BPB Reports

Report

An *in Vitro* Short-Term Treatment with Black Tea-Derived Theaflavins Reduced Infectivity of SARS-CoV-2 in Saliva

Motohiko Ogawa,[a] Mana Murae,[b,c] Shuetsu Fukushi,[a] Kohji Noguchi,[c] Hideki Ebihara,[a] and Masayoshi Fukasawa[b,*]

[a]*Department of Virology I, National Institute of Infectious Diseases, 1-23-1, Toyama, Shinjuku-ku, Tokyo 162-8640, Japan;* [b]*Department of Biochemistry and Cell Biology, National Institute of Infectious Diseases, 1-23-1, Toyama, Shinjuku-ku, Tokyo 162-8640, Japan;* [c]*Laboratory of Molecular Target Therapy, Faculty of Pharmaceutical Sciences, Tokyo University of Science, 2641, Yamazaki, Noda, Chiba 278-8510, Japan*

Received September 15, 2023; Accepted September 22, 2023

The coronavirus disease 2019 is caused by the etiological agent severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). SARS-CoV-2 is abundant in the saliva of an infected person; therefore, saliva is an important source of infection. The present study evaluated the efficacy of short-term treatment using tea-derived compounds against SARS-CoV-2 infectivity in the saliva. The antiviral efficacy of theaflavin 3,3'-gallate (TF3) and two black tea-derived theaflavin concentrates (TF35 and TF80) against a prototype Wuhan and a recent Omicron strain was evaluated using human saliva. TF3, TF35, and TF80 reduced the infectivity of both strains at high (1 mM or 1 mg/ml) and low (0.25 mM or 0.25 mg/ml) concentrations; however, antiviral efficacy against the Wuhan strain was stronger than that against the Omicron strain. Furthermore, the antiviral agents at high concentrations showed better efficacy against both strains than those at low concentrations. For example, treatment with 1 mM TF3 for 10 min decreased the infectivity of Wuhan and Omicron strains to approximately 0.05% and 3%, respectively; these reduction rates are attributable to the inactivation of large amounts of viruses (9.995×10^5 and 9.7×10^4 $TCID_{50}$, respectively). Considering these facts, it was expected that the inclusion of the main components of black tea (TF3) and the black tea-derived theaflavin concentrates (TF35 and TF80) in the oral cavity for a short time might inactivate the virus in saliva and, thus, can be considered an effective suppressor of the spread of infection.

Key words SARS-CoV-2, black tea-derived theaflavin, saliva, Wuhan, Omicron

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) primarily infects the respiratory tract, causing inflammation, and subsequently the lungs, causing severe pneumonia. SARS-CoV-2 can also infect other tissues in the body. Intravascular infections cause thrombosis. Systematic infection causes a cytokine storm and subsequent shock symptoms, followed by multiple organ failure, and finally, the fatality rate increases. SARS-CoV-2 is transmitted among people mainly by inhalation of virus-containing aerosols expelled from the mouth and nose of an infected person during coughing, sneezing, and talking. A large number of viral particles are present in an infected person's saliva,[1] which is one of the main sources of infection. We considered that reducing the infectivity of the virus in saliva would be effective for the prevention of viral transmission. Several tea derived-compounds, such as catechins and theaflavins, have been reported to decrease infectivity of SARS-CoV-2.[2,3] This study evaluated the efficacy of short-term treatment with tea-derived compounds on SARS-CoV-2 infectivity in saliva.

MATERIALS AND METHODS

Drugs Theaflavin 3,3'-gallate (TF3), tannic acid (TA), epigallocatechin gallate (EGCG), and theasinensin A (TSA) solutions as well as two black tea-derived theaflavin concentrates (TF35 and TF80) and a green tea-derived catechin concentrate (polyphenon E)[4] were tested for their antiviral efficacy against SARS-CoV-2.

Virus treatments WK-521, a prototype Wuhan strain, and TY38-873, a recently developed Omicron BA.1 strain of SARS-CoV-2 were used in this study. Viral solution (100 µl) containing 1×10^5 $TCID_{50}$ and equal volume of solution containing drugs were mixed in DMEM with 2% fetal calf serum or normal human saliva purchased from Lee BioSolutions, Inc. (Maryland Heights, MO, USA). The mixture was then incubated for 5, 10, or 20 min at room temperature. The reaction was stopped by adding 200 µl of stop solution [10% polyvinylpolypyrrolidone in PBS], which contains excess amounts of polyphenol adsorbent[5] for maintaining accurate reaction time. After incubation for 20 min, the mixture was centrifuged at 9,800 g for 5 min, and the supernatant was collected. The supernatant was diluted 10 times and was used to inoculate E6/TM2 cells (TMPRSS2-expressing VeroE6 cells);[6] the amount of virus in the culture supernatant was measured using

*To whom correspondence should be addressed. e-mail: fuka@niid.go.jp

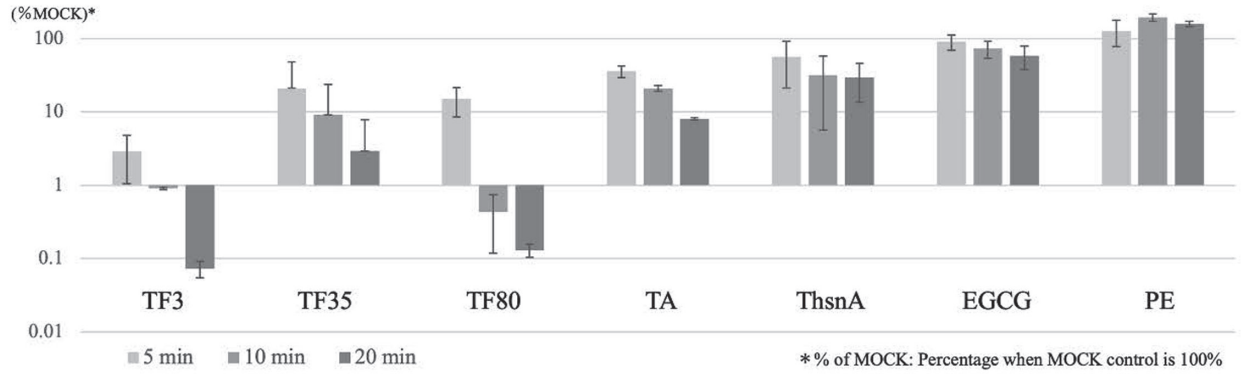

Fig. 1. Antiviral Efficacy of Tea Derived-Compounds Against Wuhan Strain of SARS-CoV-2

A mixture of the virus and the compound was incubated for 5, 10, and 20 min at room temperature. Reaction was stopped using 200 µl of 10% polyvinylpolypyrrolidone in PBS. Following incubation for 20 min, the mixture was centrifuged, and the supernatant was collected. The supernatant was used to inoculate TMPRSS2-expressing Vero E6 cells, and the amount of virus in the culture supernatant was measured after 24 h using quantitative RT-PCR. Antiviral efficacy of the drugs was evaluated using the efficacy of untreated samples as 100%. In this experiment, 1 mM theaflavin 3,3'-gallate (TF3), tannic acid, epigallocatechin gallate, and theasinensin A and 1 mg/ml of two black tea-derived theaflavin concentrates (TF35 and TF80) and a green tea-derived catechin concentrate (polyphenon E) were used. At least three experiments were performed, and one of the typical results is shown.

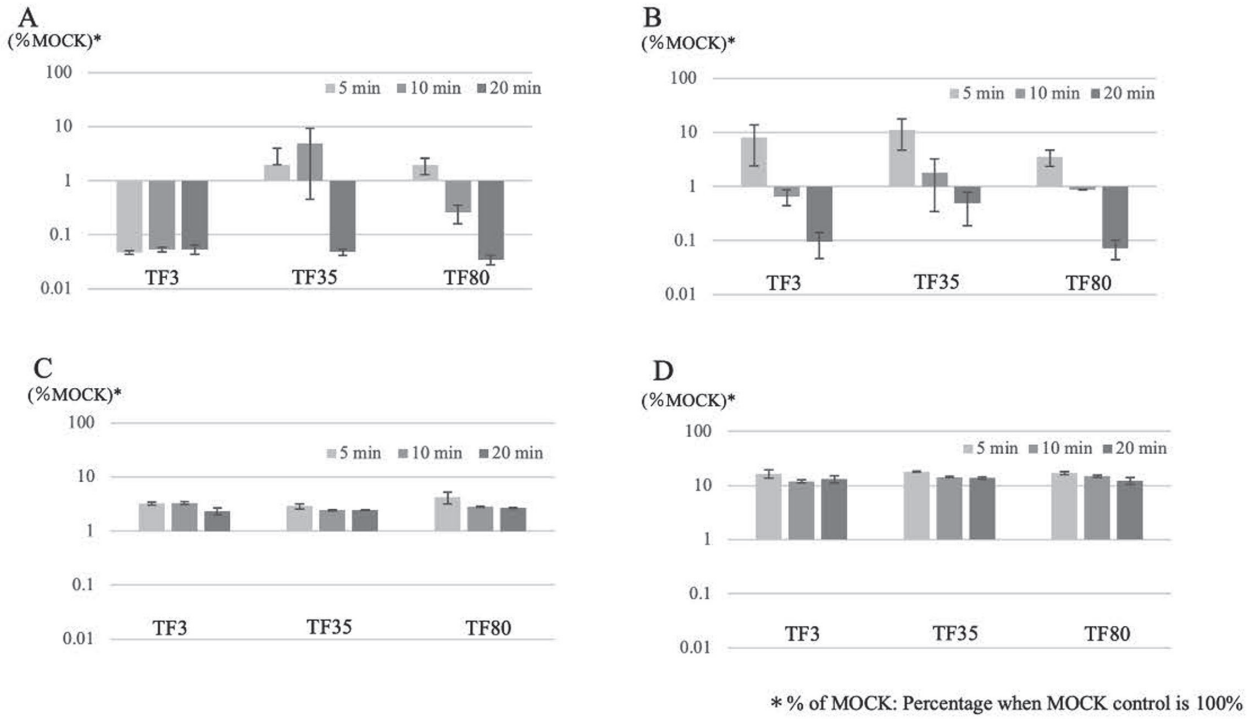

Fig. 2. Antiviral Efficacy of Theaflavin 3,3'-Gallate (TF3) and Two Black Tea-Derived Concentrates (TF35 and TF80) Against Wuhan and Omicron Strains of SARS-CoV-2 in Human Saliva

Wuhan and Omicron strains present in human saliva were treated with these drugs. Antiviral efficacy of each drug at high (1 mM TF3, 1 mg/ml TF35 and TF 80) and low (0.25 mM TF3, 0.25 mg/ml TF35 and TF 80) concentrations was evaluated. A and B show antiviral efficacy against Wuhan strain at high and low concentrations, respectively, whereas C and D show the efficacy against Omicron strain at different concentrations. At least three experimental repeats were performed in triplicate, and one of the typical results is shown.

quantitative RT-PCR 24 h post inoculation [7]. The antiviral efficacy of the drugs was evaluated by percentages of untreated samples as 100%.

RESULTS

Antiviral Efficacy in Culture Medium The antiviral efficacy of 1 mM TF3, TA, EGCG, and TSA solutions and 1 mg/ml of TF35, TF80 and polyphenon E against the Wuhan strain was evaluated. TF3, TF35, and TF80 were more effective in reducing infectivity than the other drugs (Fig. 1).

Antiviral Efficacy in Human Saliva The antiviral efficacy of TF3, TF35 and TF80 were evaluated in human saliva (Fig. 2). The antiviral efficacy of the tested components, except TF3, on the Wuhan strain became stronger with time at both high (1 mM or 1 mg/ml, Fig.2. A) and low concentrations (0.25 mM or 0.25 mg/ml, Fig.2. B). However, their effect on the Omicron strain was stable, with slight changes over time (Fig.2. C and D). Overall, the tested concentrations of the antiviral components were more effective against Wuhan than against Omicron, and the antiviral effects against both strains were higher at high concentrations than that achieved

at low concentrations. For example, after 10 min of treatment at 1 mM, the antiviral activity of TF3 against the Wuhan strain decreased to approximately 0.05%, whereas that against the Omicron strain decreased to approximately 3%.

DISCUSSION

TF3 is a theaflavin, which is the main component of black tea, and TF80 contains richer theaflavins (such as TF3) than TF35 were effective. However, catechins such as EGCG and polyphenon E were less effective, although some previous reports have shown the antiviral efficacy of green tea-derived catechins and related chemicals.[2, 8] We suggest that the difference in antiviral efficacy may depend on assay methods, such as reaction time, reagent concentration, and stop solution. Consistent with these results, TF35 containing catechins such as EGCG had a lower antiviral effect than TF80, which did not contain catechins; this result may be attributable to the difference in the amount of theaflavins, such as TF3.

The difference in the antiviral efficacy between Wuhan and Omicron strains can be attributed to the change in the mode of the Omicron strain infection, which is caused by the mutation of the spike protein,[2] although detailed analysis is needed to explain the difference. Even if the reduction rate (MOCK%) is approximately 3%, 9.7×10^4 $TCID_{50}$ equivalent numbers of viruses are inactivated. Furthermore, if the reduction rate was approximately 0.05%, the number of viruses equivalent to 9.995×10^4 $TCID_{50}$ was inactivated. These results suggested that large amounts of both viruses were inactivated.

Our results indicated that TF3, TF35, and TF80 decreased the infectivity of both the tested strains present in saliva. Furthermore, in our study, quenching catechin reactions with excess amounts of adsorbents allowed us to observe precise time-dependent effects, whereas previous studies not using a stop solution could not reveal this information.[2,3,8] Our results should not be realized for infectious respiratory aerosols. However, considering many cases suspected to be infected during eating and drinking, saliva should be considered to play an important role in transmission of infectious viral particles. TF35 used in this study is used to be commercially available as a catechin tablet containing 250 mg TF35/tablet. In our previous analysis, the tablet was placed in the oral cavity for 5 to 10 min, and approximately 10 ml saliva was secreted in this duration. Hence, in this study, treatments for 5 min and 10 min with 0.25 mg/ml TF35 were considered equivalent to the consumption of one tablet. Black tea has been reported to inactivate SARS-CoV-2;[9] however, the antiviral efficacy of drinking tea may be transient. Considering these facts, it is expected that keeping tablets or candies containing the black tea-derived theaflavin concentrates TF35 and TF80, including TF3, in the oral cavity for 5 to 20 min, can possibly inactivate the virus in the saliva and would be effective in suppressing the spread of infection through the saliva.

Acknowledgments We thank Mitsui Norin Co., Ltd. for their assistance in catechin handling and for providing TF35, TF80, and polyphenon E. We also thank Ms. Mieko Utsugi and Yoko Inamori for technical assistance.

Funding This study was supported by a research grant from the Japan Agency for Medical Research and Development (AMED).

Conflict of interest The authors declare no conflict of interest.

REFERENCES

1) Friedman M. Overview of antibacterial, antitoxin, antiviral, and antifungal activities of tea flavonoids and teas. *Mol. Nutr. Food Res.*, **51**, 116–134 (2007).
2) Nishimura H, Okamoto M, Dapat I, Katsumi M, Oshitani H. Inactivation of SARS-CoV-2 by catechins from green tea. *Jpn. J. Infect. Dis.*, **74**, 421–423 (2021).
3) Ohgitani E, Shin-Ya M, Ichitani M, Kobayashi M, Takihara T, Kawamoto M, Kinugasa H, Mazda O. Significant inactivation of SARS-CoV-2 *in vitro* by a green tea catechin, a catechin-derivative, and black tea galloylated theaflavins. *Molecules*, **26**, (2021).
4) Stockfleth E, Meyer T. Sinecatechins (Polyphenon E) ointment for treatment of external genital warts and possible future indications. *Expert Opin. Biol. Ther.*, **14**, 1033–1043 (2014).
5) Dong ZB, Liang YR, Fan FY, Ye JH, Zheng XQ, Lu JL. Adsorption behavior of the catechins and caffeine onto polyvinylpolypyrrolidone. *J. Agric. Food Chem.*, **59**, 4238–4247 (2011).
6) Shirato K, Kawase M, Matsuyama S. Middle East respiratory syndrome coronavirus infection mediated by the transmembrane serine protease TMPRSS2. *J. Virol.*, **87**, 12552–12561 (2013).
7) Shirato K, Nao N, Katano H, Takayama I, Saito S, Kato F, Katoh H, Sakata M, Nakatsu Y, Mori Y, Kageyama T, Matsuyama S, Takeda M. Development of genetic diagnostic methods for detection for novel coronavirus 2019 (nCoV-2019) in Japan. *Jpn. J. Infect. Dis.*, **73**, 304–307 (2020).
8) Henss L, Auste A, Schürmann C, Schmidt C, von Rhein C, Mühlebach MD, Schnierle BS. The green tea catechin epigallocatechin gallate inhibits SARS-CoV-2 infection. *J. Gen. Virol.*, **102**, (2021).
9) Ohgitani E, Shin-Ya M, Ichitani M, Kobayashi M, Takihara T, Kawamoto M, Kinugasa H, Mazda O. Rapid inactivation *in vitro* of SARS-CoV-2 in saliva by black tea and green tea. *Pathogens*, **10**, (2021).