Effects of Catechin-Treated Masks on the Prevention of Influenza Infection: An Exploratory Randomized Study

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Abstract
The objective of this study is to explore the effects of catechin-treated face masks on influenza prevention. We conducted a randomized controlled study in Japan. Participants included workers in a nursing home, a rehabilitation facility, and a hospital. Participants were randomly allocated into the catechin-treated (epigallocatechin gallate-treated) or non-treated face mask groups for 60 days from January to March, 2016. Incidence of laboratory-confirmed influenza infection was measured and compared between groups using Fisher's exact test. Multivariate analysis was performed to calculate adjusted odds ratios (OR) and associated 95% confidence intervals (CI). After the recruitment of participants, 234 participants were eligible for the study (catechin group, n = 118; control group, n = 116). Six participants in the full analysis set contracted influenza (catechin: 3.39%; 95% CI: 0.95–5.50%; control: 1.72%; 95% CI: 0.21–6.09%), and the incidence between the groups did not differ significantly (P = 0.68). Multivariate analysis showed a similar trend (adjusted OR: 2.35; 95% CI: 0.40–13.72; P = 0.34). Our results suggest that the use of catechin-treated face masks does not reduce influenza incidence compared with standard masks. Several limitations to the study, such as infection rates and the selected population may be responsible. Future studies will need to resolve these limitations to accurately evaluate these effects.

Key words: green tea, catechin, influenza, preventive measures, face masks, randomized controlled trial

Introduction
Influenza viruses cause an acute respiratory illness which leads to severe illness or death1, and influenza epidemics and pandemics are serious public health problem worldwide2,3. In 2014, the World Health Organization (WHO) estimated that 5–10% of adults and 20–30% of children are infected with influenza annually4. An infection rate >5% is clinically meaningful, and so the prevention of influenza is important to reduce severe illness and death.

There are several measures to prevent influenza, including vaccination and the use of the neuraminidase inhibitors oseltamivir and zanamivir5–6. Clinical trials have shown the efficacy of these measures and meta-analyses has confirmed them5–6, however, these pharmaceutical measures have several limitations. A strain-specific effect and limited supply of vaccine has been reported7,8,9, and neuraminidase inhibitor-resistant viruses are also known to exist9,10.

The mechanisms of drug resistance have been investigated and partially revealed, but novel drugs for influenza infection and/or prevention has not been developed10. Consequently, non-pharmaceutical public health measures are also important for infection control. Non-pharmaceutical public health measures include hand hygiene and the use of face masks11. These have potential as effective preventive measures, but studies have indicated that only the combination of these measures has a significant preventive effect, and the individual measures were insufficient on their own. It is therefore imperative to improve the efficacy of these non-pharmaceutical public health measures so that they may be used effectively in real-world settings.

The health benefits of catechins, the bioactive compounds in tea leaves12, have been reported from both experimental and clinical studies13–19. Epigallocatechin gallate is the most abundant catechin and has been extensively studied, and its anti-influenza effects have been reported both in experimental and clinical studies13,17–19. An experimental study using Madin-Darby canine kidney epithelial cells revealed that epigallocatechin gallate is able to inhibit the infectivity of both influenza A and B viruses19. Another study has indicated growth inhibitory effects20, and structure-activity relationships have been reported21,22. A meta-analysis suggested that gargling with tea or its ingredients may have preventive effects of influenza infection23. The anti-influenza effects of epigallocatechin gallate could contribute to the efficacy of non-pharmaceutical public health measures, but this has not been conclusively shown in clinical settings.
We have conducted a randomized controlled study to explore the effects of face masks treated with epigallocatechin gallate (hereby referred to as catechin-treated face masks) on the prevention of influenza infection in Japan.

**Methods**

**Study overview**

Workers in a nursing home, a rehabilitation facility, and a hospital participated in this randomized, double-blind, placebo-controlled study. Participants used catechin-treated or non-treated (control) face masks for 60 days from January 5, 2016 to March 4, 2016. The incidence of influenza infection among participants was compared between the two groups.

**Setting and participants**

Participants were recruited by posters and announcements at the White Cross Nursing Home, the Bannan Hakkoen (rehabilitation facility), and the Tokyo White Cross Hospital in Higashimurayama, Tokyo, Japan. Health care workers and staff who 1) had provided written informed consent, 2) were aged ≥ 20 years, 3) were willing and able to wear face masks during the study period, and 4) were willing and able to fill out questionnaires were included in the study. Anyone with tea or face mask material allergies, severe or chronic systemic immune diseases, having contracted influenza in the 6 months prior to the study or within 24 hours after participation, and subjects diagnosed by a physician as inappropriate to enroll in the study were excluded.

Eligible participants completed a self-administered questionnaire to assess baseline clinical characteristics including, age, sex, body mass index, vaccination for influenza, public transportation use, and green tea consumption habits (>200 mL/day).

**Randomization and interventions**

At the Data Management Center of Shizuoka General Hospital in Japan, a computer generated permuted block randomization was performed to randomly place eligible participants into either the catechin-treated mask or the control mask groups using a 1:1 allocation. The randomization was stratified according to the participants’ facilities, and the block size was set to two.

The participants were asked to use the masks for 60 days and to complete the questionnaire each day about their mask-wearing and the occurrence of influenza infection. As a standard protocol for using test masks, participants were asked to wear the masks during work time, and to change the masks 3 times a day. The questionnaire was collected every 30 days. Both masks were made by PROTECTEA, Ltd. (Osaka, Japan), and used the same 4-layered non-woven fabric. The catechin masks were treated with an epigallocatechin gallate solution (Sunphenon epigallocatechin gallate, 2880 mg/L) manufactured by Taiyo Kagaku Co., Ltd. (Mie, Japan) and dried at 130℃ for 22 sec before the mask production process. All participants gave written informed consent before entering the study.

**Treatment outcomes**

The primary outcome measure of this study was the incidence of laboratory-confirmed influenza infection detected using an immunochromatographic assay for the influenza antigen, performed when influenza-like symptoms were reported by participants. The pooled sensitivity of the rapid antigen detection tests, including this immunochromatographic assay, was 64.4% (95% confidence interval [CI]: 59.0–70.1%) for influenza A and 52.2% (95% CI: 45.0–59.3%) for influenza B. The secondary measure was the participants’ rate of mask-wearing over the study period. We also compared the time before influenza infection and the incidence of the common cold as secondary outcome measures.

**Statistical analysis**

Both full analysis and per protocol sets were used for efficacy analysis, and the full analysis set was used for safety analysis. An interim analysis was not planned and performed. The full analysis set was defined as all randomized participants who used a face mask at least one time, did not contract influenza within 24 hours of starting the intervention, and did not reject the data including in the statistical analysis. The per protocol set was defined as participants in the full analysis set, but excluded those with adherence rates <90%.

Continuous variables were expressed as mean ± standard deviation (SD), and categorical variables were expressed as number and percentage (%). The mask wearing rate was calculated based participants’ questionnaire on adherence. The incidence of influenza infection was compared between the catechin-treated mask group and the control mask group using Fisher’s exact test, and the 95% CI for the incidence of influenza infection was calculated. The 95% CI for the difference of influenza incidence between groups was also calculated using the exact method with the Farrington-Manning score. The time before influenza infection was compared using Cox regression analysis, and the incidence of common colds was compared between the catechin-treated mask group and the control mask group using Fisher’s exact test.

Multivariate logistic regression analysis was also performed to calculate the adjusted odds ratio (OR) and its 95% CI. Confounding variables were explored by the stepwise selection method, comprising of alternating forward selection and backward elimination. The selection and elimination criteria of...
per protocol set.

These six participants was

withdrew their consent to participate, four from the catechin
group and two from the control group. The adherence rate of

participants was 41.7 ± 11.7 years (catechin group: 40.9 ±

11.2; control group: 42.6 ± 12.2), and 95.7% of participants

in the full analysis set had been vaccinated against influenza with

the quadrivalent inactivated influenza vaccine used in the 2015–

2016 season in Japan, containing the antigens A/california/7/

2009 (X-179A) (H1N1) pdm09, A/Swiss/9715293/2013 (NIB-

88) (H3N2), B/Phuket/3073/2013, B/Texas/2/2013).

More than 55% of participants habitually used non-

pharmaceutical preventive measures, including hand washing,

hand antiseptic, and gargling (Table 1). In addition, 79.3% (92/

116) of the control group and 83.1% (98/118) of the catechin

group were regular tea drinkers.

Mask adherence rates were 99.1% and 98.9% in the
catechin and control groups, respectively, in the full analysis set,
and 99.5% and 99.7% in the per protocol set of the catechin
and control groups, respectively. The rates were not different
between groups both in the full analysis and per protocol sets
(P = 0.56 in the full analysis set, P = 0.28 in the per protocol set).

Incidence of influenza infection
In the full set analysis, four catechin group participants and two
control group participants were infected with influenza,catechin

Table 1 Baseline clinical characteristics of all participants

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Catechin group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>116</td>
<td>118</td>
</tr>
<tr>
<td>Age, mean ± SD</td>
<td>42.6 ± 12.2</td>
<td>40.9 ± 11.2</td>
</tr>
<tr>
<td>Sex, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>21 (18.1)</td>
<td>24 (20.3)</td>
</tr>
<tr>
<td>Women</td>
<td>95 (81.9)</td>
<td>94 (79.7)</td>
</tr>
<tr>
<td>Body mass index, mean ± SD</td>
<td>22.7 ± 4.4</td>
<td>22.1 ± 3.7</td>
</tr>
<tr>
<td>Vaccination, n (%)</td>
<td>112 (96.6)</td>
<td>112 (94.9)</td>
</tr>
<tr>
<td>Public transportation, n (%)</td>
<td>26 (22.4)</td>
<td>23 (19.5)</td>
</tr>
<tr>
<td>Habitual tea drinking, n (%)</td>
<td>92 (79.3)</td>
<td>98 (83.1)</td>
</tr>
<tr>
<td>Other preventive measures, n (%)</td>
<td>116 (100)</td>
<td>117 (99.2)</td>
</tr>
<tr>
<td>Hand washing</td>
<td>83 (71.6)</td>
<td>78 (66.1)</td>
</tr>
<tr>
<td>Hand antiseptic</td>
<td>68 (58.6)</td>
<td>81 (68.6)</td>
</tr>
</tbody>
</table>

Abbreviation: ± SD, standard deviation

Baseline characteristics and adherence rate of mask wearing
The baseline characteristics of the participants in the full analysis
and per protocol sets are shown in Table 1. The mean age of
the participants was 41.7 ± 11.7 years (catechin group: 40.9 ±
11.2; control group: 42.6 ± 12.2), and 95.7% of participants
in the full analysis set had been vaccinated against influenza with
the quadrivalent inactivated influenza vaccine used in the 2015–
2016 season in Japan, containing the antigens A/california/7/
2009 (X-179A) (H1N1) pdm09, A/Swiss/9715293/2013 (NIB-
88) (H3N2), B/Phuket/3073/2013, B/Texas/2/2013.

The study protocol was registered at the University hospital
Medical Information Network as #UMIN000020173.

Results

Study population
The flow diagram of this study is shown in Figure. Of the 235
recruited participants, only one was excluded; the remaining
234 participants were allocated to the catechin-treated mask
group (n = 118; 24 men and 94 women) and the control mask
group (n = 116; 21 men and 95 women). Six participants
withdrew their consent to participate, four from the catechin
group and two from the control group. The adherence rate of
these six participants was <90%, and they were excluded in the
per protocol set.
group incidence: 3.39%; 95% CI: 0.95–5.50%; control group incidence: 1.72%; 95% CI: 0.21–6.09%; % difference: −1.67%, 95% CI: −7.01–3.14%; Table 2). In the per protocol set, catechin group incidence was 3.51% (95% CI: 0.96–8.74%) and control group incidence was 1.82% (95% CI: 0.22 to 6.41%), with a % difference of −1.69% (95% CI: −7.30–3.38%; Table 2). All six participants contracted the influenza A virus. In both the full analysis and per protocol sets, the incidence of influenza infection was not significantly different between the control and catechin groups (P = 0.68 for both analyses).

**Multivariate logistic regression analysis and other secondary analysis**

After adjusting for body mass index in our stepwise multivariate logistic regression analysis, the incidence of influenza infection was also not significantly different between the control and catechin groups (full analysis set: adjusted OR: 2.35; 95% CI: 0.40–13.72; P = 0.34; per protocol set: adjusted OR: 2.45; 95% CI: 0.41–14.82; P = 0.33; Table 3). The multivariate logistic model did not show a good fit for the full analysis set data (Hosmer-Lemeshow test; full analysis set: P = 0.037; per protocol set: P = 0.51; Table 3). The time before influenza infection and the incidence of common colds also were not different in both analysis sets (Cox regression analysis; full analysis set: P = 0.33; per protocol set: P = 0.31. Fisher's exact test on common colds; full analysis set: P = 0.88; per protocol set: P = 0.75).

There were no serious adverse events associated with the intervention during the study period.

**Discussion**

This study was conducted to explore the effects of catechin-treated face masks on the prevention of influenza infection. Univariate and multivariate analyses suggested that the use of catechin-treated face masks may not reduce the incidence of influenza infection compared with the standard non-treated mask.

The principal limitation of this study is the low influenza infection rate. The rate during the study period was only 2.6% in the full analysis set, which is approximately half to one-quarter of the WHO’s estimated global rate of infection4. The WHO did not mention the specific population used to generate the estimated infection rates, but this might not reflect medical institutions4. This low rate among participants complicated the evaluation of the influenza prevention effects of the catechin-treated face mask, and also affected the results of our multivariate analysis as well as on other secondary outcomes. The poor Hosmer-Lemeshow fit in the full analysis set may be a result of larger populations or populations with higher influenza infection rates. Future

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**Table 2  Incidence of influenza infection**

<table>
<thead>
<tr>
<th></th>
<th>Influenza</th>
<th>Infection rate, % [95% CI]</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>−</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Full analysis set</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>114</td>
<td>2</td>
<td>1.72 [0.21 to 6.09]</td>
</tr>
<tr>
<td>Catechin group</td>
<td>114</td>
<td>4</td>
<td>3.39 [0.95 to 5.50]</td>
</tr>
<tr>
<td>Difference</td>
<td>−1.67</td>
<td>[−7.01 to 3.14]</td>
<td>0.68</td>
</tr>
<tr>
<td>Per protocol set</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>108</td>
<td>2</td>
<td>1.82 [0.22 to 6.41]</td>
</tr>
<tr>
<td>Catechin group</td>
<td>110</td>
<td>4</td>
<td>3.51 [0.96 to 8.74]</td>
</tr>
<tr>
<td>Difference</td>
<td>−1.69</td>
<td>[−7.30 to 3.38]</td>
<td>0.68</td>
</tr>
</tbody>
</table>

**Table 3  Results of the multivariate logistic regression analysis**

<table>
<thead>
<tr>
<th></th>
<th>Adjusted OR [95% CI]</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Full analysis set</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Catechin-treated mask</td>
<td>2.35 [0.40 to 13.72]</td>
<td>0.34</td>
</tr>
<tr>
<td>Body mass index</td>
<td>1.14 [0.98 to 1.32]</td>
<td>0.083</td>
</tr>
<tr>
<td>Hosmer-Lemeshow test</td>
<td></td>
<td>0.037</td>
</tr>
<tr>
<td>Per protocol set</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Catechin-treated mask</td>
<td>2.45 [0.41 to 14.82]</td>
<td>0.33</td>
</tr>
<tr>
<td>Body mass index</td>
<td>1.15 [0.99 to 1.34]</td>
<td>0.065</td>
</tr>
<tr>
<td>Hosmer-Lemeshow test</td>
<td></td>
<td>0.51</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; OR, odds ratio.
studies with larger populations should more accurately evaluate the preventive effects of catechin-treated face masks on influenza infection, as well as improve the prediction of confounding variables. Adjusting for these variables could also reveal other factors affecting catechin-treated face mask efficacy. In this study, the BMI was only a factor selected as a confounding variable, and it had no significant correlation with other variables. This means that the BMI had an implication in statistical models, but the point estimates of odds ratios were only 1.14 to 1.15. Therefore, this might not have any clinical meaning, and additional studies are needed to identify other variables with clinical relevance. In terms of the populations with a higher influenza infection rate, school-aged children may be a desirable population to evaluate the efficacy of the face mask.

Participants' non-pharmaceutical preventative habits may also be a potential confounder, since over 55% of participants were in the habit of hand washing, hand antisepsis, and gargling, in combination with face mask use. Aiello et al have suggested that a combination of face masks and hand hygiene may reduce the rate of influenza-like illness in young adults\(^2\,\)\(^3\). The combination of the two preventative measures may have affected or even prevented influenza infection in both groups, as well as the difference between groups. However, it is difficult to restrict the performance of non-pharmaceutical preventive measures due to ethical reasons; selecting a population that does not habitually perform these measures on a daily basis may help avoid this potential confounder.

**Conclusion**

In summary, our results suggest that the incidence of influenza infection may not be reduced by using catechin-treated face masks rather than standard face masks. However, there are several limitations to this study, such as infection rates and the selected population; therefore, future studies able to resolve these limitations will be able to more accurately evaluate the effects of catechin-treated masks on influenza prevention.

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**Conflict of Interest**

The authors declare that they have no conflicts of interest.

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**Author Contributions**

KI and YK had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: KI, HY, KK. Acquisition of data: KI, HM, HY, NT, AT. Statistical analysis and interpretation of data: KI, YK, HM. Drafting and revising of the manuscript: KI. All authors read and approved the final manuscript.

**References**


