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Enhancing cell proliferation and protection hydrogen peroxide-induced cytotoxicity in PC12 cells by rice extracts

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Abstract

Rice is well-known as source of vitamin E, beta-glucan and gamma-oryzanol which may be useful for the treatment of Alzheimer's disease. Three varieties of rice, white rice: white-hom-ma-li (MLW) and glong-hom-ma-li(MLG); red rice, Sung-yod (SY) were extracted by various methods to obtain 14 fractions. Each fraction was studied for cytotoxicity and protection neuronal cell from hydrogen peroxide-induced toxicity. By MTT reduction assay, all fractions at dose of 100 µg/ml had no cytotoxic effect on PC12 cell culture. Moreover, proliferative effect were detected only at dose of 50 µg/ml in lyophilized rinse-water from SY (SYGW), MLG (MLGW), MLW(MLWW), ethanolic extract from ML bran (MLBMEt) as 119.33 , 119.69, 120.14, 112.41 respectively by 4 independence experiments. At dose of 50 and 100 µg/ml, each fraction of MLWW, MLGW, ethanolic extract of ML bran , MLG bran and SY bran (MLBMEt, MLGMEt SYBMEt), supercritical extract from ML bran (MLBSUP), and cold-press of SY bran (SYBEX) could protect PC12 cells from oxidative stress induced by hydrogen peroxide with 83-92% effectiveness against that of vitamin E. These results concluded that various fractions from rice: rinsed water, ethanolic extract, supercritical extract and cold-press extract had protective effect on neuronal cell PC12 from oxidative stress induce by hydrogen peroxide. This neuroprotective effect may be due to the combination of more than one active principle in rice that could be potential candidates for use as nutraceutical for neurodegenerative diseases.

Keywords: neuronal cell, PC12, rice extracts, antioxidant

Introduction

Oxidative neuronal cell damage has been implicated in neurodegenerative disorders such as Alzheimer's disease (AD) (1). Many studies indicate that the brain of an AD patient is subjected to increased oxidative stress resulting from free radical damage (2). These oxidative stress-induced damages disrupt cellular function and membrane integrity, thereby leading to apoptosis (3).

Natural antioxidants have been reported to play a major role in blocking oxidative stress induced by free radicals. Rice is well-known as source of beta-glucan, gamma-oryzanol and vitamin E, especially, wheat germ and rice bran are major sources of these antioxidants (4,5). In particular, strong reduction in oxidative stress within neurone cells was one of the major role of vitamin E in brain function and in the prevention of neurodegeneration (6). A new potential antioxidant agent from rice bran, water-soluble oryzanol enzymatic extract derived from rice bran, was proved to prevent brain protein damage due to lipid peroxidation (7). In addition, there was hydrophilic antioxidants in purple rice bran which was much greater antioxidative effect than that of its lipophilic antioxidants and anthocyanins and gamma-tocols.(8).

The present study is to investigate cytotoxic and protective actions of various fractions of rice extract against hydrogen peroxide-induced rat pheochromocytoma line PC12 injury. Cell viability was measured with blue formazan that had been metabolized from colorless [3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl-tetrazolium bromide] MTT by mitochondrial dehydrogenases which are only active in live cells. This assay provides a sensitive measurement of the normal metabolic status of cells which reflects early cellular redox changes (9).

Methods

Tested materials. Three varieties of rice, white rice, White-Hom-ma-li (MLW) and Glong-hom-ma-li(MLG); red rice, Sung-yod, (SY) were extracted by various methods to obtain 14 fractions. The water soluble fractions were 3 fractions from rinsed water of 3 kinds of rice: MLWW, MLGW, SYGW and 2 fractions from freeze dried of boiled rice bran: MLBBOIL, SYBBOIL. The water non-soluble fractions obtained from maceration of rice in ethanol: MLWMEt, MLGMEt, SYGMEt and maceration of rice bran : MLBMEt, SYBMEt. Also, cold press method and supercritical extraction were used for extracting bran of ML and SY to obtain MLBEX, SYBMEX, MLBSUP and SYBSUP respectively.

Cell culture Transplantable rat pheochromocytoma, PC12 cells were purchased from American Type Culture Collection (ATCC) and grown on collagen-coated tissue plates in Dulbecco's modified minimal essential media(DMEM, high glucose formula with L-glutamate and pyruvate) supplemented with 5% (v/v) fetal bovine serum ,10% horse serum, 1% penicillin (100 U/ml) / streptomycin (100 µg/ml) under 5% CO_2 in air at 37 ºC and cells were subcultured twice a week. When the cells reached 70% confluence growth, undifferentiated and viable cells were cultured at a density of 5 $x10^4$ cells in 96-well coated plates. Cells were used for experiments 48 h. after seeding.

Intervention for cytotoxicity. Each of rice extracts at concentrations of 50, 100 µg/ml/ well was added, and incubated for 48 h.

Intervention for antioxidative stress study. After treated cells with rice extract for 24 h, hydrogen peroxide 500 µM was added into each well. New media was changed after 2 h of incubation. Vitamin E 10 mM was employed as positive control agent(9).

Cytotoxicity test. The viability measurement was assessed using MTT [3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl-tetrazolium bromide] reduction assay. After exposing the cells to an intervention, MTT (5mg/ml) was added to each culture well. Then incubation at 37 ºC for an additional 2 h, the formazan crystals were dissolved by additional 100 µl of dimethyl sulfoxide (DMSO), and the plate were shaken vigorously to ensure complete solubilization. Formazan absorbance was assessed at 570 nm by a microplate reader (biotek, power wave XS). Values are expressed as percentage of viable cells. Four independence experiments were done for each of rice extract.

Statitic analysis. Statistical analysis was performed by one way analysis of variance, ANOVA, followed by Duncan post hoc comparison. The homogenous groups with vitamin E $p < 0.05$ were demonstrated. All data are presented as mean and standard error of mean.

Results

Cytotoxicity At dose of 100 µg/ml all of 14 rice extracts had no cytotoxic effect on PC12 cell culture growing in proliferative media. Moreover, proliferative effect were detected only at dose of 50 µg/ml in lyophilized rinse-water from SY (SYGW), MLG (MLGW), MLW(MLWW), ethanolic extract from ML bran (MLBMEt) as 119.33 , 119.69, 120.14, 112.41 respectively by 4 independence experiments.(Fig.1) At dose of 100 µg/ml of these 4 mentioned groups had no significant difference in proliferation when cultured in proliferative media, but slightly showed neurite outgrowth when cultured in

differentiative media (DMEM with % 1 FBS % 5 HS; data non showed). All other water-non-soluble fractions had no effect on cell proliferation.

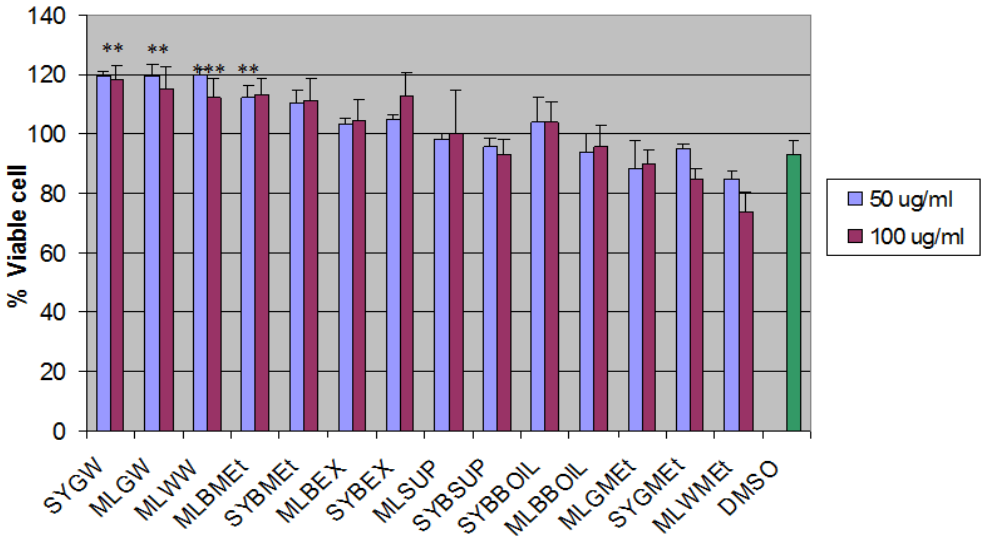

Figure 1. Percentage of PC12 cell growth (mean ± SEM) by MTT assay after treated with rice extracts exposure time 48 hours by independent experiment (N=4). SYGW, MLGW, MLWW, and MLBMEt could proliferate PC12 growth significantly. (***p< 0.001,**p< 0.01) when compared to 2% DMSO(control).

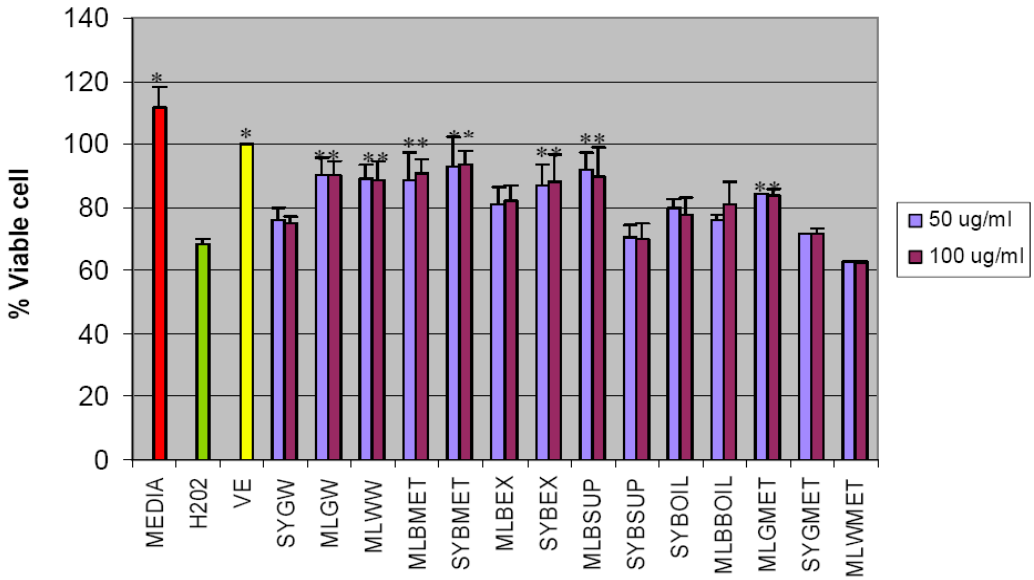

Figure 2. Percentage of viable cells in relative to 100% of vitamin E by MTT assay after treated PC12 cells with each of rice extract for 24 h followed by addition of vitamin E 10 mM for 2 h. The homogenous groups of experiment compared with vitamin E were displayed significantly (* p< 0.05).

Cell protective effect. After exposure of PC12 cells to hydrogen peroxide(H_2O_2) 500 µM for 2 h , survival cells marked decreased from 115 (media or untreated H_2O_2 group) to 68% whereas the group given vitamin E 10 nM 24 h prior to treated with H_2O_2 maintained the number of viable cells as same as the media (untreated H_2O_2) group. At dose of 50 and 100 µg/ml given, rinsed water fraction of ML and MLG (MLWW , MLGW), ethanolic extract of ML, MLGand SY bran (MLBMEt, MLGMEt, SYBMEt), supercritical extract from ML bran (MLBSUP), and cold-press of SY bran (SYBEX) could rescue cells from

oxidative stress induced by hydrogen peroxide. The number of viable cells in these mentioned groups were homogenously in range of 83 to 92 % of vitamin E ($p< 0.05$)

Discussion

After exposure each of 14 fractions of rice extract at dose of 100 µg/ml to PC12 cells in complete media, no cytotoxic effect were found. At dose of 50 µg/ml, cell proliferation was founded in 3 water-soluble fractions from SY, MLG, MLW and ethanolic extract from ML bran which may be due to hydrophilic active principle in rice that could be rinsed off. When cultured PC12 with each of these fractions in differntiative media (only supplemented with % 1 FBS, % 5 HS, and nerve growth factor 2 ng/ml), cells did not proliferate but produced slightly neurite outgrowth, belbing cell and lysis cells (data not shown). These results demonstrated that rice extracts had no cytotoxic on neuronal cell in normal condition.

The 7 fractions of rice extract that provided significant protection to the PC12 cell from H_2O_2–induced injury were 2 water-soluble fractions: MLWW, MLGW and 5 water-non- soluble fractions :MLBMEt, MLGMEt SYBMEt, MLBSUP, and SYBEX. The water-soluble fractions might composed of beta-glucan or hydrophilic antioxidant (8) whereas the water non-soluble fractions might composed of oryzanols and vitamin E in different ratio.

Conclusion

The present findings indicated that 7 rice extract fractions exert neuroprotective effects against H_2O_2 toxicity, which might be potential candidates for use as nutraceutical for neurodegenerative diseases.

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