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Research notes

Ginsenoside Rg5 prevents apoptosis by modulating heme-oxygenase-1/nuclear factor E2-related factor 2 signaling and alters the expression of cognitive impairment-associated genes in thermal stress-exposed HT22 cells

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ABSTRACT

Our results suggested that thermal stress can lead to activation of hippocampal cell damage and reduction of memory-associated molecules in HT22 cells. These findings also provide a part of molecular rationale for the role of ginsenoside Rg5 as a potent cognitive impairment preventive compound in blocking the initiation of hippocampal damage.

Thermal stress causes a number of physiological problems including heat stroke, heat cramps, and cognitive/memory impairment [1,2]. Those clinical symptoms might be the results of hippocampal formation changes due to the hippocampus neuronal cell damage [3,4]. When cell damage is caused by exogenous factors, cell cycle checkpoint genes are activated and delay the progression of cell cycles in order to allow the potential for damaged DNA repair [5]. Among the pathways involved in DNA repair process, p21 is a key gene that binds to cyclin–cyclin dependent kinase complex to induce cell cycle arrest at the transition from G0/G0 to S phase [6]. Failure to repair DNA damage occurs to trigger programmed cell death through the mitochondrial alterations and subsequently activate a caspase-poly (ADP-ribose) polymerases (PARP) cascade that induces a mitochondria-dependent apoptosis [7,8].

Thermal stress is a factor responsible for generating reactive oxygen species (ROS) production in mammals [9,10]. It is well known that ROS production is an important mechanism by which mitochondria are considered to undergo apoptosis [8]. Previous studies reported that heme oxygenase-1 (HO-1), Nrf2, and antioxidant enzymes such as SOD1, SOD2, and glutathione reductase (GR) are key factors in redox homeostasis [11–13]. In addition, a number of studies indicated that ROS is associated cognitive/memory impairment in the brain hippocampus region [14]. There are several molecular signaling pathways regulating this process including mitogen-activated protein kinase (MAPK) and cAMP-response element-binding protein (CREB) [15]. Notably, loss of CREB leads to alteration of the expression of brain-derived neurotrophic factor (BDNF) resulting in a decrease in the formation of long term memory [16,17]. Therefore, the prevention of hippocampal neuronal cell damage through the modulation of p21, PARP, CREB, and BDNF can be a solution to decrease the incidence of heat stroke, heat cramps, and cognitive impairment.

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Observation has suggested that ginsenoside Rg1 (Rg1) prevents β–amyloid peptide-induced apoptosis in primary cultured hippocampal neuron cells [18]. In addition, Rg1, Rh2, Rb, Re, and Rd attenuate exogenous factors-mediated oxidative stress in neuronal cells [19–21]. In particular, Rg5-enriched red ginseng extract has beneficial effects of memory enhancing *in vivo* [22], indicating Rg5 might be a phytochemical as therapeutic ingredient for environmental thermal stress-induced abnormalities in hippocampal neuronal cells. However, the detailed mechanisms by Rg5 contributing to prevent environmental thermal stress in hippocampal neuronal cells are still unclear. The aim of this study was to investigate whether Rg5 could suppress hippocampal neuronal cell line HT22 from thermal stress.

To determine the proper temperature which causes thermal stress in HT22 cells, we conducted the cell viability assay. As shown in Fig. 1A, HT22 cells were incubated at 37°C, 40°C, and 43°C CO_2 incubator for 24 h. We found that 40°C and 43°C decrease the cell viability in HT22 cells. We also performed cell viability assay to select the concentration of Rg5. As shown in Fig. 1B, 0 μg/mL, 20 μg/mL, and 40 μg/mL of Rg5 were nontoxic to cells. Thus, the temperature 43°C was chosen to cause thermal stress in HT22 cells and the concentration of 0 μg/mL, 20 μg/mL, and 40 μg/mL Rg5 were selected for further experiments.

To determine whether Rg5 prevents thermal stress-mediated cell cycle alteration, the expression of p21 were evaluated by western blot analysis. As shown in Fig. 2, our results showed that thermal stress markedly increased the expression of p21 in a time dependent manner, whereas Rg5 suppressed the expression of p21 in a time- and concentration-dependent manner. These data indicated that thermal stress may cause the p21-mediated cell cycle arrest in HT22 cells. Moreover, Rg5 efficiently decreased thermal stress-induced cell cycle arrest at G1/S phase of HT22 cells.

It has been reported that p21-mediated cell cycle arrest promote the progression of apoptosis [23]. We next investigated cleavage of PARP, which is the end point marker of apoptosis in the brain [24], in HT22 cells. After thermal stress, a gradual increase in cleavage of PARP were observed in a time-dependent manner as shown in Fig. 2. Interestingly, we sought that Rg5 suppressed the amount of PARP cleavage in thermal stress-exposed HT22 cells. These results provided a part of evidence that Rg5 may suppress the hippocampal neuron cell apoptosis through the regulation of p21 protein in HT22 cells.

To determine whether Rg5 altered the oxidative stress through the regulation of HO-1 and its downstream target Nrf2, we examined the HO-1 and Nrf2 protein by western blot analysis. As shown in Fig. 3A. the expression of HO-1 and Nrf2 were increased by

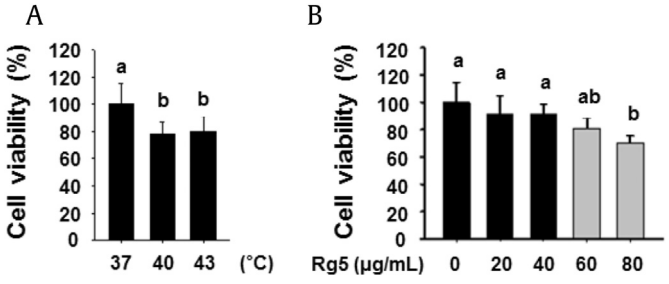

Fig. 1. Evaluation of thermal stress and Rg5 on cell viability in HT22 cells. (A) HT22 cells were treated with 37°C, 40°C, and 43°C in serum free media for 6 h ($n = 6$). The cell viability was measured after 24 h by using the MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) assay. (B) HT22 cells were treated with 0 μg/mL, 20 μg/mL, 40 μg/mL, 60 μg/mL, and 80 μg/mL of Rg5 in serum free media for 24 h ($n = 6$). The cell viability was measured after 24 h by using the MTT assay. Values with different letters are significantly different, $p < 0.05$. Rg5, ginsenoside Rg5.

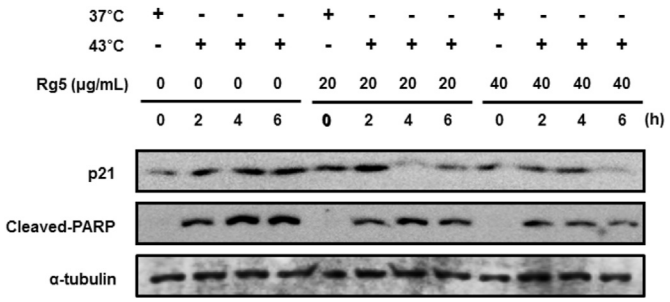

Fig. 2. Rg5 decrease the p21 and cleaved-PARP expression in thermal stress-exposed HT22 cells. HT22 cells were treated with 0 μg/mL, 20 μg/mL, and 40 μg/mL of Rg5 for 4 hand then exposed with thermal stress for 6 h in the absence or presence of Rg5. Western blot was performed using antibodies against p21 and PARP. The α-tubulin was used as the internal control. PARP, poly (ADP-ribose) polymerases; Rg5, ginsenoside Rg5.

thermal stress at 2 h and subsequently initiated the protein degradation of HO-1 and Nrf2 at 4 h in HT22 cells, whereas Rg5 effectively repressed the expression of HO-1 and Nrf2 in thermal stress-exposed HT22 cells.

Previous reports suggested that HO-1/Nrf2 regulates glutathione homeostasis by catalyzing the reduction of glutathione disulfide to glutathione through GR induction during oxidative stress [25,26]. Western blot analysis revealed that the expression of GR were increased at 2 h and remained until 6 h, whereas Rg5 strongly suppressed the expression of GR in thermal stress-exposed HT22 cells. By contrast, the reduction in the phosphorylation of CREB induced by thermal stress was reversed by Rg5 in HT22 cells. Consistent with these results, Rg5 significantly inhibited the production of nitric oxide (NO) as shown in Fig. 3B. In hippocampal neuronal cells, the phosphorylation of CREB leads to activate the expression of BDNF, which is associated with memory and cognition [27], indicating Rg5 may ameliorate the oxidative stress and cognitive-associated function in thermal stress-exposed HT22 cells.

To further investigate whether Rg5 ameliorated the expression of BDNF in thermal stress-exposed HT22 cells, BDNF and its downstream targets were examined by western blot analysis. As shown in Fig. 4A, thermal stress dramatically repressed the expression of BDNF, whereas Rg5 suppressed thermal stress-caused the BDNF alteration in HT22 cells. As expected, thermal stress markedly inhibited the phosphorylation of GSK3β, is BDNF downstream target, whereas Rg5 increased the phosphorylation of GSK3β in thermal stress-exposed HT22 cells. In particular, we observed that β-catenin, negative regulator of GSK3β, was strongly reduced by Rg5 in thermal stress-exposed HT22 cells. Our results indicated that Rg5 ameliorated thermal stress-mediated abnormal alteration of BDNF, β-catenin, and GSK3β in HT22 cells and may prevent thermal stress-induced neuronal damage. Inhibition of acetylcholinesterase (AchE) activity is being examined to determine the effect of bioactive compounds and drugs on the cognitive impairment [28,29]. Our results revealed that the activity of AchE were increased in thermal stress-exposed HT22 cells compared with HT22 cells without thermal stress. A previous study suggested that HT22 cells can release acetylcholine in a certain condition [30]. We speculated thermal stress may promote to release acetylcholine in HT22 cells. In addition, Rg5 strongly suppressed thermal stress-induced AchE activity in HT22 cells (Fig. 4B).

In summary, the aim of this study was to investigate the potential effect of Rg5 in thermal stress-exposed HT22 cells. Here, we demonstrated that thermal stress caused cell cycle arrest due to the activation of p21 and PARP cleavage in HT22 cells, whereas Rg5 retained the p21 expression as well as suppressed the PARP cleavage.

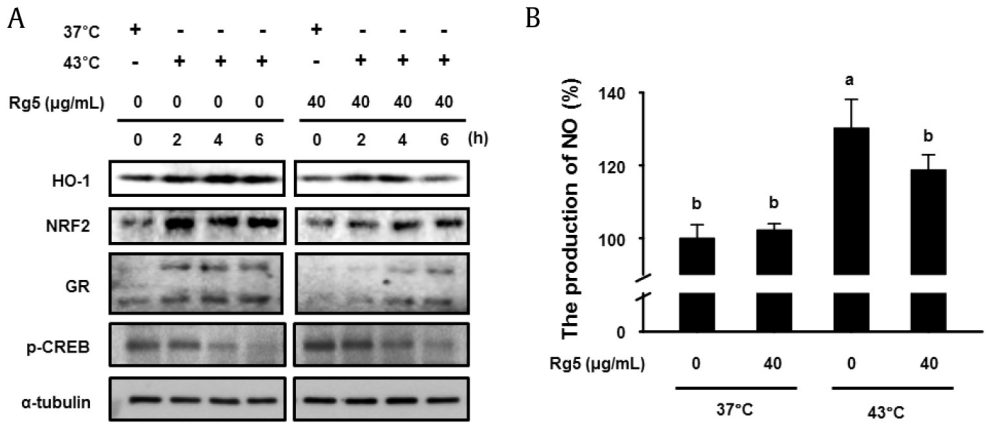

Fig. 3. Rg5 suppress the oxidative stress-associated proteins and the production of nitric oxide in thermal stress-exposed HT22 cells. (A) HT22 cells were treated with indicated concentrations of Rg5 for 4 h and then exposed with thermal stress for 6 h in the absence or presence of Rg5. Western blot was performed for HO-1, Nrf2, GR, p-CREB, and α-tubulin. (B) The production of NO in thermal stress-exposed HT22 cells with the absence or presence of Rg5 by using Griess reagent at 550 nm. Values with different letters are significantly different, $p < 0.05$. CREB, cAMP-response element-binding protein; GR, gluthathione reductase; HO-1, heme oxygenase-1; NO, nitric oxide; Rg5, ginsenoside Rg5.

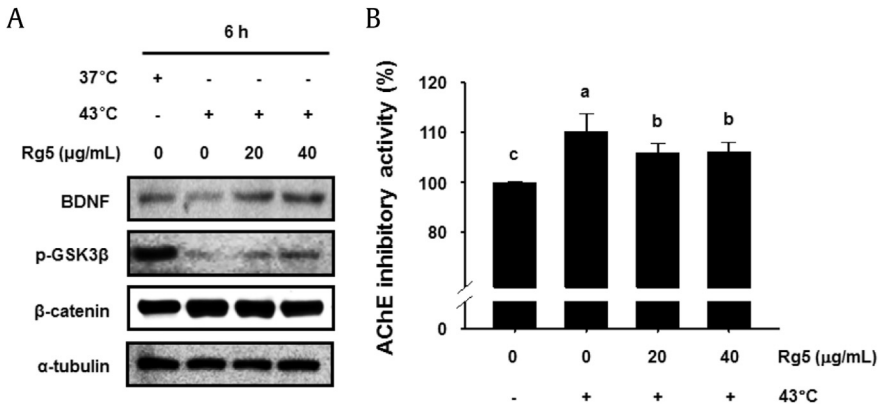

Fig. 4. Rg5 increase the expression of BDNF, GSK3β proteins, whereas decrease the expression of β-catenin in thermal stress-exposed HT22 cells. (A) HT22 cells were treated with 0 μg/mL, 20 μg/mL, and 40 μg/mL of Rg5 for 4 h and then treated with thermal stress for 6 h. Western blot was performed for BDNF, p-GSK3β, and β-catenin. The α-tubulin was used as the internal control. (B) HT22 cells were treated with 0 μg/mL and 40 μg/mL of Rg5 for 4 h and then treated with thermal stress for 6 h. The supernatant of cell were used for the evaluation of AChE inhibitory activity. Values with different letters are significantly different, $p < 0.05$. AchE, acetylcholinesterase; BDNF, brain-derived neurotrophic factor; Rg5, ginsenoside Rg5.

Moreover, Rg5 sufficiently attenuated the production of NO contents, which is an oxidative stress indicator, through regulation of antioxidant enzymes such as HO-1/Nrf2 and GR in thermal stress-exposed HT22 cells. Notably, we observed cognitive impairment-associated protein CREB and BDNF were markedly inhibited in thermal stress-exposed HT22 cell. By contrast, Rg5 ameliorated thermal stress-induced CREB, BDNF, GSK3β, and β−catenin alteration in HT22 cells. Moreover, thermal stress-mediated induction of AchE activity were efficiently inhibited by Rg5 in HT22 cells. Taken together, these findings provide a part of molecular rationale for the role of Rg5 as a neuroprotective natural compound against thermal stress-induced apoptosis via the modulation of oxidative stress and thermal stress−mediated cognitive impairment.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgments

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