Original paper

Swallowing Function Improvement Effect of Ginger (Zingiber officinale)

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Deterioration of swallowing function leads to the risk of aspiration pneumonia mortality. In elderly persons, decreased secretion of substance P (SP) from the oral and bronchial mucosae can lead to reduced swallowing function. Capsaicin has been reported to increase SP secretion and enhance swallowing function. We formulated orally disintegrating (OD) tablets consisting primarily of ginger, which contains the same vanillin derivatives as capsaicin, and performed pharmaceutical evaluation and a clinical study to assess the effect of ginger on swallowing function. OD tablets containing ginger increased the amount of SP in saliva immediately after oral ingestion, and showed a significantly higher concentration of SP in saliva between 15 and 120 min after oral ingestion as compared to placebo. Our results suggest that OD tablets containing ginger are likely to increase the amount of SP in saliva after oral ingestion and enhance swallowing function.

Keywords: ginger (Zingiber officinale), dysphagia, orally disintegrating tablets, substance P, saliva

Introduction

In addition to its association with the age-related decline in physical function, dysphagia is likely to occur in patients with neuromuscular diseases (such as cerebrovascular disease or Parkinson’s disease) or diseases of the pharynx, larynx, and surrounding areas. Japan has recently become a super-aging society, and many people develop dysphagia. Dysphagia is quite prevalent in the elderly living in rural areas. Dysphagia makes it impossible to ingest a sufficient amount of food and drink, resulting in malnutrition and dehydration. In addition, there is a risk of respiratory aspiration of food and drink, which increases the risk of developing pneumonia. Pneumonia is the leading cause of death in elderly people and in a number of cases is thought to be caused by aspiration (Teramoto et al., 2008). Dysphagia removes the pleasure from eating and greatly reduces the quality of life.

Various forms of support have been provided to people with dysphagia to help with swallowing and ingestion (Fukui et al., 2000; Morita, 2003; Morita et al., 2011; Sato et al., 2010).

Food that has been thickened or chopped has conventionally been used for people with swallowing difficulty. This changes the physical properties of the food but does not improve the patient’s deglutition reflexes. Thus, it is not a fundamental treatment for improving the swallowing reflex. Various treatment attempts have involved rehabilitation of swallowing or feeding aids, but the burden on healthcare workers and patients is large. Improving the swallowing reflex to reduce the burden on caregivers and improve

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the quality of life of patients could result in the prevention of death due to pneumonia or bronchitis.

The swallowing reflex is controlled by substance P (SP), which is released from nerve endings in the mucosa and mouth (Sasaki, 2002; Jin et al., 1994; Ujiie et al., 1993), under normal conditions. Swallowing dysfunction can be caused by a decrease in the production of dopamine and SP. In addition, the SP concentration in the sputum of elderly people who develop frequent pneumonia is significantly lower than that in healthy individuals. A correlation between the reduction of the rate of pneumonia or improvement in aspiration and the plasma SP concentration was reported in recent years (Arai et al., 2003; Ebihara et al., 1993; Ebihara et al., 2005; Nakagawa et al., 1995).

Amantadine and levodopa reportedly increase the SP concentration and markedly improve swallowing reflex disorders (Kobayashi et al., 1996; Nakagawa et al., 1999).

In addition, an angiotensin-converting enzyme participates in SP degradation. Angiotensin-converting enzyme inhibitory activity inhibits SP degradation, which normalizes the SP concentration and improves the swallowing function (Arai et al., 1998; Arai et al., 2005; Sasaki, 2002). However, it is difficult to use these agents clinically, and it is necessary to consider the risk of side effects. Therefore, to resolve these problems, attention has been focused on food components. One food component that promotes SP release in the oral cavity and improves the swallowing reflex is capsaicin (the pungent component of Capsicum annuum) (Ebihara et al., 1993; Ebihara et al., 2005; Nakagawa et al., 1995), which is an agonist of the transient vanilloid type 1 (TRPV1) receptor potential (Caterina et al., 1997; Ebihara et al., 1993; Ebihara et al., 2005; Holzer, 2011; Szallasi and Blumberg, 1999; Tominaga and Caterina, 2004). Capsaicin activates TRPV1 from unmyelinated airway C-fibers (Undem et al., 2002).

SP released from nerve endings in the mucosa and mouth is derived from the influx of Ca\(^{2+}\) into the cell because of the activation of TRPV1 (Sasaki, 2002; Jin et al., 1994; Ujiie et al., 1993), which then promotes secretion of SP (Widdicombe, 1995). However, the taste of capsaicin is not to everyone’s liking and repeated intake can result in taste desensitization (Jin et al., 1994; Tomohiro et al., 2009). Lemon juice, citric acid, and menthol reportedly improve the swallowing function and prevent pneumonia (Holzer, 2011). However, because lemon juice is acidic, invasive pulmonary problems may be caused when the acid is aspirated.

In this study, we focused on ginger (Zingiber officinale). Ginger contains a vanillin derivative as a functional ingredient, similar to capsaicin, and contains 6-, 8-, and 10-shogaol and 6-, 8-, and 10-gingerol as pungent components. The ginger components activate TRPV1 (Dedov et al., 2002; Iwasaki et al., 2006; Witte et al., 2002). The rhizome is used worldwide as a spice, and has been used in herbal medicine since ancient times (Tanabe et al., 1992). Ginger has various medicinal effects, such as hyperglycemia inhibition (Dedov et al., 2002; Sekiya et al., 2004), TRPV1 stimulation (Sekiya et al., 2004; Tominaga, 2010), and antioxidant and antithrombotic activities (Sekiya et al., 2004). We used ginger from Kochi Prefecture, the leading area for ginger production in Japan.

We selected a dosage formulation of orally disintegrating (OD) tablets, which can be taken easily by pediatric patients and elderly people with difficulty swallowing. We prepared OD tablets containing ginger, subjected them to pharmaceutical evaluation, optimized the base formulation, and conducted a clinical trial to measure their effect on the SP concentration in saliva and improvement of the swallowing function.

**Material and Methods**

*Production of OD tablets*

a) Materials and reagents

Mannitol and sucrose as excipients were purchased from Roquette (Keokuk, IA, USA) and Yoshida Pharmaceutical Co., Ltd. (Tokyo, Japan), respectively. Cornstarch binder was obtained from Nihon Shokuhin Kako Co., Ltd. (Tokyo, Japan), and magnesium stearate lubricant was obtained from Taihei Chemical Industry Co., Ltd. (Osaka, Japan). Other reagents were of the highest grade commercially available.

b) Preparation of OD tablets

A PCH-20 hand press (NPa Systems Co., Ltd., Saitama, Japan) was used to produce tablets at compression pressures of 6, 8, and 10 kN. Flat tablets (200 mg) with a diameter of 8 mm and thickness of 3 mm were prepared by changing the mixing ratio of mannitol and sucrose as excipients. The amounts of cornstarch (binder) and magnesium stearate (lubricant) were fixed at 19.9 mg and 1.0 mg, respectively. There were three types of OD tablets: A (only mannitol as an excipient), B (mixed with the same amount of mannitol and sucrose), and C (only sucrose).

*Pharmaceutical evaluation of OD tablets*

a) Hardness test

Hardness was measured using a Monsanto hardness tester (Fuji RKC, Ltd., Osaka, Japan) in the diameter dimension. The measurement was performed for 10 tablets in each batch and the mean value was calculated.

b) Disintegration test

We followed the procedure for the disintegration test specified in the 16th edition of the Japanese Pharmacopoeia, and the time required for complete disintegration and disappearance of the tablets was defined as the disintegration time using a disintegration test apparatus (Toyama Industry Co., Ltd., Osaka, Japan). Purified water, the first liquid (pH 1.2), or the second liquid (pH 6.8) was used as the test medium at 37 ± 2°C under the conditions of a temperature unassisted panel. This measurement was performed for 10 tablets of each type and the mean disintegration time was calculated.

c) Water absorption time

The water absorption time of the tablets was measured using...
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tartrazine (Yellow No. 5) aqueous solution to elucidate the disintegration mechanism of tablets. Circular filter paper with a diameter of 55 mm (Toyo Roshi Kaisha, Ltd., Tokyo, Japan) was soaked with 2 mL of 1% Yellow No. 5 and placed on a disk. A tablet was placed flat on the paper, and the time required for the color of the whole tablet to change to yellow was measured as the water absorption time. This measurement was performed for 10 tablets of each type, and the mean water absorption time was calculated.

d) Sensory test

A taste test of all tablet types was performed with 10 healthy adult male volunteers (mean age, 38 years). This test was performed as a crossover study. The volunteers rinsed their mouths sufficiently before and after the test. One sample tablet was sandwiched between the volunteer’s upper jaw and tongue (oral disintegration began at this point). The time required for complete disappearance of the tablet was defined as the oral disintegration time. The taste and sensation of the OD tablets were scored by an anonymous questionnaire as follows: (I) Sweetness (1: not strong, 2: slightly strong, 3: adequate, 4: strong, 5: too strong), (II) Bitterness (1: not strong, 2: slightly strong, 3: adequate, 4: strong, 5: too strong), (III) Melting rate (1: fast, 2: slightly fast, 3: just right, 4: slightly slow, 5: slow), (IV) Roughness (1: not rough, 2: slightly rough, 3: rough, 4: too rough), (V) Powdery rating (1: not powdery, 2: not very powdery 3: slightly powdery, 4: powdery), and (VI) Which would be your preferred tablet for daily intake (A, B, or C)?

Production of ginger-containing OD tablets

a) Materials and reagents

The materials were the same as those used in the production of the OD tablets except that ginger powder from Kochi was provided by Asano Co., Ltd. (Kochi, Japan). The 6-, 8-, and 10-gingerol and 6-shogaol were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). Acetonitrile and water were of high-performance liquid chromatography (HPLC) grade. Other reagents used were guaranteed commercial-grade products.

b) Preparation of ginger-containing OD tablets

The base formulation was optimized from the results obtained during preparation of the OD tablets. Examination of the 6-, 8-, and 10-gingerol and 6-shogaol composition of ginger was performed using the HPLC method (Wohlmuth et al., 2005). Five grams of ginger powder were placed in a centrifuge tube to which twice the sample mass of 99% ethanol was added. The preparation was sonicated for 20 min (Ultrasonic Cleaner, Yamato Scientific Co., Ltd, Tokyo, Japan) and subsequently centrifuged for 5 min at 4000 rpm (Centrifugal Separator; Kubota Corporation, Tokyo, Japan). The supernatant was transferred, stored at 4°C, and filtered through a Merck Millipore Filter (Milllex-FG Filter Unit, 0.2 μm, hydrophobic PTFE membrane; Kyoto, Japan) before being injected onto the HPLC column. HPLC was performed using a HITACHI HPLC (L-2130 PUMP; Kyoto, Japan) with a Diode Array Detector (L-7450H Plus). Chromatographic separation was performed under suitable conditions as follows: column (Cosmosil 5C18-MS-II, 4.6 mm I.D. × 150 mm; Nacalai Tesque, Inc.), mobile phase A [water/acetonitrile/trifluoroacetic acid (69.95/30/0.05)]; mobile phase B [water/acetonitrile/trifluoroacetic acid (9.95/90/0.05)], gradient: 0 – 20 min, 100% to 10% A, 20 – 30 min 10% A, flow rate: 1.0 mL/min, column temperature: 40°C, detection: UV 228 and 280 nm, and injection volume: 10 μL.

Ginger was added at 0% (placebo), 1% (the contents of 6-, 8-, and 10-gingerol and 6-shogaol in one tablet of ginger powder were 21.2, 2.2, 12.2, and 2.2 μg, respectively), 3% (the contents of 6-, 8-, and 10-gingerol and 6-shogaol in one tablet of ginger powder were 63.6, 6.6, 36.6, and 6.6 μg, respectively), and 5% (the contents of 6-, 8-, and 10-gingerol and 6-shogaol in one tablet of ginger powder were 106, 11.0, 61.0, and 11.0 μg, respectively) content. The ginger content was calculated from the capsaicin tablets used by Ebihara et al. (2005) and Sugiyama et al. (2006). Ginger-containing OD tablets were compression-molded in the same manner as the OD tablets.

c) Pharmaceutical evaluation of ginger-containing OD tablets

Hardness, disintegration, and water absorption time tests were performed as for the OD tablets. The sensory (taste) test was also performed as for the OD tablets with the exception of Part III: Stimulation (1: not strong, 2: slightly strong, 3: adequate, 4: strong, 5: too strong) and Part VI: Which would be your preferred tablet for daily intake (1%, 3%, or 5% ginger-containing)? As for the OD tablets, the questionnaires were completed anonymously.

Evaluation of the swallowing function

a) Measurement of SP in saliva

Two groups of 10 healthy adult male volunteers in their 20s and 50s were used for the swallowing function test. Saliva was collected 120 min after administration of ginger-containing OD tablets. In addition, the value at 0 min was assumed to be the same as before taking the tablet.

The SP concentration in saliva was measured using an SP ELISA kit (R&D Systems, Inc., Minneapolis, MN, USA) and a microplate reader (VersaMax; Molecular Devices, LLC., Sunnyvale, CA, USA). The saliva was collected in a saliva collection tube (Sarstedt K.K., Humbrecht, Germany). For comparison, we used placebo tablets containing no ginger. The volunteers were prohibited from eating and drinking for up to 120 min after taking the OD tablets. The coefficient of the difference between the two age groups (20s and 50s) was estimated from the following pharmacokinetic parameters: area under the curve from 0 to 120 min for saliva SP concentration (AUC0–120), maximum concentration (Cmax), time to Cmax (Tmax), and half-life (t1/2). Pharmacokinetic parameters were calculated using Microsoft Excel 2010® (Microsoft Office 2010, Microsoft, Redmond, USA). AUC0 – 120 was calculated by trapezoidal integration. The appearance of the various ginger-containing OD tablets was the same, but the tablets were not strictly blinded to the
subjects because the tastes differed. The test was performed as a crossover study with an interval of ≥2 weeks between the test periods.

b) Clinical evaluation of the swallowing function

Two groups of six healthy adult male volunteers in their 20s and 50s were used for the VideoEndoscopic examination of swallowing (VE). Endoscopy was performed by an otolaryngologist before administration and 15 min after administration of 1% ginger-containing OD tablets. A total of 3 mL of colored water with methylrosanilinium chloride was used to create blue-dyed water. The nasopharynx, hypopharynx, and larynx were observed using the endoscope. The volunteers then swallowed 3 mL of the blue-dyed water, and the nasopharynx, hypopharynx, and larynx were observed again with the endoscope. Evaluation was performed by the scoring method proposed for endoscopic swallowing evaluation (Hyodo et al., 2010).

This simple, clinically-based scoring developed for flexible endoscopic evaluation of swallowing (FEES) uses four parameters: (1) the degree of salivary pooling at the vallecula and piriform sinuses, (2) the glottal closure reflex induced by touching the epiglottis or arytenoid with the endoscope, (3) swallowing reflex initiation assessed by “white-out” timing, and (4) pharyngeal clearance after blue-dyed water is swallowed, categorized as 0 for normal, 1 for mildly impaired, 2 for moderate, or 3 for severe. Scores given by experienced otolaryngologists with expert-level experience in treating dysphagic subjects were significantly correlated with those given by nonexpert otolaryngologists and speech-language-hearing therapists. Pharyngeal clearance evaluated by VE correlated with FEES clearance scores to a statistically significant degree, as did aspiration severity with total scores.

Statistical analysis Values are expressed as the mean ± SE. After two-way analysis of variance, we performed multiple comparison tests using the unpaired Student’s t test and Dunnett’s test. Statistical evaluation of time-dependent changes in the saliva SP level was performed using the unpaired Student’s t test. Differences were considered significant at \( p < 0.05 \).

Ethical considerations This study was conducted according to the regulations and approval of the Ethical Review Board of Kochi Medical School (approval No. 23-89) and was in accordance with the principles of the Declaration of Helsinki. All volunteers gave their written and oral informed consent to participate in the study. This clinical trial was a phase 1 study involving healthy adults who freely volunteered.

Results

OD tablets Hardness, disintegration time, water absorption time, and oral disintegration time were measured for each of the three OD tablets (Table 1). As the formation pressure and mixing ratio of sucrose increased, the hardness, disintegration time, and water absorption time also increased.

The questionnaire responses on the taste and sensation for each type of OD tablet are not shown. In the sensory evaluation, there were no differences in sweetness, bitterness, roughness, or powdery rating. The melting time was prolonged for tablets A, B, and C. Four and six volunteers expressed a preference for tablets A and B, respectively (data not shown).

Ginger-containing OD tablets We prepared the ginger-

<table>
<thead>
<tr>
<th>OD tablet</th>
<th>Pressure (kN)</th>
<th>Hardness (N)</th>
<th>Disintegration time (s)</th>
<th>Water absorption time (s)</th>
<th>Oral disintegration time (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Water</td>
<td>Solution 1 (pH 1.2)</td>
<td>Solution 2 (pH 6.8)</td>
</tr>
<tr>
<td>A</td>
<td>6</td>
<td>27.2 ± 0.9</td>
<td>11.3 ± 0.5</td>
<td>16.5 ± 0.8</td>
<td>30.5 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>34.0 ± 0.69*</td>
<td>15.0 ± 0.7*</td>
<td>28.2 ± 1.3*</td>
<td>41.2 ± 1.9*</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>41.0 ± 1.67*</td>
<td>47.8 ± 1.3*</td>
<td>37.7 ± 1.9*</td>
<td>68.3 ± 0.5*</td>
</tr>
<tr>
<td>B</td>
<td>6</td>
<td>36.5 ± 0.5</td>
<td>146.7 ± 1.4</td>
<td>166.0 ± 1.9</td>
<td>152.7 ± 3.7</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>45.1 ± 1.18*</td>
<td>130.5 ± 1.3*</td>
<td>146.8 ± 4.3*</td>
<td>167.0 ± 2.7*</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>68.8 ± 0.69*</td>
<td>167.0 ± 1.5*</td>
<td>173.8 ± 1.6*</td>
<td>242.0 ± 5.3*</td>
</tr>
<tr>
<td>C</td>
<td>6</td>
<td>46.1 ± 0.6</td>
<td>231.0 ± 5.1</td>
<td>203.3 ± 0.7</td>
<td>239.5 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>59.5 ± 1.37*</td>
<td>187.3 ± 0.7*</td>
<td>229.8 ± 3.2*</td>
<td>267.0 ± 1.5*</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>95.1 ± 1.86*</td>
<td>242.0 ± 0.7*</td>
<td>257.3 ± 1.5*</td>
<td>296.0 ± 2.0*</td>
</tr>
</tbody>
</table>

A: Only mannitol as an excipient, B: Mixed with the same amount of mannitol and sucrose, C: Only sucrose. Results are expressed as mean ± SE of 30 tablets.

*p < 0.05 compared with corresponding 6-kN group (analysis of variance followed by Dunnett’s test).
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containing OD tablets at compression pressures of 8 kN and with the same amounts of mannitol and sucrose as excipients.

The hardness, water absorption time, and oral disintegration time of the ginger-containing OD tablets, and the disintegration time determined using a disintegration test apparatus are shown in Table 2. Hardness decreased significantly as the ginger content in the OD tablets increased. The water absorption time of the ginger-containing OD tablets was prolonged compared to the placebo tablets. There was no variance in the oral disintegration time among the ginger-containing OD tablets. The disintegration time of the ginger-containing OD tablets was longer than that of the placebo tablets.

The results of the sensory test are shown in Table 3. The numerical data show the mean scores of the subjects. The subjects reported no overwhelming taste of sweetness or bitterness. Spiciness increased and sweetness decreased as the ginger content increased. Six volunteers expressed a preference for the OD tablets containing 1% ginger.

The changes in saliva SP concentration in volunteers in their 20s and 50s are shown in Fig. 1. The saliva SP concentrations in the 50s group were lower than those in the 20s group for all tablets. In both age groups taking 1% and 3% ginger-containing OD tablets, the saliva SP concentrations were significantly greater than those for the placebo tablets at 15–60 min. The saliva SP concentration of subjects in the 20s group who took 5% ginger-containing OD tablets was lower than that for the placebo tablets. The saliva SP concentration of those in their 50s who took 5% ginger-containing OD tablets was greater than that for the placebo tablets. However, the concentration range was smaller than that for the 1% and 3% ginger-containing tablets.

The kinetic parameters calculated from the saliva SP concentration changes are shown in Table 4. The placebo tablets showed Cmax, Tmax, and AUC0–120 of 1210 ± 90 pg/mL, 5 min, and 1760 ± 250 pg·h/mL in the 20s group and 860 ± 90 pg/mL, 3 min, and 1360 ± 200 pg·h/mL in the 50s group.

Table 2. Effect of ginger on pharmaceutical test results.

<table>
<thead>
<tr>
<th>OD tablet</th>
<th>Hardness (N)</th>
<th>Disintegration time (s)</th>
<th>Water</th>
<th>Solution 1 (pH 1.2)</th>
<th>Solution 2 (pH 6.8)</th>
<th>Water absorption time (s)</th>
<th>Oral disintegration time (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>42.0 ± 0.7</td>
<td>194.33 ± 4.15</td>
<td>172.33 ± 2.12</td>
<td>156.50 ± 5.14</td>
<td>199.43 ± 5.57</td>
<td>78.50 ± 1.77</td>
<td></td>
</tr>
<tr>
<td>1%</td>
<td>33.6 ± 0.39*</td>
<td>241.33 ± 3.90*</td>
<td>154.17 ± 1.35*</td>
<td>236.00 ± 6.36</td>
<td>897.50 ± 2.86*</td>
<td>82.16 ± 1.97</td>
<td></td>
</tr>
<tr>
<td>3%</td>
<td>32.4 ± 0.59*</td>
<td>255.50 ± 4.81*</td>
<td>239.33 ± 2.55*</td>
<td>212.00 ± 2.92*</td>
<td>1165.67 ± 2.23**</td>
<td>90.41 ± 1.51</td>
<td></td>
</tr>
<tr>
<td>5%</td>
<td>19.6 ± 0.59**</td>
<td>240.83 ± 5.61</td>
<td>176.67 ± 1.31</td>
<td>201.17 ± 1.45*</td>
<td>1145.67 ± 11.53**</td>
<td>76.59 ± 4.33</td>
<td></td>
</tr>
</tbody>
</table>

Placebo: Mixed with the same amount of mannitol and sucrose. 1%: The content of 6-, 8-, and 10-gingerol and 6-shogaol in one tablet of ginger powder were 21.2, 2.2, 12.2, and 2.2 μg, respectively. 3%: The content of 6-, 8-, and 10-gingerol and 6-shogaol in one tablet of ginger powder were 63.6, 6.6, 36.6, and 6.6 μg, respectively. 5%: The content of 6-, 8-, and 10-gingerol and 6-shogaol in one tablet of ginger powder were 106, 11.0, and 11.0 μg, respectively.

Results are expressed as mean ± SE of 10 tablets.

*p < 0.05, **p < 0.01 compared with corresponding placebo group (analysis of variance followed by Dunnett’s test).

Table 3. Effect of ginger on sensory test results.

<table>
<thead>
<tr>
<th>OD tablet</th>
<th>Sweetness</th>
<th>Bitterness</th>
<th>Stimulation</th>
<th>Roughness</th>
<th>Powdery</th>
<th>Number of preferred panelists</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>3.14 ± 0.26</td>
<td>2.00 ± 0.38</td>
<td>1.43 ± 0.20</td>
<td>2.57 ± 0.20</td>
<td>2.43 ± 0.20</td>
<td>6</td>
</tr>
<tr>
<td>1%</td>
<td>3.00 ± 0.00</td>
<td>2.14 ± 0.34</td>
<td>3.14 ± 0.26*</td>
<td>2.57 ± 0.20</td>
<td>2.57 ± 0.20</td>
<td>6</td>
</tr>
<tr>
<td>3%</td>
<td>2.86 ± 0.34</td>
<td>1.86 ± 0.14</td>
<td>3.86 ± 0.26*</td>
<td>2.43 ± 0.20</td>
<td>2.43 ± 0.20</td>
<td>3</td>
</tr>
<tr>
<td>5%</td>
<td>1.71 ± 0.18*</td>
<td>2.86 ± 0.34</td>
<td>4.71 ± 0.18*</td>
<td>2.43 ± 0.20</td>
<td>2.43 ± 0.20</td>
<td>1</td>
</tr>
</tbody>
</table>

Placebo: Mixed with the same amount of mannitol and sucrose. 1%: The content of 6-, 8-, and 10-gingerol and 6-shogaol in one tablet of ginger powder were 21.2, 2.2, 12.2, and 2.2 μg, respectively. 3%: The content of 6-, 8-, and 10-gingerol and 6-shogaol in one tablet of ginger powder were 63.6, 6.6, 36.6, and 6.6 μg, respectively. 5%: The content of 6-, 8-, and 10-gingerol and 6-shogaol in one tablet of ginger powder were 106, 11.0, 61.0, and 11.0 μg, respectively.

Results are expressed as mean ± SE of 10 tablets.

Results are expressed as means ± SE of 10 persons.

*p < 0.05 compared with corresponding placebo Group (analysis is of variance followed by Dunnett’s test).
In the 20s group taking 1% and 3% ginger-containing OD tablets, there was no difference in Cmax, but AUC0–120 was significantly higher and Tmax was greater than for the placebo tablets. In the 20s group taking 5% ginger-containing OD tablets, there were no differences from the placebo tablets. Similar results were obtained in the 50s group taking 1% and 3% ginger-containing OD tablets. When the 50s group took 5% ginger-containing OD tablets, Cmax and AUC0 – 120 were increased, but Tmax was unchanged and t1/2 (SP disappearance) was 1 – 3 h. In the 50s group, slightly decreased swallowing function was expected. In two cases, improvements in scores were considered to be associated with the 1% ginger-containing tablets (Fig. 2). Improvements were observed in saliva stagnation in the piriform fossa, cough reflex, glottic closure reflex, pharyngeal clearance, triggering of the swallowing reflex, and pulmonary aspiration. A case in which saliva stagnation in the piriform fossa improved is shown in Fig. 3.

### Table 4. Kinetic parameters of concentration of SP in saliva after taking ginger OD tablets.

<table>
<thead>
<tr>
<th>Group</th>
<th>Ginger-containing (%)</th>
<th>Cmax (pg/mL)</th>
<th>Tmax (min)</th>
<th>AUC0–120 (pg·h/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20s</td>
<td>Placebo</td>
<td>1210 ± 90</td>
<td>5</td>
<td>1760 ± 250</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1280 ± 60</td>
<td>30</td>
<td>2310 ± 130 *</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1410 ± 240</td>
<td>30</td>
<td>2290 ± 220 *</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>1180 ± 70</td>
<td>5</td>
<td>1830 ± 60</td>
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<tr>
<td>50s</td>
<td>Placebo</td>
<td>860 ± 90 a</td>
<td>5</td>
<td>1540 ± 200</td>
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<tr>
<td></td>
<td>1</td>
<td>1100 ± 160 #</td>
<td>15</td>
<td>1940 ± 140 *</td>
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<td>3</td>
<td>1080 ± 120 a</td>
<td>15</td>
<td>1940 ± 160 *</td>
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<tr>
<td></td>
<td>5</td>
<td>1010 ± 40 a</td>
<td>5</td>
<td>1830 ± 30</td>
</tr>
</tbody>
</table>

20s: Healthy adult male volunteers aged 20 – 29 years. 50s: Healthy adult male volunteers aged 50 – 59 years.

Placebo: Mixed with the same amount of mannitol and sucrose. 1%: The content of 6-, 8-, and 10-gingerol and 6-shogaol in one tablet of ginger powder were 21.2, 2.2, 12.2, and 2.2 μg, respectively. 3%: The content of 6-, 8-, and 10-gingerol and 6-shogaol in one tablet of ginger powder were 63.6, 6.6, 36.6, and 6.6 μg, respectively. 5%: The content of 6-, 8-, and 10-gingerol and 6-shogaol in one tablet of ginger powder were 106, 11.0, 61.0, and 11.0 μg, respectively.

Results are expressed as mean ± SE of five persons.

*p < 0.05 compared with corresponding placebo group (analysis of variance followed by Dunnett’s test).

#p < 0.05 compared with corresponding 20s group (analysis is of variance followed by unpaired Student’s t test).

![Fig. 1. Change in SP concentration in saliva of volunteers in their 20s (A) and 50s (B).](image)

◇: placebo  ●: 1% ginger-containing OD tablets ▲: 3% ginger-containing OD tablets ■: 5% ginger-containing OD tablets

Results are expressed as mean ± SE of five persons.

*p < 0.05 compared with corresponding placebo group (analysis is of variance followed by Dunnett’s test).

#p < 0.05 compared with corresponding time 0 (analysis is of variance followed by paired Student’s t test).
Discussion

Pneumonia is reportedly the third leading cause of death and primarily affects those >65 years of age, among whom aspiration accounts for approximately 70% of cases (Yamaya, 2011). Aspiration more frequently occurs in elderly people because the swallowing reflex declines with age. The swallowing reflex is controlled by SP, which is synthesized in the sensory ganglia of the pharynx (Ujiie et al., 1993). However, evaluation of the swallowing function is performed by endoscopy or angiography. SP is secreted from nerve endings in the oropharyngeal mucosa and is involved in swallowing; decreased secretion of SP is reportedly a cause of dysphagia (Arai et al., 2003; Ebihara et al., 2005; Nakagawa et al., 1995).

However, SP measurements are not used clinically. Activation of TRPV1 promotes secretion of SP (Widdicombe, 1995). Capsaicin is a component of chili peppers and a TRPV1 agonist. It induces strong secretion of SP from sensory nerve endings in the pharynx and esophageal mucosa (Ebihara et al., 2005). The gingerol and shogaol components of ginger also have a stimulating effect on TRPV1 (Iwasaki et al., 2006).

The swallowing reflex improvement action of 6-gingerol has been already reported (Sugiyama et al., 2006). However, this study was conducted in rats, and the effects in humans were unknown.

In this study, we attempted to develop a ginger-containing formulation to improve swallowing and aspiration. Therefore, we prepared a new OD tablet in a dosage form capable of maintaining high oral concentrations, which is the site of action of the main component, and that can be taken easily by pediatric patients and elderly patients with difficulty swallowing.

We evaluated the saliva concentration of SP in volunteers after taking the OD tablets.

The OD tablets showed different properties depending on the

Fig. 2. VE score by swallowing endoscopy of 1% ginger-containing OD tablets
Results are expressed as mean ± SE of eight persons in their 50s.
*p < 0.05 compared with corresponding before administration (analysis of variance followed by paired Student’s t test).

Fig. 3. Clinical evaluation by swallowing endoscopy of the 50s age group. Saliva stagnation in the piriform fossa showed improvement from the 1% ginger-containing OD tablet.
(A) Before placebo administration, Score 1. (B) 15 minutes after placebo administration, Score 1. (C) Before administration of the 1% ginger-containing OD tablet, Score 1. (D) 15 minutes after administration of the 1% ginger-containing OD tablet, Score 0.
mixing ratio of the excipients. The hardness of the OD tablets tended to increase as the sucrose content increased, and the disintegration time, water absorption time, and oral disintegration time tended to increase. Water solubility and disintegration when sucrose was used as an excipient were lower than those when mannitol was used, and the tablets showed high viscosity when sucrose was dissolved in water. In addition, because of the hardness of the sugar crystals, tablet hardness increased when sucrose was used. These features are thought to extend the disintegration time, water absorption time, and oral disintegration time (Kikoshi et al., 2010; Nishiura, 2008; Sugimoto et al., 2001).

In clinical trials of OD tablets, there were no problems with volunteers taking each type of OD tablets. Many participants expressed a preference for tablet B because of its moderate disintegration. We propose that taste was immediately sensed because of the more rapid collapse of tablet A than of tablets B and C and that the remaining time in the oral cavity was long and uncomfortable, as for tablet C. The above findings will help to establish the optimal composition of the OD tablets.

In vitro, the reported EC50 for capsaicin, 6-gingerol, 8-gingerol, and 6-shogaol TRPV1 were 0.0039, 3.3, 0.11, and 0.32 μM, respectively (Morera et al., 2012). On the other hand, in vivo, it was reported that capsaicin troche (1.5 μg/tablet) improved the swallowing reflex (Ebihara et al., 2005); moreover, 1 μM capsaicin and 13.7 μM 6-gingerol were reported to show the same level of effect on swallowing improvement (Sugiyama et al., 2006). We referred to the studies by Ebihara et al. (2005) and Sugiyama et al. (2006) regarding in vivo conditions and set the contents of the ginger-containing OD tablets at 1%, 3%, and 5%; our study showed high correlations between in vivo and in vitro studies.

Based on the manufacturing method of OD tablets, the ginger-containing OD tablets were prepared in accordance with the composition of the B tablets (mannitol:sucrose = 1:1). The ginger-containing tablets showed a tendency toward lesser hardness and prolonged disintegration and water absorption times compared to the placebo tablets. The latter two features were thought to be due to the poor water solubility of some of the ginger components. With respect to taste, stimulation increased because of the increased ginger content, and the volunteers stated that they would find it difficult to continue taking the OD tablets containing 5% ginger. Thus, as with capsaicin, not everyone finds the spicy taste of ginger acceptable, and the ginger content is therefore an important factor in continued compliance.

In the clinical test, to evaluate the swallowing function, we compared volunteers in their 20s and 50s. Changes in the saliva SP concentration over time in the 50s group was lower than that in the 20s group when taking any tablet. The Cmax and AUC0–120 values calculated from the saliva SP concentration changes when taking the placebo tablets were 1210 ± 90 pg/mL and 1760 ± 250 pg·h/mL in the 20s group and 860 ± 90 pg/mL and 1540 ± 200 pg·h/mL in the 50s group, respectively. We confirmed that the saliva SP concentration decreased with age.

In the 20s group, the saliva SP concentration at 15 and 60 min after taking 1% and 3% ginger-containing OD tablets was significantly higher than that after taking the placebo tablets; in contrast, it was lower after taking the 5% ginger-containing OD tablets. For the 1% and 3% ginger-containing OD tablets, Cmax did not differ significantly from that of the placebo tablets, but AUC0–120 was significantly higher and Tmax was prolonged. There were no differences in AUC0–120, Cmax, or Tmax between the 5% ginger-containing OD and placebo tablets. In the 50s group, the saliva SP concentration 15 and 60 min after taking the 1% and 3% ginger-containing OD tablets was significantly higher than with the placebo tablets. Capsaicin was reported to increase the concentration of salivary SP in people > 25 years old (Abe et al., 2013). We suggest that ginger-containing OD tablets improve the swallowing function as effectively as capsaicin.

However, the saliva SP concentration of the 1% and 3% ginger-containing OD tablets increased to a lesser degree than for the 5% ginger-containing OD tablets. Tmax, AUC0–120, and Cmax were similar to those in the 20s group. AUC0–120, Cmax, and Tmax were higher for the 1% and 3% ginger-containing OD tablets than for the placebo tablets. AUC0–120 and Cmax were higher but Tmax was unchanged for the 5% ginger-containing OD tablets. High concentrations of capsaicin have been linked to a desensitization phenomenon (Ebihara et al., 1993; Ebihara et al., 2005; Szallas and Blumberg, 1999; Caterina et al., 1997). As for capsaicin, the results of the 5% ginger-containing OD tablets in both the 20s and 50s groups suggested a similar desensitization phenomenon.

In the 1% and 3% ginger-containing OD tablets, the saliva SP concentrations were significantly greater than those for the placebo tablets from 15 to 60 min. Based on this result, the dosage of the ginger-containing OD tablets was considered to be reasonable. The 5% ginger-containing OD tablets require a further, detailed study.

Future studies will investigate this phenomenon. Cmax and AUC0-120 in the 50s group taking the 1% and 3% ginger-containing OD tablets increased to a level similar to that in the 20s group taking placebo tablets. Hence, we inferred that the saliva SP concentration in the elderly can be increased to the level of people in their 20s by the intake of ginger-containing OD tablets. The t1/2 of SP is 1–3 h. Therefore, we can expect improvement in the swallowing function by taking ginger-containing OD tablets before every meal. It is difficult to take capsaicin before every meal because it is spicy, hot, and uncomfortable to the taste. On the other hand, among gingerols and shogaols, 8-gingerol, 10-gingerol, and shogaols retain TRPV1 activity and lower pungency because of their long alkyl chains (Iwasaki et al., 2006). Capsiate has no pungency and has been reported to act on TRPV1 and improve the swallowing function as a TRPV1 agonist (Iida et al., 2003; Yamasaki et al., 2010). 8-Gingerols, 10-gingerols and shogaols, having low pungency, are believed to act on TRPV1 and improve
the swallowing function as capsiate. Therefore, ginger powder, which has weaker pungency than capsaicin, was considered. Blood SP levels have been used as a clinical indicator for the swallowing reflex (Arai et al., 1998). However, evaluation of the saliva SP level, as performed in the present study, is less invasive. We suggest that the salivary SP concentration alone, without measurement of blood SP concentration, represents a potentially new biomarker for the swallowing reflex. During endoscopic clinical evaluation of the swallowing function, volunteers showed improved FEES scores upon taking the ginger-containing OD tablets. The improvement in swallowing function is suggested to be associated with the gingerols and shogaol (TRPV1 agonists) contained in ginger. Here, we investigated the correlation between the amount of SP in saliva and the swallowing function in elderly volunteers expected to have an age-associated decrease in swallowing function.

Here, we have shown that ginger-containing OD tablets increase the concentration of SP in saliva after oral ingestion and enhance the swallowing function during clinical evaluation. These findings suggest that ginger-containing OD tablets will be beneficial for the prevention of dysphagia. We would like to elucidate the validity and safety of ginger for the swallowing function in repeat-dose studies. The development of a dosage form that is suitable for patients with dysphagia is the final endpoint of our studies.

Conflict of Interest
The authors declare that they have no conflicts of interest.

References


**URL cited**