Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.e-jds.com

Short Communication

SARS-CoV-2 recombinant spike protein induces cell apoptosis in rat taste buds

Toru Yamamoto*, **Yuhei Koyama**, **Tomoaki Ujita**, **Emi Sawada**, **Naotaka Kishimoto**, **Kenji Seo**

Division of Dental Anesthesiology, Faculty of Dentistry & Graduate School of Medicine and Dental Sciences, Niigata University, Japan

Received 12 August 2022; Final revision received 17 August 2022
Available online ■ ■ ■

KEYWORDS
SARS-CoV-2;
COVID-19;
Apoptosis;
TNF-α;
Taste dysfunction;
Rat

Abstract Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections can cause loss or alteration of taste and smell as early symptoms or sequelae, but the detailed mechanism behind this phenomenon remains unclear. Here, we investigated whether the SARS-CoV-2 spike protein induces taste cell apoptosis and expression of the apoptosis-related cytokine TNF-α in male Sprague–Dawley rats. Terminal deoxynucleotidyl transferase (TdT)-mediated deoxyuridine triphosphate (dUTP)-fluorescein nick end labeling (TUNEL) assay results revealed a significantly higher apoptosis index for taste cells in the SARS-CoV-2 group than for those in the control group. An immunohistochemistry analysis indicated significantly more TNF-α-positive cells in the SARS-CoV-2 group compared with the control group. These data suggest that the SARS-CoV-2 spike protein promotes taste cell apoptosis and the release of apoptosis-related cytokine TNF-α, implicating its contribution to the taste malfunction caused by coronavirus disease 2019 (COVID-19).
© 2022 Association for Dental Sciences of the Republic of China. Publishing services by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Introduction

Several nonspecific signs and symptoms, including not only respiratory symptoms but also dysgeusia, have been reported as being related to coronavirus disease 2019 (COVID-19), which is caused by infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Patients with COVID-19 often experience smell and taste disorders, mainly a decrease or loss of smell (hyposmia and anosmia, respectively) and taste (hypogeusia and ageusia,

* Corresponding author. Division of Dental Anesthesiology, Faculty of Dentistry & Graduate School of Medicine and Dental Sciences, Niigata University, 2-5274 Gakkocho-dori, Chuo-ku, Niigata, 951-8514, Japan.
E-mail address: toruyamamoto@dent.niigata-u.ac.jp (T. Yamamoto).

https://doi.org/10.1016/j.jds.2022.08.016
1991-7902/© 2022 Association for Dental Sciences of the Republic of China. Publishing services by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Please cite this article as: T. Yamamoto, Y. Koyama, T. Ujita et al., SARS-CoV-2 recombinant spike protein induces cell apoptosis in rat taste buds, Journal of Dental Sciences, https://doi.org/10.1016/j.jds.2022.08.016

respectively).[1] We reported previously that SARS-CoV-2 recombinant proteins induced the degeneration of taste buds in rat circumvallate papillae.[2] However, the detailed mechanism underlying taste dysfunction due to SARS-CoV-2 infection remains unclear.

This study investigated whether the SARS-CoV-2 spike protein promotes taste cell apoptosis and expression of the apoptosis-related cytokine TNF-α, which could be related to COVID-19-related taste impairment.

Materials and methods

All experimental procedures were reviewed and approved by the Animal Research Committee of Niigata University (approval number: SA01149) and were performed in accordance with the guidance of the National Institutes of Health on animal care and use.

Animal experiments

Male Sprague–Dawley rats (5 weeks old, 150–200 g; The Jackson Laboratory, Yokohama, Japan) were housed in pairs and maintained in a facility with a 12/12-h light/dark cycle at approximately 23 °C with free access to food and water for 1 week before their use in our experiments.

Total 6 animals were utilized in the present experiments. Animals were randomly divided into two groups and anesthetized with a mixture of three anesthetics (2.5 mg/kg butorphanol, 0.375 mg/kg medetomidine, and 2 mg/kg midazolam, *i. p.*). In the albumin group (n = 2), albumin (diluted in saline, 10 μg/50 μl per rat; albumin from bovine serum; Wako, Tokyo, Japan) was applied to the circumvallate papillae as a control treatment. The recombinant SARS-CoV-2 spike protein (AG30689; Proteintech, Rosemont, IL, USA) was diluted in saline at concentration of 10 μg/50 μl per rat and the small thin filter paper soaked in liquid was applied to the circumvallate papillae for 30 min as the SARS-CoV-2 group (n = 4).

At 48 h after the application of albumin or recombinant SARS-CoV-2 spike, the animals were again anesthetized, first as described above and then injected with chloral hydrate (600 mg/kg, *i. p.*). The deeply anesthetized rats were transcardially perfused with 0.02 M phosphate-buffered saline (PBS; pH 7.2) and then with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2). Circumvallate papillae were carefully harvested and fixed with 4% paraformaldehyde in 0.1 M phosphate buffer for at least 48 h. After being soaked in 20% sucrose in PBS overnight for cryoprotection, the tissues were embedded in Tissue Tek OCT compound (Sakura Finetechnical, Tokyo, Japan) and frozen at −80 °C. Frozen sections were cut transversely into 15-μm-thick sections using a cryostat (Leica Biosystems, Nussloch, Germany).

Assessment of cell apoptosis in circumvallate papillae by TUNEL assay

The terminal deoxynucleotidyl transferase (TdT)-mediated deoxyuridine triphosphate (dUTP)-fluorescein nick end labeling (TUNEL) method was utilized via a TUNEL assay apoptosis detection kit (R&D Systems Inc., Minneapolis, MN, USA) to assess the possible occurrence of cell apoptosis in rat taste buds of the circumvallate papillae. After being briefly incubated with 4',6-diamino-2-phenylindole (DAPI) for 10 min and subsequently washed in PBS, the sections were mounted on glass slides and covered with a cover slip. A typical taste bud contains elongated cells that form a rosebud-shaped structure. An individual taste bud from an intact tongue was generally found every 30–60 μm in preliminary experiments. To avoid double counting, one out of four consecutive sections (because 60 μm/15 μm = 4) from a tongue specimen was used for measurements. Thus, taste buds were non-repetitively counted across images of all serial sections. The TUNEL-positive cells were counted and analyzed in each of 10 randomly selected sections from both groups. All the DAPI-positive cells and the TUNEL-positive cells were counted with a fluorescence microscope (BZ-X800; Keyence, Itasca, IL, USA) and ImageJ Fiji (NIH, Bethesda, MD, USA). The apoptosis index was calculated by using the following formula (the number of apoptotic cells/the total number of nucleated cells) $\times$ 100%.

Assessment of TNF-α expression in rat circumvallate papillae

Specimens of the circumvallate papillae were collected at 48 h after treatment and incubated overnight at 4 °C with rabbit anti-TNF-α primary antibody (1:500, GTX110520; GeneTex, Irvine, CA, USA). The sections were washed three times with PBS and then incubated with donkey anti-rabbit secondary antibody (1:1000, Alexa Fluor 594, The Jackson Laboratory) for 1 h at room temperature in the dark. Ten randomly selected fields ($\times$ 20) of whole circumvallate papillae from each group were assessed using ImageJ Fiji to quantify the TNF-α-positive cells.

Statistical analysis

Data are expressed as the mean $\pm$ SD. Unpaired *t*-tests were used to compare differences in the apoptosis index and TNF-α-positive rate. Statistical analyses were performed using GraphPad Prism 8 (GraphPad, San Diego, CA, USA). Values of $P < 0.05$ were taken to indicate a significant difference.

Results

More cell apoptosis of taste buds in the SARS-CoV-2 group

TUNEL analyses revealed a statistically significant higher apoptosis index in the SARS-CoV-2 group, as compared with the Albumin (control) group (19.4 $\pm$ 6.1% vs 6.7 $\pm$ 2.2%, respectively; $P < 0.05$) (Fig. 1).

Higher numbers of TNF-α -positive cells in the SARS-cov-2 group

An immunohistochemical analysis indicated that there were significantly more TNF-α-positive cells in the circumvallate

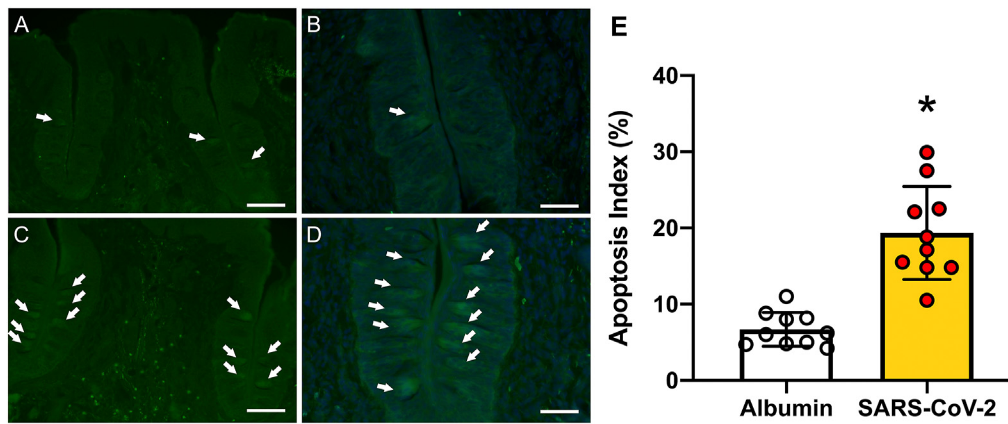

Figure 1 **Cell apoptosis in rat taste buds of circumvallate papillae.** TUNEL staining of taste buds in the Albumin (control) (A, B) and SARS-CoV-2 (C, D) groups; arrows indicate TUNEL positive cells; scale bars = 100 μm (A, C) or 50 μm (B, D) (E): Apoptosis index in the Albumin and SARS-CoV-2 groups. *: $P < 0.05$ vs Albumin group, unpaired t-test; n = 10/group.

papillae of the SARS-CoV-2 group than in that of the Albumin (control) group (24.8 ± 5.3% vs 6.8 ± 2.5%, respectively; $P < 0.05$) (Fig. 2).

Discussion

We previously reported that a local application of recombinant SARS-CoV-2 spike protein to the circumvallate papillae of rats led to reductions in the number of taste buds and in the number and area of taste cells, implying potential contribution for taste impairment.[2] Recently, it has also been reported that the SARS-CoV-2 spike protein triggers inflammatory responses and apoptosis through cell death signaling in mouse lung epithelial cells as well as in the cells of several cultured cell lines.[3,4] Therefore, the present study investigated whether the SARS-CoV-2 spike protein induces apoptosis of taste bud cells. Its results demonstrate that a local application of recombinant SARS-CoV-2 spike protein to the circumvallate papillae of rats induced taste cell apoptosis along with an increase in the local TNF-α level.

Ordinarily, taste bud cells undergo apoptosis and turnover at a constant cycle of 11–14 days.[5] We conducted the apoptosis detection test using albumin at the same concentration used for the recombinant SARS-CoV-2 spike protein (control treatment) to clarify whether the observed taste cell apoptosis was due to stimulation provided by simple protein contact or by the SARS-CoV-2 spike protein specifically. Although a few apoptotic cells

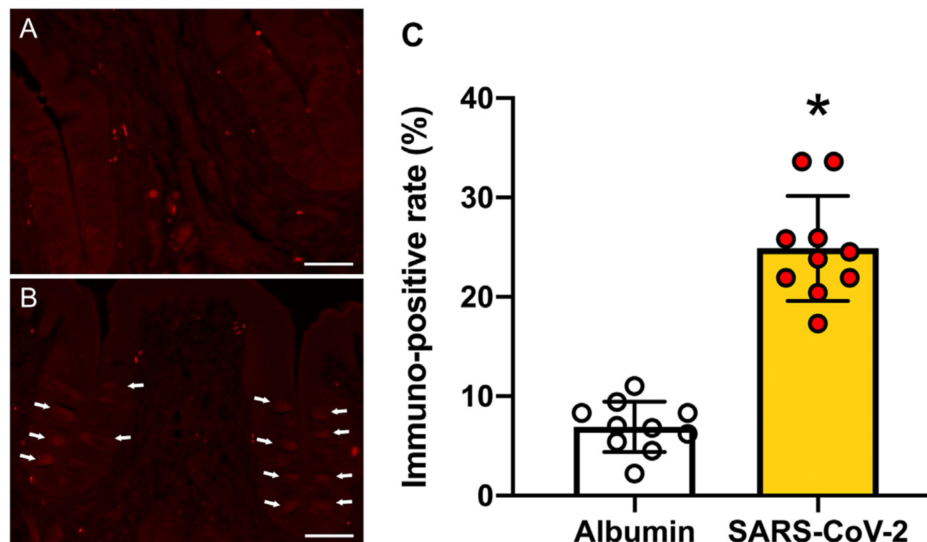

Figure 2 **Immunohistochemical analysis of TNF-α in taste bud cells (A–B).** Representative immunohistochemistry images of taste buds in the Albumin (control) (A) and SARS-CoV-2 (B) groups; arrows indicate immunoreactivity for TNF-α; scale bar = 100 μm (C): Percentage of TNF-α-positivity (%) among the cells in the Albumin and SARS-CoV-2 groups. *: $P < 0.05$ vs Albumin group, unpaired t-test; n = 10/group.

were observed in the control group, significantly more taste cell apoptosis was observed in the SARS-CoV-2 group. Emerging evidence suggests that the SARS-CoV-2 spike protein induces apoptosis and necrosis in macrophages,[6] peripheral blood mononuclear cells,[6] and adipocytes.[7] Recent reports using several cell lines also revealed that the SARS-CoV-2 spike protein promotes apoptosis in SARS-CoV-2-infected cells.[8,9] Our results support a similar occurrence in taste cells.

It is thought that apoptosis is triggered by extracellular environment changes induced by the SARS-CoV-2 spike protein, i.e., the inflammatory-immune response, and by cell death signaling induced by SARS-CoV-2 infection. Macrophages recognize infected cells that are about to undergo apoptosis, and, in response, they release various inflammatory cytokines to attract other innate immune cells. The TNF-α released from these macrophages and IFN-γ from T cells not only induces the migration of more immune cells to expand the inflammatory-immune response but also leads to the apoptosis of infected cells.[10] Therefore, we investigated whether the stimulation of rat circumvallate papillae with a recombinant SARS-CoV-2 spike protein would promote the release of TNF-α. An immunohistochemical analysis revealed a significantly higher immunoreactivity of TNF-α in the taste cells of recombinant SARS-CoV-2 spike protein-treated rats. It is known that infected cells and their post-apoptosis remnants are phagocytosed by migrated macrophages. Thus, the results of this work and the findings from our previous study together suggest that the SARS-CoV-2 spike protein acts directly on taste bud cells via AEC2 receptors to induce intracellular death signaling, along with a local inflammation-immune response, which leads to TNF-α-mediated apoptosis, subsequently resulting in the phagocytosis of infected cells and consequent tissue degeneration of the taste buds. The limitation of the study is that a small sample size with only six animals in total.

This study demonstrated that a topical application of recombinant SARS-CoV-2 spike protein promoted a higher local level of the apoptosis-related proinflammatory cytokine TNF-α, and the occurrence of taste cell apoptosis in response to this treatment implicates the SARS-CoV-2 spike protein in having a potential contribution to the taste dysfunction often exhibited in persons with COVID-19. Future investigations should further explore the details of the mechanisms responsible for the taste disorders caused by COVID-19.

Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

Acknowledgments

This work was supported by JSPS KAKENHI [grant numbers: 21K21036] and Nishiyama Dental Academy General Foundation to Toru Yamamoto and NAGAI Promotion Foundation for Science of Perception to Kenji Seo.

References

1. Jesada K, Songklot A, Kornkiat S, Kachorn S, Supinda C. Smell and taste dysfunction in patients with SARS-CoV-2 infection: a review of epidemiology, pathogenesis, prognosis, and treatment options. *Asian Pac J Allergy Immunol* 2020;38:69—77.
2. Yamamoto T, Koyama Y, Imai Y, Sawada E, Kishimoto N, Seo K. SARS-CoV-2 recombinant proteins-induced degeneration of taste buds in rat circumvallate papillae. *J Dent Sci* 2022;17:1450—3.
3. Li S, Zhang Y, Guan Z, et al. SARS-CoV-2 triggers inflammatory responses and cell death through caspase-8 activation. *Signal Transduct Targeted Ther* 2020;5:235.
4. Ren Y, Shu T, Wu D, et al. The ORF3a protein of SARS-CoV-2 induces apoptosis in cells. *Cell Mol Immunol* 2020;17:881—3.
5. Ueda K, Ichimori Y, Maruyama H, et al. Cell-type specific occurrence of apoptosis in taste buds of the rat circumvallate papilla. *Arch Histol Cytol* 2008;71:59—67.
6. Tlili B, Bandar A, Hayat S, et al. SARS-CoV-2 coronavirus spike protein-induced apoptosis, inflammatory, and oxidative stress responses in THP-1-like-macrophages: potential role of angiotensin-converting enzyme inhibitor (perindopril). *Front Immunol* 2021;12:728896.
7. Gema F, Victoria C, Victor V, et al. FNDC4 and FNDC5 reduce SARS-CoV-2 entry points and spike glycoprotein S1-induced pyroptosis, apoptosis, and necroptosis in human adipocytes. *Cell Mol Immunol* 2021;18:2457—9.
8. Li F, Li J, Wang PH, et al. SARS-CoV-2 spike promotes inflammation and apoptosis through autophagy by ROS-suppressed PI3K/AKT/mTOR signaling. *Biochim Biophys Acta (BBA) - Mol Basis Dis* 2021;1867:166260.
9. Rittibet Y, Patompong K, Ratana B. Coronavirus infection-associated cell death signaling and potential therapeutic targets. *Molecules* 2021;26:7459.
10. Rajendra K, Bhesh RS, Shraddha T, et al. Synergism of TNF-α and IFN-γ triggers inflammatory cell death, tissue damage, and mortality in SARS-CoV-2 infection and cytokine shock syndromes. *Cell* 2021;184:149—68.