Anthocyanin Extracts from Black Soybean (**Glycine max** L.) Protect Human Glial Cells Against Oxygen-Glucose Deprivation by Promoting Autophagy

Yong Kwan Kim^1,a, Hye Hyeon Yoon^1,a, Young Dae Lee^1, Dong-Ye Youn^1, Tae Joung Ha^2, Ho-Shik Kim^1 and Jeong-Hwa Lee^1,*

^1Department of Biochemistry, The Catholic University of Korea College of Medicine, Seoul 137-701, ^2Department of Functional Crop, National Institute of Crop Science (NICS), Rural Development Administration (RDA), Miryang 627-803, Republic of Korea

**Abstract**

Anthocyanins have received growing attention as dietary antioxidants for the prevention of oxidative damage. Astrocytes, which are specialized glial cells, exert numerous essential, complex functions in both healthy and diseased central nervous system (CNS) through a process known as reactive astrogliosis. Therefore, the maintenance of glial cell viability may be important because of its role as a key regulator of neuropathological events. The aim of this study was to investigate the effect of anthocyanin on the survival of glial cells exposed to oxidative stress. Our results demonstrated that anthocyanin extracts from black soybean increased survival of U87 glioma cells in a dose dependent manner upon oxygen-glucose deprivation (OGD), accompanied by decrease levels of reactive oxygen species (ROS). While treatment cells with anthocyanin extracts or OGD stress individually activated autophagy induction, the effect was significantly augmented by pretreatment cells with anthocyanin extracts prior to OGD. The contribution of autophagy induction to the protective effects of anthocyanin was verified by the observation that silencing the Atg5 expression, an essential regulator of autophagy induction, reversed the cytoprotective effect of anthocyanin extracts against OGD stress. Treatment of U87 cells with rapamycin, an autophagy inducer, increased cell survival upon OGD stress comparable to anthocyanin, indicating that autophagy functions as a survival mechanism against oxidative stress-induced cytotoxicity in glial cells. Our results, therefore, provide a rationale for the use of anthocyanin as a preventive agent for brain dysfunction caused by oxidative damage, such as a stroke.

**Key Words:** Anthocyanin, Oxygen-glucose deprivation, Glial cells

**INTRODUCTION**

Anthocyanins are water-soluble pigments that belong to the large class of polyphenols and are responsible for the reddish-blue color in a variety of plant tissues (Clifford, 2004). Although the absorption and metabolism-based pharmacokinetics of anthocyanin in serum, subsequent to their bioavailability, are dependent on their nature of chemical structure (Prior and Wu, 2006; McGhie and Walton, 2007), an increasing number of studies provide evidences for health-benefits of anthocyanin including anti-atherogenic activity, vision improvement, anticancer and anti-inflammatory activities (Kamei et al., 1995; Wang et al., 1999; Matsumoto et al., 2003; Xia et al., 2006). These physiological functions of anthocyanin are largely based on their anti-oxidant function as a free radical scavenger but recent studies have revealed that anthocyanins regulate the expression of several genes related to atherosclerosis, and induce apoptosis or autophagy (Longo et al., 2008; Lee et al., 2009b; Mauray et al., 2010; Paixão et al., 2011). These observations suggest that anthocyanin may play a role in the modulation of signal pathways involved in cell death and inflammation upon exposure to oxidative stress.

In the brain, supplementation of blueberries in the diets of mouse or rats resulted in enhanced short-term memory and improvements in motor behavior (Casadesus et al., 2004; Papandreou et al., 2009). Furthermore, dietary supplements of blueberry fed for 8 weeks to 19-month-old rats were shown to be effective in reversing the course of neuronal and behavioral aging (Joseph et al., 1999; Ramirez et al., 2005). As a possible mechanism for this neuroprotective effect, it was suggested...
that the induction of hippocampal heat shock protein (Hsp70) in response to lipopolysaccharide (LPS) challenge was re-
stored in the blueberry-fed old rats, to comparable response
levels as those observed in young rats (Galli et al., 2006). The
neuroprotective effect of anthocyanin was demonstrated in vi-
tro by showing that cyanidin-3-glucoside (C3G) extracted from
mulberry has cytoprotective effects on PC12 cells exposed to
oxidative stress such as oxygen-glucose deprivation (OGD) or
hydrogen peroxide. In addition, these extracts were also effect-
tive in the decrease of infarction volume observed in an in vivo
mouse model of ischemia with transient middle cerebral artery
occlusion (Kang et al., 2006).

It has long been assumed that glial cells including astrocytes
serve merely as structural supports for neurons in the central
nervous system (CNS). However, accumulating evidence has
proposed that astrocytes provide microenvironments for ho-
meostasis throughout the normal CNS by secretion of vari-
ous neurotrophic factors, cytokines, and neurotransmitters, in
response to various signals via specific receptors (Markiewicz
and Lukomska, 2006). In pathological conditions, astrocytes
become reactive in response to most forms of CNS injury, in-
cluding infection, trauma, ischemia, and neurodegenerative
diseases. The basic process of reactive astrocytes involves
the formation of hypertrophy, changes in gene expression profile,
and induction of astrocyte proliferation. In contrast to neuro-
supportive effects of astrocytes in normal CNS, reactive as-
trocytes exhibit both beneficial as well as harmful effects on
neuronal survival and function (Hamby and Sofroniew, 2010;
Sofroniew and Vinters, 2010). Therefore, astrocyte survival
could be considered a key determinant for CNS outcome,
neuronal degeneration or repair of neuronal activity. While the
protective effect of anthocyanin in response to oxidative stress
have been demonstrated in a variety of cells, its effect on glial
cells, which are prone to oxidative stress exposure, has not
been sufficiently investigated. Several tumor cell lines of gli-
oma origin are used for model of astrocytes in vitro (Jung et al.,
2010; Pouillet et al., 2011) although glioma cell lines and
normal astrocytes exhibit different responses in survival rate
to deregulation of proliferative signals (Senger et al., 2002;
Sick et al., 2011) but show similar susceptibilities to oxidative
stress-induced apoptosis or autophagy (Bonini, et al., 2004;
Hwang et al., 2010). In this study, therefore, we examined the
effect of anthocyanin extracts from black soybeans on the sur-
vival of U87 glioma cells under OGD, which mimics the isch-
emic condition in vivo. We found that anthocyanin exhibited a
protective effect on the survival of U87 cells in response to
oxidative stress, which is associated with an increase in au-
tophagy induction under conditions of hypoxic stress.

**MATERIALS AND METHODS**

**Extraction and purification of anthocyanins**

Black seed coated soybean (*Glycine max* L.) cultivar
Cheongna 3 developed by the National Institute of Crop Sci-
ence (NICS) was selected for the source of anthocyanin in this
study. The extraction of anthocyanin contents was performed
as in the previous studies (Ha et al., 2009; Lee et al., 2009a).
Anthocyanin contents were determined by means of high
performance liquid chromatography using a Dionex Ultimate
3000 series (Dionex Softron GmbH, Germering, Germany).
Among the anthocyanins in Cheongna 3, cyanidin-3-O-glu-
coside was the major anthocyanin constituent, representing
68.3% of anthocyanin, followed by delphinidin-3-O-glucoside
(25.2%), and petunidin-3-O-glucoside (6.5%).

**Cells culture and OGD treatment**

Human glioblastoma cells (U87) from American Tissue Cul-
ture Collection (Manassas, VA, USA) were maintained in mini-
um essential medium (MEM) supplemented with 10% heat
inactivated fetal bovine serum and antibiotics solution (penicil-
in G 100 unit/ml and streptomycin 100 mg/ml) at 37°C in a
humidified incubator with 5% CO₂. Before exposure to OGD,
U87 cells were seeded onto 35 mm culture dish (Iwaki, Tokyo,
Japan) at the density of 5×10⁴ cells /ml and incubated over-
night. OGD treatment was performed as previously described
(Jung et al., 2010). Briefly, the cells were washed twice with
degassed DMEM without glucose and serum and immediately
treated with various concentration of anthocyanin extracts
from black soybeans or rapamycin (Sigma-Aldrich, St. Louis,
MO, USA) as indicated. Afterwards, the cells were incubated in
anaerobic chamber containing 85% (v/v) N₂, 10% (v/v) H₂ and
5% (v/v) CO₂ (Thermo Forma, Marietta, OH, USA) at
37°C for 5 h.

**Cell viability**

Cell viability was determined by the colorimetric assay
which measures the reducing activity of mitochondrial en-
zymes using 2-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetra-
zolium bromide (MTT, Duchefa, Haarlem, The Netherlands)
dyes. After OGD stress in the absence or presence of antho-
cyanin or rapamycin, cells were incubated with MTT (0.5 mg/
ml) and incubated for 2 h at 37°C. After removing the medium,
the formazan crystals were dissolved by acid isopropyl alcohol
and subsequently distilled water. The extent of reduction of
MTT was quantified by measuring the absorbance at 570
nm using a Victor 3 spectrophotometer (PerkinElmer, Turku,
Finland). The relative viability was expressed as a percentage
of control cells.

**Flow cytometric analysis of ROS**

ROS generation was determined by flow cytometry us-
ing 2′, 7′-dichlorodihydrofluorescein diacetate (DCF-DA, Molecu-
lar probes, Eugene, OR, USA). DCF-DA is hydrolyzed by intracel-
ular esterase to yield a reduced, non-fluorescent compound,
DCFH. The ROS produced by cells oxidized the DCFH to
highly fluorescent DCF. After exposure to OGD stress, cells
were incubated with 10 μM DCF-DA for 30 min at 37°C and
then washed twice with ice-phosphate buffered saline. Quan-
tification of ROS levels from each sample was measured us-
ing a FACSDiCalibur™ (Becton Dickinson, San Jose, CA, USA)
with excitation at 488 nm and emission at 525 nm.

**Western blotting**

Whole cell lysates were prepared using RIPA buffer (150
mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS,
50 mM Tris-HCl, pH7.5) with protease inhibitors (Roche,
Mannheim, Germany) and immediately sonicated three times
for 15 sec each on ice followed by centrifugation at 13.200
rpm at 4°C for 20 min. The concentration of protein from each
sample was measured by the BCA assay kit (Pierce, Rock-
ford, IL, USA). Equal amount of proteins was separated on
12% SDS-PAGE and transferred to polyvinylidene difluoride
membrane (Millipore, Bedford, MA, USA). After incubation
with 0.1% TTBS (20 mM Tris-HCl, pH 7.6, 137 mM NaCl, 0.1% Tween-20) containing 5% non-fat dried milk for 30 min, the membranes were incubated with the antibodies against LC3 (Sigma-Aldrich) (1:10,000), ATG5 (Epitomics, Burlingame, CA, USA) (1:1,000) or beta-actin (Sigma-Aldrich) (1:15,000). Following washing and incubation with G-horseradish peroxidase-conjugated secondary antibodies (Millipore) (1:2,000), the immunoreactive bands were visualized by enhanced chemiluminescence kit from Pierce. Quantification of intensities for each band was determined by multi gauge 2.2 software (Fuji Photo Film Co, Tokyo, Japan).

Small interfering RNA (siRNA) transfection
To inhibit autophagy induction, cells were transfected with a total 100 nM of ATG5 siRNA (Genolution, Seoul, Korea) using the Neon™ Transfection system kit (Invitrogen, Paisley, UK) at 1,300 V, 30 ms in antibiotic-free medium, according to the manufacturer’s instructions. The targeted sequences of ATG5 were GGAAUAUCCUGCAGAAGAA and sequences of negative control were CCUACGCCACCAAUUUCGU. After 48 h of transfection, cells were subjected to expose to OGD stress.

Statistical analysis
The data are presented as means ± SD obtained from at least three independent experiments. The significant differences between groups were determined using unpaired Student’s t-test. Values with \( p < 0.05 \) was considered significant.

RESULTS
Protective effect of anthocyanin extracts from black soybeans on OGD susceptibility
As shown in Fig. 1A, the dose of anthocyanin extracts we used in this study did not affect on the survival or growth rate of U87 glioma cells, up to 100 \( \mu \text{g/ml} \), as determined by MTT assay. To examine the effect of anthocyanin on the survival of glioma cells upon oxidative stress, U87 cells were exposed to OGD stress in the presence or absence of anthocyanin extracts. As shown in Fig. 1B, the pretreatment of anthocyanin extracts prior to OGD exposure increased the viability of U87 cells in dose-dependent manner. Cell viability determined after oxidative stress was 13.4% in control cells, but were 29.4%
and 40% in the cells treated with anthocyanin extracts at 50 μg/ml and 100 μg/ml, respectively. Furthermore, the DCF fluorescence intensity representing reactive oxygen species (ROS) levels was attenuated by pretreatment with anthocyanins before OGD induction over 10-fold in cells pretreated with anthocyanin extracts compared to untreated cells (Fig. 1C). Therefore, the protective effect of anthocyanin in response to OGD stress in U87 cells is associated with its free radical scavenging activity.

Induction of autophagy under hypoxic condition was enhanced in the presence of anthocyanin

It has been well known that oxidative stress induces cell death involving apoptotic pathway. However, oxidative stressors such as hydrogen peroxide and 2-methoxyestradiol (2-ME) were shown to induce autophagic cell death in several cell lines including U87 glioma cells (Chen et al., 2008; Byun et al., 2009). Thus, we examined whether autophagy is induced under OGD condition in U87 cells and whether the induction is affected by the presence of anthocyanin. Autophagy is characterized by the formation of an autophagosome requiring the participation of microtubule-associated protein light chain 3 (LC3) which is normally localized to the cytoplasm (Tanida et al., 2004). Thus, the conversion of the cytoplasmic form of LC3 (LC3-I, 16 kDa) to the preautophagosomal membrane-bound form of LC3 (LC3-II, 16 kDa) is used as a marker for the measurement of degree of autophagy induction. Western blotting assay for LC3 revealed an increase in the density of LC3-II bands by anthocyanin treatment alone as well as by OGD stress. The lipidation of LC3 was markedly increased by OGD in the presence of anthocyanin extracts (Fig. 2A). The mean ratio of band density of LC3-II to LC3-I was increased to 9-fold compared to control in response to OGD, and pretreat-

Fig. 2.

**Fig. 2.** Anthocyanin extracts promotes autophagy induction under OGD stress. (A) Western assay for LC3 proteins were performed with cell lysates after OGD with or without black soybean anthocyanin extracts (BSAE,100 μg/ml). Beta-actin was detected as a loading control. (B) The intensities of LC-II over LC-I was measured by densitometric analysis. The mean values from three independent experiments (± S.D) were shown after arbitrarily setting the value from control cells as 1.0. (*p<0.05, **p<0.01).

**Fig. 3.** Inhibition of autophagy reverses the protective effect of anthocyanin extracts on the OGD-induced cell death. (A) U87 cells were transfected with control siRNA (siCon) or Atg5 siRNA (siAtg5) for 48 h and the expression levels of endogenous Atg5 were examined by western blot analysis. (B) The relative survival of U87 cells transfected with control siRNA or Atg5 siRNA with or without BSAE under OGD stress was determined by MTT assay. The value from control cells treated with control siRNA not exposed to OGD was set as 100%. (C) The effect of rapamycin on the survival of U87 cells upon OGD was examined. Prior to exposure to OGD, indicated dose of rapamycin was added to U87 cells for 1 h. After 5 h of OGD stress, the viability was determined by MTT assay. Bars represent means ± S.D from three independent experiments. (**p<0.001).
ment with anthocyanin extracts significantly further increased the ratio to 2.3-fold of OGD, which is almost 20-fold greater than control (Fig. 2B). It should be noted that LC3 conversion by anthocyanin treatment in the absence of OGD stress, was about 3-fold greater than control.

Inhibition of autophagy reverses the protective effect of anthocyanin

Although induction of autophagy was potentiated by anthocyanin extracts under OGD condition, it is uncertain whether the autophagy induction by anthocyanin contributes the protective effect of anthocyanin or not. To clarify this point, we investigated the effect of inhibition of autophagy on cell viability after OGD stress. The expression of Atg5, an essential protein for autophagosome formation (Klionsky, 2007; Mizushima, 2007), was suppressed by siRNA transfection prior to exposure to OGD (Fig. 3A). Silencing of Atg5 expression resulted in the reduction of cell viability after OGD in the absence of anthocyanin from 20.9% to 16.3%. In addition, the increase in the viability provided by treatment anthocyanin extracts was significantly decreased by Atg5 silencing, from 34.2% to 22.7% (Fig. 3B). This result suggests that enhancement of autophagy by anthocyanin confer a survival advantage against cell death by oxidative stress. To confirm the protective effect of autophagy upon oxidative stress-induced cell death, we pretreated U87 cells with rapamycin, an autophagy inducer (Ravikumar et al., 2006), before the application of OGD stress. Fig. 3C shows that pretreatment with rapamycin increased cell viability dose dependently, which was comparable to anthocyanin. Thus, autophagy induction during oxidative stress might confer an advantage to cells to sustain their viability during unfavorable environmental conditions.

DISCUSSION

Anthocyanins have been reported to possess potent antioxidant activity and subsequent protective effect from oxidative damage in vitro and in vivo. However, it has not yet been clearly determined whether anthocyanins exhibit a protective role on the survival of glial cells which determines neuronal fate and subsequent clinical outcome upon oxidative stress. In our study, we showed that pretreatment with anthocyanins extracted from black soybeans prior to OGD stress in U87 glioma cells significantly increased cell viability (Fig. 1B), which is related to greater ROS clearance (Fig. 1C). Consistent with our results, it has been previously reported that pretreatment with proanthocyanidin extracts from grape seeds results in the elevation of the hydrogen peroxide tolerance in primary glial cells as measured by lactate dehydrogenase release assay (Roychowdhury et al., 2001). Although different oxidative stressors were employed in our study and previous study, the protective effect of anthocyanin on glial cells may be related by the inhibition of ROS generation as demonstrated by in the decrease of DCF fluorescence signal in both studies. The DCF signals in our study increased by 15-fold and 2-fold in the absence and presence of anthocyanin, respectively, compared with control (Fig. 1C). While ROS levels were suppressed close to normal levels, the recovery of viability by anthocyanin extracts was insufficient to be explained by ROS levels, from 13% to 40. Thus, the protective effect of anthocyanin on the survival of glial cells upon OGD may be mediated by the complex mechanisms that are not directly correlated to ROS levels.

Oxidative stress has been known to induce cell death through ROS generation, which involves caspase-dependent apoptotic pathways. Recent studies, however, have suggested that ROS can induce autophagic cell death in glioma cells, which is independent of apoptotic cell death (Chen et al., 2008; Byun et al., 2009). In the present study, we also demonstrated that autophagy was induced in response to OGD stress, which is a metabolic as well as oxidative stress condition, as shown by activation of the autophagosomal marker LC3 (Fig. 2). Moreover, the level of autophagosome-associated LC3-II was significantly further increased by anthocyanin pretreatment in response to OGD stress. The physiological role of autophagy is essentially to remove long-lived protein and damaged organelles, thus autophagy can rescue cells in distressed conditions such as anticancer treatment or nutrient deprivation by providing molecules necessary for metabolism to sustain cell survival (Meijer and Codogno, 2009). Accordingly, OGD-mediated autophagy or its promotion by anthocyanin may either promote or impair cell death. Considering the restoration of viability by anthocyanin upon OGD stress, the enhancement of autophagy may contribute to the protective ability of anthocyanin upon OGD stress. In support of this hypothesis, the siRNA-mediated inhibition of Atg5 expression led to a decrease in the survival of U87 cells provided by pretreatment of anthocyanin extracts under OGD condition (Fig. 3A, B). Therefore, the promotion of autophagy induction by anthocyanin confers protection of U87 cells exposed to OGD stress. The importance of promotion of autophagy in the protective role of anthocyanin was confirmed by showing that viability upon OGD stress was recovered by the treatment with rapamycin, an autophagy inducer (Fig. 3C). The Silencing Atg5 also reduced the viability of U87 cells against OGD stress in the absence of anthocyanin, although to a lesser degree than in the presence of anthocyanin, suggesting that activation of autophagy by OGD stress itself may not participate to the induction of oxidative stress-mediated cell death (Fig. 3B).

It has been reported previously groups that anthocyanin-rich extract from P. lentiscus berry or delphinidin, an anthocyanin, possess the ability to induce autophagy in hepatoma cells (Longo et al., 2008; Feng et al., 2010). We also observed that only the treatment of anthocyanin extract without OGD stress resulted in the activation of autophagy in U87 glioma cells as evidenced by increase in LC3 lipidation (Fig. 2A). Therefore, autophagy induction of anthocyanin is not a unique feature limited in hepatoma cells. Previous groups show that autophagy was induced under conditions in which an anthocyanin mixture or delphinidin exhibit cytotoxic effect or growth retardation effect on hepatoma cells. The present study demonstrated the activation of autophagy by anthocyanin could occur without affecting growth rate or viability of U87 cells (Fig. 1A, Fig. 2). The discrepancy for the autophagy induction and cytotoxic effect of anthocyanin in this study and previous studies may be attributed to the dose or relative composition of anthocyanin extract. The previous group showed that autophagy was induced with 200 μg/ml of anthocyanin extracts from berries (P. Lentiscus L.), at which survival rate of hepatoma cells were about 60% (Longo et al., 2008), while 100 μg/ml anthocyanin extracts from black soybean were used in the present study. Furthermore, the relative composition of two major anthocyanins, cyanidin 3-O-glucoside and delphinidin 3-O-glucoside, was 4:1 in the extracts from berries in the pre-
vious study whereas the ration was 2.7:1 in the extracts from blue seed-coated soybean. In the present study, depending the chemical nature of anthocyanins, different patterns in absorption and degradation as well as distinct biological responses were observed in vivo in vitro (Prior and Wu, 2006). Thus, difference in the relative composition of these two anthocyanins and their degradation products may affect on the cell survival and autophagy induction. It has been shown that, in the presence of autophagy inhibitors, delphinidin or anthocyanin mixture was shown to induce necrosis or enhance induction of apoptosis. In the present study, Atg5 silencing did not affect the anthocyanin-treated cells but did reverse the protective effect of anthocyanin on OGD stress (data not shown and Fig. 3B).

Taken together, anthocyanin-induced macroautophagy, which is observed in response to the cytotoxic dose of anthocyanin or under hypoxic condition, was required for the survival of cells in unfavorable environment conditions. It has not been determined however, whether the same mechanism of autophagy induction by anthocyanin is employed under the cytotoxic conditions in addition to viable conditions, as well as in the presence or absence of oxidative stress.

Upon ischemic injury, astrocytes are subjected to undergo swelling and the astrocyte gap junction may remain unfastened, leading to deterioration in the balance of uptake and release of glutamate, and the diffusion of proapoptotic factors, which may contribute to acceleration of cell death in surrounding neurons (Budd and Lipton, 1998; Lin et al., 1998). However, considering that astrocytes are a reservoir for a high amount of antioxidants and that astrocytes release essential neurotrophic factors (Dringen et al., 1999; Chen et al., 2001), they may also play a protective role in the survival of neurons against oxidative stress. Supporting this presumption, failure of astrocyte functions contributed to neuronal degeneration and disruption of the astroglial scar is associated with the spread of inflammation and infarction volume after stroke in mouse models (Rossi et al., 2007; Li et al., 2008; Takano et al., 2009). Therefore, the preservation of the viability of glial cells including astrocytes in response to oxidative stress may be crucial for the reduction of neuronal death, thereby limiting the infarction area. Based on our results, autophagy induction could be developed as a beneficial approach to promote glial cell survival in response to oxidative stress. In the present study, anthocyanin is a potential candidate to be an effective adjuvant for the prevention or reduction of glial cell death, and subsequent neuronal death during conditions of hypoxic status, such as stroke.

ACKNOWLEDGMENTS

This research has been supported by the Biogreen 21 Program (No.PJ007186), Rural Development Administration, Republic of Korea.

REFERENCES

Bonini, P., Cicioni, S., Cardinale, A., Vitale, C., Serafini, A. L., Ciotti, M. T. and Marlier, L. N. (2004) Oxidative stress induces p53-mediated apoptosis in gliar: p53 transcription-independent way to die. J. Neurosci. Res. 75, 83-95.

Budd, S. L. and Lipton, S. A. (1998) Calcium tsunami: do astrocytes transmit cell death messages via gap junctions during ischemia? Nat. Neurosci. 1, 431-432.

Byun, Y. J., Kim, S. K., Kim, Y. M., Chae, G. T., Jeong, S. W. and Lee, S. B. (2009) Hydrogen peroxide induces autophagic cell death in C6 glioma cells via BNIP3-mediated suppression of the mTOR pathway. Neurosci. Lett. 461, 131-135.

Casadesus, G., Shukitt-Hale, B., Stellwagen, H. M., Zhu, X., Lee, H. G., Smith, M. A. and Joseph, J. A. (2004) Modulation of hippocampal plasticity and cognitive behavior by short-term blueberry supplementation in aged rats. Nutr. Neurosci. 7, 309-316.

Chen, Y., Vartikainen, N. E., Ying, W., Chan, P. H., Koistinaho, J. and Swanson, R. A. (2001) Astrocytes protect neurons from nitric oxide toxicity by a glutathione-dependent mechanism. J. Neurochem. 77, 1601-1610.

Chen, Y., McMillian-Ward, E., Kong, J., Israels, S. J. and Gibson, S. B. (2008) Oxidative stress induces autophagic cell death independent of apoptosis in transformed and cancer cells. Cell Death Differ. 15, 171-182.

Clifford, M. N. (2004) Diet-derived phenols in plasma and tissues and their implications for health. Planta Med. 70, 1103-1114.

Dringen, R., Pfeffer, B. and Hamprecht, B. (1999) Synthesis of the antioxidant glutathione in neurons: supply by astrocytes of CysGly as precursor for neuronal glutathione. J. Neurosci. 19, 562-569.

Feng, R., Wang, SY., Shi, Y. H., Fan, J. and Yin, X. M. (2010) Delphinidin Induces Necrosis in Hepatocellular Carcinoma Cells in the Presence of 3-Methyladenine, an Autophagy Inhibitor. J. Agric. Food Chem. 58, 3957-3964.

Gall, R. L., Bielinsky, D. F., Szprengiel. A., Shukitt-Hale, B. and Joseph, J. A. (2006) Blueberry supplemented diet reverses age-related decline in hippocampal HSP70 neuroprotection. Neurobiology of Aging. 27, 344-350.

Ha, T. J., Lee, J. H., Shin, S. O., Shin, S. H., Han, S. I., Kim, H. T., Ko, J. M., Lee, M. H. and Park, K. Y. (2009) Changes in anthocyanin and isoflavone concentrations in black seed-coated soybean at different planting locations. J. Crop Sci. Biotech. 12, 79-86.

Hamby, M. E. and Sofroniew, M. V. (2010) Reactive astrocytes as therapeutic targets for CNS disorders. Neurotherapeutics. 7, 494-506.

Hwang, J., Lee, S., Lee, J. T., Kwon, T. K., Kim, D. R., Kim, H., Park, H. C. and Suk, K. (2010) Gangliosides induce autophagic cell death in astrocytes. Br. J. Pharmacol. 159, 586-603.

Joseph, J. A., Shukitt-Hale, B., Denisova, N. A., Bielinsky, D., Martin, A., McEwen, J. J. and Bickford, P. C. (1999) Reversals of age-related declines in neuronal signal transduction, cognitive, and motor behavioral deficits with blueberry, spinach, or strawberry dietary supplementation. J. Neurosci. 19, 8114-8121.

Jung, S. E., Kim, Y., Yoon, D. Y., Lim, M. H., Ko, J. H., Ahn, Y. S. and Lee, J. H. (2010) Down-modulation of Bis sensitizes cell death in C6 glioma cells induced by oxygen-glucose deprivation. Brain Res. 1349, 1-10.

Kamei, H., Kojima, T., Hasegawa, M., Koide, T., Umeda, T., Yukawa, T. and Terabe, K. (1995) Suppression of tumor cell growth by anthocyanins in vitro. Cancer Invest. 13, 590-594.

Kang, T. H., Hur, J. Y., Kim, H. B., Ryu, J. H. and Kim, S. Y. (2006) Neuroprotective effects of the cyanidin-3-O-beta-d-glucopyranoside isolated from mulberry fruit against cerebral ischaemia. Neurosci. Lett. 391, 122-126.

Klionsky, D. J. (2007) Autophagy: from molecular to cellular under-standing in less than a decade. Nat. Rev. Mol. Cell Biol. 8, 931-937.

Lee, J. H., Kang, N. S., Shin, S. O., Shin, S. H., Lim, S. G., Suh, D. Y., Baek, I. Y., Park, K. Y. and Ha, T. J. (2009a) Characterisation of anthocyanins in the black soybean (Glycine max L.) by HPLC-DAD-ESI/MS analysis. Food Chemistry 112, 226-231.

Lee, S. H., Park, S. M., Park, S. M., Park, J. H., Shin, D. Y., Kim, G. Y., Ryu, C. H., Shin, S. C., Jung, J. M., Kang, H. S., Lee, W. S. and Choi, Y. H. (2009b) Induction of apoptosis in human leukemia U937 cells by anthocyanins through down-regulation of Bcl-2 and activation of caspases. Int. J. Oncol. 34, 1077-1083.

Li, L., Lundkvist, A., Andersson, D., Wilhelmsson, U., Nagai, N., Pardó, A. C., Nodin, C., Stålberg, A., Aprico, K., Larsson, K., Yabe, T., Moons, L., Fotheringham, A., Davies, I., Carmeliet, P., Schwartz, J. P., Pekna, M., Kubista, M., Blomstrand, F., Maragakis, N., Nilsson, 73
M. and Pekny, M. (2008) Protective role of reactive astrocytes in brain ischemia. J. Cereb. Blood Flow Metab. 28, 468-481.
Lin, J. H., Weigel, H., Cotrina, M. L., Liu, S., Bueno, E., Hansen, A. J., Hansen, T.W., Goldman, S. and Nedergaard, M. (1998) Gap-junction-mediated propagation and amplification of cell injury. Nat. Neurosci. 1, 494-500.
Longo, L., Platini, F., Scardino, A., Alabiso, O., Vasapollo, G. and Tessitore, L. (2008) Autophagy inhibition enhances anthocyanin-induced apoptosis in hepatocellular carcinoma. Mol. Cancer Ther. 7, 2476-2485.
Markiewicz, I. and Lukomska, B. (2006) The role of astrocytes in the physiology and pathology of the central nervous system. Acta. Neurobiol. Exp. 66, 343-358.
Matsumoto, H., Nakamura, Y., Tachibanaki, S., Kawamura, S. and Hirayama, M. (2003) Stimulatory effect of cyanidin 3-glycosides on the regeneration of rhodopsin. J. Agric. Food Chem. 51, 3560-3563.
Mauray, A., Felgines, C., Morand, C., Mazur, A., Scalbert, A. and Milenkovic, D. (2010) Nutrigenomic analysis of the protective effects of bilberry anthocyanin-rich extract in apo E-deficient mice. Genes Nutr. 5, 343-353.
McGhie, T. K. and Walton, M. C. (2007) The bioavailability and absorption of anthocyanins; towards a better understanding. Mol. Nutr. Food Res. 51, 702-713.
Meijer, A. J. and Codogno, P. (2009) Autophagy: regulation and role in disease. Crit. Rev. Clin. Lab. Sci. 46, 210-240.
Mizushima, N. (2007) Autophagy: process and function. Genes Dev. 21, 2861-2873.
Paido, J., Canes, TC. and Almeida, L. M. (2011) Dietary anthocyanins protect endothelial cells against peroxynitrite-induced mitochondrial apoptosis pathway and Bax nuclear translocation: an in vitro approach. Apoptosis 16, 976-989.
Papandreou, M. A., Dimakopoulou, A., Linardaki, Z. I., Cordopatis, P., Klimis-Zacas, D., Margarity, M. and Lamari, F. N. (2009) Effect of a polyphenol-rich wild blueberry extract on cognitive performance of mice, brain antioxidant markers and acetylcholinesterase activity. Behav. Brain Res. 198, 352-358.
Pouliette de Gannes, F., Haro, E., Hurtier, A., Taxile, M., Ruffié, G., Billaudel, B., Veyret, B. and Lagroye, I. (2011) Effect of exposure to the edge signal on oxidative stress in brain cell models. Radiat. Res. 175, 225-230.
Prior, R. L. and Wu, X. (2006) Anthocyanins: structural characteristics that result in unique metabolic patterns and biological activities. Free Radic. Res. 40, 1014-1028.
Ramírez, M. R., Izquierdo, I., do Carmo Bassols, Raseira, M., Zuanazzi, J. A., Barros, D. and Henriques, AT. (2005) Effect of lyophilised Vaccinium berries on memory, anxiety and locomotion in adult rats. Pharmacol. Res. 52, 457-462.
Ravikumar, B., Berger, Z., Vacher, C., O’Kane, C. J. and Rubinsztein, D. C. (2006) Rapamycin pre-treatment protects against apoptosis. Hum. Mol. Genet. 15, 1209-1216.
Rossi, D. J., Brady, J. D. and Mohr, C. (2007) Astrocyte metabolism and signalling during brain ischemia. Nat. Neurosci. 11, 1377-1386.
Roychowdhury, S., Wolf, G., Keilhoff, G., Bagchi, D. and Horn, T. (2001) Protection of primary glial cells by grape seed proanthocyanidin extract against nitrosative oxidative stress. Nitric. Oxide 5, 137-149.
Senger, D. L., Tudan, C., Guiot, M. C., Mazzoni, I. E., Molenkamp, G., LeBlanc, R., Antel, J., Olivier, A., Snipes, G. J. and Kaplan, D. R. (2002) Suppression of Rac activity induces apoptosis of human glioma cells but not normal human astrocytes. Cancer Res. 62, 2131-2140.
Sick, E., Boukhari, A., Deramaudt, T., Rondé, P., Bucher, B., André, P., Gies, J. P. and Takeda, K. (2011) Activation of CD47 receptors causes proliferation of human astrocytoma but not normal astrocytes via an Akt-dependent pathway. Glia. 59, 308-319.
Sofroniew, M. V. and Vinters, H. V. (2010) Astrocytes: biology and pathology. Acta. Neuropathol. 119, 7-35.
Takano, T., Oberheim, N., Cotrina, M. L. and Nedergaard, M. (2009) Astrocytes and ischemic injury. Stroke. 40, S8-12.
Tanida, I., Ueno, T. and Komnami, E. (2004) LC3 conjugation system in mammalian autophagy. Int. J. Biochem. Cell Biol. 36, 2503-2518.
Wang, H., Nair, M. G., Strasburg, G. M., Chang, Y. C., Booren, A. M., Gray, J. I. and DeWitt, D. L. (1999) Antioxidant and antiinflammatory activities of anthocyanins and their aglycon, cyanidin, from tart cherries. J. Nat. Prod. 62, 294-296.
Xia, X., Ling, W., Ma, J., Xia, M., Hou, M., Wang, Q., Zhu, H. and Tang, Z. (2006) An anthocyanin-rich extract from black rice enhances atherosclerotic plaque stabilization in apolipoprotein E-deficient mice. J. Nutr. 136, 2220-2225.