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Research Note

Brown Rice (*Oryza sativa* L. cv. Hiami) Extract Promotes Cellular Growth by Upregulation of GH and IGF-1 Expression and Secretion

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Abstract The effects of the ethanolic extract of brown rice (BRE, *Oryza sativa* L. cv. Hiami) on cellular growth were investigated. Gene expression analysis by qPCR showed that the expression of the mRNA expressions of growth hormone (GH) and insulin-like growth factor (IGF)-1 genes was significantly induced in cells stimulated with BRE compared with controls, and the effects were dose dependent. In addition, the hormone secretion of GH and IGF-1 was significantly induced in GH3 and HepG2 cells stimulated with BRE in line with the qPCR results. Collectively, these results suggest that BRE induces the expression and secretion of GH and IGF-1 *in vitro*; therefore, application may promote cellular growth.

Keywords: brown rice, growth, growth hormone, IGF-1, IGFBP-1

Introduction

Cellular growth is a complicated biological process in which two growth hormones play critical roles. Growth hormone (GH), secreted from the pituitary gland, is a key peptide that stimulates cell proliferation, growth, reproduction, and regeneration in mammals, including humans. Insulin-like growth factor (IGF), another key growth hormone, is predominantly expressed in the liver, and its gene expression and secretion are positively regulated by GH through the JAK-STAT pathway by GH binding to a GH-receptor in the hepatocytes. The liver is a major target of GH and the principal site of IGF-1 production (1,2). Accordingly, IGF-1, a hormone homologous to proinsulin (3), is secreted from the liver and transported to target tissues such as bones and muscles (4), where it induces growth-promoting effects by regulating cell proliferation and metabolic pathways (5). Thus, the induction of GH and IGF-1 expression could stimulate cellular and organismal growth.

It has been reported that several amino acids promote cellular growth. For example, the stimulatory effects of arginine on the secretion of GH has been reported to occur by suppressing the endogenous secretion of somatostatin, which inhibits GH release (6). Arginine also directly increases GH synthesis in pituitary (7). However, evidence suggests that agents with an anti-oxidant effect could facilitate growth (8) because reactive oxygen species (9) inhibit growth. Therefore, removal of ROS can promote cell proliferation and differentiation (10).

Rice is a staple food worldwide. It has been reported that non-polished brown rice contains a significant amount of high-quality protein and phytochemicals and has multiple health benefits compared with polished rice. Brown rice also contains essential amino acids, vitamins, and minerals including magnesium and zinc compared with polished white rice, giving it a high nutritional value (9,11). The amino acid profile of the rice and wheat flour are comparable, but the nutritive value of protein in rice is higher than that of wheat flour, suggesting that the quality of rice proteins may be better than those of other grains (12,13). The overall nutritional profile of brown rice indicates that it is likely to promote cellular growth. In this study, we investigated whether brown rice (*Oryza sativa* L. cv. Hiami) could induce the expression and secretion of two critical growth hormones from cultured cell lines.

Materials and Methods

Sample extraction Brown rice (*Oryza sativa*L. cv. Hiami) and wheat flour were provided by the Rural Development Administration

(Suwon, Korea). Two hundred grams of powder was added to 1 L of 80% ethanol and mixed for 18 h. The ethanolic extract was obtained by filtration using a 0.45 μm nylon membrane filter (Whatman, Maidstone, England) and was vacuum evaporated by an N-1000 rotary evaporator (Eyela, Tokyo, Japan) at 40°C, then freeze dried for 72 h after freezing at −20°C. The dried extracts (brown rice extract; BRE, wheat flour extract; WFE) were stored at −20°C until the experiment.

Cell culture Rat pituitary GH3 and human hepatocyte HepG2 cells were obtained from the Korean Cell Line Bank (Seoul, Korea) and cultured in the Dulbecco's modified medium (DMEM; Hyclone, Logan, UT, USA) containing 10% heat-inactivated fetal bovine serum (FBS; Hyclone) and 1% penicillin/streptomycin (PEST; Welgene Inc., Seoul, Korea). Cells were maintained at 37°C in a humidified atmosphere of 5% CO_2.

Cell viability assay The viability of GH3 and HepG2 cells cultured in 96-well plate at concentration of $3x10^4$ cells/mL was determined based on the amount of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) that was reduced to formazan. After treatment with various concentrations of BRE and WFE for 24 h, culture medium containing MTT (0.5 mg/mL) was added to each well, and the cells were incubated at 37°C for 3 h before mixing with dimethyl sulfoxide (DMSO) to dissolve the formazan crystals. The absorbance at 570 nm was then measured. Values were normalized to the sample protein concentration.

Antioxidant effect assay Di(phenyl)-(2,4,6-trinitrophenyl)imino-azanium (DPPH) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Twenty-five microliters of BRE or WFE (10 mg/mL) and 1.475 mL of DPPH solution (0.1 mM in 80% methanol) were mixed and incubated for 30 min at room temperature. Absorbance was measured at with Smartspec™ plus spectrophotometer (Bio-Rad, Hercules, CA, USA), and antioxidant activity was calculated as follows:

$$(\%)=\left(1-\frac{A_{samples}-A_{revision}}{A_{standard}}\right)\times 100$$

Quantitative PCR analysis GH3 and HepG2 cells were cultured in 6-well plates and incubated with tested samples for 24 h. Total RNA was extracted from GH3 and HepG2 cells with the RNA iso plus kit (Takara, Otsu, Japan). cDNA was synthesized from 2 μg of total RNA using M-MLV reverse transcriptase (Mbiotech, Seoul, Korea) and oligo (1). Gene expression levels were measured using a Bio-Rad iQ5 iCycler system with RealMasterMix SYBR ROX (5 Prime, Hamburg, Germany). The primer sequences were as follows: rGH forward 5'-AGGGCATCCAGGCTCTGAT-3' and reverse 5'-GCATGTTGGCGTCAAACT TG-3'; rβ-actin forward 5'-CATGTACGTTGCTATCCAGGC-3' and reverse 5'-CTCCTTAATGTCACGCACGAT-3'; hIGF-1 forward 5'-GCTCTTCAGTTC GTGTGTG-3' and reverse 5'-GACTTGGCAGGCTTGAGG-3'; hIGFBP-1 forward 5'-TATGATGGCTCGAAGGCTCT-3' and reverse 5'-TAGACGCAC CAGCAGAGTC-3'; and hβ-actin forward 5'-CCTTCTTGGGTATGGAA TCC-3' and reverse 5'-TCTGCATCCTGTCAGCAATG-3'. The reaction conditions for the rat primers were 95°C for 4.5 min followed by 40 cycles of 95°C for 10 s, 55°C for 30 s, and 68°C for 20 s. Conditions for human primers were 95°C for 10 min followed by 40 cycles of 95°C for 15 s, 60°C for 1 min, and 68°C for 20 s. Gene expression levels were calculated by the iQ5 Optical System software (version 2; Bio-Rad) using β-actin as the reference gene.

Quantification of GH and IGF-1 GH and IGF-1 concentrations were quantified with commercial ELISA kits from Invitrogen (Camarillo, CA, USA) and Koma Biotech (Seoul, Korea), respectively. Experiments were performed according to the manufacturer's protocol. In brief, cells were incubated with tested samples for 24 h, and GH and IGF-1 concentrations were quantified in culture media from GH3 and HepG2 cells, respectively, using a microplate reader (Bio-Rad) at 450 nm.

Statistical analysis Data are presented as the mean±SEM. The student's t-test was used to compare means between two groups. A value of $p<0.05$ was considered statistically significant.

Results and Discussion

Cell viability and antioxidant activity of BRE Cell viability was assessed with the MTT assay in cells stimulated with BRE and WFE for 24 h in GH3 and HepG2 cells. The results showed that BRE and WFE did not significantly affect cell viability compared with the control (Fig. 1A and 1B), suggesting that BRE and WFE are not cytotoxic. The free-radical scavenging activity was used to measure the antioxidant activity using ascorbic acid (VitC) as the positive control. BRE showed a significant free-radical scavenging effect via the removal of 41.6% of free radicals (Fig. 1C). However, WFE showed significantly lower levels of antioxidant activities than BRE; it only removed 2.2% of free radicals found in the control (Fig. 1C). High levels of ROS, including hydrogen peroxide (H_2O_2), the superoxide ion (O_2), and the hydroxide radical (OH), damage cellular proteins, lipids and DNA. ROS are known to inhibit cellular growth, so reducing cellular oxidative stress could promote cellular proliferation and differentiation (10). Our results suggest that BRE is effective in the removal of free radicals such as ROS and may enhance cellular growth by removing cytosolic free radicals (8).

GH and IGF-1 expression in HepG2 and GH3 cells BRE induces the expression of GH and IGF-1 genes and reduces IGFBP-1 expression *in vitro* GH and IGF-1 are two key hormones that stimulate cellular and organismal growth (14). IGFBP-1 binds to IGF-1 to mask IGF-1 activity, so IGFBP-1 levels are inversely associated with IGF-1 activity (15). We next investigated the effects of BRE on the gene expression of GH,

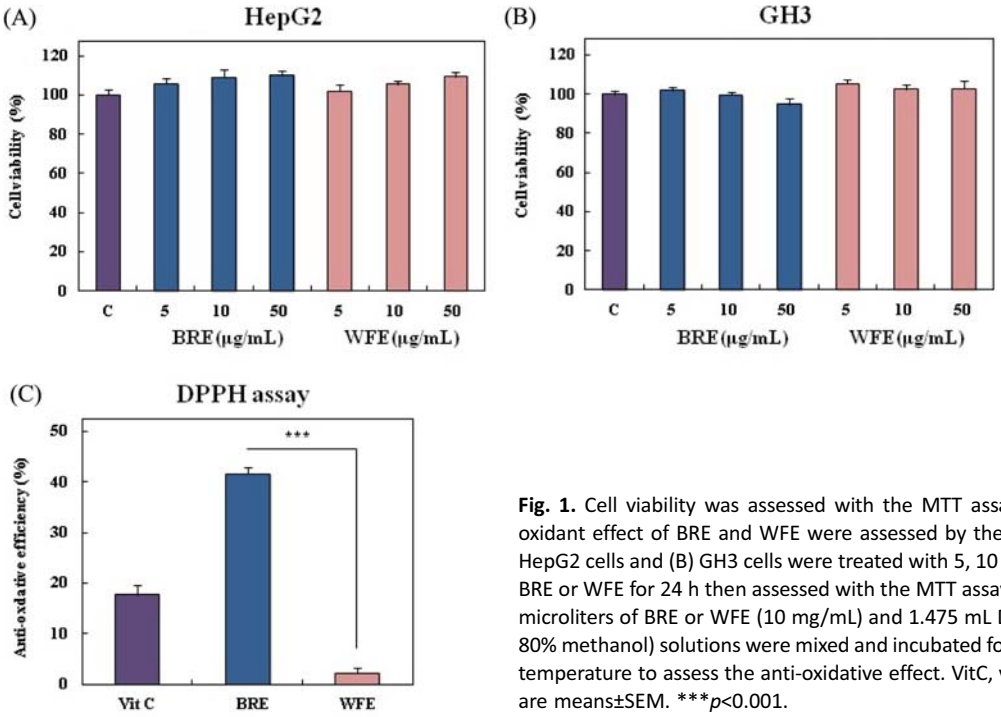

Fig. 1. Cell viability was assessed with the MTT assay, and the anti-oxidant effect of BRE and WFE were assessed by the DPPH assay. (A) HepG2 cells and (B) GH3 cells were treated with 5, 10 and 50 μg/mL of BRE or WFE for 24 h then assessed with the MTT assay. (C) Twenty-five microliters of BRE or WFE (10 mg/mL) and 1.475 mL DPPH (0.1 mM in 80% methanol) solutions were mixed and incubated for 30 min at room temperature to assess the anti-oxidative effect. VitC, vitamin C. Values are means±SEM. ***p<0.001.

IGF-1 and IGFBP-1 by qPCR analysis. GH expression was assessed in GH3 rat pituitary gland cells. The BRE group showed a significant increase of mRNA expression of GH in a concentration-dependent manner. GH mRNA was 85 and 215% at 10 and 50 μg/mL BRE, respectively, compared with controls. In contrast, WFE did not affect GH mRNA expression (Fig. 2A). Arginine (10 mM) was used as the positive control and augmented the GH mRNA level as much as 2.69-fold compared with the control (Fig. 2A). IGF-1 and IGFBP-1 gene expressions were also measured in the HepG2 human hepatoma cell line. IGF-1 mRNA levels increased significantly in cells stimulated with BRE by 156 and 194% at 10 and 50 mM, respectively (Fig. 2B). However, WFE did not affect IGF-1 expression (Fig. 2B). BRE (50 μg/mL) reduced IGFBP-1 mRNA expression by 77.8% compared with control HepG2 cells (Fig. 2C). WFE did not affect the expression of IGFBP-1, while arginine (10 mM), a positive control, reduced IGFBP-1 expression by 77.5% in HepG2 cells (Fig. 2C). These data suggest that BRE may promote cellular and organismal growth by inducing both GH and IGF-1 gene expression and reducing IGFBP-1 expression.

GH and IGF-1 secretion in HepG2 and GH3 cells We next quantified GH and IGF-1 secretion from cultured cells using ELISA. Treatment with arginine, a positive control, and BRE significantly increased GH concentrations in the culture media of GH3 cells. The GH concentration was increased by 25.6, 15.0, and 28.2% at 10 mM of arginine and 5 and 10 μg/mL BRE, respectively, compared with control GH3 cells (Fig. 3A). However, the GH concentration was not altered in cells treated with WFE (Fig. 3A).

The IGF-1 concentration was also measured in the culture media of HepG2 cells. The result showed that IGF-1 concentrations were increased in the BRE group. In cells treated with BRE, IGF-1 concentrations were significantly increased by 51.7% compared with the controls. WFE had no effect (Fig. 3B). These data confirmed that BRE induced GH and IGF-1 secretion in cultured cells, suggesting that BRE can provide a growth-promoting effect.

Cellular and organismal growth are primarily controlled by GH and IGF-1 through inducing cell proliferation, differentiation and development. Somatotropic cells within the lateral wings of the anterior pituitary gland synthesize, store and secrete GH. Circulating GH binds to the GH receptor in the liver and stimulates the synthesis and secretion of IGF-1. Secreted IGF-1 acts as a major mediator of GH to promote growth by acting on target organs through the blood stream. IGF-1 induces cellular growth via activating the phosphatidylinositol-3 kinase (PI3K) signaling pathway, which is upstream of the serine/threonine-specific protein kinase (Akt) and mammalian target of rapamycin (mTOR), which promote cell proliferation and differentiation (16).

We confirmed that BRE increased the gene expression of GH and IGF-1 in GH3 and HepG2 cells, respectively, whereas WFE did not affect the gene expressions of either GH or IGF-1. These data were consistent with the ELISA results, which confirm the induction of GH and IGF-1 secretion, indicating that BRE either directly or indirectly stimulated the gene and protein expression and subsequent secretion of GH and IGF-1. Meanwhile, IGFBP-1, a member of the IGFBP family, interrupts the release of IGF-1 from hepatocytes by direct binding (15). Thus, the reduction of IGFBP-1 increases hepatic IGF-1 secretion and its plasma concentration, which promotes the cellular growth of target tissues. Our study demonstrated that BRE not only induced IGF-1 but also reduced IGFBP-1. These results suggested that the

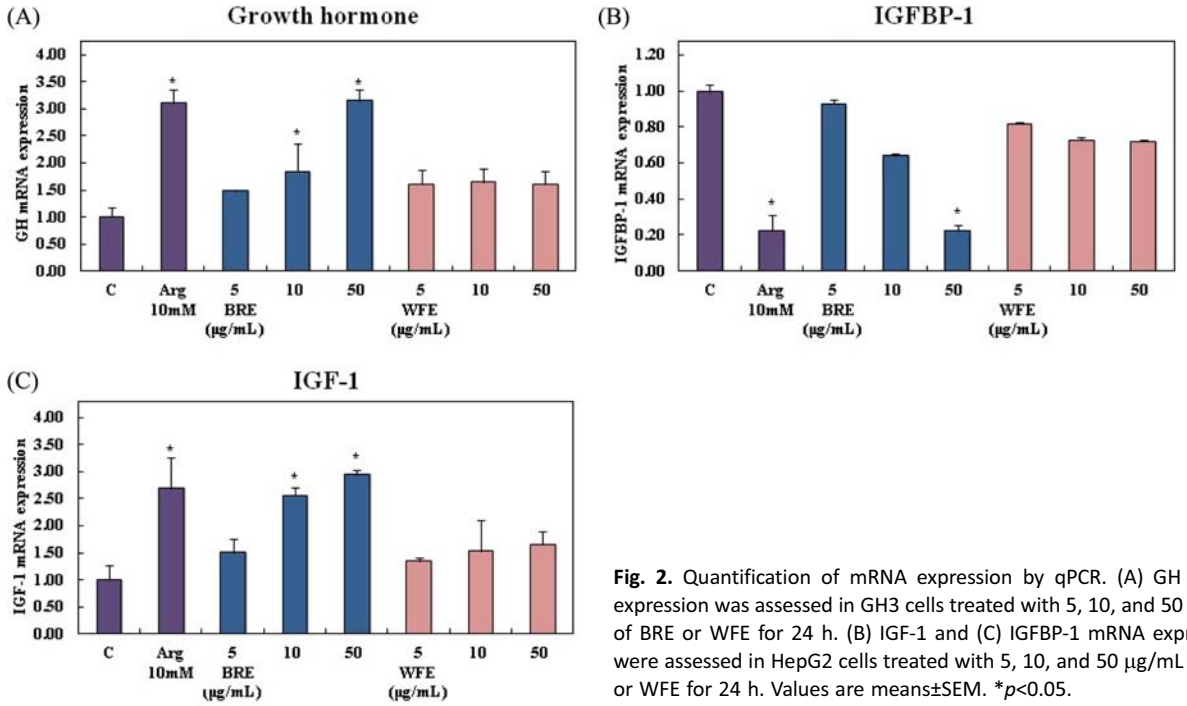

Fig. 2. Quantification of mRNA expression by qPCR. (A) GH mRNA expression was assessed in GH3 cells treated with 5, 10, and 50 μg/mL of BRE or WFE for 24 h. (B) IGF-1 and (C) IGFBP-1 mRNA expression were assessed in HepG2 cells treated with 5, 10, and 50 μg/mL of BRE or WFE for 24 h. Values are means±SEM. **p*<0.05.

increase of plasma IGF-1 concentration by BRE was achieved by a dual mechanism of induction of IGF-1 expression and reduction of IGFBP-1 expression.

Various benefits of anti-oxidant phytochemicals have been intensively investigated. Interestingly, oxidative stress is deleterious to cell growth, which includes proliferation and differentiation (10). Therefore, reduction of the cellular oxidative stress can activate the synthesis of growth factors and subsequent induction of growth. Anti-oxidant efficacy is associated with growth-promoting activities (8). The anti-oxidant effect of WFE was marginal in our result, potentially because the wheat lost active anti-oxidant substances during the wheat flour processing. Our data indicates that brown rice contains much stronger antioxidants than wheat flour.

Brown rice is a less-refined rice than white rice. It is nutrient rich and has high contents of phytochemical, polyphenols, folic acid, and minerals such as zinc (9). The biological activity of brown rice has been intensively studied, including its preventive effects on cancer and type 2 diabetes (17,18). It is also known that brown rice contains higher concentrations of phenolic compounds than white rice and thus is more health promoting.

In the present study, BRE significantly induced growth hormone expression and secretion from cultured GH3 and HepG2 cells compared with those of WFE. Grains lose their beneficial contents during refining. The nutritional excellence of whole grains is well known (19,20). The optimum proportion of beneficial ingredients in natural brown rice may also trigger growth stimulation. Actually, although the protein content of wheat is generally higher than rice, the rice had an amino acid score of 62 compared with the wheat flour score of 38, suggesting that the protein quality of rice is better

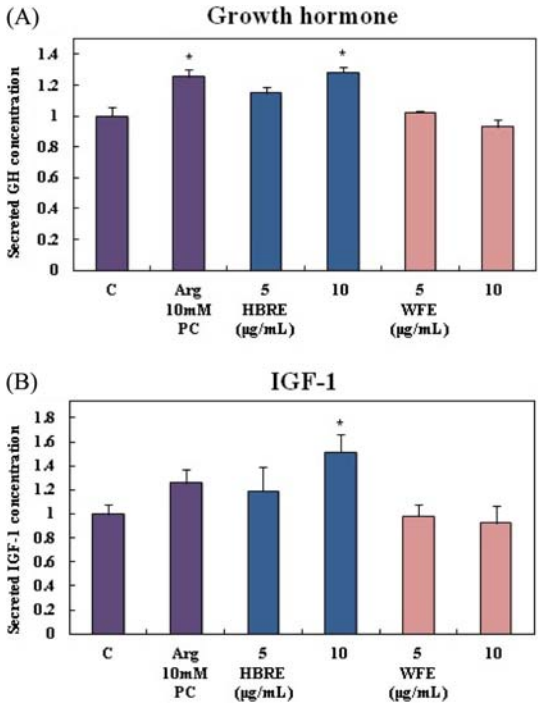

Fig. 3. Quantification of GH and IGF-1. (A) GH and (B) IGF-1 were measured by ELISA from media cultured with GH3 cells and HepG2 cells, respectively, treated with 5 and 10 μg/mL of BRE or WFE for 24 h. Values are means±SEM. **p*<0.05.

than the protein quality in other grains (12,13), which may, at least in part, explain the growth-promoting activity of BRE in our study. Further study will determine the active compound(s) of BRE that affect cellular growth.

In conclusion, we demonstrated that BRE, a newly developed rice variety, has better growth-promoting effects than WFE through analyzing the expression of GH, IGF-1, and IGFBP-1, which are major proteins involved in growth promotion. The qPCR results showed that BRE induced mRNA levels of both GH and IGF-1 and reduced IGFBP-1 mRNA expression in treated cells. In contrast, WFE-treated cells exhibited no difference compared with the control. This tendency was consistent with the result of the ELISA protein determination. The anti-oxidant effect of BRE was also superior to that of WFE. These data lead to the conclusion that BRE intake will provide greater cellular and animal growth-promoting effects compared with WFE.

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Disclosure The authors declare no conflict of interest.

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