Brief Report

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Continuous ingestion of sodium chloride solution promotes allergen absorption and may exacerbate allergy symptoms on ovalbumin-induced food allergy in mice

Mamoru Tanaka*, Rui Lu, Hana Kozai

Department of Food and Nutrition Sciences, College of Bioscience and Biotechnology, Chubu University, Kasugai, Aichi, Japan.

SUMMARY Various studies have reported relationships between salt intake and diseases, such as hypertension, cardiovascular disease, stroke, gastric cancer, and bronchial asthma. However, no reports exist on the relationship between salt intake and food allergies. In this study, we investigated the effect of continuous ingestion of sodium chloride (NaCl) on allergy symptoms using a mouse model of food allergy. BALB/c mice were divided into four groups of 6-8 animals each. The control-water group (CW) and sensitization-water group (SW) groups were provided free access to water, and the control-1% NaCl group (CS) and sensitization-1% NaCl group (SS) groups were provided a 1% NaCl solution. The SW and SS groups were sensitized with 50 μg ovalbumin (OVA) at 2 timepoints by intraperitoneal injection. After oral administration of OVA, anaphylactic response was measured and blood was collected. The mice were sacrificed, and serum levels of OVA and anti-OVA immunoglobulin (Ig)E and IgG1 were measured by enzyme-linked immunosorbent assays. The sodium ion (Na^+) concentrations in duodenal and jejunal extracts were measured using a Na^+ ion meter. The results suggested that continuous ingestion of a 1% NaCl solution for 36 days promoted allergen absorption and may have aggravated allergy symptoms in the mice. However, NaCl ingestion did not affect Na^+ concentrations in the small intestine or OVA-specific antibody production.

Keywords Food allergy, sodium chloride, BALB/c mice, absorption, allergy symptoms

1. Introduction

Excessive salt intake may induce hypertension, and, if the blood pressure remains high, the blood vessels and heart may be strained, resulting in the progression of arteriosclerosis and cardiac hypertrophy. Consequently, studies on the relationship between dietary salt intake and disease have focused mainly on life-style related conditions, such as stroke, myocardial infarction, and heart failure (*1*). The sodium ion (Na^+) attracts water, which increases the amount of water and blood flow in the human body (*1*). Therefore, it is postulated that the absorption of allergens may be promoted. In addition, it has been suggested that the small-intestinal epithelial cells may be damaged by an increase in Na^+ concentration in the gastrointestinal tract. Damage to epithelial cells in the small intestine induced by wheat gliadin (*2*), acute exercise (*3*), and aspirin intake (*4*) has been reported to increase absorption of allergens. However, to our knowledge, there have been no studies on the relationship between salt intake and allergies. We hypothesized that, when food is digested, Na^+ will damage small-intestinal epithelial cells and increase the absorption of undigested and partially digested allergens, which may exacerbate allergy symptoms. In this study, we investigated the effect of continuous ingestion of a sodium chloride (NaCl) solution on allergy symptoms using a mouse model of food allergy.

2. Materials and Methods

2.1. Animal and sensitization

All experiments were performed in accordance with the guidelines for animal experimentation and approved by the Animal Experimental Committee of the Chubu University (authorization number: 201910003). Female, 5-week-old BALB/cCrSlc mice (15-20 g in weight) were obtained from Japan SLC, Inc. (Hamamatsu, Shizuoka, Japan). Mice were housed at 22 ± 2°C with 50 ± 10% relative humidity and a 12-h light-dark cycle in a specific pathogen-free facility. The animals were provided a standard diet and water *ad libitum*. After acclimating for 6 days, the mice (n = 29) were divided into four groups

and provided with either water or 1% NaCl (prepared in water). The mice were grouped as follows: control-water group (CW), control-1% NaCl group (CS), sensitization-water group (SW), and sensitization-1% NaCl group (SS). The sensitized mice were injected intraperitoneally with 50 µg of ovalbumin (OVA; A5503, Sigma-Aldrich, St. Louis, MO, USA) and 4 mg of aluminum hydroxide (Imject™ Alum; Thermo Fisher Scientific, Waltham, MA, USA) emulsified in 0.2 mL of phosphate-buffered saline (PBS), pH 7.0, on days 14 and 21. The unsensitized animals received aluminum hydroxide in PBS as the vehicle control.

2.2. Animal protocol

Thirty-six days after the beginning of the experiment, all mice (11 weeks old) were orally administered 10 mg of OVA in 0.5 mL of PBS each. Thirty minutes after the oral administration, rectal temperatures were measured using an endorectal probe and AD-1687 Weighing Environment Logger (A&D Co., Toshima-Ku, Tokyo, Japan) to evaluate allergic responses. The mice were anesthetized with isoflurane to collect whole blood from the portal vein and then sacrificed to collect the duodenal and jejunal contents. Serum was separated from the blood by centrifugation at 700 × *g* for 15 min. The small intestines were divided into six equal parts. The duodenum (upper one-sixth of the intestine) and jejunum (second and third sections of the upper intestine) were extracted with 1 mL of deionized water. Supernatants were prepared from the duodenal and jejunal extracts by centrifugation at 700 × *g* for 15 min. All samples were stored at - 20°C or lower.

2.3. Enzyme-linked immunosorbent assay (ELISA)

The OVA-specific immunoglobulin (Ig)E and IgG1 levels in mouse serum were evaluated by ELISA as described previously (*2*). Flat-bottomed microtiter plates were precoated with 100 µL of OVA (10 µg/mL) in a carbonate buffer (pH 9.6) and incubated overnight at 4°C. After the wells were washed with PBS containing 0.05% Tween 20 (PBS-T), 1% bovine serum albumin (BSA) in PBS-T was added to each well, and the plate was incubated for 1 h at 37°C. BSA and Tween 20 were obtained from Wako Pure Chemical Industries (Chuo-ku, Osaka, Japan). Each serum sample was diluted 100-fold (specific IgE) or 1000-fold (specific IgG1) with 1% BSA/PBS-T and 100 µL aliquots were added to each well. The plate was incubated for 1 h at 37°C and each well was washed five times with PBS-T. Aliquots (100 µL) of either horseradish peroxidase (HRP)-conjugated IgG anti-mouse IgE (diluted 1:1,000; Santa Cruz Biotechnology, Dallas, Texas, USA) or HRP-conjugated IgG anti-mouse IgG1 (diluted 1:5000; Santa Cruz) were added to the appropriate wells and incubated for 1 h at 37°C. The wells were washed five times with PBS-T, and 100 µL of *o*-phenylendiamine (0.4 mg/mL) in a citrate-phosphate buffer (pH 5.0) containing 0.006% H_2O_2 (aq.) was added to each well. Color development was measured by colorimetric photometry at 490 nm.

The FASPEK Egg (Ovalbumin) ELISA Kit (OVA) was purchased from Morinaga Institute of Biological Science, Inc. (Yokohama, Kanagawa, Japan). The levels of OVA in mouse serum were measured according to the manufacturer's instructions.

2.4. Measurement of Na^+ concentration

The Na^+ concentration was determined using a LAQUAtwin Na-11 Na^+ ion meter (HORIBA Scientific, Minami-Ku, Kyoto, Japan).

2.5. Statistical analysis

Data are expressed as the mean ± SEM. All statistical analyses were performed using SPSS Statistics software, version 25.0 (IBM Japan, Chuo-Ku, Tokyo, Japan). Analysis of variance (ANOVA) with Tukey's post hoc test was used to determine differences between the control and treated groups of mice. Data were considered to be significantly different with p-values < 0.05.

3. Results and Discussion

In the present study, we used a mouse model of OVA-induced food allergy to investigate the effect of continuous ingestion of NaCl solution on allergy symptoms, allergen concentration and anti-OVA antibodies in the serum, and Na^+ concentration in the small intestine. In this study, we did not measure water intake; however, there was no difference in body weight observed in the presence or absence of sensitization or 1% NaCl solution (data not shown).

This mouse model for food allergies is characterized by excessive production of allergen-specific IgE and IgG1 antibodies (*5,6*). Therefore, IgE and IgG1 levels are often used as indicators of a food allergy. The concentrations of OVA-specific IgE and IgG1 produced by the mice injected with OVA were significantly greater than the antibody levels produced by the unsensitized mice (Figures 1A and 1B). However, a significant difference in OVA-specific IgE and IgG1 levels was not observed between the SW and SS groups (Figures 1A and 1B). Our results suggest that the sensitization with OVA promoted antibody production, but there was no effect on antibody production after ingestion of 1% NaCl solution.

Next, we investigated the effect of continuous intake of 1% NaCl on allergy symptoms and the gastrointestinal tract of unsensitized and sensitized mice. It has been reported that histamine released from mast cells of allergen-sensitized mice increased vascular permeability and decreased body temperature (*7*). Therefore, changes

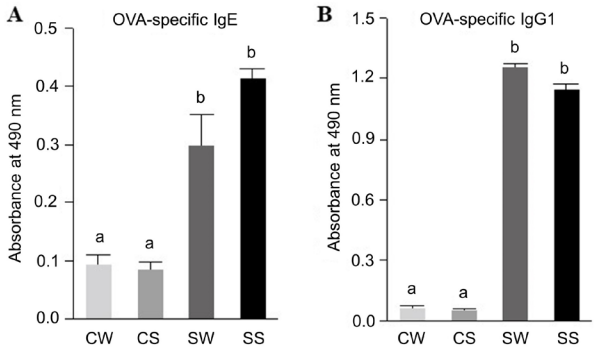

Figure 1. OVA-specific (A) IgE and (B) IgG1 levels in sera from unsensitized and OVA-sensitized mice after ingestion of water or 1% NaCl solution. CW, control-water group (n = 6); CS, control-1% NaCl group (n = 8); SW, sensitization-water group (n = 8); SS, sensitization-1% NaCl group (n = 7); OVA, ovalbumin. Data are expressed as the mean ± SEM, $p < 0.05$ (a *vs.* b).

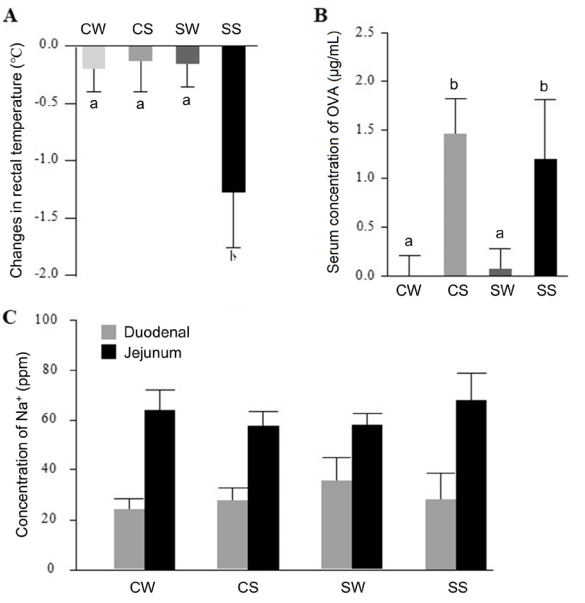

Figure 2. Effect of NaCl on changes in (A) rectal temperatures, (B) serum concentration of OVA, and (C) Na^+ concentration of duodenal and jejunal contents after oral administration of OVA in OVA-unsensitized and sensitized mice. CW, control-water group (n = 6); CS, control-1% NaCl group (n = 8); SW, sensitization-water group (n = 8); SS, sensitization-1% NaCl group (n = 7); OVA, ovalbumin. Data are expressed as the mean ± SEM. $p < 0.05$ (a *vs.* b).

in body temperature are used commonly in the evaluation of allergies (*6,7*). In our study, body temperature was determined to assess anaphylaxis in the mice. The SS group had a significant decrease in rectal temperature compared with the other groups (Figure 2A). Our data suggest that NaCl ingestion exacerbates allergy symptoms after sensitization with OVA, in contrast to sensitization alone.

The concentration of OVA was significantly higher in the portal blood of mice who ingested the 1% NaCl solution compared with that in the portal blood of mice who ingested water (Figure 2B). These results indicated that NaCl intake promoted allergen absorption with or without prior sensitization with OVA. However, there were no significant differences in Na^+ concentration in the duodenal and jejunal contents between the four groups (Figure 2C).

The mechanism by which salt raises blood pressure has not been completely elucidated; however, increases in blood volume and Na^+ concentration in body fluids after salt intake may be related. It has been suggested that endogenous digitalis-like substances are involved as humoral factors and vasoactive substances. These substances are present in the adrenal gland and central nervous system, have natriuretic, vasoconstrictive, and sympathetic effects, and are increased by salt intake. In addition, it has been reported that other substances and conditions are involved in regulating high blood pressure caused by salt intake, including aldosterone, angiotensin, vasopressin, nitric oxide and vascular endothelium, oxidative stress, and Na^+ storage in the skin (*1*).

In our study, the difference in Na^+ concentration in the upper part of the small intestine and the degree of damage to the small intestine could not be observed. However, we propose that allergens are more easily absorbed by damaged epithelial tissue than under normal conditions. Several factors have also been reported to be involved in increased absorption of allergens (*2-4*). Yokooji *et al.* (*4*) investigated the absorption mechanism of food allergens using fluorescein-labeled OVA in rats and found that the undigested allergen was absorbed through intercellular transport pathways, even in a healthy gastrointestinal tract. In addition, these authors reported that aspirin promoted the absorption of OVA by disrupting the intestinal barrier (*4*). The increased absorption of OVA after NaCl ingestion may be mediated by a similar mechanism involving damage to the intestinal barrier.

In conclusion, using a mouse model for food allergy, we have shown that continuous ingestion of 1% NaCl solution for 36 days promoted allergen absorption and may have exacerbated allergy symptoms. NaCl ingestion did not affect the Na^+ concentration in the small intestine or the production of allergen-specific antibodies.

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**Address correspondence to:*
Mamoru Tanaka, Department of Food and Nutrition Sciences, College of Bioscience and Biotechnology, Chubu University, Kasugai, Aichi, Japan, 1200 Matsumoto-cho, Kasugai-city, Aichi 487-8501, Japan.
E-mail: m-tanaka@isc.chubu.ac.jp

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