Short Communication

Epigallocatechin-3-gallate (EGCG): a potential molecule for the development of therapeutics against emerging SARS-CoV-1, MERS-CoV and SARS-CoV-2 coronaviruses ☆

Jeswin Joseph, T. Karthika, V.R. Akshay Das, V. Stalin Raj*

Virology Scientific Research (VSR) Laboratory, School of Biology, Indian Institute of Science Education and Research Thiruvananthapuram (IISER TVM), India

ARTICLE INFO

Sir,

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has emerged as a contagious coronavirus following the two major outbreaks of SARS-CoV-1 in 2003 and Middle East respiratory syndrome coronavirus (MERS-CoV) in 2012 [1]. To date, approximately 147 million laboratory-confirmed cases of SARS-CoV-2 infection and 3 million deaths have been reported globally, driving the need to develop rapid and effective therapeutics. Coronaviruses initiate the infection cycle by binding the receptor-binding domain (RBD) of their spike glycoprotein to its cellular receptor [1]; thus, interfering in spike–receptor interactions would aid in the early prevention of virus–host interactions.

Epigallocatechin-3-gallate (EGCG), the primary component in green tea (*Camellia sinensis*), has unique 3-galloyl and 5′-OH groups critical for binding to envelope glycoproteins and inhibiting the cellular entry of several viruses, including Zika virus, hepatitis C virus and influenza virus [2]. In this study, we report the antiviral properties of EGCG against SARS-CoV-1, SARS-CoV-2 and MERS-CoV. EGCG prevents the entry of pseudotyped coronaviruses (CoV-PVs) at micromolar concentrations. Moreover, EGCG binds to the RBD and interferes with the interaction of SARS-CoV-1 and -2 with its receptor [angiotensin-converting enzyme 2 (ACE2)] as well as MERS-CoV with its receptor [dipeptidyl peptidase 4 (DPP4/CD26)].

EGCG and epicatechin (EC) are polyphenolic flavonoids (Fig. 1a,b) predominantly found in green tea and have a wide range of medicinal benefits [2]. To investigate the antiviral activity of EGCG and EC against coronaviruses, in this study we first treated Vero E6 cells, susceptible to SARS-CoV-1, SARS-CoV-2 [3] and MERS-CoV [4], with either EGCG or EC (5–100 μM), followed by infection with CoV-PVs. Similarly, CoV-PVs were treated prior to infection of Vero E6 cells. The level of inhibition was quantified by calculating the number of green fluorescent protein (GFP)-positive cells per well. Cells pre-treated with EGCG or EC did not significantly reduce the level of viral infection (Fig. 1c). In contrast, a considerable decrease of CoV-PV infection was found with EGCG-treated CoV-PVs but not with EC-treated CoV-PVs or non-enveloped adenovirus type 5. A dose-dependent inhibition was observed in EGCG-treated viruses (Fig. 1d) with 90% reduction in infectivity of SARS-CoV-1, SARS-CoV-2 and MERS-CoV-PVs at 85, 38 and 98 μM of EGCG, respectively.

Furthermore, we measured the cytotoxicity of EGCG and EC on Vero E6 cells by the standard MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium-bromide] assay. None of the compounds showed cytotoxicity even at the highest concentrations tested or at extended incubation periods (1 h or 24 h), whereas cells treated with floxuridine (positive control) showed 30% cell death after 24 h of treatment (Fig. 1e). Our data indicate that EGCG inhibits the entry of CoV-PVs without inducing cytotoxicity.

Next, to test whether EGCG interacts with the spike protein of SARS-CoV-1, SARS-CoV-2 and MERS-CoV, we generated recombinant RBD proteins and performed fluorescence emission spectrophotometry. We incubated RBD proteins with varying concentrations of EGCG (25–100 μM) at either 25°C or 37°C and measured the emission spectra. We observed a gradual decrease in fluorescence emission of the RBD proteins treated with EGCG (Fig. 1f), consistent with previous reports showing that interaction of EGCG with the envelope proteins of herpes simplex virus 1 or reovirus decreases the fluorescence emission of these proteins [5]. These data indicate that EGCG interacts with the RBD of CoVs, and a
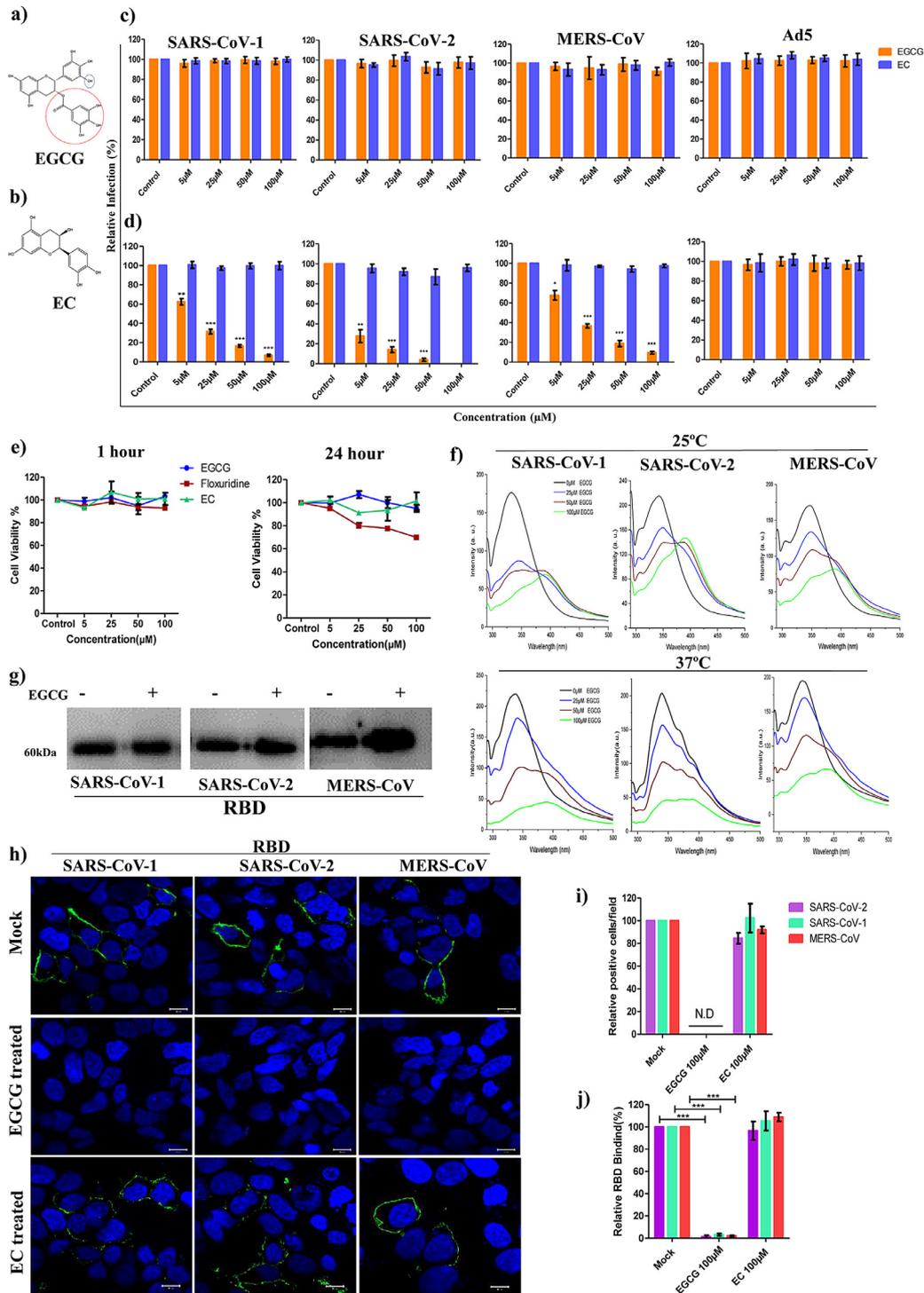
Fig. 1. Antiviral activity of epigallocatechin-3-gallate (EGCG) against emerging severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), SARS-CoV-1 and Middle East respiratory syndrome coronavirus (MERS-CoV).
(a,b) Chemical structure of EGCG (a) and epicatechin (EC) (b). The 3-galloyl (red circle) and 5′-OH group (blue circle) in EGCG are highlighted. (c) Vero E6 cells were treated with different concentrations of EGCG and EC, followed by infection with pseudotyped SARS-CoV-1, SARS-CoV-2, MERS-CoV or adenovirus type 5 (Ad5). (d) Pseudotyped coronaviruses (CoV-PVs) and Ad5 were treated with EGCG or EC, followed by infection of Vero E6 cells. Data represent the mean ± S.D. of an experiment repeated three times. (e) Cytotoxicity of EGCG and EC in Vero E6 cells was measured at 1 h and 24 h post-treatment with the compounds. Floxuridine was used as a positive control for the experiment. Data represent the mean ± S.D. of an experiment with duplicates repeated twice. (f) Binding of EGCG to the receptor-binding domain (RBD) of SARS-CoV-1, SARS-CoV-2 and MERS-CoV was analysed by fluorescence emission spectroscopy. (g) Western blot analysis of the recombinant RBD proteins after treatment with EGCG. (h) Binding assay of recombinant RBD treated with or without either EGCG or EC on HEK293T cells transiently expressing either angiotensin-converting enzyme 2 (ACE2) or dipeptidyl peptidase 4 (DPP4). (i) RBD-positive cells were quantified by calculating the total positive cells per field. (j) FACS analysis of RBD-positive cells, normalised to 100% relative to the control. Statistical significance was determined by two-tailed *t*-test: *** $P < 0.001$. S.D., standard deviation.

complete quenching was observed at 100 µM of EGCG at 37°C (Fig. 1f), which correlates with our CoV-PVs infection (Fig. 1d). Next, we ruled out that the observed inhibition was due to protein degradation by western blot analysis (Fig. 1g), which indicates that EGCG could bind to the RBD of CoVs without affecting its stability.

Furthermore, to check whether the binding of EGCG with the RBD inhibits the spike–receptor interaction, we performed a binding assay on HEK293T cells expressing ACE2/DPP4. RBD proteins were incubated independently with either EGCG or EC and the complex was then allowed to bind on the surface of the cells. Binding of RBD proteins was analysed by confocal microscopy and fluorescence-activated cell sorting (FACS). EC-treated RBD did bind, but EGCG-treated proteins did not bind on their respective receptors (Fig. 1h). Moreover, no RBD-bound cells were observed with EGCG-treated proteins in either of the assays (Fig. 1i, j). These data clearly show that EGCG-bound RBD protein is unable to interact with either ACE2 or DPP4. We speculate that inhibition of viral entry by EGCG might be due to binding to the RBD–receptor interface or elsewhere in the RBD, causing conformational changes hindering the receptor interactions.

Detailed information regarding the materials and methods are given in the Supplementary material.

In summary, we demonstrated the antiviral activity of EGCG on SARS-CoV-1, SARS-CoV-2 and MERS-CoV in vitro and elucidated the interaction of EGCG to the RBD of emerging CoVs. This study puts forward a potential lead molecule for the development of broad-spectrum antivirals against emerging CoVs.

Competing interests

None declared.

Acknowledgments

Funding

Ethical approval

This study was approved by the Institute Biosafety Committee (IBSC) [No. BT/BS/17/447/2011-PID].

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jgar.2021.05.005.

References

[1] V'kovski P, Kratzel A, Steiner S, Stalder H, Thiel V. Coronavirus biology and replication: implications for SARS-CoV-2. Nat Rev Microbiol 2021;19:155–70. doi:10.1038/s41579-020-00468-6.
[2] Xu J, Xu Z, Zheng W. A review of the antiviral role of green tea catechins. Molecules 2017;22:1337. doi:10.3390/molecules22081337.
[3] Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell 2020;181:271–80 .e8. doi:10.1016/j.cell.2020.02.052.
[4] Raj VS, Mou H, Smits SL, Dekkers DHW, Müller MA, Dijkman R, et al. Dipeptidyl peptidase 4 is a functional receptor for the emerging human coronavirus-EMC. Nature 2013;495:251–4. doi:10.1038/nature12005.
[5] Colpitts CC, Schang LM. A small molecule inhibits virion attachment to heparan sulfate- or sialic acid-containing glycans. J Virol 2014;88:7806–17. doi:10.1128/jvi.00896-14.