Chemical Papers (2019) 73:1135–1144
https://doi.org/10.1007/s11696-018-0664-7

ORIGINAL PAPER

Attenuation of hydrogen peroxide-induced oxidative stress in SH-SY5Y cells by three flavonoids from *Acer okamotoanum*

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Received: 22 August 2018 / Accepted: 3 December 2018 / Published online: 10 December 2018
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Abstract

The flavonoids quercitrin, isoquercitrin, and afzelin were isolated from the ethyl acetate fraction of *Acer okamotoanum*. The effectiveness of these flavonoids in protecting human cells was investigated using SH-SY5Y neuronal cells. Oxidative stress was induced by hydrogen peroxide (H_2O_2) and cells treated with flavonoids had an increased viability as compared with untreated cells. The flavonoid-treated cells showed significantly less reactive oxygen species production and lactase dehydrogenase release than the untreated cells. Especially, quercitrin and isoquercitrin showed the strongest protective effects against oxidative stress among several tested flavonoids as determined by the results for cell viability, reactive oxygen species (ROS) production, and lactate dehydrogenase release. Furthermore, we measured the expression of inflammation- and apoptosis-related proteins. These flavonoids attenuated inflammation by downregulating the expression of cyclooxygenase-2, inducible nitric oxide synthase, and apoptotic signaling via inhibiting caspase activation. The present results suggest that three flavonoids of *A. okamotoanum* protect against cellular oxidative stress possibly through regulating inflammation, apoptosis, and ROS-scavenging.

Keywords *Acer okamotoanum* · Afzelin · Isoquercitrin · Oxidative stress · Quercitrin

Introduction

Oxidative stress due to the accumulation of reactive oxygen species (ROS) is a risk factor for the development of Alzheimer's disease and other neurodegenerative diseases (Facheris et al. 2004). ROS including free radicals [e.g., hydroxyl radicals (•OH) and superoxide radicals ($^{\bullet}O_2^{-}$)] as well as hydrogen peroxide (H_2O_2) are associated with neuronal cell death in various neurodegenerative diseases (Gella and Durany 2009; Kwon et al. 2015; Valko et al. 2007). The overproduction of ROS promotes lipid peroxidation, protein oxidation, and DNA damage, resulting in neuronal apoptosis in brain cells (Facheris et al. 2004; Gella and Durany 2009). In addition, over-accumulation of ROS can also trigger inflammation reactions as well as aggravate brain damage in patients with neurodegenerative disease (Gella and Durany 2009; Smith et al. 2000). H_2O_2 is the critical ROS for inflammation and apoptosis in neuronal cells and the major precursor of highly reactive free radicals (Rhee 1999). To protect against oxidative stress such as H_2O_2-induced neuronal damage, some studies have focused on bolstering cellular antioxidant defenses by supplying exogenous antioxidants. Antioxidant compounds derived from plant sources can protect the human body against oxidative stress and attenuate the progression of several neurodegenerative diseases (Hu et al. 2015; Nirmaladevi et al. 2014; Zhou et al. 2018). To identify natural substances that can have potential neuroprotective activities, several studies have reported the assessment of candidate compounds or extracts from various

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11696-018-0664-7) contains supplementary material, which is available to authorized users.

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plant species using cell lines induced to undergo oxidative stress in culture (Ju et al. 2012; Park et al. 2015).

Acer okamotoanum (AO) has been demonstrated to exert biological activities such as cognitive improvement, antioxidant, and anti-cancer effects (Choi et al. 2017; Jin et al. 2008; Takayama et al. 2013; Qadir et al. 2007). Several studies have reported that AO contains active compounds such as β-amyrin as well as cleomiscosin A and C (Jin et al. 2007; Kim et al. 1998). In addition, we previously isolated and identified flavonoids such as quercitrin (QU quercetin-3-rhamnoside), isoquercitrin (IQ quercetin-3-glucoside), and afzelin (AF kaempferol-3-rhamnoside) from the aerial parts of AO (Fig. 1) (Lee et al. 2018).

Furthermore, we previously demonstrated that QU, IQ, and AF had anti-diabetic effects through the inhibition of aldose reductase activity (Lee et al. 2018). These three flavonoids have been reported to exert anti-inflammatory, antioxidative, and anti-cancer effects (Ma et al. 2016; Li et al. 2016a; Vellosa et al. 2015; Yin et al. 2013; Zhu et al. 2015). However, the potential neuroprotective effects of three flavonoids (QU, IQ, and AF) against oxidative stress-induced inflammation and apoptosis in neuronal cells have not been previously studied.

Therefore, this study aimed to examine the potential protective effects of the flavonoids QU, IQ, and AF isolated from AO against H_2O_2-induced oxidative stress in human neuronal SH-SY5Y cells.

Materials and methods

Preparation of Plant Materials

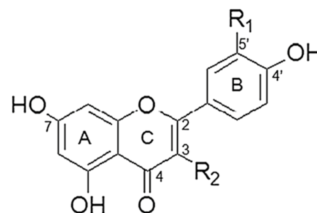
QU, IQ, and AF were previously isolated from the ethyl acetate fraction of AO (Lee et al. 2018).

Reagents

Dulbecco's modified eagle medium (DMEM), fetal bovine serum (FBS), penicillin–streptomycin, and trypsin-ethylenediaminetetraacetic acid (EDTA) solution were obtained from Welgene (Daegu, Korea). Dimethyl sulfoxide (DMSO) and 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) were purchased from Bio Pure (Ontario, Canada) and Bio Basic (Toronto, Canada), respectively. Dichlorofluorescein diacetate (DCF-DA) was purchased from Sigma–Aldrich (St. Louis, MO, USA), and H_2O_2 was purchased from Junsei (Tokyo, Japan). Radio-immuno-precipitation assay (RIPA) buffer was purchased from Elpics Biotech (Daejeon, Korea), protease inhibitor cocktail from Calbiochem (Cambridge, MA, USA), polyvinylidene fluoride (PVDF) membranes from Millipore (Bedford, MA, USA), and enhanced chemiluminescence (ECL) substrate solution from Bio-Rad Laboratories (Hercules, CA, USA). The primary antibodies cyclooxygenase-2 (COX-2, #sc-7951), inducible nitric oxide synthase (iNOS, #sc-8310), B cell lymphoma 2 associated X (Bax, #sc-493), and B cell lymphoma 2 (Bcl-2, #-sc-492) were purchased from Santa Cruz Biotechnology (Dallas, TX, USA). Poly-ADP ribose polymerase (PARP, #9532), β-actin (#8457), caspase-3 (#9662), caspase-9 (#9508), and secondary antibodies (#7074, #7076) were purchased from Cell Signaling Technology (Beverly, MA, USA).

Cell culture

The SH-SY5Y human neuronal cells were acquired from American Type Culture Collection (Manassas, VA, USA). Cells were maintained in a T-75 flask containing DMEM supplemented with FBS (10%) and penicillin (5%) at 37 °C in a humidified atmosphere of CO_2 (5%) in air. The cells



Compound	R_1	R_2
QU	OH	*O*-Rham
IQ	OH	*O*-Glc
AF	H	*O*-Rham

Fig. 1 The structures of flavonoids extracted from AO

were washed with phosphate-buffered saline (pH 7.4) and sub-cultured with 0.05% trypsin–EDTA.

Cell viability

The MTT colorimetric assay was used to determine cell viability (Mosmann 1983). Five milligrams per milliliter of MTT solution was added to each well of a 96-well plate, followed by incubation for 4 h at 37 °C. Next, the medium containing the MTT was removed. The formazan crystals incorporated into the cells were solubilized with DMSO (200 µL) for 30 min, and then the absorbance was read at 540 nm using a microplate reader (Thermo Fisher Scientific, Waltham, MA, USA).

Measurement of ROS production

Intracellular ROS production was determined using the DCF-DA fluorescence assay (Wang and Zhu 2003). DCF-DA solution (80 µM) was added to each well of a black 96-well plate, followed by incubation for 30 min. Next, the fluorescence emission from each well was read at an excitation of 480 nm and emission of 535 nm using a fluorescence spectrophotometer (BMG Labtech., Ortenberg, Germany).

Measurement of lactate dehydrogenase (LDH) release

Lactate dehydrogenase (LDH) release was determined using an LDH cytotoxicity detection kit (Clontech Laboratories, Mountain View, CA, USA) according to the manufacturer's protocol. The cell culture supernatant from each well was mixed with LDH solution, followed by incubation for 30 min at 25 °C. Next, the absorbance of each well was read at 540 nm using a microplate reader (Thermo Fisher Scientific).

Western blotting

The cells were lysed with RIPA buffer containing a protease inhibitor cocktail. Equal amounts of protein were loaded into each lane of the gel. After electrophoretic separation, the proteins were transferred to a PVDF membrane. The membranes were blocked with skim milk (5%) for 1 h at room temperature, and then probed with primary antibodies against iNOS, COX-2, caspase-3, PARP, Bax, caspase-9, and Bcl-2. Next, the membrane was washed with PBS containing Tween®-20 and then incubated with the corresponding secondary antibody for 1 h at room temperature. Finally, the immunoreactive blots were detected using ECL solution and visualized using a Davinch-Chemi™ chemiluminescence imaging system (Core Bio, Seoul, Korea).

Statistical analysis

Data are presented as mean ± standard deviation (SD). The data were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test. $p < 0.05$ was considered statistically significant.

Results

Protective effects of flavonoids from AO against H_2O_2-induced growth inhibition

The effect of three flavonoids from AO such as QU, IQ, and AF on the cell viability of SH-SY5Y neuronal cells under H_2O_2-induced oxidative stress was determined using the MTT assay (Fig. 2).

We initially examined the potential cytotoxic effects of the flavonoids using SH-SY5Y cells. Various concentrations (1–10 µg/mL) of the three flavonoids had no effect on the viability of SH-SY5Y cells (Supplemental data). Treatment with 300 µM H_2O_2 decreased the cell viability to 48.60 ± 0.93% relative to that of untreated cells. However, treatment with QU, IQ, and AF at 10 µg/mL increased the cell viability to 64.63 ± 1.19%, 69.86 ± 3.40%, and 61.48 ± 3.14%, respectively. In summary, H_2O_2-induced neuronal cell death was significantly attenuated by these three flavonoids.

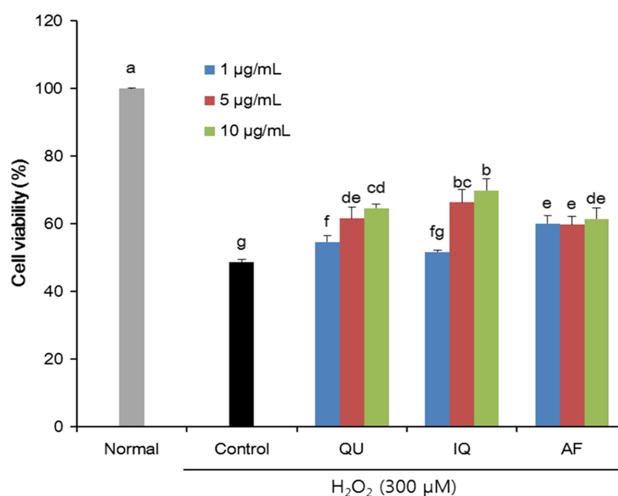


Fig. 2 Effects of flavonoids from AO on the cell viability of SH-SY5Y cells treated with H_2O_2. Cells were treated for 2 h with the three flavonoids from AO (QU, IQ, and AF) at 1, 5, and 10 µg/mL, followed by the addition of H_2O_2 for 24 h. Values are mean ± SD. [a–g]Means with different letters were significantly different ($p < 0.05$) by Duncan's multiple range test

Inhibitory effects of flavonoids from AO against H_2O_2-induced ROS formation

To investigate the protective effects of the three flavonoids from AO, their ROS scavenging activities were measured by the DCF-DA assay (Fig. 3).

Cells treated with 1 mM H_2O_2 exhibited a steady increase in ROS production as compared to untreated cells. However, treatment of the cells with QU, IQ, and AF significantly inhibited H_2O_2-induced ROS production. Furthermore, the IQ-treated cells showed an inhibition of ROS formation in a concentration-dependent manner. These results indicated that H_2O_2-induced ROS generation was attenuated by flavonoids from AO.

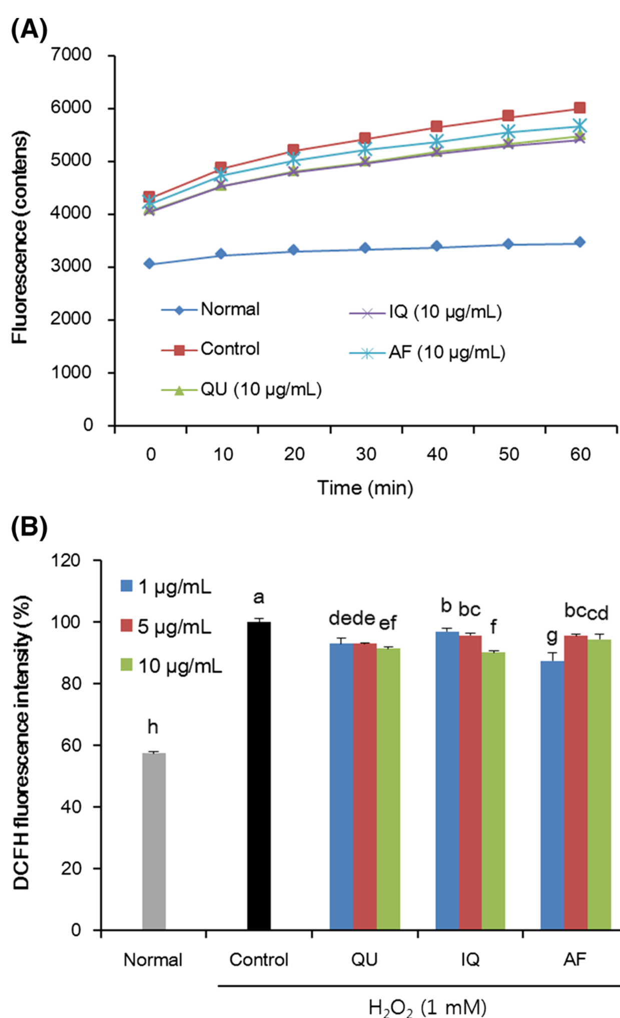


Fig. 3 Effects of flavonoids from AO on ROS generation in SH-SY5Y cells treated with H_2O_2. **a** Change of ROS fluorescence during 60 min of treatment. **b** The intensity of ROS fluorescence at 60 min. Cells were treated for 2 h with three flavonoids from AO (QU, IQ, and AF) at 1, 5, and 10 μg/mL, followed by the addition of H_2O_2 for 24 h. Values are mean ± SD. $^{a–h}$Means with different letters were significantly different ($p < 0.05$) by Duncan's multiple range test

Effect of flavonoids from AO against H_2O_2-induced LDH release

The exposure of SH-SY5Y cells to 300 μM H_2O_2 -elevated LDH release as compared with that in untreated cells (Fig. 4), while IQ treatment of the H_2O_2-induced cells suppressed the H_2O_2-induced LDH release.

Cells treated with IQ at 5 and 10 μg/mL showed a significant inhibition of LDH release by 85.47 ± 0.74% and 86.74 ± 4.73%, respectively, as compared to the H_2O_2-treated control group. These findings demonstrate that among these AO-derived flavonoids, IQ was particularly effective in protecting neuronal stress against H_2O_2-induced damage.

Protective effects of flavonoids from AO against H_2O_2-induced inflammation

To investigate the protective effects of flavonoids from AO against H_2O_2-induced oxidative stress in neuronal cells and asses the underlying mechanisms, the abundances of inflammation-related proteins were measured by western blotting. As shown in Fig. 5, cells treated with 300 μM H_2O_2 showed higher abundances of iNOS and COX-2 than untreated cells.

Furthermore, cells treated with 10 μg/mL QU or IQ showed significant reductions in the abundances of iNOS and COX-2, while the AF-treated group showed a significant downregulation of COX-2 only. These results showed that the three flavonoids from AO regulated inflammatory pathways in the H_2O_2-induced SH-SY5Y neuronal cells.

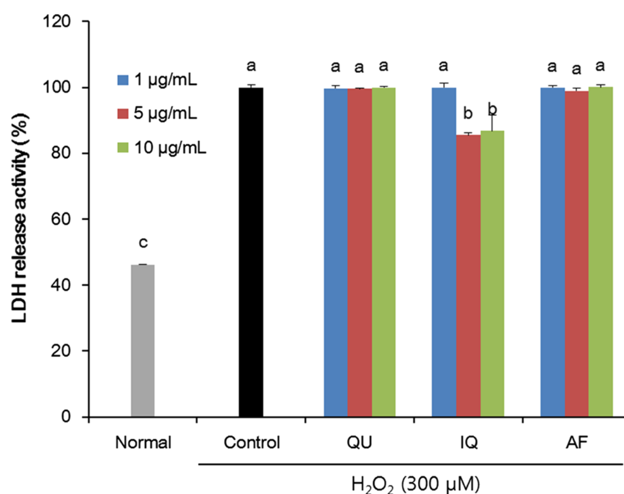


Fig. 4 Effects of flavonoids from AO on LDH release from SH-SY5Y cells treated with H_2O_2. Cells were treated for 2 h with three flavonoids from AO (QU, IQ, and AF) at 1, 5, and 10 μg/mL, followed by the addition of H_2O_2 for 24 h. Values are mean ± SD. $^{a–c}$Means with different letters were significantly different ($p < 0.05$) by Duncan's multiple range test

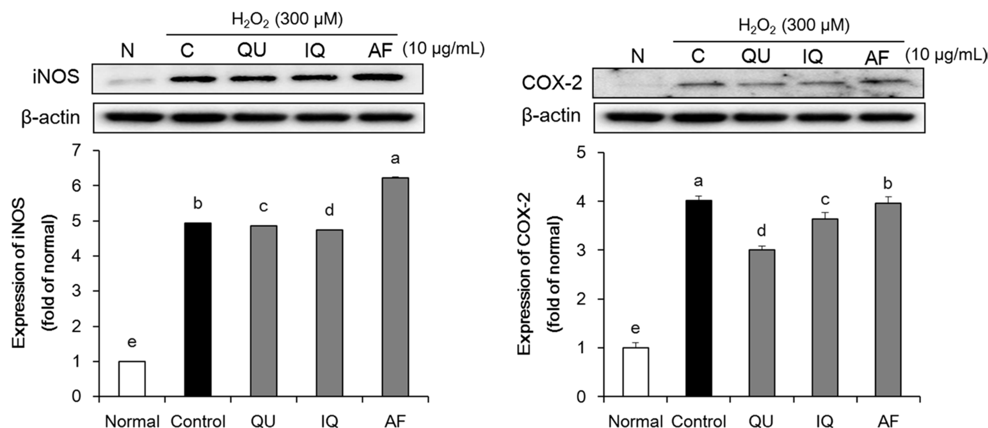


Fig. 5 Effects of flavonoids from AO on the abundances of iNOS and COX-2 in SH-SY5Y cells treated with H_2O_2. Cells were treated for 2 h with three flavonoids from AO (QU, IQ, and AF) at 10 µg/mL, followed by the addition of H_2O_2 for 24 h. β-Actin was used as loading control. Values are mean ± SD. [a–e]Means with different letters were significantly different ($p < 0.05$) by Duncan's multiple range test

Protective effects of flavonoids from AO against H_2O_2-induced neuronal apoptosis

We further investigated the effects of these three flavonoids on H_2O_2-induced apoptosis in SH-SY5Y neuronal cells. The results showed that cells treated with 300 µM H_2O_2 had significantly increased the abundances of cleaved caspase-9, caspase-3, and PARP as compared with untreated cells, indicating neuronal cell apoptosis via the activation of caspases (Fig. 6).

However, the groups treated with H_2O_2 followed by the three flavonoids showed significant reductions in the abundances of cleaved caspase-9, caspase-3, and PARP as compared with the cells treated with H_2O_2-only. Additionally, H_2O_2-treated control group showed an increase in the abundance of pro-apoptotic protein Bax/anti-apoptotic protein Bcl-2 (Fig. 7).

However, the flavonoid-treated group showed a down-regulation of Bax/Bcl-2 ratio. These results showed that the three flavonoids attenuated oxidative stress-induced neuronal cell dysfunction by regulating proteins involved in apoptosis.

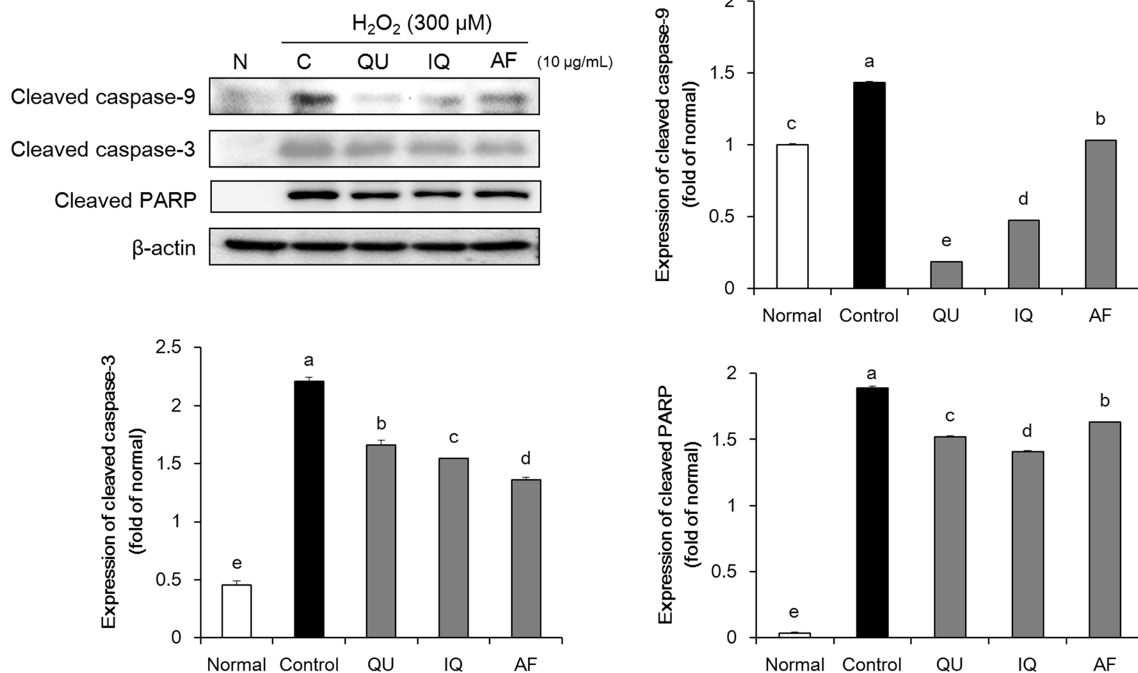


Fig. 6 Effects of flavonoids from AO on the abundances of cleaved caspase-9, caspase-3, and poly-ADP ribose polymerase in SH-SY5Y cells treated with H_2O_2. Cells were treated for 2 h with three flavonoids from AO (QU, IQ, and AF) at 10 µg/mL, followed by the addition of H_2O_2 for 24 h. β-Actin was used as loading control. Values are mean ± SD. [a–e]Means with different letters were significantly different ($p < 0.05$) by Duncan's multiple range test

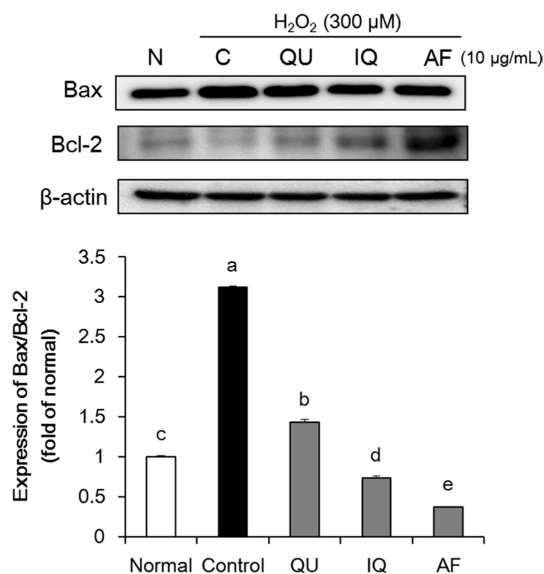


Fig. 7 Effects of flavonoids from AO on the abundances of Bax and Bcl-2 in SH-SY5Y cells treated with H_2O_2. Cells were treated for 2 h three flavonoids from AO (QU, IQ, and AF) at 10 μg/mL, followed by the addition of H_2O_2 for 24 h. β-Actin was used as loading control. Values are mean ± SD. [a–e]Means with different letters were significantly different ($p < 0.05$) by Duncan's multiple range test

Discussion

The brain contains high contents of polyunsaturated fatty acids as well as redox-active iron and copper; therefore, brain tissue is more susceptible to oxidative stress than other organs (Garbarino et al. 2015). Recent reports have focused on the identification of potential compounds that protect central nervous tissue against oxidative stress (Facheris et al. 2004; Smith et al. 2000). To study the potential neuroprotective activities of candidate compounds, the SH-SY5Y neuronal cell line has been widely used (Agholme et al. 2010; Park et al. 2015). For the induction of neuronal damage, H_2O_2 has been used to trigger inflammation and cell apoptosis (Rhee 1999).

Flavonoids, such as quercetin and kaempferol, are natural phenolic compounds, and their glycosides are present in a wide range of plants. QU and IQ are quercetin derivatives containing rhamnoside and glucoside, respectively, while AF is a kaempferol derivative containing rhamnoside (Lee et al. 2014; Li et al. 2016b). Three flavonoids (QU, IQ, and AF) are chemically but different from sugar binding and number of −OH group. Three flavonoids have a double bond between C-2 and C-3 position and a ketone group at C-4 on the C ring in the backbone structure. In addition, they have link hydroxyl group (−OH) at C-7 on the A ring and C-4′ position on the B ring. QU and IQ bind −OH group at C-5′ on the B ring, but AF binds − H at same position. Therefore, QU and IQ have a catechol moiety (4′,5′-di-OHs on B ring); while AF has no catechol moiety. In addition, quercetin and kaempferol have −OH group at C-3 on the C ring, it commonly glycosylated. QU and AF glycosylated rhamnoside, and IQ glycosylated glucoside at C-3 on the C ring (Panche et al. 2016). Previous study reported catechol moiety (4′,5′-di-OHs on B ring) in the flavonoids molecule is higher anti-oxidant ability by attributing to the stability of its oxidized product form (Li et al. 2014). Several studies reported that flavonoids linked by −OH at C-7 and C-4′ showed higher anti-oxidant activity on the renal cellular membrane by radical scavenging activity and inhibition of lipid peroxidation than a lack of −OH at C-7 and C-4′ (Yokozawa et al. 1999; Zhang et al. 2014). In addition, three flavonoids constituted sugar such as glucose or rhamnose attached at C-3 (R2) position. Previous study investigated that sugar linked at C-3 in the structural backbone would higher anti-oxidation capability among other flavonoids (Yokozawa et al. 1999). More phenolic hydroxyl groups linked to the flavonoid structure backbone possess high anti-oxidant activity (Zhang et al. 2014). The present study indicated that the catechol moiety in B ring played the anti-oxidative role from oxidative stress. In addition, three flavonoids bind different sugar. QU and AF are linked with rhamnoside, but IQ is linked with glucoside at C-3 on the C ring. It indicated that the biological activity of flavonoids depends on the number of −OH, binding position of −OH and presence of sugar.

These flavonoid glycosides can exert several biological activities. QU reportedly has anti-diabetic (Babujanarthanam et al. 2011), anti-inflammatory (Ma et al., 2016), and anti-oxidant (Yin et al. 2013) effects.; IQ has shown hepatoprotective (Xie et al. 2016), anti-oxidant (Li et al. 2016b), and anti-inflammatory (Li et al. 2016a) effects; and AF has been reported to exert anti-oxidant (Vellosa et al. 2015), anti-cancer (Zhu et al. 2015), and anti-bacterial (Lee et al. 2014) effects. However, the neuroprotective activities of flavonoids isolated from AO in H_2O_2-induced SH-SY5Y neuronal cells had not previously been investigated. In this study, we tested the three flavonoids (QU, IQ, and AF) and found that these compounds protected SH-SY5Y neuronal cells against H_2O_2-induced oxidative stress by regulating inflammation and apoptosis.

In our results, SH-SY5Y cells treated with H_2O_2 showed a decreased cell viability and increased ROS generation, indicating that oxidative stress was induced by H_2O_2 in these cells. However, treatment with the flavonoids from AO significantly increased cell viability and attenuated ROS production in SH-SY5Y cells exposed to H_2O_2, demonstrating that the flavonoids exerted protective effects against H_2O_2-induced oxidative stress in these neuronal cells. Previous study demonstrated that presence of catechol moiety in the flavonoids higher ROS scavenging activity (Li et al. 2014). In our results (Figs. 2, 3), QU and IQ showed higher

protective effect from oxidative stress by increasing cell viability and decreasing ROS production than AF, indicating the role of catechol moiety on anti-oxidative activity. In addition, QU inhibited the production of ROS (such as H_2O_2 and O_2^-) in ultraviolet B radiation-exposed epidermal cells (Yin et al. 2013), while IQ reduced intracellular lipid and protein oxidation via its ROS scavenging effects in yeast cells (Silva et al. 2009). AF has also been examined in vitro for its anti-oxidant effects on free radical and ROS (Vellosa et al. 2015). These studies demonstrated protective effects conferred by QU, IQ, and AF against cellular oxidative stress.

LDH is a stable cytoplasmic enzyme (López et al. 2003) and it is rapidly secreted through the cell membrane upon damage to the membrane via H_2O_2-induced lipid peroxidation (Garcimartín et al. 2014). Our results demonstrated that H_2O_2-treated cells showed increased LDH release as compared with untreated cells. However, treatment with IQ significantly attenuated the LDH release as compared with H_2O_2-treated cells. When comparing the antioxidant activity of QU and IQ in vitro, the 6″-OH group in IQ conferred a higher ROS-scavenging activity than that of QU (Li et al. 2016b). The present results suggest that IQ protected against H_2O_2-induced LDH leakage from neuronal cells, and the −OH group in IQ probably contributed to its protective activity.

Exposure to oxidative stress via H_2O_2 can trigger inflammatory reactions in the neurons (Rhee 1999). Major inflammatory proteins such as iNOS and COX-2 play critical roles in the development and progression of neurodegenerative diseases. Furthermore, iNOS can directly influence neuronal apoptosis by inducing the synthesis and release of nitric oxide (Lyman et al. 2014), while the upregulation of COX-2 in the brain could induce synaptic dysfunctions and memory impairment (Cowley et al. 2008; Lyman et al. 2014). To determine the mechanisms underlying the inflammatory effects of the flavonoids from AO, we assessed the abundances of iNOS and COX-2 in the H_2O_2-exposed SH-SY5Y neuronal cells. In our present study, QU and IQ significantly down-regulated the expressions of iNOS and COX-2. Consistent with these findings, previous reports also demonstrated that QU downregulated inflammatory cytokines (TNF-α and IL-1β) and pro-inflammatory genes such as iNOS via inhibiting NF-κB signaling in the inflammation-induced macrophages (Comalada et al. 2005; Satué et al. 2013). Several studies indicated that QU ameliorated iNOS expression in vivo as well as in vitro system under oxidative stress and inflammation (Camuesco et al. 2004; Dai et al. 2013; Jo et al. 2008). IQ also suppressed the production of inflammatory cytokines (IL-1B, IL-6, and IL-8) in human basophilic cells (Li et al. 2016a). In addition, the anti-inflammatory effect of IQ was also demonstrated on lipopolysaccharide-induced nitrite production in rat macrophages and acetaminophen-induced oxidative stress in liver injury by down-regulation of the iNOS protein (Xie et al. 2016; Lee et al. 2008). In the oxidative damage, inflammatory reaction leads to two inducible enzymatic pathways such as iNOS and COX-2. The over-expression of iNOS produces NO from oxygen and L-arginine and COX-2 is up-regulated by prostaglandins from arachidonic acid, during the inflammatory process (Needleman and Manning 1999). In our results, treatment of AF showed down-regulated protein expression of COX-2, but not iNOS, in the H_2O_2-treated SH-SY5Y cells. Rho et al. (2011) demonstrated anti-inflammatory effects of kaempferol and its derivatives including AF, but AF is lower NO inhibitory activity among kaempferol and other kaempferol derivatives, consistent with our results. Therefore, we suggest that QU and IQ have protective effect from inflammatory reaction via down-regulations of both iNOS and COX-2, whereas the anti-inflammatory activity of AF would be only related to COX-2 down-regulation and other mechanisms. Therefore, these results indicated that the three flavonoids can help to ameliorate oxidative stress-induced inflammation reactions.

Oxidative stress induced upon exposure to H_2O_2 stimulates mitochondrial membrane injury in the brain, leading to apoptotic neuronal cell death by the regulation of Bcl-2 family proteins and caspase-dependent factors (Grutter 2000). The activation of caspase-3, caspase-9, and PARP can result in neuronal cell death, thus contributing to the development of neurodegenerative diseases such as Alzheimer's disease (Waldmeier and Tatton 2004). Bax and Bcl-2 are apoptotic and anti-apoptotic proteins, respectively, in the Bcl-2 family. An increase in the abundance of Bax can lead to neuronal cell death by caspase activation and neuronal cell death in patients with neurodegenerative diseases (Hartmann et al. 2001). Therefore, the inactivation of caspases and lowering of the Bax/Bcl-2 ratio play protective roles against neurodegenerative diseases. To investigate the effects of flavonoids from AO on apoptosis, we investigated the abundances of caspases and Bcl-2 family proteins in H_2O_2-treated SH-SY5Y cells. Our results indicated a downregulation of Bcl-2 and up-regulation of Bax, cleaved caspase-9, -3, and PARP by H_2O_2 in SH-SY5Y neuronal cells, leading to apoptotic cell death. However, the flavonoids from AO suppressed the H_2O_2-induced upregulation of pro-apoptotic cleaved caspase-3, -9, PARP, and Bax in SH-SY5Y neuronal cells. This indicates that flavonoids from AO can suppress apoptosis in cells exposed to oxidative stress. The previous studies reported anti-apoptosis effects of three flavonoids from AO (QU, IQ, and AF) in the oxidative stress-induced apoptosis (Zhu et al. 2016; Chen et al. 2006; Shin et al. 2013). IQ showed down-regulation of apoptotic protein expression such as cleaved caspase-9, -3, PARP, and p53 under H_2O_2-induced apoptotic cell (Zhu et al. 2016). In addition, IQ inhibited H_2O_2-induced apoptosis in the cellular system

and the treatment of IQ showed attenuation of apoptotic rate in the Hoechest 33342/PI double staining and Annexin V-FITC/PI staining (Zhu et al. 2016). QU also inhibited pro-apoptotic protein expressions by down-regulation of caspase activity and MAPK pathway in the western blot analysis and attenuated H_2O_2-induced cytotoxicity through anti-apoptotic morphological observations in the flow cytometric analysis (Chen et al. 2006). AF also has cellular anti-apoptotic effects at morphological level in the TUNEL assay and H&E staining and down-regulation of apoptotic protein expressions such as caspase-8, -3, -9, and PARP under the oxidative stress-induced cell damage (Shin et al. 2013). Moreover, we will further investigate the anti-apoptotic mechanisms of three flavonoids from AO under oxidative stress-induced neuronal dysfunction.

Various flavonoids have been reported to exert neuroprotective effects. QU attenuated amyloid β-induced neurotoxicity in hippocampal neuronal cells by inhibiting lipid peroxidation and activating anti-oxidant enzymes such as glutathione peroxidase (Rattanajarasroj and Unchern 2010). In addition, IQ has been reported to protect against hydroxyl dopamine-induced neurotoxicity in PC12 cells by activating anti-oxidant enzymes including superoxide dismutase, catalase, glutathione, and glutathione peroxidase (Magalingam et al. 2014). However, the neuroprotective activities of three flavonoids from AO (QU, IQ, and AF) in SH-SY5Y neuronal cells exposed to H_2O_2 have not yet been fully understood. Taken together, our findings indicated that the AO-derived flavonoids, QU, IQ, and AF have protective effects against oxidative stress-induced inflammation and apoptosis in SH-SY5Y neuronal cells.

Conclusion

Our study has demonstrated that flavonoids from AO protect against H_2O_2-induced cytotoxicity via reducing ROS generation and inhibiting LDH release. In addition, three flavonoids from AO (QU, IQ, and AF) attenuated the activation of inflammation and apoptosis. We propose that these flavonoids from AO may have protective effects against oxidative stress-induced neurodegenerative diseases. In addition, we suggest that flavonoids from AO could be useful as preventing and therapeutic agents for neurodegenerative diseases including AD, although further clinical studies have to be supported to elucidate clearly protective mechanisms of flavonoids from AO against neurodegenerative diseases.

Acknowledgements This work was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2015R1D1A1A01058868), Republic of Korea. This research was supported by Global PH.D Fellowship Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2016_H1A2A1906940).

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