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Note

Evaluation of the Antiviral Activity of a Green Tea Solution as a Hand-Wash Disinfectant

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Based on the broad-spectrum antiviral effect of green tea catechins, we established an experimental skin contact model for influenza virus transmission and evaluated the use of a green tea solution as a first-hand disinfectant. The infectivity of the virus on the skin cell layer became obsolete when washed with the green tea solution. The skin contact model could be applied to develop non-pharmaceutical intervention measures for reducing human transmission of the influenza virus.

Key words: influenza virus; green tea; epigallocatechin gallate; antiviral; artificial skin

Green tea, a product of the plant *Camellia sinensis*, has gained increased attention for its biological effects on health. Well-known effects of green tea on human health include anti-diabetic, hypocholesterolemic, anti-inflammatory and anticarcinogenic activities, as well as antiviral properties.[1–7)] In respect of its antiviral properties, a green tea extract has been shown to have inhibitory effects on the human immunodeficiency virus, influenza virus, Epstein-Barr virus, herpes virus, and human T-cell lymphotropic virus type 1, and many other viruses. This broad-spectrum antiviral activity is expected to be found among enveloped viruses in general.[4,8)]

The first influenza pandemic of the 21st century emerged in Mexico in 2009, with numerous clinical and fatal cases.[9)] Vaccines and antivirals remain the most effective options for the prevention or therapeutic intervention against influenza infection.[10)] Vaccines for influenza are thought to be the most preferable means for controlling influenza virus infection in terms of reducing the morbidity and mortality, although there are regulatory issues concerning vaccine manufacture and approval.[9)] However, the therapeutic potency of most of the antiviral agents has been compromised by side effects on humans and the emergence of drug-resistant viral strains.[11,12)] The search for antiviral substances with high efficacy, low toxicity, and minor side effects must therefore be continued from both natural and synthetic resources.[3–7,13–15)]

In parallel with prevention and therapy using vaccines and antivirals, much simpler and probably lower-cost intervention would be possible for reducing the transmission of epidemic respiratory viruses among the public. Enhanced hygiene, including wearing face masks and keeping the hands clean continue to be proposed as the first-hand measures against the spread of respiratory viral infection, because influenza viruses are transmitted through both skin contact and through aerosol transfer.[16)] Although animal models for the aerosol transmission of influenza have been well established,[17–19)] few studies have been conducted to establish a skin contact model for developing intervention measures. We establish in this present study a skin contact model, and based on the potent antiviral activity of green tea catechins,[4–6,20)] we show the effectiveness of a green tea solution as a hand wash for effectively reducing viral transmission through skin contact.

The green tea extracts (both water and ethanol extracts) and green tea bags were provided by Amore Pacific Co. (Seoul, Korea). The green tea bag solution was prepared by adding 120 mL of 70 °C water to a tea bag, leaching for 10 min and filtering with a 0.2 μm syringe filter. A 1x green tea bag solution (GTS) represents the concentration of 1 tea bag (1.2 g of green tea) in 120 mL of water. Two-fold and three-fold concentrated GTS (GTS 2x and GTS 3x) was made by adding one or two additional tea bags in 120 mL of water. EGCG was purchased from Taiyo Kagaku Co. (Mie, Japan). Human influenza viruses A/Puerto Rico/8/34 (H1N1) and A/Sydney/5/97 (H3N2), and avian influenza virus A/Aquatic bird/Korea/w81/05 (H5N2) were propagated in embryonated chicken eggs as described elsewhere.[13)] The cold-adapted influenza vaccine donor strain, X-31 ca (H3N2) virus, was generated after 92 passages at a lower temperature in embryonated chicken eggs.[21)] Madin-Darby canine kidney (MDCK) cells were obtained from American Type Culture Collection (ATCC) and were cultured as monolayers in a minimal essential medium (MEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 50 units of penicillin, 50 μg of streptomycin, and 25 μg of amphotericin. Artificial skin cells (Neoderm® E) were purchased from Tego Science, Seoul, Korea.

To extend the antiviral effect of the green tea extract on influenza viruses as reported previously,[6)] the effect

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Abbreviations: ATCC, American Type Culture Collection; EGCG, epigallocatechin gallate; FBS, fetal bovine serum; GTE, green tea extract; GTS, green tea bag solution; MDCK, Madin-Darby canine kidney; MEM, minimal essential medium

Table 1. Analysis of Chemical Components of Tested Green Tea Extracts

	Caffeine	Gallic acid	GC	EGC	Catechin	EGCG	EC	GCG	ECG	CG	Total catechin
GTE A[a,c]	5.48	0.22	1.95	10.22	0.35	9.11	2.51	0.88	—	—	25.02
GTE B[a,c]	3.75	0.2	2.02	8.6	0.35	17.72	2.27	1.97	3.29	0.15	36.19
GTS[a,d]	2.67[b]	—	0.15	3.42	0.83	11.08	3.42	0.13	2.32	—	21.35

[a]GTE A stands for green tea water extract; GTE B stands for green tea ethanol extract; GTS stands for green tea bag solution.
[b]Units for all the numerical values are % of total green tea.
[c]1 g of both water and ethanol extract diluted in 10 mL of DW, followed by filtration and then measured by HPLC.
[d]1 tea bag (1.2 g) in 120 mL of DW was filtrated and measured by HPLC.

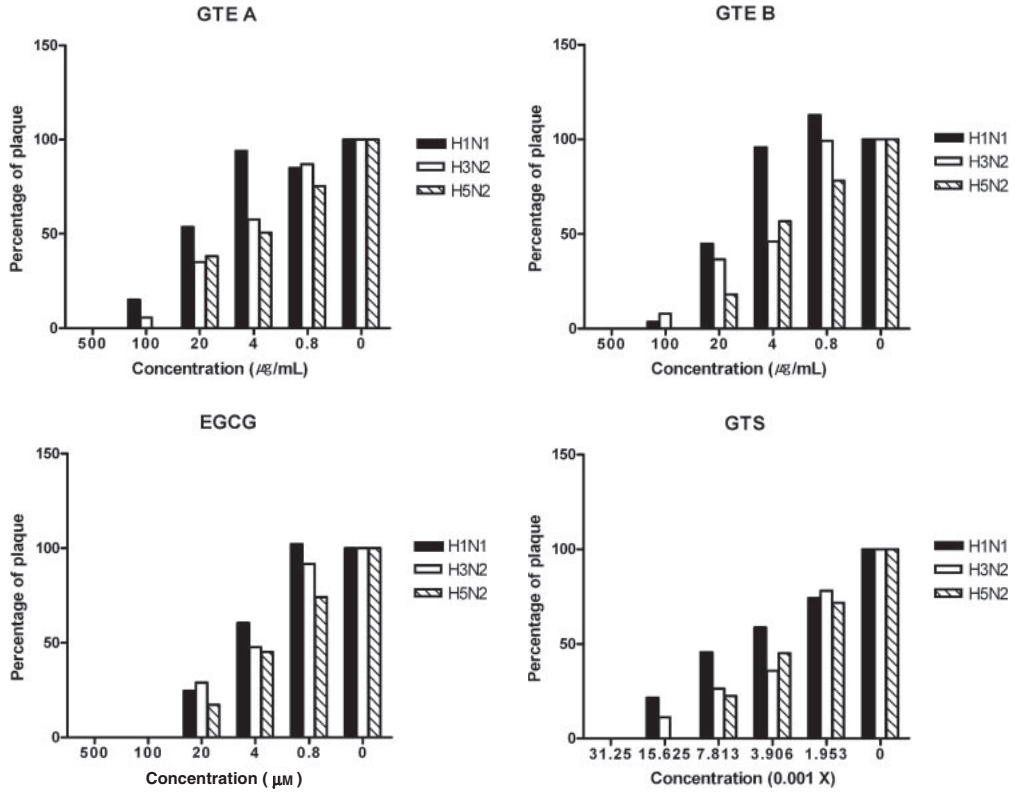

Fig. 1. Inhibitory Effects of the Tested Materials on Plaque Formation.
Six-well plates containing monolayers of MDCK cells were infected with equal volumes of a virus suspension (100 pfu/mL). The overlaid medium, mixed with 2% low-melting-point agarose and DMEM and containing different concentrations of the test materials, was added to each plate. The plates were incubated at 37 °C for 48 h, and the resulting plaque was stained with crystal violet and counted. GTE A stands for the green tea water extract; GTE B stands for the green tea ethanol extract; and GTS stands for the green tea bag solution. The 1x green tea bag solution represents 1 tea bag (1.2 g of green tea) in 120 mL of water.

of the green tea ethanol extract on the anti-influenza activity was compared with that of the water extract, the green tea bag solution and purified EGCG. An analysis of the chemical components of these tested green tea extracts is summarized in Table 1. The antiviral effect on MDCK cells was measured by a plaque reduction assay against the three subtypes of influenza A virus, human subtypes H1N1 and H3N2 and avian subtype H5N2. Figure 1 shows that plaque was completely inhibited at a concentration of 500 μg/mL of the green tea extract and 100 μM of EGCG. The green tea bag solution completely inhibited plaque at a concentration of 0.03x. IC_{50} values for the tested green tea extracts were as follow: 27.85 μg/mL (H1N1), 9.43 μg/mL (H3N2), and 4.73 μg/mL (H5N2) for the green tea water extract; 18.38 μg/mL (H1N1), 3.76 μg/mL (H3N2), and 6.75 μg/mL (H5N2) for the green tea ethanol extract; 8.67 μM (H1N1), 3.83 μM (H3N2), and 3.47 μM (H5N2) for EGCG; and 64.82 μg/mL (H1N1), 32.48 μg/mL (H3N2), and 35.51 μg/mL (H5N2) for the green tea bag solution. It is interesting that the H5N2 avian influenza virus exhibited greater sensitivity to both the green tea extract and EGCG than the H1N1 human virus, the IC_{50} values for the H5N2 avian virus being 2–5 fold lower than for the H1N1 virus.

Based on the present results (Fig. 1) as an extension of previous work,[4–7,22] we chose green tea as a disinfectant from natural resources to further evaluate the viral transmission inhibition by physical contact, using artificial skin cells as a simplified model. Figure 2A shows a schematic representation of the tray in which artificial skin cells were grown. The layer of skin cells was maintained on the support pad through which the essential components for cell growth were provided from the medium in this tray.

The potential infiltration of a virus into the maintenance medium was evaluated as an initial test. The artificial skin layer was initially infected with 1×10^4 pfu of a virus, and after 45 min of incubation, the skin was washed, and the virus titer of the washing solution

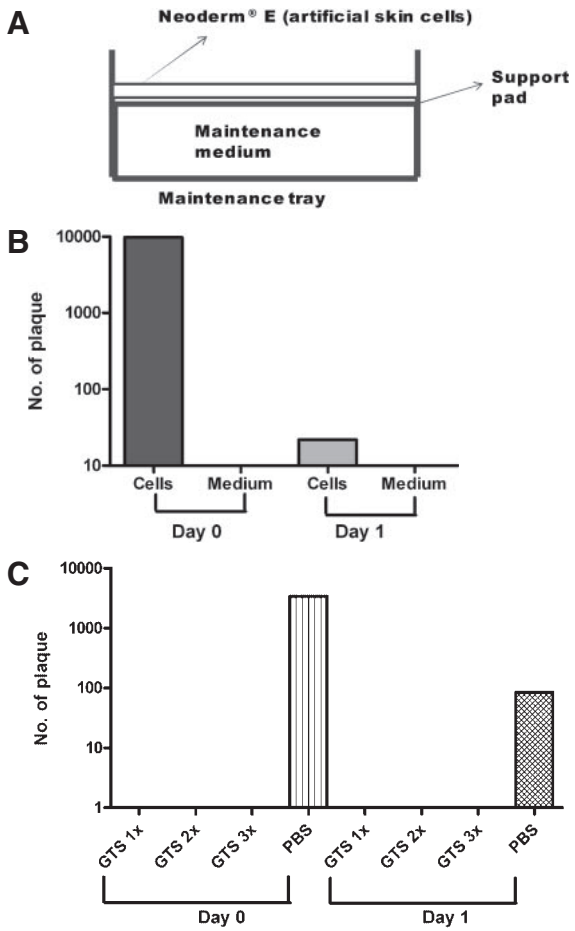

Fig. 2. Establishment of the Skin Contact Model for Evaluating the Anti-Infection Agents.

A, Schematic illustration of the artificial skin cells. The support pad was placed over 13 mL of the maintenance medium. After 5 min, Neoderm® E artificial skin cells were placed on the support pad and incubated for 24 h at 37 °C in a CO_2 atmosphere prior to the assays. B, Suitability test for Neoderm® E in the viral clearance assay. The Neoderm® E artificial skin cells were infected with 1×10^4 pfu of the X-31 ca virus (H3N2). After 45 min of shaken incubation at room temperature, each well was washed with PBS for viral titration (day 0). MEM was then added to each well and incubated for 24 h at 37 °C in a CO_2 atmosphere to further examine the presence of viruses, each well being washed with PBS for viral titration after the incubation (day 1). C, Viral clearance assay using artificial skin cells. The skin cells were infected with 5×10^4 pfu of the X-31 ca virus. After 45 min of shaken incubation at room temperature, each well was washed with either the green tea solution or PBS and the plaque assay was conducted (day 0). MEM was then added to each well and incubated for 24 h at 37 °C in a CO_2 atmosphere to further examine the presence of viruses. Each well was washed with PBS for viral titration after the incubation (day 1). GTS stands for the green tea bag solution; GTS 2x and GTS 3x respectively stand for two-fold and three-fold concentrated doses of the GTS 1x solution.

was evaluated by a plaque assay. We found almost quantitative recovery of the virus in the skin washing solution. After infecting with the influenza virus and incubation, the maintenance medium was tested to detect the virus (Fig. 2B). None of the viruses could be detected in the maintenance medium, suggesting that the virus, after contact and infection of the skin cells, had failed to infiltrate the maintenance medium. The lack of infiltration into the skin layer and the quantitative recovery of viruses in the washing solution show the suitability of the artificial skin cells as a virus skin contact model, and further simplified the calculation of viral clearance mediated by the direct contact of a green tea extract with the skin cells.

The inhibitory effect of green tea on viral transmission through skin contact was tested by using the artificial skin model. The skin-washing solution was serially diluted up to 10^5 before the plaque assay of MDCK cells for the potential presence of infectious virus particles. As shown in Fig. 2C, after cells had been washed with the green tea solution, none of the viruses could be detected by the plaque assay. In contrast, 3.4×10^4 pfu of the virus was detected when washed with PBS (control group). To further detect if any viruses remained on the artificial skin cells after washing, the cells were incubated overnight with MEM at 37 °C in a 5% CO_2 atmosphere, and the plaque assay was conducted. Figure 2C shows that none of the viruses could be detected in the green tea solution group, whereas 8.4×10 pfu of viruses were detected in the PBS control group. The failure to detect any viruses remaining on the artificial skin cells after an overnight culture in MEM confirmed the strong virucidal activity of the green tea solution. This result indicates that the virucidal activity of the green tea solution[6] has the potential for inhibiting the transmission of the influenza virus by physical contact and highlights its potential usefulness as a first-hand skin-washing disinfectant.

The avian influenza viruses currently circulating in poultry farms with pandemic potential include the H2, H5, H7, and H9 subtypes.[23] Furthermore, the novel swine-origin A (H1N1) virus has recently emerged as the closest to pandemic level in the 21st century.[22,24,25] The broad-spectrum antiviral activity of catechins in the green tea extract toward both human and avian influenza viruses merits its further evaluation as both a means for managing avian influenza outbreaks in poultry farms and as a convenient disinfectant for minimizing the avian-to-human or human-to-human transmission of influenza viruses. It should be noted, however, that the oxygen-reactive nature of catechins, while being beneficial as an anti-oxidant,[26] could compromise the anti-viral activity.[4,6] The appropriate combination of green tea and such an antioxidant as vitamin C could increase the half-life of the antiviral activity of catechins[27] and the shelf-life of a green tea-based hand-washing solution. We believe that the skin contact model for viral transmission presented here would be useful for developing such a first-hand hygienic measure as a hand-washing disinfectant solution to act against viral transmission among humans.

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