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Sodium Cromoglycate Prevents Exacerbation of IgE-Mediated Food-Allergic Reaction Induced by Aspirin in a Rat Model of Egg Allergy

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Key Words
Anaphylaxis · Aspirin · Food allergy · Intestinal permeability · Ovalbumin · Sodium cromoglycate

Abstract
Background: Aspirin (ASP)-facilitated absorption of ingested allergens is considered an exacerbating factor in the development of food allergy. Sodium cromoglycate (SCG) is used for the treatment of atopic dermatitis with food allergy, but the efficacy of SCG in ASP-exacerbated food-allergy reactions is unclear. In this study, we evaluated the effect of SCG on ASP-exacerbated food-allergic reactions, as well as allergen absorption, in egg-allergic model rats. ***Methods:*** Plasma concentrations of ovalbumin (OVA) and fluorescein isothiocyanate-labeled dextran (FD-40), a marker for non-specific-absorption pathways, were measured after oral administration of mixtures of OVA and FD-40 in OVA-unsensitized and OVA-sensitized rats. IgE-mediated allergic reactions were evaluated by measuring changes in rectal temperature and Evans blue dye (EBD) extravasation in the intestine and liver after oral challenge with OVA. The effects of ASP and SCG on such absorption and allergic reactions were also evaluated kinetically. ***Results:*** In OVA-sensitized rats, plasma concentrations of OVA and FD-40 were significantly higher than those in unsensitized rats after oral administration. ASP increased the intestinal absorption of OVA and FD-40 via the paracellular pathway, and a lower rectal temperature and higher EBD extravasation were detected in the intestine and liver of OVA-sensitized rats. SCG ameliorated these ASP-facilitated absorptions and allergic reactions in a dose-dependent manner. In particular, high-dose SCG (195.2 μmol/kg) completely inhibited these absorptions and reactions. ***Conclusion:*** SCG can prevent ASP-exacerbated allergic reactions in patients with food allergy resulting from inhibition of increases in allergen absorption.

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Introduction

Food allergy is a serious healthcare issue because it can result in life-threatening anaphylaxis. The prevalence of food allergy is increasing – epidemiologic studies have shown that food allergy affects ~2–5% of adults and 8% of children [1, 2]. Several foods appear to be responsible for allergy, including eggs, milk, wheat, and peanuts [1]. Symptoms of food allergy include urticaria, dyspnea, diarrhea, and systemic anaphylaxis [3, 4]. These type-I allergic symptoms develop as a result of degranulation of mast cells after crosslinking of the immunoglobulin (Ig)E antibody bound to the surface of mast cells with allergens [3]. Thus, ingested food allergens must be absorbed across intestinal epithelial cells before they have access to mast cells in subepithelial regions. Several studies have suggested that the symptoms of IgE-mediated food allergy are de-

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pendent on the amounts of allergen absorbed into the blood [5–8]. Strait et al. [7] demonstrated, using passive and active models of anaphylaxis in mice, that ingested allergens must be absorbed systemically to induce anaphylaxis. Brockow et al. [8] reported that plasma concentrations of gliadin [a major allergen for wheat-dependent exercise-induced anaphylaxis (WDEIA)] were increased markedly in parallel with allergic symptoms on provocation tests in patients with WDEIA. Thus, the amount of allergen absorbed via the intestine is thought to be related to the severity of symptoms of IgE-mediated food allergy.

Aspirin (ASP) is used widely as an anti-inflammatory and anticoagulant agent. The pharmacologic actions of ASP result from its inhibition of cyclooxygenase (COX), which produces prostaglandins and thromboxanes from arachidonic acid [9]. Two isoforms of COX have been identified: COX-1 and COX-2 [10]. COX-1 is involved in the physiologic production of prostaglandins, whereas COX-2 is responsible for the induction of inflammation. Studies have shown that ASP aggravates allergy symptoms, including urticaria and anaphylaxis, in patients with IgE-mediated food allergy [11–13]. The pathogenesis of ASP-exacerbated food allergy symptoms is not known. Previously, we reported that increased serum levels of ingested allergen were required to elicit allergic reactions in provocation tests in patients with WDEIA and that ASP increased the absorption of ingested allergens after impairment of paracellular pathways in rats and humans [13–16]. These findings indicate that IgE-mediated food-allergic symptoms are exacerbated by ASP through facilitation of allergen absorption from the intestinal tract. On the other hand, ASP enhanced the allergic reaction on skin prick tests in patients with food-dependent exercise-induced anaphylaxis, suggesting that ASP can also directly activate the inflammatory cells [17]. With respect to the ASP-activating mechanisms of inflammatory cells, we reported that ASP enhanced the histamine release from human basophils via increased activation of Syk kinase [18]. In addition, Suzuki and Ra [19] demonstrated that ASP also enhanced IgE-mediated leukotriene C_4 secretion through the facilitating L-type Ca^{2+} channels at low concentrations (≤0.3 mM) in rat basophilic leukemia RBL-2H3 cells. Thus, increases in the absorption of allergens via the intestinal tract and activation of inflammatory cells are considered to be the underlying mechanisms for the pathogenesis of ASP-exacerbated food-allergic symptoms.

The most reliable therapy for food allergy is strict elimination of causative foods from dietary sources. However, patients may occasionally ingest foods containing causative allergens without recognizing them. Sodium cromoglycate (SCG) has been reported to be effective in preventing the symptoms of food allergy if used alone and/or in a supportive role with an elimination diet [20–24]. Several studies have shown that SCG decreases the symptoms of food allergy by inhibiting the release of chemical mediators (including histamine) and cytokines (including tumor necrosis factor-α) from inflammatory cells including activated mast cells [25, 26]. In addition, SCG diminishes the absorption of ingested macromolecules such as allergens, polyethylene glycols, and lactulose, indicating that SCG also decreases allergic symptoms through prevention of allergen absorption [27–29]. Based on these results, we hypothesized that SCG could also ameliorate ASP-exacerbated allergic symptoms by preventing ASP-facilitated allergen absorption from the intestinal tract. Sugimura et al. [24] reported that SCG administered orally at a dose of 100 mg (195.2 μmol, a recommended dose for atopic dermatitis with food allergy) 20 min before meals suppressed the development of anaphylaxis in 2 children with WDEIA. In contrast, Takahashi et al. [30] reported, in a WDEIA case, that intake of a single dose of SCG at 100 mg 1 h before ingestion of bread did not reduce increased serum levels of wheat allergen and did not improve the anaphylactic symptoms provoked by a combination challenge of ASP and exercise. The prophylactic effect of SCG on the anaphylactic symptoms facilitated by ASP is incompletely understood because the optimal doses and effectiveness of SCG given via the oral route are controversial [27, 31, 32]. Shin et al. [33] reported that high-dose SCG (195.2 μmol/kg) decreased compound 48/80-induced mortality by anaphylaxis more than normal-dose SCG (19.5 μmol/kg). Thus, it is expected that high-dose SCG can also ameliorate the ASP-exacerbated food-allergic symptoms mediated by IgE. In this study, we examined the effect of SCG (19.5 and 195.2 μmol/kg) on ASP-facilitated intestinal absorption of an egg allergen, i.e. ovalbumin (OVA), and anaphylaxis in OVA-sensitized rats.

Materials and Methods

Materials

OVA (grade V), SCG, and fluorescein isothiocyanate-labeled dextran 40 (FD-40; average molecular weight, 40 kDa) were purchased from Sigma-Aldrich (St. Louis, Mo., USA). ASP was obtained from Wako Pure Chemicals (Osaka, Japan). Evans blue dye (EBD) and formamide were purchased from Nacalai Tesque (Kyoto, Japan). Alum adjuvant (Imject® Alum) and ELISA plates (F8 MaxiSorp Loose Nunc-Immuno™ Modules) were from Thermo Fisher Scientific (Waltham, Mass., USA). The blocking reagent (Block Ace®), horseradish peroxidase (HRP)-conjugated mouse

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anti-rat IgE (MARE-1), and 3, 3′, 5, 5′-tetramethylbenzidine (TMB) solution (TMB Microwell Peroxidase Substrate System) were purchased from DS Pharma Biomedical (Osaka, Japan), GeneTex (Irvine, Calif., USA), and KPL (Gaithersburg, Md., USA), respectively. All chemicals used were of the highest purity available.

Animals

Male Sprague-Dawley (SD) rats (4 weeks old) were obtained from Japan SLC (Shizuoka, Japan). Rats were fed a standard laboratory diet (MF; Oriental Yeast Company, Tokyo, Japan) and water ad libitum. Rats were maintained in a temperature- and light-controlled environment for 1 week before OVA sensitization. All experiments involving animals were carried out in accordance with the Guide for Animal Experimentation of the Committee of Research Facilities for Laboratory Animal Sciences of Hiroshima University (Hiroshima, Japan).

Sensitization Protocol

Rats were sensitized by intraperitoneal injection with 1 ml of physiologic (0.9%) saline containing 22.2 nmol of OVA and Imject® Alum [128.2 μmol $Al(OH)_3$ and 171.5 μmol of $Mg(OH)_2$] at weekly intervals for 4 weeks (fig. 1) according to a previous report with slight modifications [34]. Four weeks after the first immunization, blood (0.2 ml) was collected from the jugular vein to check plasma levels of OVA-specific IgE using an enzyme-linked immunosorbent assay (ELISA). Rats with low levels of OVA-specific IgE were given an OVA injection every week for an additional 2 weeks, and IgE levels were checked again. At 4 or 6 weeks, rats with OVA-specific IgE were used as the OVA-sensitized group (n = 48) and divided randomly into the following 6 groups for studies on absorption and anaphylaxis: control (vehicle alone), normal-dose SCG (SCG 19.5 μmol/kg), high-dose SCG (SCG 195.2 μmol/kg), ASP (ASP alone), ASP + normal-dose SCG (ASP + SCG 19.5 μmol/kg), and ASP + high-dose SCG (ASP + SCG 195.2 μmol/kg). Unsensitized rats (n = 16) received physiologic saline containing adjuvant alone at weekly intervals for 4 weeks and were randomized to the control group or the ASP group.

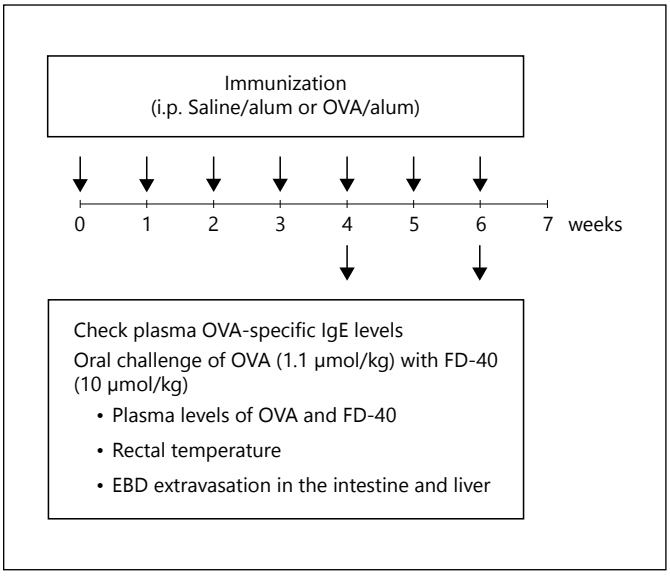

Fig. 1. Sensitization and oral challenge with OVA. SD rats (5 weeks) were sensitized by intraperitoneal injection of 22.2 nmol of OVA and Imject® Alum [128.2 μmol of $Al(OH)_3$ and 171.4 μmol of $Mg(OH)_2$] at weekly intervals for 4 weeks. At week 4, plasma levels of IgE specific for OVA were measured. Rats with low levels of OVA-specific IgE were given an OVA injection every week for an additional 2 weeks. At 4 or 6 weeks, rats with OVA-specific IgE were used as the OVA-sensitized group. Rats in the unsensitized group were injected (intraperitoneally) with saline and Imject® Alum [128.2 μmol of $Al(OH)_3$ and 171.4 μmol of $Mg(OH)_2$] at weekly intervals for 4 weeks. Absorption of ingested OVA and FD-40, rectal temperatures, and vascular permeability were evaluated after oral challenge with OVA at week 4 or 6. i.p. = Intraperitoneal.

Measurement of Plasma Levels of OVA-Specific IgE

To confirm the sensitization to OVA, plasma levels of IgE specific to OVA were determined using ELISA according to our previous report with slight modifications [35]. Briefly, the wells of the ELISA plates were coated with 100 μl of OVA (222 nM) dissolved in phosphate-buffered saline (PBS, pH 7.4) at 4°C. After washing with PBS containing 0.1% Tween 20 (PBS-T) 6 times, plates were incubated with 1% Block Ace® for 2 h at room temperature. Then, 100 μl of each sample of rat plasma (diluted 1:10 in 1% Block Ace®) was added to each well and incubated for 2 h at room temperature. After washing with PBS-T, the wells were incubated with 100 μl of MARE-1 (diluted 1:1,000 in PBS) for 2 h at room temperature. The wells were washed with PBS-T and then incubated with 100 μl of TMB at room temperature. After incubation, the reaction was terminated with 100 μl of 1 M phosphoric acid. Absorbance was measured at 450 nm against 630 nm as reference using Multiskan GO (Thermo Fisher Scientific).

Absorption of OVA and FD-40 after Oral Administration

To evaluate the effects of ASP and SCG on absorption of the ingested allergen, absorptions of OVA and FD-40 in rats were examined as reported previously [14, 15]. Briefly, OVA-unsensitized and OVA-sensitized rats were fasted overnight and anesthetized with pentobarbital (30 mg/kg, intraperitoneally). The anesthetized rats were cannulated with polyethylene tubing (PE-50) at the femoral artery for blood sampling. Vehicle alone (PBS at pH 7.4), ASP (166.5 μmol/kg), and/or SCG (19.5 or 195.2 μmol/kg) were administered using a stainless-steel feeding tube. OVA (1.1 mM) and FD-40 (10 mM) were dissolved in PBS, and the mixture was administered (orally) at 1.0 ml/kg 30 min after treatment. Blood (0.25 ml each) was collected at a designated time interval for 3 h via a cannula inserted in the femoral artery, and centrifuged to collect plasma. Plasma concentrations of OVA and FD-40 were determined using a sandwich ELISA kit (Morinaga Institute of Biological Science, Yokohama, Japan) and a Microplate Fluorometer (PerkinElmer, Waltham, Mass., USA) at 500 nm for excitation and 520 nm for emission, as described previously [14, 15].

Evaluation of Systemic Anaphylaxis

To evaluate IgE-mediated systemic anaphylaxis in allergic animal models, a standardized anaphylaxis score system and measurements of serum histamine levels, rectal temperature, blood pressure, and vascular permeability are frequently used after the allergen challenge [36, 37]. In this study, systemic anaphylaxis was

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Table 1. Plasma levels of OVA-specific IgE in rats assigned to 8 groups

	Unsensitized	Sensitized		
SCG, μmol/kg:	0	0	19.5	195.2
Control	0.00±0.00	1.70±0.33[a, b]	1.49±0.22[a, b]	1.45±0.10[a, b]
+ASP	0.01±0.00	1.69±0.20[a, b]	1.45±0.06[a, b]	1.58±0.18[a, b]

Levels of OVA-specific IgE in the plasma of rats were measured by ELISA. Optical densities at 450 nm in 10-fold-diluted plasma are shown. Rats were given an intraperitoneal injection of vehicle alone or OVA every week for 4 or 6 weeks. Each value represents the mean ± SEM for 8 rats. +ASP = ASP treatment; SCG = sodium cromoglycate. [a] $p < 0.01$: significantly different from week 0. [b] $p < 0.01$: significantly different from unsensitized rats.

evaluated by monitoring changes in rectal temperature and vascular permeability after oral challenge with OVA, quantitatively. Rectal temperature was measured using a specific rectal thermometer for rats (Shibaura Electronics, Saitama, Japan) before and 30 min after OVA challenge during the absorption study described above according to a previous report [38]. The increased vascular permeability induced by vasoactive mediators such as histamine is evidence of the systemic anaphylaxis caused by an allergen challenge. Changes in vascular permeability in the intestine and liver were evaluated via the EBD extravasation method according to the method of Miyasaka et al. [39]. Briefly, EBD (136.0 μmol/kg) was injected (intravenously) via a cannula inserted in the femoral vein 20 min before the oral OVA challenge (1.1 μmol/kg). Thirty minutes later, the peritoneal cavity was opened and the vascular system exsanguinated by cutting of the abdominal aorta. The intestine and liver were isolated and half of each tissue was immersed into formamide (4 ml/g of wet tissue weight) to extract EBD at 20°C for 24 h. The other half of each tissue was dried at 60°C for 24 h. The concentration of EBD extracted was determined at 620 nm using Multiskan GO and is reported in micromoles per gram of dry tissue weight.

Statistical Analyses

Data are reported as means ± SEM. Differences in mean values between groups were assessed using the Kruskal-Wallis test or analysis of variance, followed by a post hoc Tukey or Student t test. For plasma levels of OVA-specific IgE, differences in mean values among each group were analyzed using the Kruskal-Wallis test with Scheffe's F test. $p < 0.05$ was considered statistically significant.

Results

Plasma Levels of IgE Specific for OVA

Plasma levels of IgE specific for OVA were determined to confirm sensitization to OVA. Before the first immunization of OVA (0 weeks), OVA-specific IgE was not detected in the plasma of all rats. At 4 or 6 weeks, plasma levels of OVA-specific IgE were significantly increased in OVA-sensitized rats, whereas levels of OVA-specific IgE were marginal in unsensitized rats (table 1). Significant differences were not observed in plasma levels of IgE among the 6 sensitized groups.

Effect of SCG on ASP-Facilitated Oral Absorption of OVA

We examined the effect of SCG on ASP-facilitated absorption of OVA and FD-40 into blood after oral administration of their mixture (fig. 2), whereby FD-40 was used as a marker for nonspecific pathways (i.e. paracellular and fluid-phase endocytic pathways) [40]. The areas under the concentration-time curves from 0 to 3 h ($AUC_{0-3\ h}$) of OVA and FD-40 are summarized in table 2. In unsensitized control rats, OVA and FD-40 given via the oral route were absorbed gradually with time and reached a peak plasma concentration (C_{max}) of 14.78 ± 2.24 pM at 1.5 h for OVA and 1.28 ± 0.45 nM at 1.0 h for FD-40 after administration of these mixtures, respectively (fig. 2a, c). ASP treatment significantly increased the C_{max} and $AUC_{0-3\ h}$ values of OVA and FD-40 by ~2.8- and ~3.1-fold in relation to those for OVA, and by ~1.5- and ~1.9-fold in relation to those for FD-40, compared to the controls (fig. 2b, d; table 2). In OVA-sensitized rats, higher $AUC_{0-3\ h}$ values of OVA and FD-40 were observed compared to values in unsensitized rats. These higher $AUC_{0-3\ h}$ values were increased further by ASP treatment, i.e. ~2.2- and ~2.1-fold more than those in sensitized control rats, respectively (table 2). These results suggested that sensitization with OVA increases intestinal permeability, and that ASP further facilitates the permeability of OVA and FD-40. SCG treatment decreased OVA absorption in sensitized control and ASP-treated rats in a dose-dependent manner. In particular, SCG (195.2 μmol/kg, orally) decreased ASP-facilitated absorptions of OVA and FD-40 in sensitized rats to the same levels as those seen in unsensi-

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Table 2. Effect of SCG on $AUC_{0-3\,h}$ values of OVA and FD-40 after oral administration of a mixture of OVA and FD-40 in OVA-unsensitized and sensitized rats

	Unsensitized	Sensitized		
SCG, μmol/kg:	0	0	19.5	195.2
OVA, pmol·h/l				
Control	27.7±4.2	103.3±9.6[d]	69.4±18.6	32.4±4.2[f]
+ASP	86.9±3.9[a]	232.1±13.9[b, d]	186.4±15.1	81.7±1.0[f]
FD-40, nmol·h/l				
Control	2.30±0.24	4.28±0.13[c]	3.74±0.26	2.26±0.22[e]
+ASP	4.35±0.18[a]	8.89±0.87[b, d]	7.99±0.60	4.25±0.06[f]

ASP (166.5 μmol/kg) and SCG (19.5 or 195.2 μmol/kg) were administered (orally) 30 min before oral administration of a mixture of OVA (1.1 μmol/kg) and FD-40 (10 μmol/kg). Each value represents the mean ± SEM for 4 rats. +ASP = ASP treatment. [a] $p < 0.05$, [b] $p < 0.01$: significantly different from controls (non-ASP treatment). [c] $p < 0.05$, [d] $p < 0.01$: significantly different from unsensitized rats, [e] $p < 0.05$, [f] $p < 0.01$: significantly different from OVA-sensitized rats not treated with SCG.

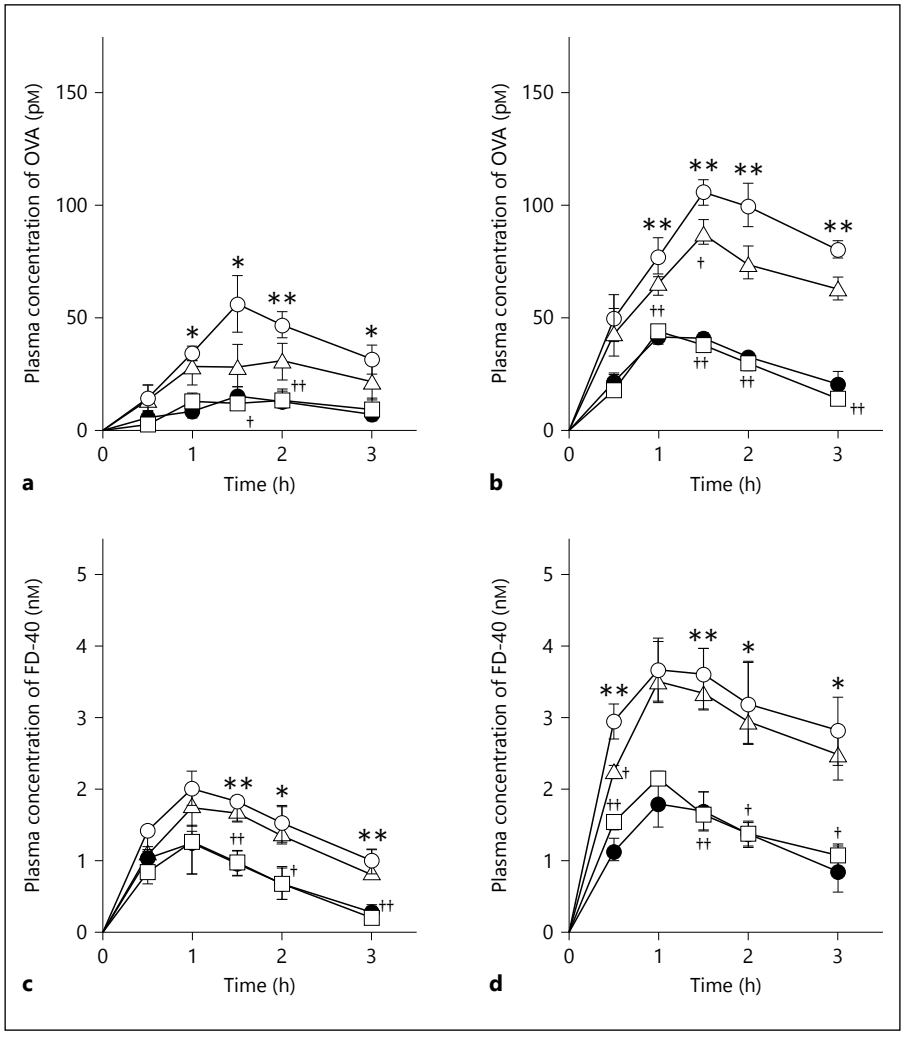

Fig. 2. Effect of SCG on plasma concentrations of OVA (**a**, **b**) and FD-40 (**c**, **d**) after oral administration of a mixture of OVA and FD-40 in OVA-unsensitized and OVA-sensitized rats. Unsensitized: closed circles. Sensitized: open circles (0 μmol/kg), triangles (19.5 μmol/kg), and squares (195.2 μmol/kg). **a**, **c** Untreated control rats. **b**, **d** ASP-treated rats. ASP (166.5 μmol/kg) and SCG (19.5 or 195.2 μmol/kg) were administered (orally) 30 min before oral administration of a mixture of OVA (1.1 μmol/kg) and FD-40 (10 μmol/kg). Each value represents the mean ± SEM for 4 rats. * $p < 0.05$, ** $p < 0.01$: significantly different from OVA-sensitized rats not treated with SCG. † $p < 0.05$, †† $p < 0.01$: significantly different from OVA-unsensitized rats.

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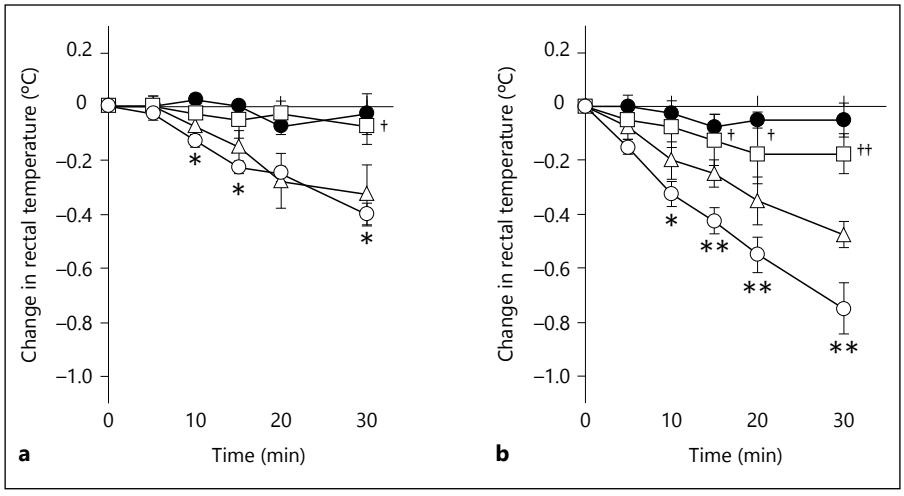

Fig. 3. Effect of SCG on changes in rectal temperature after oral administration of a mixture of OVA and FD-40 in unsensitized and sensitized rats. Unsensitized: closed circles. Sensitized: open circles (0 μmol/kg), triangles (19.5 μmol/kg), and squares (195.2 μmol/kg). **a** Untreated control rats. **b** ASP-treated rats. ASP (166.5 μmol/kg) and SCG (19.5 or 195.2 μmol/kg) were administered (orally) 30 min before oral administration of OVA (1.1 μmol/kg) and FD-40 (10 μmol/kg). Each value represents the mean ± SEM for 4 rats. * $p < 0.05$, ** $p < 0.01$: significantly different from OVA-sensitized rats not treated with SCG. † $p < 0.05$, †† $p < 0.01$: significantly different from OVA-unsensitized rats.

tized rats (fig. 2; table 2). These results demonstrated that high-dose SCG given via the oral route completely ameliorates ASP-facilitated absorption of OVA.

Effect of SCG on ASP-Exacerbated Anaphylaxis after Oral Challenge with OVA

The effect of SCG on ASP-exacerbated anaphylaxis was examined by monitoring the rectal temperature 30 min after oral challenge with OVA (fig. 3). This strategy was used because the reduction in rectal temperature is related to systemic anaphylaxis after elicitation of immediate hypersensitivity reactions according to previous reports [38, 41]. In unsensitized rats, there was no significant change in rectal temperature after oral challenge with OVA irrespectively of ASP treatment, whereas an immediate and significant reduction in rectal temperature was observed in sensitized control rats. Furthermore, ASP caused a significant decline in rectal temperature in sensitized rats (fig. 3). Normal-dose SCG slightly improved the ASP-exacerbated reduction in rectal temperature, and high-dose SCG ameliorated the reduction in rectal temperature to the normal levels seen in unsensitized rats.

Next, we examined the effect of SCG on ASP-exacerbated anaphylaxis via a vascular permeability assay using EBD (table 3). After oral challenge with OVA, EBD extravasation in the liver as well as the proximal and distal intestine of sensitized rats was increased slightly compared to that of unsensitized rats in the control and ASP-treated groups. The vascular leakage of EBD in the proximal and distal intestine of sensitized rats treated with ASP was significantly higher than that of the controls, though there was no significant difference in extravasation in the liver between control and ASP-treated rats. SCG decreased EBD extravasation in the intestine and liver of sensitized rats in a dose-dependent manner. In particular, high-dose SCG ameliorated EBD extravasation to the same levels as those seen in unsensitized rats. These results suggest that the severity of systemic anaphylaxis is closely related to plasma levels of OVA, and that SCG can prevent the development of ASP-exacerbated anaphylaxis resulting from the reduction of OVA absorption.

Discussion

The severity of ingested allergen-induced anaphylaxis is known to depend on the density of intestinal mast cells [42]. Previous reports have shown that ASP exacerbates type-I allergic symptoms due to the stimulation of histamine release from mast cells and basophils via the activation of Syk kinase [18] and suppression of prostaglandin E_2 production [43, 44]. In addition, the severity of symptoms of type-I food allergy appears to be correlated with serum levels of allergens, and allergen absorption facilitated by ASP is an exacerbating factor of IgE-mediated food allergy [5–8]. Several studies have shown that SCG (orally) can prevent symptoms of food allergy by inhibiting the activation of mast cells and the absorption of ingested allergens [20–26]. In contrast, several reports have shown that SCG (orally) confers no benefit in the treatment of food hypersensitivity [27, 31, 32]. Thus, the effectiveness and optimal doses of SCG (orally) needed to prevent IgE-mediated anaphylaxis because of food allergy remain controversial [27, 31, 32]. In terms of the oral dose, 100 mg (195.2 μmol) of SCG has been recommended to treat atopic dermatitis in subjects aged ≥2 years. However,

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Table 3. Effect of SCG on EBD extravasation induced by anaphylactic reactions in the intestine and liver of OVA-unsensitized and OVA-sensitized rats after oral administration of OVA

	Unsensitized	Sensitized		
SCG, μmol/kg:	0	0	19.5	195.2
Proximal intestine				
Control	0.53±0.04	0.85±0.10b	0.73±0.03	0.31±0.02[e]
+ASP	0.67±0.07	1.40±0.16[a, c]	1.16±0.10	0.54±0.04[e]
Distal intestine				
Control	0.57±0.06	0.93±0.18b	0.65±0.05	0.44±0.03[d]
+ASP	0.68±0.08	1.21±0.14[b]	0.96±0.14	0.52±0.06[e]
Liver				
Control	0.73±0.10	1.09±0.14	1.00±0.03	0.55±0.04[e]
+ASP	0.86±0.07	1.08±0.06	1.14±0.10	0.64±0.13[d]

EBD was injected intravenously 20 min before oral administration of OVA (1.1 μmol/kg) and extracted by formamide from each tissue. ASP (166.5 μmol/kg) and SCG (19.5 or 195.2 μmol/kg) were administered (orally) 30 min before oral administration of OVA, respectively. Each value represents the mean ± SEM (μmol/g dry tissue) for 4 rats. +ASP = ASP treatment. [a] $p < 0.01$: significantly different from controls. [b] $p < 0.05$, [c] $p < 0.01$: significantly different from unsensitized rats. [d] $p < 0.05$, [e] $p < 0.01$: significantly different from OVA-sensitized rats not treated with SCG.

Edwards [20] suggested that the optimal dose for a single challenge is 50–150 mg (97.6–292.8 μmol) for infants, 100–200 mg (195.2–390.4 μmol) for children, and 400–600 mg (780.7–1,171.1 μmol) for adults with food allergy. Moreover, Shin et al. [33] reported that high-dose SCG (195.2 μmol/kg) decreased the mortality by anaphylaxis induced by compound 48/80 more than normal-dose SCG (19.5 μmol/kg) in a murine model. These reports suggest that high-dose SCG can modulate ASP-exacerbated allergic symptoms in IgE-mediated food allergy. In this study, we investigated the effect of SCG on ASP-facilitated absorption of OVA and IgE-mediated anaphylaxis at 2 doses (19.5 and 195.2 μmol/kg) in egg-allergic model rats.

We found that ASP exacerbated anaphylaxis with increasing intestinal absorption of OVA. Previously, we reported that OVA was absorbed via 2 pathways (i.e. paracellular and receptor- and clathrin-mediated endocytic) and that ASP induced disruption of the intestinal barrier followed by OVA absorption through the paracellular pathway [15]. Matsubara et al. [45] reported that intact (45 kDa) and truncated (40 kDa) forms of OVA were detected in portal and peripheral blood after oral administration, indicating that OVA absorbed into blood from the intestinal lumen possesses its allergenicity. With regard to the mechanism of ASP-induced intestinal barrier disruption, suppression of prostaglandin synthesis by COX-1 [46], oxidative stress [47], and modulation of tight junctional proteins such as zonula occludens (ZO)-1 [48] and claudin-7 [49] have been reported. The specific sensitization is known to increase intestinal absorption of the allergen in several animal models of allergy [50–54]. In the present study, a higher absorption of OVA was also observed in OVA-sensitized rats compared to unsensitized rats (fig. 2; table 2). Intestinal absorption of allergens has been reported to occur in 2 distinct phases at different times in sensitized rats. In phase I, allergens are transported via the transcellular pathway within the first 2 min after the allergen challenge and before the activation of mast cells; the paracellular pathway is used after the activation of mast cells in phase II [53]. Berin et al. [54] demonstrated that sensitization to HRP enhanced its absorption from the rat intestinal tract via both transcellular and paracellular pathways and the former pathway was dominantly facilitated. In good agreement with this report, the absorption of FD-40 (marker of nonspecific pathways) and OVA was increased in sensitized rats (fig. 2; table 2). Yang et al. [55] demonstrated that no HRP was observed in paracellular regions in mast cell-deficient Ws/Ws rats sensitized by HRP, indicating that activation of mast cells causes increased permeability of specific allergens in sensitized rats. With respect to the molecular mechanisms of increased permeability, modulations of epithelial function by certain inflammatory mediators, including histamine, prostaglandins, nitric oxide, and proteases released from mast cells, have been reported [56–58]. In particular, rat mast cell protease II can increase the expression of

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the epithelial paracellular pathway as a result of the reduction of type-IV collagen in basal membranes and tight-junction proteins such as ZO-1 and occludin [57, 58]. In addition, mast cells may enhance the paracellular permeability of the allergen by activating enteric nerves because some nerves are activated by chemical mediators including histamine from mast cells, and paracellular transport of HRP was reportedly increased by stimulation with a cholinergic agonist, i.e. carbachol [59, 60].

Normal-dose SCG (19.5 μmol/kg) slightly decreased the ASP-facilitated absorption of OVA and FD-40 in sensitized rats, and high-dose SCG (195.2 μmol/kg) ameliorated absorption to the same levels as those seen in unsensitized rats (fig. 2; table 2). SCG decreases the symptoms of food allergy by inhibiting the release of chemical mediators and cytokines from activated mast cells [25, 26], suggesting that the inhibitory mechanism of SCG for intestinal absorption of OVA is considered to be related to inhibition of the release of chemical mediators from activated mast cells [25, 26]. In addition, Weangsripanaval et al. [61] reported that SCG inhibited the uptake of a soybean allergen, i.e. Gly m Bd 30K, in human colon adenocarcinoma (Caco-2) cells with a half-maximal inhibitory concentration (IC_{50}) of 16.1 mM, suggesting that SCG also has an inhibitory effect of allergen absorption in a mast cell-independent manner. The physiologic volume of fluid in the human small intestine under fasting conditions has been reported to be ~500 ml [62]. Thus, it could be speculated that the initial concentration of SCG in the intestinal lumen exceeded the IC_{50} of 16.1 mM when high-dose SCG (195.2 μmol/kg) was given via the oral route. Taken together, high-dose SCG could have inhibited OVA absorption via mast cell-dependent and mast cell-independent mechanisms. High-dose SCG (195.2 μmol/kg) also prevented ASP-exacerbated anaphylaxis in sensitized rats (fig. 3; table 3), in which the plasma concentrations of OVA were similar to those of sensitized control rats in which allergic symptoms had been induced. We could not elucidate the relationship between plasma levels of OVA and anaphylactic symptoms in control and ASP-treated rats. However, SCG might inhibit allergic symptoms via unknown mechanisms in addition to its preventive effects on intestinal absorption of allergens.

The product information leaflet of SCG (Intal®; Sanofi, Paris, France) shows that SCG does not exhibit chronic or subacute toxicities upon repeat oral administrations of 195.2 μmol/kg in rats. High-dose SCG may also be safe in humans because the oral bioavailability of SCG is <1% [61]. Strait et al. [7] suggested that the severity of systemic anaphylaxis appeared to be closely related to the initial plasma concentration of allergens to which mast cells are sensitized rather than to how long the allergen concentration remains elevated. That finding suggested that inhibition of the initial plasma concentration of allergens by SCG can prevent IgE-mediated systemic anaphylaxis.

In conclusion, we showed that high-dose SCG (195.2 μmol/kg) is a prophylactic agent for ASP-exacerbated anaphylaxis after oral challenge with OVA because it prevents ASP-facilitated intestinal absorption of OVA in egg-allergic model rats. When patients with food allergy take ASP, intake of high-dose SCG (195.2 μmol/kg) before meals can prevent ASP-exacerbated anaphylaxis in IgE-mediated food allergy.

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