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

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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 worldwide^{2,3)}. 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 infection, including vaccination and the use of the neuraminidase inhibitors oseltamivir and zanamivir^{5,6)}. Clinical trials have shown the efficacy of these measures and meta-analyses has confirmed them^{5,6)}, however, these pharmaceutical measures have several limitations. A strain-specific effect and limited supply of vaccine has been reported^{5,7,8)}, and neuraminidase inhibitor-resistant viruses are also known to exist^{9,10)}.

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 masks^{11,12)}. 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 leaves¹³⁾, have been reported from both experimental and clinical studies^{14–17)}. 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 studies^{13,18–21)}. 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 reported^{22,23)}. A meta-analysis suggested that gargling with tea or its ingredients may have preventive effects of influenza infection¹⁷⁾. 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.

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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 because the masks were for disposable use. 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°C 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,

Figure Study flow diagram

EGCG, epigallocatechin gallate ; FAS, full analysis set ; PPS, per protocol set.

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 B²⁴⁾. 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 $\pm$ 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 test²⁵⁾, 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 score²⁶⁾. 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 test²⁵⁾.

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

Table 1 Baseline clinical characteristics of all participants

	Control group	Catechin group
Full analysis set		
Number of subjects	116	118
Age, mean $\pm$ SD	42.6 $\pm$ 12.2	40.9 $\pm$ 11.2
Sex, n (%)		
Men	21 (18.1)	24 (20.3)
Women	95 (81.9)	94 (79.7)
Body mass index, mean $\pm$ SD	22.7 $\pm$ 4.4	22.1 $\pm$ 3.7
Vaccination, n (%)	112 (96.6)	112 (94.9)
Public transportation, n (%)	26 (22.4)	23 (19.5)
Habitual tea drinking, n (%)	92 (79.3)	98 (83.1)
Other preventive measures, n (%)		
Hand washing	116 (100)	117 (99.2)
Hand antisepsis	83 (71.6)	78 (66.1)
Gargling	68 (58.6)	81 (68.6)
Per protocol set		
Number of subjects	110	114
Age, mean $\pm$ SD	43.4 $\pm$ 12.1	41.1 $\pm$ 11.1
Sex, n (%)		
Men	20 (18.2)	23 (20.2)
Women	90 (81.8)	91 (79.8)
BMI, mean $\pm$ SD	22.5 $\pm$ 4.5	22.0 $\pm$ 3.4
Vaccination, n (%)	106 (96.4)	108 (94.7)
Public transportation, n (%)	26 (23.6)	21 (18.4)
Habitual tea drinking, n (%)	88 (80.0)	95 (83.3)
Other preventive measures, n (%)		
Hand washing	110 (100)	113 (99.1)
Hand antisepsis	79 (71.8)	75 (65.8)
Gargling	65 (59.1)	78 (68.4)

Abbreviation : SD, standard deviation

significance was set to 0.15. The control group was used as the reference for this analysis. The Hosmer-Lemeshow test was also performed to assess the fit of the logistic models²⁷⁾. The significance level was set at $P < 0.05$, and all statistical analyses were performed using SAS version 9.4 for Windows (SAS Institute Inc., Cary, NC, USA).

Ethics statement

The study protocol was approved by the Ethics Committee of the University of Shizuoka (No. 26-29; September 29, 2016), and was conducted in accordance with the Declaration of Helsinki. 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.

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/2/2013²⁸⁾.

More than 55% of participants habitually used non-pharmaceutical preventive measures, including hand washing, hand antisepsis, 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 2 Incidence of influenza infection

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

Abbreviation : CI, confidence interval

Table 3 Results of the multivariate logistic regression analysis

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

Abbreviations : CI, confidence interval ; OR, odds ratio

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 caused by the low incidence of influenza infection, indicating that larger populations or populations with higher influenza infection rates are needed to construct better models²⁷⁾. Future

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 mask⁴⁾.

Participants' non-pharmaceutical preventative habits may also be a potential confounder, since over 55% of participants were in the habit of hand washing, hand antiseptis, 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 adults¹²⁾. 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.

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