,6] of the mucosal absorption of some drugs and peptides, on the decrements of plasma calcium levels following oral mucosal administration of HCT in rats at pH 7.4. The plasma calcium levels were little affected by the administration of HCT without additives to rats (no additive in Table I and Fig. 2 (A)). But the coadministration of surfactants such as sodium deoxycholate, sodium tauroglycocholate, SLS, quillajasaponin (Quillayanin P-20®), sodium myristate and sucrose palmitate (Ryoto Sugar Ester® P-1670), decreased the plasma calcium levels ($p < 0.05$) significantly. These additives have been demonstrated to be effective promoters for the oral mucosal absorption of HCT. However, α-cyclodextrin, γ-cyclodextrin, saponin (tea nut), sodium salts of straight chain-fatty acids, except myristic acid, and bile acids, except deoxycholic acid and tauroglycocholic acid, had no promoting effect.

Effects of Sugar Esters

The changes in plasma calcium levels following oral mucosal administration of HCT preparation (pH 7.4) contaning 2.3 mg/ml of sucrose palmitate (Ryoto Sugar Ester® P-1670) are shown in Fig. 1. The plasma calcium levels were decreased by increasing the administered amounts of HCT up to 12.75 μg/head. The plasma calcium level reached the minimum at

TABLE I. Effects of Various Additives on the Decrements of Plasma Calcium Levels Following Oral Administration of HCT in Rats at pH 7.4[a)]

Additives	ΔA (%) [b)]
No additive	1.6±1.4
Saponin group	
Saponin (tea nut)	3.4±2.5
Quillayasaponin (Quillayanin P-20®)	15.6±2.9 [c)]
Sodium straight-chain fatty acid group	
Sodium hexanate	−1.0±2.2
Sodium octanoate	2.8±2.5
Sodium nonanoate	4.2±1.4
Sodium myristate	7.4±1.2 [c)]
Sodium palmitate	−0.7±0.5
Cyclodextrin group	
α-Cyclodextrin	−1.7±1.9
γ-Cyclodextrin	−0.2±0.8
Bile acid group	
Ursodeoxycholic acid	3.2±3.4
Sodium deoxycholate	21.6±2.1 [d)]
Sodium tauroglycocholate	14.2±2.7 [c)]
Sodium glycodeoxycholate	6.0±2.6
Sodium taurodeoxycholate	5.4±1.0
Other surfactant group	
Polyoxyethylene (20) sorbitan monoolate	−1.1±1.9
Sodium lauryl sulfate	32.2±1.8 [d)]
Sucrose palmitate (Ryoto Sugar Ester® P-1670)	8.3±1.2 [c)]

a) Amounts of HCT and each additive applied to rats were 12.75 μg and 1.125 mg (22.5 mg/ml), respectively.
b) Each value is expressed as the mean ±S.E. of 3−5 animals. Significantly different from control (no additive) within each group: *c)*, $0.01 < p < 0.05$; *d)*, $p < 0.01$.

1 h after dosing and then returned gradually to its initial level. Figure 2 shows the effects of the concentration of sucrose palmitate (Ryoto Sugar Ester® P-1670) on changes in the plasma calcium levels following oral mucosal administration of HCT. The marked decrements of the plasma calcium level were obtained at a concentration greater than 2.3 mg/ml. The extent of ΔA (8.3 ± 1.2%) at a concentration of 22.5 mg/ml was slightly lower than that (10.8 ± 1.8%) at a concentration of 2.3 mg/ml, but there was no significant difference ($0.05 < p$) in the plasma calcium level at every sampling point between both groups. Figure 3 shows the effects of pH on changes in the plasma calcium levels following oral mucosal administration of HCT. The decrement of plasma calcium level at pH 7.4 was larger than that at pH 3.6, but there was no significant difference in the decrements between pH 7.4 and 11.3.

Table II shows the effects of the ratio of monoester and hydrophilic-lipophilic balance (HLB) values of sugar esters on the decrements of the plasma calcium levels following oral mucosal administration of HCT. The promoting effects were found with Ryoto Sugar Ester® S-1170, S-1570, S-1670, P-1570 and P-1670, which had HLB values ranging from 11 to 16, and contained monoesters in the range of approximate 60 to 80%. P-1570 and P-1670 (the ratio of palmitic acid to stearic acid was approximately 8 : 2) were equal to or more effective

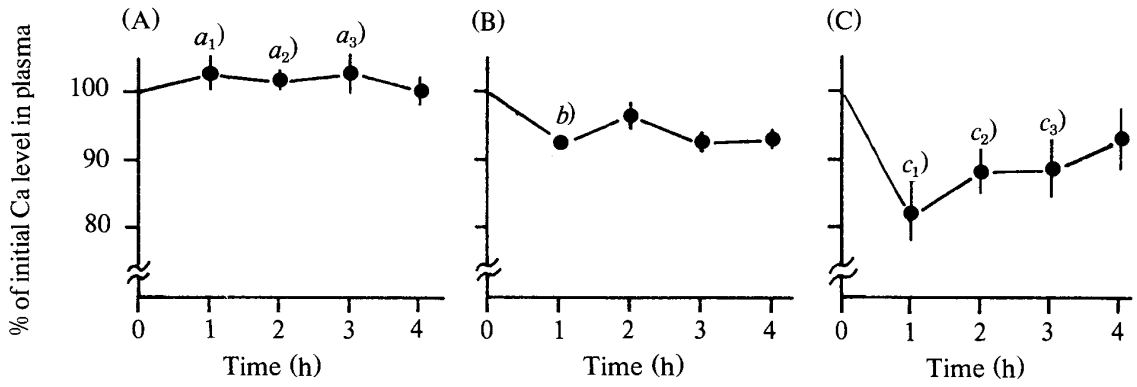

Fig. 1. Effects of HCT Dose on Changes in Plasma Calcium Levels Following Oral Mucosal Administration of HCT with Sucrose Palmitate (Ryoto Sugar Ester® P-1670, 2.3mg/ml) in Rats at pH 7.4
The doses of HCT were (A), 0 μg/head; (B), 5.00 μg/head; (C), 12.75 μg/head. Each value is expressed as the mean ±S.E. of 3 or 4 animals. Significant differences: $a_1) - c_1)$, $b) - c_1)$, $p < 0.01$; $a_1) - b)$, $a_2) - c_2)$, $a_3) - c_3)$, $0.01 < p < 0.05$.

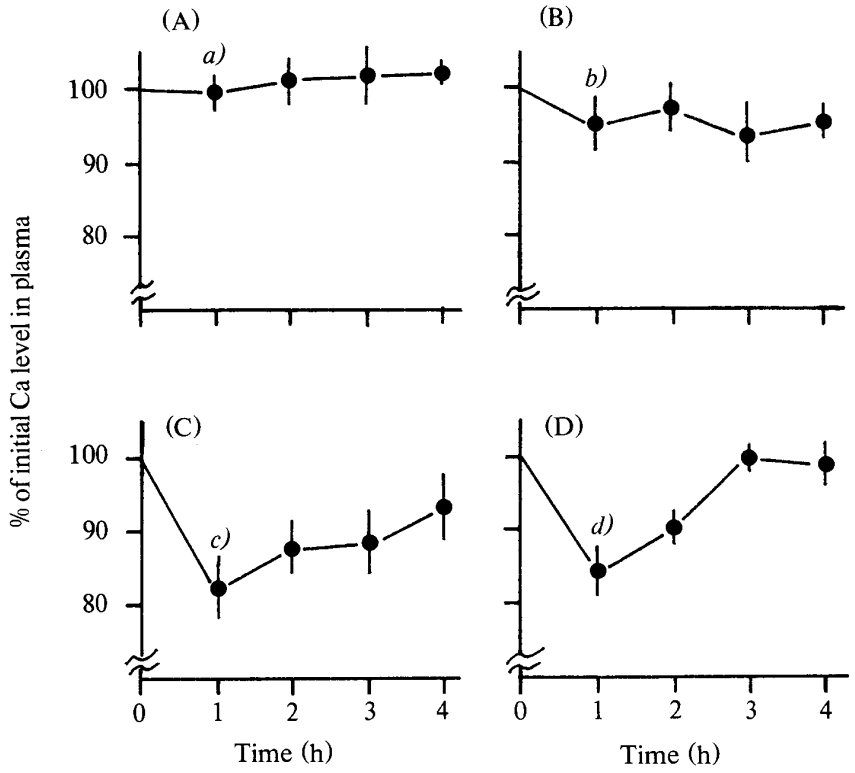

Fig. 2. Effects of the Concentration of Sucrose Palmitate (Ryoto Sugar Ester® P-1670) on Changes in Plasma Calcium Levels Following Oral Mucosal Administration of HCT in Rats at pH 7.4

Dose of HCT; 12.75 μg/head. The concentrations of Ryoto Sugar Ester® P-1670 were (A), 0 mg/ml; (B), 0.2 mg/ml; (C), 2.3 mg/ml; (D), 22.5 mg/ml. Each value is expressed as the mean±S.E. of 3 or 4 animals. Significant differences: *a*)—*c*), *a*)—*d*), *b*)—*c*), $p < 0.01$; *b*)—*d*), $0.01 < p < 0.05$.

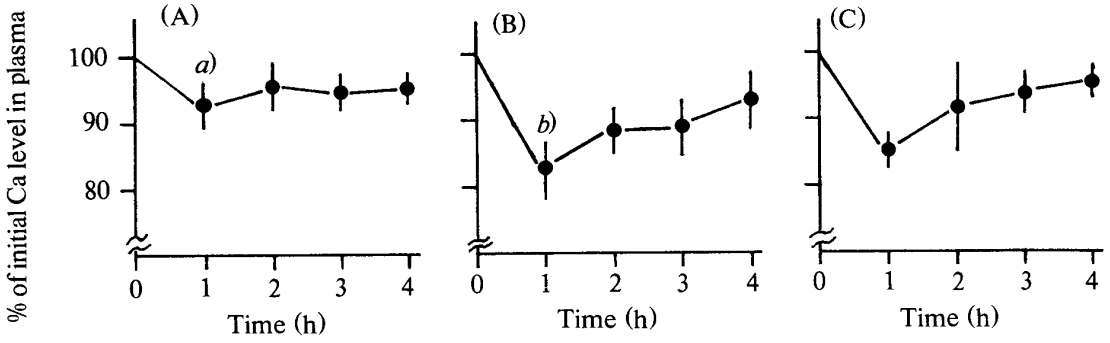

Fig. 3. Effects of pH on Changes in Plasma Calcium Levels Following Oral Mucosal Administration of HCT with Sucrose Palmitate (Ryoto Sugar Ester® P-1670, 2.3 mg/ml) in Rats.

Dose of HCT; 12.75 μg/head. The pH values of HCT preparation were (A), pH 3.6; (B), pH 7.4; (C), pH 11.3. Each value is expressed as the mean±S.E. of 4 animals. Significant differences: *a*)—*b*), $p < 0.01$.

than S-1570 and S-1670 (the ratio of palmitic acid to stearic acid was approximately 3 : 7).

Table III shows the effects of the carbon number of the constituent fatty acid in sugar esters on the decrements of the plasma calcium levels following oral mucosal administration of HCT. Sugar esters composed of myristic acid (Ryoto Sugar Ester® M-1695) and palmitic acid (Ryoto Sugar Ester® MO-C16) possessed promoting effects. The other sugar esters composed of capric acid, lauric acid and stearic acid did not show any promoting effect on the absorption of HCT.

Effects of Sodium Myristate

TABLE II. Effects of Sugar Ester on the Decrements of Plasma Calcium Levels Following Oral Mucosal Administration of HCT in Rats at pH 7.4[a)]

Sugar ester [b)]	HLB values [c)]	Constituent fatty acid and its ratio [d)]	Ratio of monoester (%) [c)]	ΔA (%) [e)]
No additive				1.6±1.4
S-270	2	C14:C16:C18 (2:31:68)	15	3.5±1.5
S-370F	3	C14:C16:C18 (2:31:68)	18	2.2±1.0
S-570	5	C14:C16:C18 (2:31:68)	30	2.0±1.6
S-770	7	C14:C16:C18 (2:31:68)	41	2.5±0.6
S-970	9	C14:C16:C18 (2:31:68)	47	−0.9±2.0
S-1170	11	C14:C16:C18 (2:31:68)	59	6.6±0.7 *f)*
S-1570	15	C14:C16:C18 (2:31:68)	72	7.1±0.8 *f)*
S-1670	16	C14:C16:C18 (2:31:68)	75	7.0±1.7 *f)*
P-1570	15	C14:C16:C18 (2:80:18)	70	6.7±1.3 *f)*
P-1670	16	C14:C16:C18 (2:80:18)	79	10.8±1.8 *g)*

a) Amounts of HCT and each sugar ester applied to rats were 12.75 μg and 0.1125 mg (2.25 mg/ml), respectively. *b)* Ryoto Sugar Ester®. *c)* Analyses were performed by Mitsubisi-Kasei Food Corporation, Tokyo, Japan. *d)* C14, myristic acid; C16, palmitic acid; C18, stearic acid. Analyses were performed by Mitsubisi-Kasei Food Corporation, Tokyo, Japan. *e)* Each value is expressed as the mean ± S.E. of 3 or 4 animals. Significantly different from control (no additive): *f)*, $0.01 < p < 0.05$; *g)*, $p < 0.01$.

TABLE III. Effects of Carbon Number of Fatty Acid in Sugar Esters on the Decrements of Plasma Calcium Levels Following Oral Mucosal Administration of HCT in Rats at pH 7.4[a)]

Sugar ester [b)]	Fatty acid [c)]	Ratio of monoester (%) [d)]	ΔA (%) [e)]
No additive			1.6±1.4
Sucrose monocaprate (MO-C10)	C10	100	2.0±1.8
Sucrose laurate (L-1695)	C12	81	1.9±1.9
Sucrose monolaurate (MO-C12)	C12	100	−1.6±1.2
Sucrose myristate (M-1695)	C14	82	7.4±1.6 *f)*
Sucrose monopalmitate (MO-C16)	C16	100	6.9±1.4 *f)*
Sucrose monostearate (MO-C18)	C18	100	−0.6±1.4

a) Amounts of HCT and each sugar ester applied to rats were 12.75 μg and 0.1125 mg (2.25 mg/ml), respectively. *b)* Ryoto Sugar Ester®. *c)* The component of fatty acids in sugar esters. C10, capric acid; C12, lauric acid; C14, myristic acid; C16, palmitic acid; C18 stearic acid. *d)* Analyses were performed by Mitsubisi-Kasei Food Corporation, Tokyo, Japan. *e)* Each value is expressed as the mean ± S.E. of 3 or 4 animals. *f)* Significantly different from control (no additive) $0.01 < p < 0.05$.

Table IV shows the effects of the concentration of sodium myristate on the oral mucosal absorption of HCT in rats. A marked decrement of the plasma calcium level was obtained at a concentration of 22.5 mg/ml and there was no promoting effect on the absorption of HCT at 2.3 mg/ml.

TABLE IV. Effects of Concentration of Sodium Myristate on the Decrements of Plasma Calcium Levels Following Oral Mucosal Administration of HCT in Rats at pH 7.4[a)]

Concentation (mg/ml)	ΔA [b)]
0	1.6±1.4
2.3	−1.3±0.6
22.5	7.4±1.2 [c)]

a) Amount of HCT applied to rats was 12.75 μg.
b) Each value is expressed as the mean ± S.E. of 3 or 4 animals. *c)* Significantly different from control (no additive) $0.01 < p < 0.05$.

Discussion

To evaluate the oral mucosal absorption of HCT, plasma calcium level was determined in rats. Although a decrement of plasma calcium level was rarely found by the administration of HCT without additives, the coadministration of HCT and various surfactants, for example, sodium myristate, sodium deoxycholate, sodium tauroglycocholate, SLS, quillajasaponin and sugar esters, resulted in a lowering of plasma calcium levels (Table I). The reasons for the difference in the promoting effect between the structual analogs may be obtained by additional studies. There was a report claiming the promoting effect of straight-chain fatty acids on the rectal absorption of sodium ampicillin in rats was related to the length of their chains.[7)]

Furthermore, it was reported that the promoting effect of bile salts on the rectal absorption of sodium ampicillin in rats was also related to their chelating abilities and the promoting effect of sodium deoxycholate on rectal absorption of sodium ampicillin was markedly decreased in the presence of Ca^{2+}.[8)] But this mechanism was contradicted by Hirasawa *et al.*[9)] who suggested the contribution of solvent drag in the rectal absorption of antipyrine. The affinity of calcium ion for sodium deoxycholate and sodium taurodeoxycholate was reported to be 0.083 and 0.163 (gram ion/M), respectively.[8)] The decrements of the plasma calcium level (ΔA) with sodium deoxycholate and sodium taurodeoxycholate in our experiments were 21.6 and 5.4%, respectively. The present results appear to support the mechanism of Hirasawa *et al.* for oral mucosal absorption of HCT.

The promoting effect of sucrose palmitate (Ryoto Sugar Ester® P-1670) on the oral mucosal absorption of HCT was also demonstrated as shown in Table I. Sugar esters might be expected to be less toxic.[10)] Furthermore, they are commercially available and have a wide range of HLB values depending upon the kind and ratio of the constituent fatty acids in sugar esters. Hence, experiments on the effect of sugar esters were performed in some detail.

When sucrose palmitate (Ryoto Sugar Ester® P-1670) was added to an HCT preparation as a promoter, a significant decrement of plasma calcium level was obtained with 12.75 μg of HCT and 0.115 mg of sugar ester (2.3 mg/ml × 50 μl) per rat at pH 7.4 (Figs. 1, 2 and 3). As the change in the plasma calcium level reached maximum at 1 h after dosing, the absorption of HCT through the rat oral mucosa appeared to be relatively rapid. There was no statistically significant difference in the response between the sugar ester's concentration of 2.3 and 22.5 mg/ml and between pH 7.4 and 11.3. Therefore, the above conditions may to be favorable on the oral mucosal absorption of HCT. The addition of the sugar ester having a HLB value from 11 to 16 resulted in the promoting effect of oral mucosal absorption of HCT (Table II). This result was similar to that previously reported on the nasal absorption of insulin in rats, in which polyoxyethylene lauryl ether was added as a non-ionic surfactant.[5)] The ratio of palmitic acid to stearic acid in sugar esters appeared not to be important in the oral mucosal absorption of HCT (Table II). The type of fatty acid of sugar esters, that is, the length of the carbon chain of the fatty acid moiety was found to be an important factor for promoting the absorption of HCT. This phenomenon that both myristate ester and palmitate ester enhanced the oral mucosal absorption of HCT (Table III) was most intersting and should be studied in detail.

It is known that sugar esters are decomposed to sucrose and fatty acid under physiological conditions.[11)] The present study demonstrated that sodium myristate was effective on the oral mucosal absorption of HCT. However, it is likely that sugar esters themselves predominant-

ly promote the absorption of HCT for the following reasons ; 1) Sugar esters appeared to have a promoting effect at a lower concentration than that of sodium myristate (Tables III and IV). 2) Sodium palmitate had no promoting effect in comparison with sucrose monopalmitate (Ryoto Sugar Ester® MO-C16).

It has been reported that surfactants appear to increase the permeability of the nasal mucosa and thereby promote the nasal absorption of insulin.[12] Another possible explanation is that the surfactants reduced the proteolytic enzyme activity in the nasal mucosa.[12] The preliminary examination showed that sucrose palmitate (Ryoto Sugar Ester® P-1670) did not affect the decomposition of HCT in the 9000 × ***g*** supernatant fluid of the rat oral mucosal homogenate (pH 7.4). Therefore, in the presence of the surfactants such as sugar esters, the oral mucosal absorption of HCT seems to be promoted by the increasing of the permeability of oral mucosa.

In conclusion, sodium deoxycholate, sodium tauroglycocholate, quillajasaponin, SLS, sodium myristate and sugar esters had a promoting effect on the oral mucosal absorption of HCT in rats, and the results of the present study suggest that oral mucosal administration will be one possible route for therapy with HCT.

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