A Randomized Controlled Study on the Effects of Gargling with Tea Catechin Extracts on the Prevention of Influenza Infection in Healthy Adults

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Experimental studies have revealed that tea catechins prevent influenza virus infection; however, the clinical effects have been inconclusive. At the onset of the influenza season, a randomized, double-blind, placebo-controlled study was conducted from December 2005 to March 2006 in Japan. A total of 404 healthy volunteers, 20–65 years of age, were enrolled and randomly assigned to two groups: the catechin group gargling with tea catechin extract solution (approximately 400 µg/mL catechins) or the placebo group gargling without tea catechin extracts. In both groups, gargling was performed three times daily for 90 days. All participants were inoculated with the influenza vaccine before participating in the study. The primary outcome measure was the incidence rate of influenza infection during the study identified by a rapid assay for influenza virus antigens. On an intention to treat basis, 195 participants in the catechin group and 200 in the placebo group who started the intervention were included in the analysis. Of the participants, 6 (1.5%) were infected with influenza. The incidence rate of influenza infection in the catechin group (1.0%, 2 participants) was half that in the control group (2.0%, 4 participants), but not significant between the two groups. We could not find significant effects of gargling with tea catechin on prevention of influenza in the healthy adults inoculated with the influenza vaccine of the 2005–2006 season. However, the effects in more susceptible groups, i.e., those not vaccinated against the influenza virus, children, elderly or immunosuppressed people remain inconclusive.

Trial registration: ClinicalTrials.gov ID, NCT00239213

Key words: catechin, gargling, influenza infection, prophylaxis, healthy adults

Introduction

Influenza virus infection occurs as both a pandemic and interpandemic, and in all age groups worldwide. In order to reduce morbidity and mortality, several potential strategies such as vaccination, antiviral medication, and hygienic procedures are in place. Vaccines are the most widely used for the prophylaxis of influenza infection, but the uptake of immunization varies substantially and vaccine supply continues to be a problem. Thus far, evidence for the effects of antivirals, such as amantadine or neuraminidase inhibitor on the prophylaxis of influenza infection has not been established. Upper respiratory tract infections are also the most common source of morbidity worldwide, and they are reported to be expensive for the healthcare systems. Therefore, it is important to find ways to reduce the frequency of both influenza infection and common colds.

Catechins are the major components of tea flavonoids. Experimental studies have demonstrated that catechins possess various physiological activities such as antioxidative, anticancer,
Methods

1. Study participants

The study was conducted as a randomized, double-blind, placebo-controlled trial, for 90 days at the onset of the influenza season from October 2005 to May 2006. It was conducted in central Japan in three cities, namely, Shizuoka, Hamamatsu, and Higashi-Murayama city. From the three study areas, 458 healthy adult volunteers were recruited for the study that was to be conducted from October 2005 to November 2005. The participants were initially screened by a self-administered questionnaire for inclusion and exclusion criteria. The criteria for inclusion comprised the following: either gender; age, 20 to 65 years; subjectively healthy, and vaccinated against influenza prior to participation in the study. The criteria for exclusion comprised the following: gargling with liquids other than water during the study; allergy to tea; and medical conditions such as a low immune state (e.g., collagen diseases, poorly controlled diabetes, tuberculosis, HIV/AIDS, or cancer), an allergic state (e.g., bronchial asthma, severe hay fever, a severe hypersensitivity to food ingestion), or severe dysfunctions (cardiac, respiratory, renal, or hepatic). Further, participants were excluded by the study physician responsible for each area if they were on medication that would interfere with the evaluation, such as immunosuppressive drugs, corticosteroids, antibiotics, or other herbal products (e.g., echinacea, American ginseng) that may interfere with the study.

The self-administered questionnaire used to collect the participants’ baseline characteristics including year of birth, gender, body mass index, smoking and alcohol consumption, and tea or health food consumption. Between late October and early December 2005, prior to participating in the study, all of the volunteers were vaccinated with an influenza vaccine from the same batch. In accordance with the recommendations of the World Health Organization (WHO) for the 2005-2006 northern hemisphere influenza season, the vaccine used in the study contained the following viral strains: A/New Caledonia/20/99 (H1N1)-like virus, A/New York/55/2004 (H3N2)-like virus, and B/Shanghai/361/2002-like virus. The study was approved by the Ethics Committee of the University of Shizuoka and was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all the participants prior to conduction of the study.

2. Intervention

Participants were randomly selected to receive either the catechin extract or placebo. Randomization was performed using a scheme that was generated using the Statistical Analysis System (SAS) for Windows, version 9.1.3 (SAS Institute, Inc., Cary, NC, USA). The randomization codes were not broken until all the data were analyzed.

Participants in both groups were instructed to gargle three times daily for 90 days from December 2005 to March 2006. The participants were instructed to dissolve 1.0 g of the tea catechin extract or placebo in half a cup (approximately 100 mL) of sterilized tap water and gargle for approximately 15 seconds, three times consecutively, thrice a day. The final concentration received by the catechin group was approximately 400 μg/mL catechins, which is the same as that of commercially sold common green tea beverages in Japan. The concentration of total catechins was determined by the in vitro study, to be a sufficient amount for the inhibition of the infectivity of influenza virus9. The catechins were formulated as polyphenon 70A (Mitsui Norin Co., Ltd., Tokyo), and the total catechin content was 82.8%, including 59.3% (−)-epigallocatechin gallate, 15.1% (−)-epicate-
chinate gallate, 3.8% (−)-galloclatechin gallate, 1.8% (−)-epicatechin, 1.7% (−)-epogalloclatechin, 0.5% (−)-catechin gallate, 0.3% (−)-galloclatechin, and 0.3% (−)-catechin. The placebo was formulated to be almost the same color and taste of catechins, and the quality of double-blinding was ascertained by the clinical research coordinators before the start of the study.

During the follow-up period, all of the participants received a 30-day supply of either the catechin extract or placebo, and they were requested to fill in the prescribed form (gargling diary) every day. The participants had to report the frequency of gargling and the severity of their cold-related symptoms including nasal (rhinorrhea and sneezing), pharyngeal (soreness and itching), bronchial (cough and phlegm), and general symptoms (feverishness, arthralgia, and malaise) scored on a 4-point scale according to the Jackson method (0= no symptoms, 1= mild symptoms, 2= moderate symptoms, and 3= severe symptoms)\(^{20}\). This assessment was further verified by the study physician of each area or clinical research coordinators via telephone or e-mail. Each symptom score was reported at the maximum severity of a cold event, and the total symptom score was calculated by adding the symptom scores. Participants were also asked to report any secondary complications, hospital admission, job absence, or adverse events. The study physician of each area attended to the adverse events and identified the causal relationship for each.

During the study of prophylaxis, all participants were asked not to take any other cold medication, gargle with povidone iodide or tea, or change their hygiene related habits such as hand-washing except for the necessity of use at the treatments. Every month, during the follow-up period, the study physician and clinical research coordinator from each area monitored the participants’ health condition and compliance with gargling instructions and encouraged them to maintain the prescribed intervention.

Influenza infection was defined as certified by a commercially available rapid assay for influenza virus antigens. The assay could not distinguish between mere carriage of the virus and the presence of actual infection. Therefore, the assay was performed only if a participant had an influenza-like illness. The symptoms of an influenza-like illness were a minimum temperature of 37.8°C accompanied by a recent or aggravated cough and one or more of the following signs or symptoms: chills, myalgia, malaise, sore throat, new or increased rhinorrhea or headache, and loss of appetite or diarrhea. If an influenza infection or influenza-like illness was detected, antiviral therapy was administered based on the physician’s decision. Upper respiratory tract infection was defined by the presence of cold-related symptoms but not influenza-like illness.

3. Outcome measures

With regard to the primary outcome, the incidence rates of influenza infection, detected by a rapid assay for influenza virus antigens, were measured, and the incidence of infection during the study was compared between the two groups. Secondary outcome measures comprised the incidence rates of upper respiratory tract infections, the severity of the symptoms and the duration of the cold among incident cases, and the incidence-free time for influenza or upper respiratory tract infections after the intervention. The outcomes were assessed with a self-administered questionnaire, and the participants who suffered from influenza or upper respiratory infections on the first day of intervention were excluded from the analysis. For the safety evaluation, adverse events such as throat and respiratory tract irritation and obstruction or allergic bronchial spasm were examined at each gargling session during the study.

All analyses were performed by a statistician under blinded conditions. The data for the participants who had been randomly assigned to a group were analyzed, and those who provided only baseline information on an intention-to-treat basis were excluded. Participants who withdrew or emigrated were censored (i.e., data were included in analysis up to the point of withdrawal, even though they did not stay for the complete observational period).

4. Statistical analysis

Our previous report demonstrated that the incidence of influenza in elderly nursing home residents was 2% when they gargled with catechin extract solution and 10% when gargling was not perfor-
med. Consequently, the sample size was calculated as 190 for each group at a power level of 0.90, and a significance level of 0.05. Expecting 5% dropouts, we set the total sample size at 400.

All the statistical analyses were performed using SAS for Windows, version 9.1.3. The data for continuous variables was expressed as the mean ± standard deviation (SD). Differences in quantitative data between the groups were assessed using the Student's t-test. The Fisher's exact test was used to compare the differences in the incidence rates of influenza or upper respiratory infections, and other qualitative data. The generalized Wilcoxon test was used to compare the differences in the incidence-free time of influenza or upper respiratory tract infections, and cumulative incidence rates at 30, 60, 90 days were determined by the Kaplan-Meier method. The Wilcoxon test was used to compare the differences in the ordinal data, such as the severity of symptoms and duration of the cold among the incident cases. A P value of less than 0.05 was used to indicate statistical significance.

Results

Figure 1 illustrates the flowchart of the study. After the screening process, a total of 404 volunteers (39.9 ± 11.4 years, mean ± standard deviation; 88 men, 316 women) were enrolled and randomly assigned to the catechin or placebo group. Of these, 200 were assigned to receive the catechin extract and 204, to receive placebo. Of the participants in the catechin group, 5 did not start treatment, and in the placebo group, 4 did not start their treatment because of refusal to consent. The 195 participants in the catechin group and 200 in the placebo group who started intervention were included in the analysis on an intention-to-treat basis. Treatment was discontinued for 7 participants in the catechin group and 9 in the placebo group for various reasons such as refusal to participate (6 participants), severe flu (8 participants), or throat irritation (2 participants).

The baseline characteristics of the participants are shown in Table 1. Between the two groups, there were no significant differences in age, gender, body mass index, smoking and alcohol consumption, and tea and health food consumption. Although all the participants received an influenza vaccine, 1.5% of the participants (6 participants) were infected with influenza that was identified as type A using the antigen assay. No influenza pandemic or related use of antiviral prophylaxis occurred during the study.
Table 1  Baseline characteristics of the participants in the group gargling with tea catechin extracts solution (catechin group) and the placebo (control) group*

<table>
<thead>
<tr>
<th></th>
<th>Catechin group (N = 200)</th>
<th>Control group (N = 204)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>39.6±11.4</td>
<td>40.2±11.5</td>
<td>0.5849</td>
</tr>
<tr>
<td>Men/Women</td>
<td>36/164</td>
<td>52/152</td>
<td>0.0718</td>
</tr>
<tr>
<td>Body mass index¹</td>
<td>21.1±2.77</td>
<td>21.5±2.77</td>
<td>0.1476</td>
</tr>
<tr>
<td>Smoking (+/past/−)</td>
<td>39/12/149</td>
<td>47/14/143</td>
<td>0.6147</td>
</tr>
<tr>
<td>Alcohol (+/past/−)</td>
<td>117/4/79</td>
<td>114/1/89</td>
<td>0.3080</td>
</tr>
<tr>
<td>Tea drinking (mL/day)</td>
<td>607±333</td>
<td>591±341</td>
<td>0.6471</td>
</tr>
<tr>
<td>Taking health foods (+/−)</td>
<td>33/167</td>
<td>41/159</td>
<td>0.5820</td>
</tr>
</tbody>
</table>

*Plus-minus values are means±SD. There were no significant differences between the groups.

¹The body mass index is the weight in kilograms divided by the square of the height in meters.

The incidence rate of influenza infection in the catechin group (1.0%, 2 participants) was half that in the placebo group (2.0%, 4 participants), but no significant difference was observed between the two groups. The incidence rate of upper respiratory infections was also not significantly different between the two groups (48.2%, 94 participants in the catechin group vs. 51.5%, 103 participants in the placebo group). Figure 2 shows the cumulative incidence-free time curves of influenza or upper respiratory infections in each group. The incidence-free time was not significantly different (p value, 0.1720). As determined by the Kaplan-Meier method, the cumulative incidence rate at 30, 60, and
Table 2 The comparison of the incidence rates of influenza and respiratory tract infection, severity and duration of the respiratory tract infection between the group gargling with tea catechin extract solution (catechin group) and the placebo (control) group

<table>
<thead>
<tr>
<th></th>
<th>Catechin group (N = 195)</th>
<th>Control group (N = 200)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influenza illness</td>
<td>2 (1.0%)</td>
<td>4 (2.0%)</td>
<td>0.8423</td>
</tr>
<tr>
<td>Influenza-like illness</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Upper respiratory infections</td>
<td>94 (48.2%)</td>
<td>103 (51.5%)</td>
<td>0.4515</td>
</tr>
<tr>
<td>(1 time)</td>
<td>64</td>
<td>76</td>
<td></td>
</tr>
<tr>
<td>(2 times)</td>
<td>26</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>(3 times)</td>
<td>4</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Severity (total Jackson scores)</td>
<td></td>
<td></td>
<td>0.8934</td>
</tr>
<tr>
<td>(1-5)</td>
<td>31</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>(6-10)</td>
<td>44</td>
<td>46</td>
<td></td>
</tr>
<tr>
<td>(10&lt;)</td>
<td>19</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Duration</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (Quartile)</td>
<td>7 (11)</td>
<td>9 (10)</td>
<td>0.1886</td>
</tr>
<tr>
<td>(Min.- Max.)</td>
<td>(2-55)</td>
<td>(2-41)</td>
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</tbody>
</table>

90 days was 23.5%, 38.2%, and 47.9%, respectively, in the placebo group. On the other hand, this value was 13.4%, 32.7%, and 43.7% respectively, in the catechin group. The severity of the symptoms and duration of the cold among incident cases did not differ significantly between the two groups (Table 2).

In each group, there were no severe complications such as bronchopneumonia or encephalitis and no cases that required hospital admission. No significant differences in job absence were observed between the groups. The interventions were well tolerated by the participants. Only 2 participants experienced an adverse event, i.e., mild throat irritation. The frequency of throat irritation was similar in the 2 groups (0.5% in each group), and the symptoms disappeared after discontinuation of the gargling despite the continued consumption of tea. No serious adverse events such as respiratory tract irritation and obstruction or allergic bronchial spasm were observed during the study.

Discussion

The present study is the first randomized, double-blind, placebo-controlled study to investigate the effects of gargling with tea catechin extracts on the prevention of influenza infection in healthy adults. Contrary to a previous report on elderly nursing home residents, we could not confirm that the positive effects of catechin extracts on the prevention of influenza infection in healthy adults inoculated with the influenza vaccine. However, the discrepancy in the results of the two studies might be due to the low incidence rate of influenza infection in our study (1.5%) that was insufficient to obtain the required statistical power. In a typical epidemic season, approximately 5%-15% of adults and children develop symptomatic influenza. The 2005-2006 season that we studied was interepidemic in the United States as well as in Japan, and no remarkable pandemic outbreak occurred also in the study areas. Further, the selection of healthy adults inoculated with influenza vaccine lowered the frequency of influenza infection. The rate of influenza infection is higher in people not vaccinated against the influenza virus and in children, elderly, or immunosuppressed people. Therefore, it can be speculated that the incidence of influenza infection is higher if the study population comprised people who are highly vulnerable to influenza, and hence, the results would vary.

In experimental studies, catechins bind to the hemagglutinin of the influenza virus, and they inhibit viral adsorption to Madin-Darby canine kidney (MDCK) cells; these results provide an insight into the mechanisms by which tea catechin extracts inhibit the influenza virus. Although evidence from basic experiments is accumulating, data from randomized, controlled clinical trials...
that are linked to the basic experimental results are not yet established. Further studies are required to clarify the effects of tea catechins in humans in order to recommend their use against influenza infection.

As shown in the results, we did not observe any significant effects of catechin extracts on the prevention of upper respiratory tract infections, although the cumulative incidence rate in the catechin group was slightly lower than that in the control group. In Japan, gargling is generally recommended as a preventive modality for upper respiratory tract infections. Recently, Satomura et al reported that mere gargling with water was effective in preventing upper respiratory tract infections compared to the usual hygienic care. Therefore, it should be considered that gargling itself has a placebo effect which is similar to that of gargling with tea catechin extracts.

Tea catechins are reported to be well tolerated, except in tea factory workers with occupational asthma induced by the inhalation of green tea dust. During the three months of gargling, no serious side effects were observed in the participants, except for throat irritation in 0.5% of the participants in each group. The symptoms disappeared after discontinuation of gargling despite the continued consumption of tea. Therefore, the adverse effects were believed to be related to the gargling itself, not to catechins.

In summary, we could not find significant effects of gargling with tea catechin on the prevention of influenza or upper respiratory tract infections in the healthy adults who had been inoculated with the influenza vaccine. However, the effect on more susceptible groups, i.e., those not vaccinated against influenza, children, elderly, or immunosuppressed people remain inconclusive.

Acknowledgments
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