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Rice bran-derived protein fractions enhance sulforaphane-induced anti-oxidative activity in gingival epithelial cells

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ABSTRACT

Objective: Food-derived bioactive peptides have been reported to exhibit various beneficial effects, including antimicrobial, anti-inflammatory, and anti-oxidant properties. Oxidative stress has been implicated in the development of several inflammatory diseases such as periodontal disease. However, the anti-oxidative effect of food-derived bioactive peptides in gingival epithelial cells (GECs) is unknown. Therefore, we examined the bioactivity of the peptides in GECs.

Design: Food-derived peptide fractionations derived from rice bran, rice endosperm, corn, and soy were screened for anti-oxidative effects using anti-oxidant response element (ARE)-luciferase–transfected HEK 293 cells. The induction of anti-oxidation–related genes and proteins in GECs by the fractions were examined by quantitative PCR and Western blotting, respectively. Then, the fraction-mediated anti-oxidative effects were examined by measuring intracellular reactive oxygen species (ROS) levels using flow cytometry. Furthermore, the anti-oxidative response-related cellular signaling pathways were analyzed via Western blotting.

Results: Although treatment with the food-derived peptides alone did not activate anti-oxidative responses, co-treatment with sulforaphane (SFN; a potent anti-oxidant) and certain food-derived peptides enhanced anti-oxidative responses in ARE-luciferase–transfected HEK 293 cells. The fractions augmented heme oxygenase-1 mRNA and protein expression in GECs. The percentage of ROS-positive cells was significantly decreased by co-treatment with SFN and peptide fractions derived from rice bran. Furthermore, the involvement of both nuclear factor erythroid 2-related factor 2 (Nrf2) and extracellular signal-regulated kinase (ERK) in the enhancement of anti-oxidative responses was demonstrated by Western blotting.

Conclusions: Peptides derived from rice bran enhances SFN-induced anti-oxidative responses in GECs through ERK–Nrf2–ARE signaling.

1. Introduction

Oxidative stress is a phenomenon caused by an imbalance between oxidative and anti-oxidative events in the body, and this imbalance is attributable to excessive free radical levels (Pizzino et al., 2017). Elevated intracellular levels of reactive oxygen species (ROS), a major free radical derived from molecular oxygen, can cause DNA, protein, and lipid damage, leading to impaired cellular biological properties (Schieber & Chandel, 2014). ROS-mediated impairment has been implicated in the pathology of several human diseases including cancer, atherosclerosis, and rheumatoid arthritis (Quinonez-Flores, Gonzalez-Chavez, Del Rio Najera, & Pacheco-Tena, 2016; Reuter, Gupta, Chaturvedi, & Aggarwal, 2010).

Anti-oxidants are substances that prevent oxidation and protect cells from damage caused by free radicals, and they play a pivotal role in suppressing or slowing the progression of oxidative stress-related

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Table 1
Oligonucleotide sequences.

Gene	Forward	Reverse
GAPDH	ACCAAATCCGTTGACTCCGAC	TTCGACAGTCAGCCGCATCT
HO-1	GAAGAGGCCAAGACTGCGTT	AGTGTAAGGACCCATCGGAGA

GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HO-1, heme oxygenase-1.

diseases (Young & Woodside, 2001). Sulforaphane (SFN), a well-known potent anti-oxidant that functions as a natural free radical scavenger, can reduce oxidative stress (Guerrero-Beltran, Calderon-Oliver, Pedraza-Chaverri, & Chirino, 2012; Mazarakis, Snibson, Licciardi, & Karagiannis, 2020). SFN-induced anti-oxidation exerts beneficial effects on cardiovascular diseases (Bai et al., 2015), arthritis (Moon et al., 2021), and cancer (Yeh & Yen, 2009). SFN-induced anti-oxidative effects are mainly regulated by nuclear factor erythroid 2-related factor 2 (Nrf2), a transcription factor facilitating the expression of anti-oxidant substances including heme oxygenase-1 (HO-1). Nrf2 is normally bound to the adaptor protein Kelch-like ECH associated protein 1 (Keap1) in the cytoplasm, which leads to the degradation of Nrf2 by the proteosome. Anti-oxidants induce the dissociation of Nrf2 from Keap1, its translocation into the nucleus, and its subsequent binding bind to anti-oxidant response elements (AREs) in the nucleus, resulting in the expression of anti-oxidation–related genes (Loboda, Damulewicz, Pyza, Jozkowicz, & Dulak, 2016). The Keap/Nrf2/ARE-mediated anti--oxidative response is mainly regulated by mitogen-activated protein kinase (MAPK) signaling pathways including extracellular signal-regulated kinase (ERK), c-Jun NH2-terminal kinase (JNK), and p38 pathways (Zipper & Mulcahy, 2003). Previous reports demonstrated that the phosphorylation and activation of ERK induced Keap/Nrf2/ARE-mediated anti-oxidative responses in gingival epithelial cells (GECs) (Yokoji-Takeuchi et al., 2020).

Periodontitis, a chronic inflammatory disease of the oral cavity, is triggered by gram-negative microbial infection in tooth-supporting tissues. Without appropriate treatment, inflammation destroys the bone around the teeth, resulting in tooth loss (Kinane, Stathopoulou, & Papapanou, 2017; Lovegrove, 2004). A variety of studies investigated the involvement of ROS and the resultant oxidative stress in the onset/progression of periodontitis (Chapple & Matthews, 2007). The association of oxidative stress and periodontitis has been extensively demonstrated in clinical studies, which revealed that greater oxidative stress and lower anti-oxidant activity can be detected in the plasma, saliva, and gingival crevicular fluid of patients with periodontitis (Tothova & Celec, 2017). In addition, intervention studies illustrated that periodontal treatment improved the status of oxidative damage, suggesting a pathobiological association between oxidative stress and periodontitis (Bostanci, Toker, Senel, Ozdemir, & Aydin, 2014). An animal study using knockout mice clearly demonstrated the importance of Nrf2 in the pathogenesis of periodontitis (Sima et al., 2016). Tamaki et al. reported that the induction of experimental periodontitis in rats increased systemic oxidative marker levels, and these effects were reversed by the administration of an anti-oxidant compound (Tamaki et al., 2014). The gingival epithelium acts as a physical barrier in the oral cavity, and GECs are exposed to a large number of exogenous stimulants that can induce oxidative stress (Takahashi et al., 2019). Although ROS production by *Porphyromonas gingivalis* in GECs has been reported previously (H. Wang et al., 2014), the detailed dynamics of ROS in GECs remain to be elucidated.

Food-derived bioactive peptides have attracted significant interest for their health benefits (Karami & Akbari-Adergani, 2019). Accumulated evidence indicates that these bioactive peptides possess several activities including anti-microbial, ant-inflammatory, wound-healing, and angiogenic properties (Mansour, Pena, & Hancock, 2014). Some reports detailed the anti-oxidative effect of rice-derived peptides in hepatic epithelial cells (Moritani et al., 2017, 2020). However, the effects of these peptides in GECs remain to be identified. Therefore, this study examined the effect of food-derived peptides on human GECs, focusing

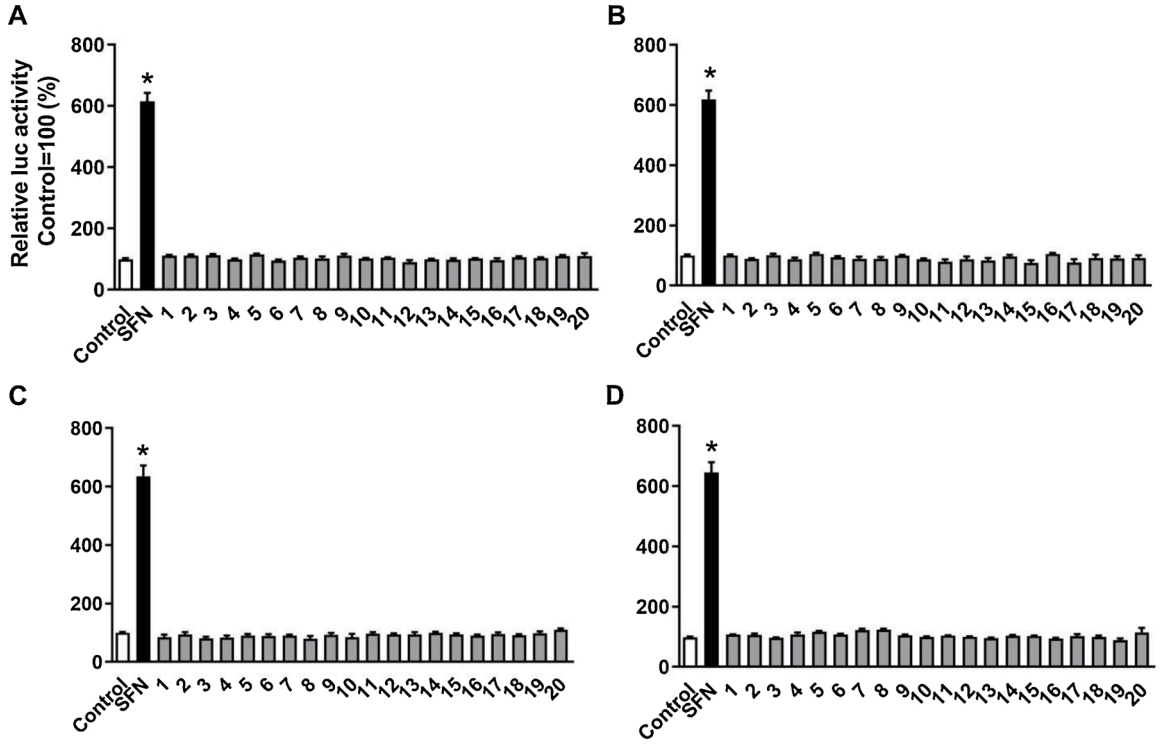

Fig. 1. No induction of anti-oxidant responses by treatment with food-derived peptides alone.
Relative luciferase activity in anti-oxidant response element-luciferase reporter plasmid-transfected HEK293 cells 4 h after treatment with various food-derived fractionated peptides at a concentration of 0.5 mg/mL (A: rice bran, B: rice endosperm, C: corn, D: soy). n = 5 per group. Sulforaphane (SFN, 5 μM) served as the positive control. Data are indicated as the mean ± SEM. *$p < 0.05$ compared to control via analysis of variance.

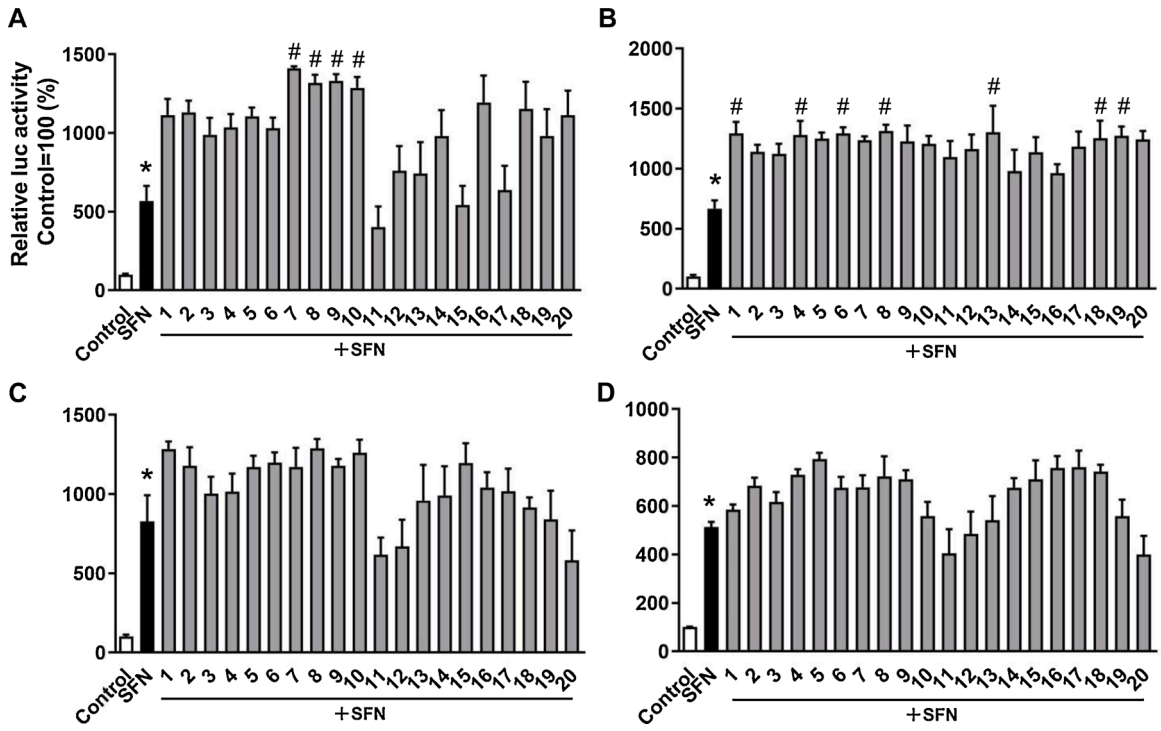

Fig. 2. Peptides from rice bran and rice endosperm enhanced sulforaphane (SFN)-induced anti-oxidative responses.
Relative luciferase activity in the anti-oxidant response element-luciferase reporter plasmid-transfected HEK293 cells 4 h after treatment with the combination of the indicated food-derived fractionated peptides (0.5 mg/mL) and 5 μM SFN (A: rice bran, B: rice endosperm, C: corn, and D: soy). n = 5 per group. SFN (5 μM) served as a positive control. Data are presented as the mean ± SEM. *$p < 0.05$ compared to control via analysis of variance.

on the anti-oxidant stress response.

2. Materials and methods

2.1. Reagents and antibodies

Anti-Nrf2, anti-ERK, anti-phosphorylated ERK, anti-p38, anti-phosphorylated p38, anti-JNK, and anti-phosphorylated JNK antibodies were purchased from Cell Signaling Technology (Danvers, MA, USA). Anti-HO-1 antibody was obtained from GeneTex, Inc. (San Antonio, TX, USA). For Western blotting, we used rabbit anti-mouse glyceraldehyde 3-phosphate dehydrogenase (GAPDH; Cell Signaling Technology) and peroxidase-labeled anti-rabbit IgG antibodies (Cell Signaling Technology). Tert-butyl hydroperoxide (TBHP) and ERK kinase inhibitor, PD98059 was purchased from Wako Pure Chemical Industries (Osaka, Japan). SFN was acquired from Cayman Chemical (Ann Arbor, MI, USA). Food-derived peptides (rice bran, rice endosperm, corn, and soy) used in this study were prepared in accordance with previously published methods (Taniguchi et al., 2017). Briefly, powdered food-derived protein was dissolved in ultrapure water and homogenized using a POLYTRON homogenizer (KINEMATICA; Bohemia, NY). The pH of the suspension was adjusted to 7.8 using 1 M NaOH. Then the homogenized mixture was digested by adding enzymes and incubated at 37 °C for 5 h. After inactivating the enzyme by incubating it at 90 °C for 10 min, the mixture was then centrifuged at 12,000 × g for 30 min at 4 °C. After dialysis against ultrapure water using Spectra/Por dialysis tubing (MWCO, 500–1000 Da; Spectrum Laboratories, Inc., Rancho Dominguez, CA), peptides with low molecular weights were removed and the supernatants were freeze-dried and stored for subsequent separation stages. Freeze-dried hydrolysates were dissolved in ultrapure water and fractionated by ampholyte-free isoelectric focusing (autofocusing) using a Rotofor (Bio-Rad, Richmond, CA). Autofocusing is a preparative isoelectric focusing technique based on the ampholytic properties of peptide mixtures, containing tryptic hydrolysates of food proteins. The constant power and run times were adjusted to 12 W and 2 h, respectively; samples were collected in 20 fraction tubes and used for the experiments.

2.2. Cell preparation and cultures

A human GEC line (Ca9-22) was purchased from the RIKEN Bioresource Center (Tsukuba, Japan) and cultured in Dulbecco's modified Eagle's medium (DMEM, Thermo Fisher Scientific, Waltham, MA, USA) in the presence of 10 % fetal bovine serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin (Thermo Fisher Scientific). A human embryonic kidney cell line (HEK293) was obtained from DS Pharma Biomedical Co., Ltd. (Osaka, Japan) and grown in DMEM in the presence of 10 % FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin. All cell lines were grown in an incubator at 37 °C in an atmosphere of 5 % CO_2. For the treatment of cells with food-derived peptides alone, the peptides at a concentration of 0.5 mg/mL were added to the culture plate and further analysis was performed after the indicated incubation time. For the co-treatment of cells with food-derived peptides and SFN, the peptides (0.5 mg/mL) and SFN (5 μM SFN) were added at the same time. GECs were pre-incubated with PD98059 (5 μM) for 30 min before adding the indicated stimulants.

2.3. Cell viability assay

The 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide (MTT) assay was performed to examine the cytotoxicity of the peptides according to the manufacturer's protocol (Sigma-Aldrich, St. Louis, MO, USA). Briefly, GECs were seeded onto flat-bottomed 96-well plates at a density of 5 × 10^4 cells/mL and incubated overnight with complete culture medium at 37 °C in an atmosphere of 5 % CO_2. Then, the GECs were treated with fractions derived from rice bran for 4 h and subjected to the MTT assay. MTT solution (1 mg/mL) in PBS was added into each well, and the cells were incubated for 2 h. After cell lysis using

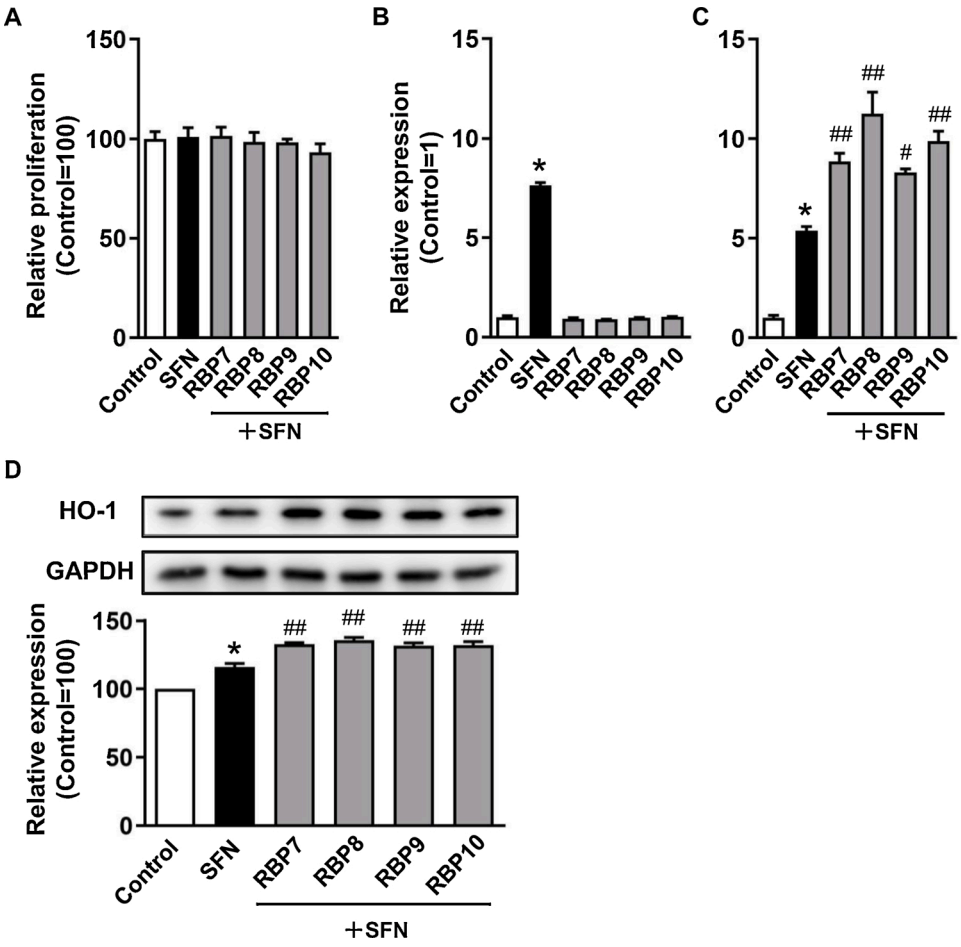

Fig. 3. Rice bran peptides (RBPs) enhanced sulforaphane (SFN)-induced anti-oxidation–related gene and protein expression in gingival epithelial cells (GECs).
(A) Cell viability in GECs. GECs were treated for 4 h with the indicated substances. Relative mRNA (B, C) and protein (D) expression of heme oxygenase-1 (HO-1) in GECs 4 h after treatment with the indicated substances. GAPDH served as a loading control. The protein signal was standardized to GAPDH. n = 3 per group. Data are indicated as the mean ± SEM. $^{*}p < 0.05$ versus control, $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ vs. SFN only via analysis of variance.

DMSO solution, the optical density (OD) of the wells was measured using a Spectra Max ABS Plus plate reader (Molecular Devices, San Jose, CA, USA) at a wavelength of 540 nm, and relative proliferation rate was calculated on the basis of the OD.

2.4. Real-time quantitative PCR

TRI Reagent® (Molecular Research Center, Inc., Cincinnati, OH, USA) was used to isolate total RNA from the cells. Then, a Universal cDNA Master Transcriptor (Roche Molecular Systems, Inc., Branchburg, NJ, USA) was used to synthesize cDNA. Following this, FastStart Essential DNA Green Master (Roche Molecular Systems) was used to quantify gene expression on a LightCycler® *480* (Roche Molecular Systems). *GAPDH* mRNA levels were used to normalize the level of each mRNA sample using the ΔΔCt method, as reported previously (Livak & Schmittgen, 2001). The primer sequences used in this study are displayed in Table 1 (Thermo Fisher Scientific).

2.5. Western blotting

Whole protein was extracted from the cells using M-PER Mammalian Protein Extraction Reagent (Thermo Fisher Scientific) with the Halt Protease Inhibitor Cocktail and Halt Phosphatase Inhibitor Cocktail (Pierce Biotechnology, Rockford, IL, USA). A protein assay was performed using a Pierce Bicinchoninic Acid Protein Assay Kit (Pierce Biotechnology). Then, extracted proteins were subjected to SDS-polyacrylamide gel electrophoresis and transferred to polyvinylidene fluoride membranes (EMD Millipore Corporation, Burlington, MA, USA). The membranes were incubated with primary (HO-1, 1:500; Nrf2, 1:1000; ERK, 1:1000; p-ERK, 1:1000; p38, 1:1000; p-p38, 1:1000; JNK, 1:1000; p-JNK, 1:1000; GAPDH, 1:5000) and secondary antibodies (peroxidase-labeled anti-rabbit IgG antibody, 1:10,000). ECL Plus Western blotting detection reagents (GE Healthcare, Chicago, IL, USA) and ImageQuant LAS 4000 (GE Healthcare) were used to observe the targeted proteins. The intensity of the signal was converted using ImageJ software (NIH, Bethesda, MD, USA).

2.6. Establishment of a stably transfected cell line and luciferase reporter assay

HEK293 cells were transfected with ARE-luciferase reporter plasmids (pGL4.37[luc2P/ARE/Hygro]; Promega, Southampton, UK) using Lipofectamine 2000 (Invitrogen) reagent in accordance with the manufacturer's guideline. Then, a stably transfected cell line was generated via positive selection with hygromycin. For the screen, the cells were treated with substances as indicated in each experiment for 4 h, and firefly luciferase activities were monitored in their lysates. Luciferase bioluminescence measurements were performed at room temperature using a GloMax luminometer (Promega). Activity was expressed as relative light units emitted from total assays, and it was calculated with respect to background activity.

2.7. Flow cytometric analysis of ROS detection

ROS production was assessed using CellROX Green reagent (Thermo Fisher Scientific) according to the manufacturer's instructions. CellROX reagent was added to culture plates, which were incubated for 30 min and then washed three times with PBS. ROS levels were quantified using flow cytometry (NovoCyte Flow Cytometer; ACEA Biosciences, Inc., CA, USA).

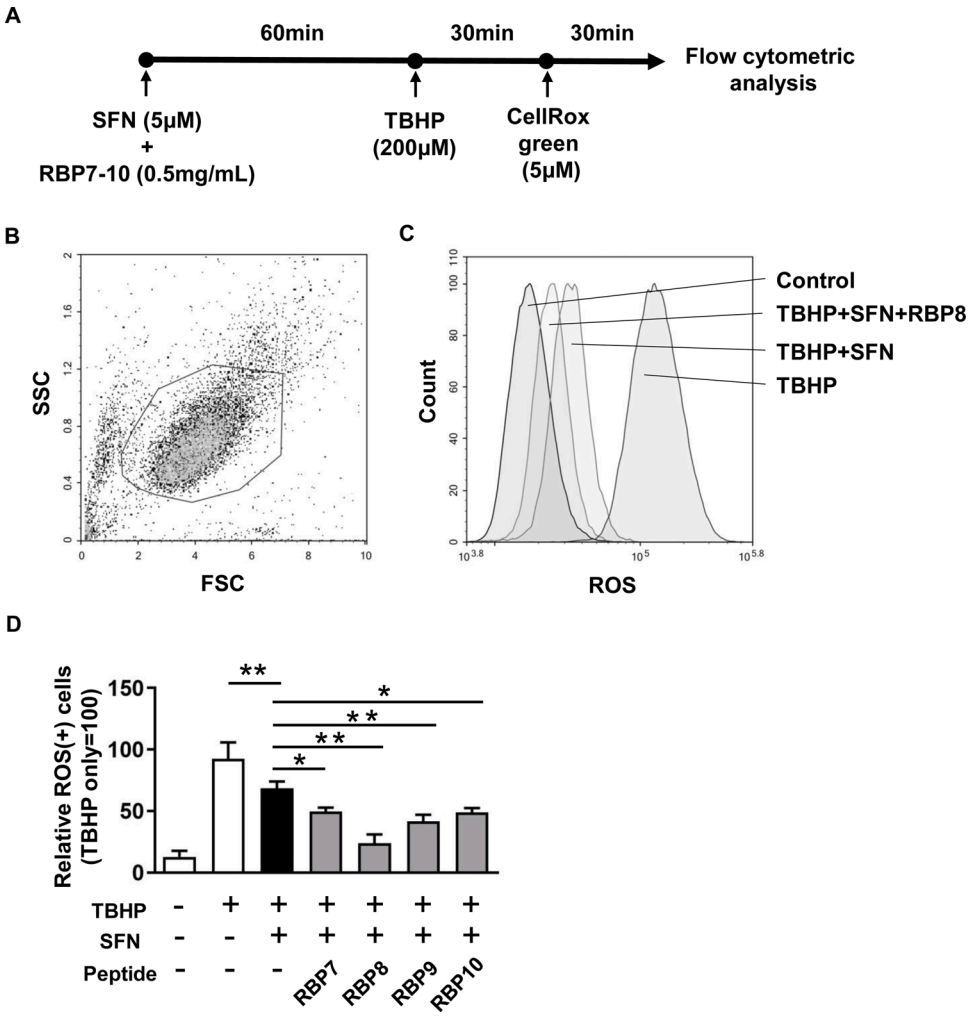

Fig. 4. Rice bran peptides (RBPs) increased the inhibitory effect against tert-butyl hydroperoxide (TBHP)-induced ROS generation.
(A) Experimental design for flow cytometry. (B) A representative image presented the gated cells following flow cytometry. (C) Representative flow cytometry histograms as an overlay of the indicated groups. (D) Quantification of reactive oxygen species (ROS) production in gingival epithelial cells. The population of ROS-positive cells was measured by flow cytometry. n = 4 per group. Data are indicated as the mean ± SEM. $^{*}p < 0.05$ $^{**}p < 0.01$ as indicated via analysis of variance.

2.8. Statistical analysis

Data are presented as the mean ± SEM. Statistical analysis was performed using one-way analysis of variance followed by post hoc tests for multiple comparisons. All statistical analyses were performed using GraphPad Prism (GraphPad Software, Inc., La Jolla, CA, USA). Significance was indicated by $p \leq 0.05$.

3. Results

3.1. Peptides derived from rice bran and rice endosperm enhanced SFN-induced anti-oxidative responses

First, we examined the activation of anti-oxidant responses following the treatment of ARE-luciferase reporter plasmid-transfected HEK293 cells with various food-derived peptides (rice bran, rice endosperm, corn, and soy). Unexpectedly, none of them induced anti-oxidant responses (Fig. 1). However, co-treatment with SFN and several peptides derived from rice bran and rice endosperm significantly increased luciferase activity compared to the effects of SFN alone (Fig. 2). These findings suggested that several food-derived peptides, especially rice bran peptides (RBPs), enhanced anti-oxidative responses induced by SFN.

3.2. RBPs enhanced SFN-induced anti-oxidation–related gene and protein expression in GECs

Next, we explored the involvement of RBPs in the induction of anti-oxidation–related gene and protein expression in GECs. After confirming that RBPs were not cytotoxic to GECs (Fig. 3A), we examined the induction of HO-1 mRNA and protein expression by RBPs. Consistent with the previous findings in transfected HEK293 cells, co-treatment with SFN and several RBPs (RBP7–10) significantly increased HO-1 mRNA expression, but HO-1 mRNA expression was not observed following exposure to RBPs alone (Fig. 3**B, C**). Similarly as the alterations of mRNA levels, HO-1 protein expression was higher in the SFN + RBP7–10 groups than in the SFN only group (Fig. 3D). Altogether, several RBPs in combination with SFN augmented HO-1 expression at both the mRNA and protein level in GECs.

3.3. RBPs increased the inhibitory effect against TBHP-induced ROS generation

To examine the functional effects of RBPs on SFN-induced anti-oxidative responses in GECs, we performed a fluorescent probe-based ROS production assay using flow cytometry *in vitro* (Fig. 4A). After gating the GEC area, the population of ROS-positive cells in each group was plotted on a histogram (Fig. 4**B, C**). The dramatic ROS generation induced by TBHP, a strong oxidizing agent, was suppressed by SFN treatment, and further suppression was observed in the SFN + RBP groups (Fig. 4C). Quantification illustrated the significant inhibitory

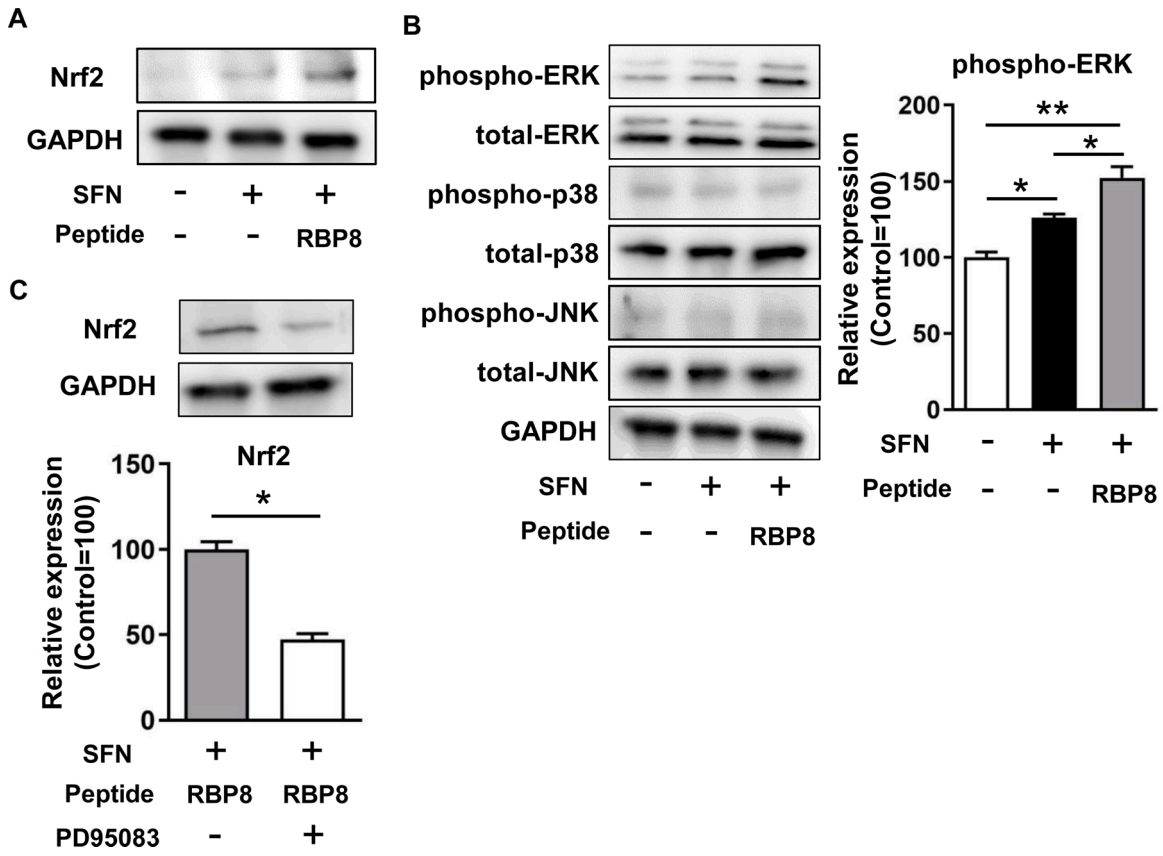

Fig. 5. Extracellular signal-regulated kinase (ERK)–nuclear factor erythroid 2-related factor 2 (Nrf2) axis is responsible for the rice bran peptide (RBP)-induced augmentation of anti-oxidative responses in gingival epithelial cells (GECs).
(A) The cell lysate was evaluated via Western blots using Nrf2-specific antibody. GECs were stimulated with the indicated treatments for 4 h. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) served as the loading control. (B) Western blots of mitogen-activated protein kinase (MAPK) phosphorylation and quantification of the phosphorylated ERK protein in GECs. GECs were stimulated with the indicated treatments for 4 h. GAPDH served as a loading control. The protein signal was standardized to GAPDH. n = 3 per group. Data are indicated as mean ± SEM. *$p < 0.05$ as indicated using analysis of variance. (C) Western blots and quantification of Nrf2 with or without ERK inhibitor. GECs were pre-treated in the presence or absence of ERK inhibitor (5 μM) for 30 min before the indicated treatment. The protein signal was standardized to total ERK. n = 3 per group. Data are indicated as mean ± SEM. *$p < 0.05$ as indicated using analysis of variance.

effect of SFN + RBP treatment on TBHP-induced ROS production; in particular, remarkable inhibition was observed in the SFN + RBP8 group (Fig. 4D). These results indicated the enhancement of anti-oxidative responses by RBPs in combination with SFN.

3.4. ERK–Nrf2 axis is responsible for the enhancement of SFN-induced anti-oxidative responses by RBP8

To elucidate the intracellular signaling pathway involved in SFN-induced anti-oxidative responses, we focused on the ERK–Nrf2 cascade, which was previously reported to regulate anti-oxidative responses by phosphorylating ERK (Sun et al., 2015; Yokoji-Takeuchi et al., 2020). As expected, Nrf2 was induced by SFN treatment, and further induction was observed following SFN + RBP8 treatment (Fig. 5A). In addition, SFN treatment resulted in the phosphorylation of ERK, but not p38 and JNK, and combined treatment with SFN and RBP8 increased the level of ERK phosphorylation (Fig. 5B). The Nrf2 induction on SFN + RBP8 treatment diminished significantly by pretreating it with a selective ERK inhibitor (Fig. 5C). Taken together, these results suggested that RBP8 enhances SFN-induced anti-oxidative responses through the ERK–Nrf2–ARE pathway in GECs.

4. Discussion

In this study, we revealed that RBPs enhanced SFN-induced anti-oxidative responses in GECs. To our knowledge, this is the first report describing the involvement of food-derived bioactive peptides in anti-oxidant effects in GECs and their cellular signaling pathways. These findings suggested the possible application of food-derived bioactive peptides in the prevention/treatment of oxidative stress-related chronic diseases, including periodontitis.

As demonstrated in this study, stimulation with food-derived peptides alone unexpectedly did not induce anti-oxidative responses. Most previous pubs demonstrated in this study, stimulation with food-derived peptides alone unexpectedly did not induce anti-oxidative responses. Most previous publications demonstrated that food-derived peptides alone function as bioactive substances (Kawakami et al., 2017; Matsugishi et al., 2021; Tamura et al., 2019). Moritani et al. reported that hydrolyzed peptides from rice bran protein directly exert a protective effect against oxidative stress by modulating anti-oxidative enzyme expression via the Nrf2 pathway in hepatic epithelial cells (Moritani et al., 2017). The discrepancy might be attributable to the differences in the types of target cells and/or technical methods of preparation of the substances.

Regarding the molecular mechanisms by which the combination of SFN and RBPs enhanced anti-oxidative activity, the following two mechanisms are suggested: i) increased cell membrane permeability and ii) enhancement of cellular signal transduction by the peptides. SFN possesses a lipophilic nature and exhibits relatively low molecular weight, permitting its flux into cells via passive diffusion (Houghton, Fassett, & Coombes, 2016). Destabilization of the cell membrane by specific peptides has been proposed, leading to an increasing influx of

SFN into the cytosol through the formation of transient pores (Herce et al., 2009; Yesylevskyy, Marrink, & Mark, 2009). In addition, specific peptide fractions called cell-penetrating peptides (CPPs) have been widely investigated, and the CPP-conjugated drug delivery system is gaining increasing attention as a therapeutic modality in medical fields (Guidotti, Brambilla, & Rossi, 2017; Patel et al., 2019). Given the fact that most rice peptides are cationic, binding of the peptides to SFN might increase the affinity of the complex for negatively charged cell membranes, resulting in the enhancement of influx to cells and subsequent induction of anti-oxidative responses by SFN in this present study. Conversely, enhancement of cellular signal transduction by the peptides could be another possible mechanism. Ranjan et al. reported that a chemically conjugated protein with a specific peptide augmented apoptotic signaling in intestinal epithelial cells (Ranjan, Waghela, Vaidya, & Pathak, 2020). A specific peptide is known to inhibit the DNA-binding and transcriptional activities of the p65 NF-κB subunit as well as the production of inflammatory mediators in macrophages upon stimulation (Wang et al., 2011). Furthermore, Fotin-Mleczek et al. demonstrated that three types of cationic peptides modulate tumor necrosis factor (TNF) receptor-mediated signal transduction upon TNF stimulation (Fotin-Mleczek et al., 2005). Of note, this article mentioned that the peptides themselves did not activate signal transduction, suggesting the augmentation of cellular response without any direct induction of signaling activation by the peptides themselves. Given the possible manipulation of a peptide in the Nrf2/Keap1 interaction in oxidative response signaling (Steel, Cowan, Payerne, O'Connell, & Searcey, 2012), RBPs might function in the same manner.

Two limitations in this study could be addressed in future research. First, this study lacked proof of bioactive peptide-mediated anti-periodontitis effects *in vivo*. Several studies described the involvement of oxidative stress in the pathogenesis of periodontitis in mice using Nrf2-knockout or oxidative stress detector-luciferase models (Kataoka et al., 2016; Sima et al., 2016). Ikeda et al. reported that the anti-oxidant resveratrol, which is likely to directly inhibit the effects of ROS, promotes the healing of periodontal destruction in a mouse ligature-induced experimental periodontitis model (Ikeda et al., 2018). Elucidation of the anti-periodontitis property of SFN though epithelial anti-oxidative effects can strengthen our hypothesis. Second, this study revealed the suppression of ROS accumulation by co-treatment with SFN and RBPs; however, we did not demonstrate the biological cellular responses resulting from ROS suppression. Excess ROS accumulation via oxidative stress affects proliferation, differentiation, and apoptosis in epithelial cells (Aw, 2003). The effects of the combination of SFN and RBPs in epithelial biology must be clarified in future research.

5. Conclusions

A specific peptide derived from rice bran enhances SNF-induced anti-oxidative responses in GECs through ERK–Nrf2–ARE signaling. Given the previous findings of anti-microbial and anti-inflammatory of bioactive food-derived peptides, RBPs could be a possible application in the prevention/treatment of periodontal diseases.

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Author contributions statement

M.S. and N.T. designed the research, and wrote the manuscript with the support of K.T., Y.A-N. The *in vitro* experiments were performed by M.S., M.Y-H., and T.T.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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