PIBB 生物化学与生物物理进展
Progress in Biochemistry and Biophysics
2010, 37(7): 779~785
www.pibb.ac.cn

Protective Effect of Anthocyanin Against The Oxidative Stress in Neuroblastoma N2a Cells*

LIU Ling-Ling, SHENG Bai-Yang, YAN Yu-Fang, GONG Kai, MA Tuo,
ZHAO Nan-Ming, ZHANG Xiu-Fang, GONG Yan-Dao**
(*State Key Laboratory of Biomembrane and Membrane Biotechnology, School of Life Sciences, Tsinghua University, Beijing* 100084, China)

Abstract Oxidative stress is proved to play an important role in the pathogenesis of Alzheimer's disease (AD). The protective effect of anthocyanin against endogenous Aβ was investigated in N2a/Swe.Δ9 cells, which are widely used as AD model cells. Anthocyanin belongs to the family of flavonoids extracted from plants. It was demonstrated that anthocyanin at 100 μmol/L significantly inhibited the oxidative stress by decreasing the vulnerability, intracellular ROS and $[NO]_i$ in N2a/Swe.Δ9 cells. Oxidative stress induces increased activation of C-JunN-terminal kinase (JNK). It was demonstrated that anthocyanin can decrease the activation of JNK in N2a/Swe.Δ9 cells, suggesting that anthocyanin exerts its protective effect by inhibiting the activation of JNK. Therefore, anthocyanin could act as an oxidative stress suppressor in protecting the N2a/Swe.Δ9 cells against Aβ induced cell injury and it is a promising candidate for AD treatment in the future.

Key words Alzheimer's disease, oxidative stress, anthocyanin, c-Jun N-terminal kinase (JNK)
DOI: 10.3724/SP.J.1206.2009.00773

Alzheimer's disease (AD) is one of the most common neurodegenerative diseases characterized by extracellular amyloid plaques and intracellular neurofibrillary tangles[1–3]. The major protein component of amyloid plaques is beta-amyloid peptide (Aβ), which is generally believed to play a causative role in the progression of AD[4].

There are lots of hypotheses about how Aβ induced the AD pathology including Aβ forming ion channels resulting toxicity[5] and others[6–7]. Among the mechanisms on Aβ-mediated toxicity, oxidative stress hypothesis, suggesting that Aβ generates free radical species or decreases the endogenous antioxidants[8–12], has drawn more and more attention. It is believed that the Aβ-mediated oxidative stress is an important factor in the pathology of AD[13–14]. Aβ-mediated oxidative stress in AD was furthermore proved by other evidence: inhibition of Aβ production resulted in decreased oxidative stress[15]. The levels of reactive oxygen species (ROS) and intracellular NO level ($[NO]_i$) are both essential parameters of oxidative stress[16]. ROS is highly reactive with biomolecules, including proteins, lipids, carbohydrate, DNA and RNA.

There have been many therapeutic researches aimed at the oxidative stress in AD. Some antioxidants, such as vitamine E and some compounds isolated from plants, have been proved to be effective in treating AD models[17–18]. Antioxidant strategy has been proved as a promising alternative therapy for AD[17–19].

Anthocyanin ($C_{27}H_{31}O_{16}$) is a naturally occurring water-soluble pigment present widely in many plants[20]. It belongs to the family of compounds known as flavonoids and is particularly abundant in berries and other fruits[21]. Besides its functions in plants[22], anthocyanin can be used as an antioxidant for the purpose of therapy[23]. Previous studies showed that anthocyanin was neuroprotective[24], anti-inflammatory[25] and helpful in heart disease[26] with its property of

*This work was supported by grants from Tsinghua-Yue-Yuen Medical Sciences Fund (20240000514), and The State Key Laboratory of Biomembrane and Membrane Biotechnology.
**Corresponding author.
Tel: 86-10-62785049, E-mail: gongyd@tsinghua.edu.cn
Received: December 31, 2009 Accepted: March 18, 2010

antioxidant.

In the present study, anthocyanin was examined to clarify whether it can protect the N2a/Swe.Δ9 cells against endogenous Aβ induced oxidative stress. The intracellular ROS and $[NO]_i$ were determined as indicators of oxidative stress. The vulnerability to H_2O_2 was examined to demonstrate its ability in protecting N2a/Swe. Δ9 cells from oxidative stress. C-Jun N-terminal kinase (JNK) is an important member of the mitogen-activated protein kinase superfamily [27]. Activation of JNK is a key result of oxidative stress including ROS and $[NO]_i$ overproduction [28]. In the present study, we detected the expression of pJNK, the activated JNK, to investigate whether anthocyanin protects cells by inhibiting the JNK activation. AD model cells used here were N2a/Swe. Δ9 cells which produce more endogenous Aβ and suffer severer oxidative stress than their wild type control(N2a/wt)[15].

1 Materials and methods

1.1 Materials

1.1.1 Cell culture. Mouse neuroblastoma N2a cells and N2a cells stably co-expressing a human APP695 harboring the "Swedish" double mutation (K670M/N671L) and PS1ΔE9 mutation (N2a/Swe.Δ9)[29] were kindly presented by Dr.Huaxi Xu (the Burnham Institute, SD, USA). The cells were maintained in 50% Dulbecco's modified Eagle's medium (DMEM), 50% OPTI-MEM plus 5% fetal bovine serum (Gibco) with 200 mg/L G418.

1.1.2 Reagents. Anthocyanin (M_r = 611 of purity > 95%) was obtained from Shan Xi Sciphar Biotechnology Co., Ltd (China). Modified Dulbecco's Eagle's medium (DMEM) and OPI-MEM supplement were obtained from Gibco Invitrogen Corporation (USA). Methyl thiazolyl tetrazolium (MTT) and DMSO were purchased from Amresco (USA). NO indicator 3-Amino, 4-aminomethyl-2', 7'-difluorescein, diacetate (DAF-FM DA) and 2, 7-dichlorofluorescin-diacetole (DCFH-DA) were purchased from Beyotime (Jiangsu, China). Antibody against JNK and pJNK were obtained from Cell Signal Pathway Corporation.

1.2 Methods

1.2.1 Cell viability. MTT assay was used to assess cell viability. Briefly, the N2a cells (10^4 cells/well) were seeded in 24-well plates. 24 h later, cells were incubated with 100 or 200 μmol/L anthocyanin for 24 h. Subsequently, cells were treated with or without 50 μmol/L H_2O_2 for 24 h. Then MTT was added to each well with a final concentration of 1 g/L and the plates were incubated at 37℃ for 4 h. MTT solution was removed and 1 ml DMSO was added to each well to dissolve the insoluble formazane crystal. The absorbance at 570 nm was measured by a microplate reader using DMSO as the blank.

1.2.2 Measurement of intracellular ROS. To estimate the intracellular ROS production, cells were seeded in 35 mm dishes at a density of 1×10^5 cells. 24 h later, 100 or 200 μmol/L anthocyanin was added to cells and the incubation was lasted for 48 h then for ROS detection. Cells were rinsed with Krebs-Ringer solution (100 mmol/L NaCl, 2.6 mmol/L KCl, 25 mmol/L $NaHCO_3$, 1.2 mmol/L $MgSO_4$, 1.2 mmol/L KH_2PO_4 and 11 mmol/L glucose), and 10 μmol/L DCFH-DA was loaded. After incubation at 37℃ in a 5% CO_2 incubator for 1 h, cells were washed five times with the Krebs-Ringer solution and examined under a confocal fluorescence microscope (FV500, Olympus, Japan) equipped with an argon laser. The digital images were analyzed with Image-Pro Plus software. The average fluorescent intensity of intracellular areas was measured to index the ROS level.

1.2.3 Intracellular NO detection. The intracellular NO level ($[NO]_i$) was measured by a NO-sensitive fluorescence probe DAF-FM DA by confocal microscopy as described previously[30]. Cells incubated with or without anthocyanin as above were loaded with DAF-FM DA (10 μmol/L) at 37℃ for 30 min in Krebs-Ringer solution (100 mmol/L NaCl, 2.6 mmol/L KCl, 25 mmol/L $NaHCO_3$, 1.2 mmol/L $MgSO_4$, 1.2 mmol/L KH_2PO_4 and 11 mmol/L glucose). After washed three times, cells were incubated for another 30 min to ensure complete cleavage of DAF-FM DA by the intracellular ester enzyme that releases the NO-sensitive probe (DAF-FM). Fluorescence was detected with a laser scanning confocal microscope (FV 500, Olympus, Japan). The digital images were analyzed with Image-Pro Plus software. The average fluorescent intensity of intracellular areas was measured to index the NO level.

1.2.4 Western blot. About 1×10^7 cells were collected. Cell lysates were prepared in 200 μl RIPA buffer containing 50 mmol/L Tris-HCl, pH 7.4, 150 mmol/L NaCl, 0.5% Nonidet P-40 and 1 mmol/L each of EDTA, EGTA, phenylmethyslysulfonyl fluoride and Na_3VO_4, and 10 mg/L each of the protease inhibitors

leupeptin, aprotinin and pepstatin then sonicated for 10 s on ice. The lysates were then centrifuged at 10 000 r/min for 15 min at 4℃. The protein content of the supernatants was determined with the Bio-Rad protein assay reagent (bicinchoninic acid). Then the samples were mixed with 4× sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer (60 mmol/L Tris, pH 6.8, 10% glycerol, 2% SDS, 10% β-mercaptoethanol and 0.005% bromophenol blue) and boiled for 5 min at 100℃. The supernatants used for immunoblotting proteins were separated by 10% SDS-PAGE and electrotransferred onto nitrocellulose membrane. Immunoblots were analyzed using specific primary antibodies. After washing three times with TBST, the nitrocellulose membrane was incubated with secondary anti-rabbit antibody conjugated with alkaline phosphatase which reacts with BCIP/NBT substrate. The bands on the membrane were scanned, and then analyzed using the Pro-Plus imaging software.

1.2.5 Statistical analysis. All data are represented as $\bar{x} \pm s$. The data were analyzed by a one-way ANOVA. $P < 0.05$ was considered to be statistically significant.

2 Results

2.1 Anthocyanin decreases the vulnerability of N2a/Swe.Δ9 cells

As reported before [15], N2a/Swe.Δ9 cells show more vulnerability than N2a/wt ones indicated by MTT evaluated cell viability after exposure to H_2O_2. Here in N2a/Swe.Δ9 cells, we examined the protective effect of anthocyanin by measuring cell viability after exposure to H_2O_2. Cells were incubated with 100 or 200 μmol/L anthocyanin for 24 h and then exposed to 50 μmol/L H_2O_2 for another 24 h. The results of MTT assay showed that H_2O_2 caused significant decrease in cell viability to (41.2 ± 3.0)% in N2a/Swe.Δ9 cells. When pretreated with 100 and 200 μmol/L anthocyanin for 24 h, cell viability was increased to (64.4 ± 4.5)% and (54.6 ± 3.0)% respectively (Figure 1), which suggested the anthocyanin can effectively prevent the damage of oxidative stress induced by H_2O_2. The results showed that when the concentration of anthocyanin was increased to 200 μmol/L, it will show a little toxicity to cells and decreased ability in cell protection from H_2O_2. The results of MTT assay showed that anthocyanin could protect cells against H_2O_2 induced damage and effectively decrease the vulnerability of N2a/Swe.Δ9.

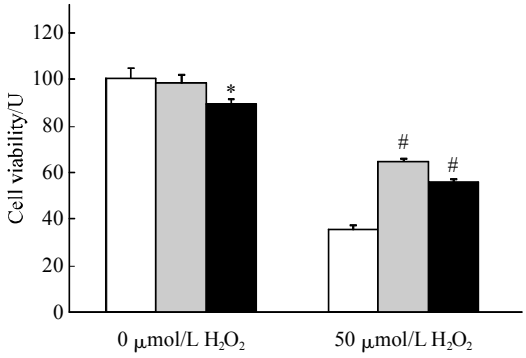

Fig. 1 Protective effects of anthocyanin on vulnerability of N2a/Swe.Δ9 cells to H_2O_2 measured with MTT assay

N2a/Swe.Δ9 cells were pretreated with anthocyanin for 24 h and further treated with H_2O_2 for 24 h. Cell viability was assessed by MTT assay. The result showed that anthocyanin can effectively improve the cell viability against H_2O_2. □: 0 μmol/L anthocyanin; ▨: 100 μmol/L anthocyanin; ■: 200 μmol/L anthocyanin. $^{*\#}P < 0.05$, significantly different from the control group.

2.2 Anthocyanin decreases the intracellular ROS of N2a/Swe.Δ9 cells

As shown in Figure 2, the level of ROS in N2a/Swe.Δ9 cells was about 2 times higher than that in N2a/wt cells. To investigate whether anthocyanin can block the abnormal increased production of ROS in N2a/Swe.Δ9 cells, we measured the intracellular ROS levels in cells treated with or without anthocyanin using the MDCFH-DA fluorescent dye. It was demonstrated that treatment of N2a/Swe.Δ9 cells with 100 or 200 μmol/L anthocyanin can significantly decrease intracellular ROS level (Figure 2). When the N2a/Swe.Δ9 cells were treated with 200 μmol/L anthocyanin, the intracellular ROS level in N2a/Swe.Δ9 cells was even lower than the level in N2a/wt cells. Thus, anthocyanin can effectively decrease the ROS accumulation in N2a/Swe.Δ9 cells at proper concentration.

2.3 Anthocyanin decreases the intracellular NO of N2a/Swe.Δ9 cells

$[NO]_i$ is an important parameter of intracellular oxidative stress. $[NO]_i$ was measured here as an indicator of oxidative stress with a NO probe named DAF-FM DA. The results demonstrated the level of $[NO]_i$ in N2a/Swe.Δ9 cells was about 2 times higher than that in N2a/wt cells. Anthocyanin was used to treat the N2a/Swe.Δ9 cells for 48 h, then the $[NO]_i$ was measured. The results showed that, similar to the ROS results, the $[NO]_i$ was significantly depressed when the

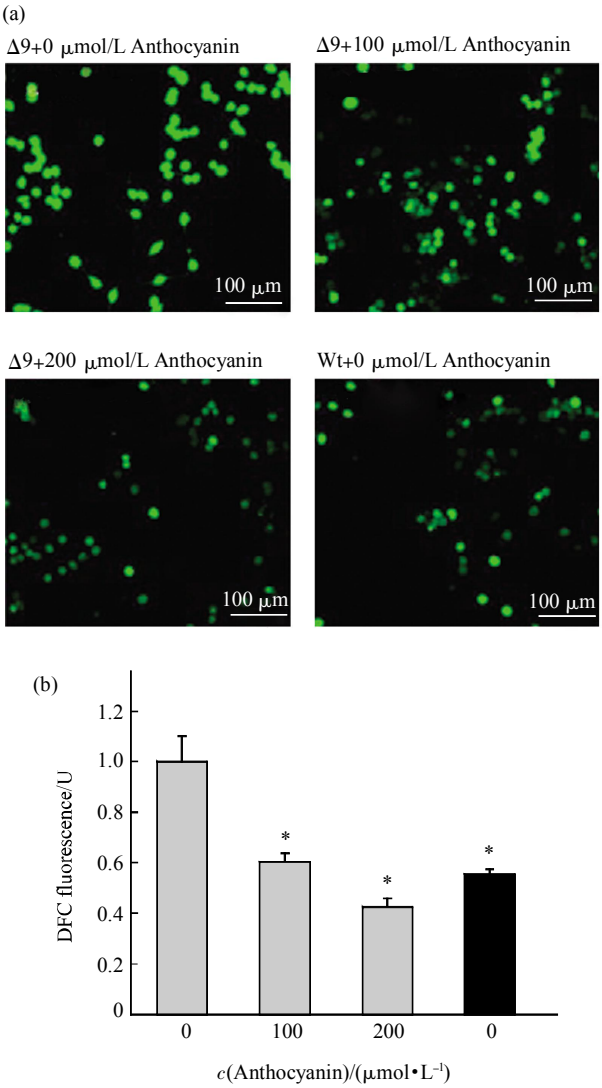

Fig. 2　Protective effects of anthocyanin on endogenous Aβ induced ROS in N2a/Swe.Δ9 cells

N2a/Swe.Δ9 cells were treated with anthocyanin for 48 h. ROS production was measured by the MDCFH-DA fluorescent dye. (a) Representative confocal microscopic images of DFC fluorescence in N2a/Swe.Δ9 and N2a/wt cells with or without anthocyanin. (b) The relative units of DFC fluorescence in cells with or without anthocyanin treatment according to the confocal images. The result showed that anthocyanin can decrease the ROS in N2a/Swe.Δ9 cells. □: Δ9; ■: wt. *$P < 0.05$, significantly different from the non-treated cells. Scale bar = 100 μm.

N2a/Swe.Δ9 cells were treated with anthocyanin in N2a/Swe.Δ9 cells (Figure 3). When the cells were treated with 200 μmol/L anthocyanin, the level of $[NO]_i$ was lower than that in N2a/wt cells. Thus, anthocyanin of proper concentration can effectively decrease the $[NO]_i$ in N2a/Swe.Δ9 cells. Combined with the cell viability and the ROS results, it seems that the appropriate concentration would be about 100 μmol/L and at this concentration, anthocyanin can significantly decrease the vulnerability as well as the abnormal ROS and $[NO]_i$ accumulation in N2a/Swe.Δ9 cells. Therefore, anthocyanin would be a promising candidate against abnormally increased oxidative stress for AD treatment.

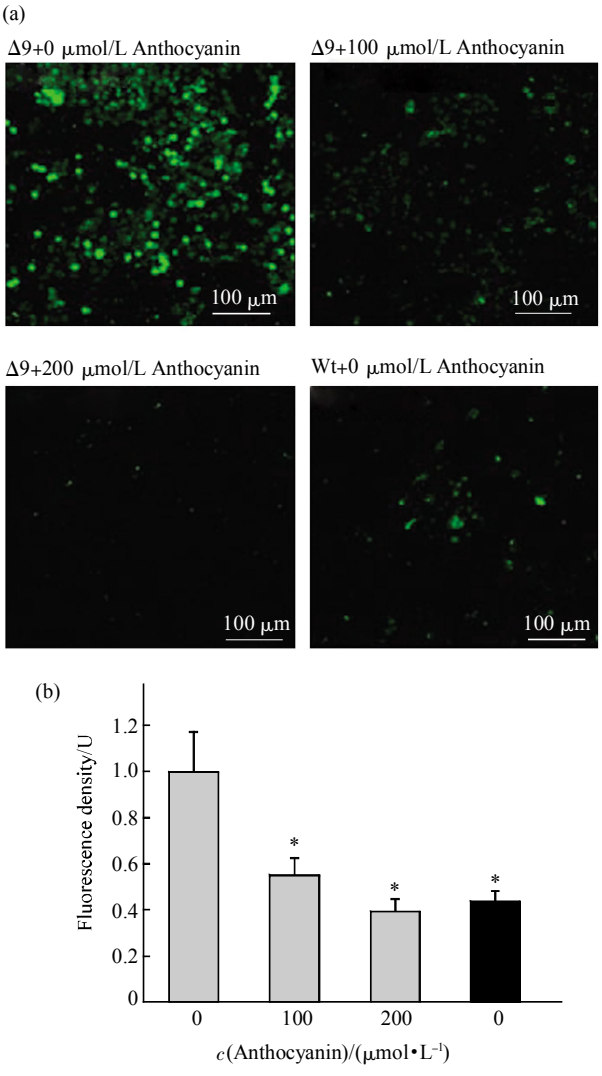

Fig. 3　Protective effects of anthocyanin on endogenous Aβ induced $[NO]_i$ in N2a/Swe.Δ9 cells

N2a/Swe.Δ9 cells were treated with anthocyanin for 48 h. $[NO]_i$ production was measured by the DAF-FM DA fluorescent dye. (a) Representative confocal microscopic fluorescence images of N2a/Swe.Δ9 and N2a/wt cells with or without anthocyanin treatment. (b) The relative units of fluorescence intensity in cells with or without anthocyanin treatment measured by the confocal images. The result showed that anthocyanin can decrease the $[NO]_i$ in N2a/Swe.Δ9 cells. □: Δ9; ■: wt. *$P < 0.05$ significantly different from the non-treated cells. Scale bar = 100 μm.

2.4 Anthocyanin protects cells against oxidative stress by depressing the JNK activation

In the following experiments, the possible mechanism on how anthocyanin protects the N2a/Swe.Δ9 cells from oxidative stress was investigated. It was demonstrated that N2a/Swe.Δ9 cells suffered increased oxidative stress of ROS and $[NO]_i$, thus the activated JNK was increased, compared with N2a/wt cells. Expression of pJNK, the activated JNK, was detected by Western blot analysis in N2a/Swe.Δ9 cells with or without anthocyanin pretreatment. Total JNK was loaded as control and N2a/wt cells were used as control. The result was shown in Figure 4. 100 μmol/L anthocyanin can effectively decrease the activated JNK in N2a/Swe.Δ9 cells, which suggested that anthocyanin exerts its protective effect by modulating the JNK signal pathway.

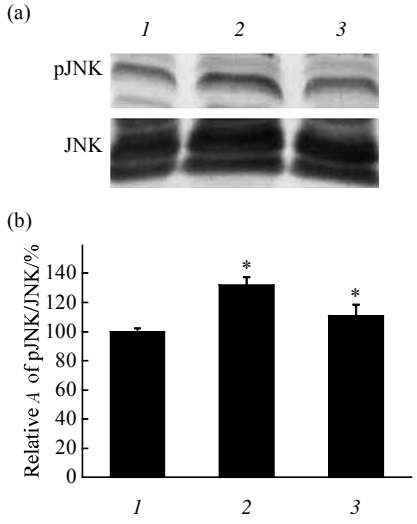

Fig. 4 Anthocyanin can depress the activation of JNK

N2a/wt and N2a/Swe.Δ9 cells treated with or without anthocyanin were analyzed by Western blot with antibody against pJNK and JNK. (a) pJNK and JNK expression in cells treated with or without anthocyanin. (b) The relative optical intensity of pJNK/JNK in N2a/Swe.Δ9 and N2a/wt cells. The result showed that anthocyanin can decrease the pJNK activated by oxidative stress in N2a/Swe.Δ9. $*P < 0.05$ significantly different from the pre-adjacent group. *1*: wt; *2*: Swe.Δ9; *3*: Swe.Δ9+ Anthocyanin.

3 Discussion

Elevated oxidative stress in AD brain has been reported by many researches in cell models and in transgenic mouse models [31]. Therefore, decreasing oxidative stress became a target for alternative therapy of AD. The present study is aimed to investigate whether anthocyanin can protect the N2a/Swe.Δ9 cells by decreasing the oxidative stress.

AD model cells used here were N2a/Swe.Δ9 cells. Genetic mutations in genes such as APP, presenilin-1 or presenilin-2 have been reported to cause early onset of AD [32]. There have been some transgenic cells used as AD model cells such as cells stably expressing APP695 (N2a/APP695), cells expressing human Swedish mutation (K670M/N671L) APP695 gene (N2a/APPswe), cells cotransfected with APP695 harboring the Swedish mutant and wt human PS1 or mutants (N2a/Swe.Δ9). We chose the N2a/Swe.Δ9 cells as cell models here because among these model cells, the N2a/Swe.Δ9 cells were suffering the highest level of endogenous Aβ and severest oxidative stress [15]. The cell model is considerably reliable as it is a stably line. N2a cells transfected with empty plasmid were used as wild type control.

In the present study, it was shown that anthocyanin can exert its protective effects against endogenous Aβ induced oxidative stress. Pretreatment with anthocyanin can significantly decrease the cell vulnerability, intracellular ROS and $[NO]_i$ in N2a/Swe.Δ9 cells. Then we investigated whether JNK signal pathway was involved in the protection against oxidative stress, considering that activation of JNK is a key consequence of oxidative stress in cells [33]. It was shown from the results that pretreatment with anthocyanin can inhibit the excessive activation of JNK in N2a/Swe.Δ9 cells, which provides a clue for clarifying mechanism of the protective effects.

In this study, it was found that anthocyanin can also protect N2a/wt cells against H_2O_2, but the protect effect was less remarkable on N2a/wt cells than that on N2a/Swe.Δ9 cells. The ROS and $[NO]_i$ were not decreased much in N2a/wt cells after anthocyanin treatment (data not shown). As reported before [15], N2a/Swe.Δ9 cells demonstrate more vulnerability, intracellular ROS and $[NO]_i$ than N2a/wt cells. Therefore, we can conclude that anthocyanin can effectively decrease the abnormally increased oxidative stress in N2a/Swe.Δ9 cells while it does not affect the normal ROS and $[NO]_i$ much in N2a/wt cells. Anthocyanin can protect particularly the N2a/Swe.Δ9 cells from oxidative injure induced by the AD pathological factors.

On the other hand, it was also shown that when the N2a/Swe.Δ9 cells were pretreated with a relative

high concentration of anthocyanin, some side-effects were observed. As shown in Figure 1, the cell viability of N2a/Swe.Δ9 cells was decreased when treated with 200 μmol/L anthocyanin. Furthermore, 200 μmol/L anthocyanin treatment protected the cells against H_2O_2 less. When treated with excessive anthocyanin, N2a/Swe.Δ9 cells showed decreased ROS and $[NO]_i$ which were even less than N2a/wt cells, which may not be an optimal condition for the cells. ROS play their important physiological role in regulating cell signal, programmed cell death, stress response and so on[34]. All theses results demonstrated that anthocyanin can effectively protect the N2a/Swe.Δ9 cells against oxidative stress, while excessive anthocyanin could be a little toxic. The moderate dose would be around 100 μmol/L for our model cells. The proper dose might be different for various AD model cells. For practical application, the dose should be carefully determined by further systematic experiments.

In conclusion, this study demonstrated that anthocyanin, as an effective antioxidant extracted from plants, can suppress the oxidative stress induced by AD pathology. The protective effect was exerted by its ability in decreasing vulnerability, intracellular ROS and $[NO]_i$. Mechanism of the protection is involved in the inhibition of activated JNK induced by oxidative stress. However, the concentration of anthocyanin administered should be determined carefully. In summary, anthocyanin is promising as an oxidative stress defender and a candidate for alternative therapy of AD.

Acknowledgements We thank Dr. Xu HX from the Burnham Institute for providing the N2a cell lines.

References

[1] Yankner B A. The pathogenesis of Alzheimer′s disease: is amyloid beta protein the beginning or the end?. Ann N Y Acad Sci, 2000, **924**: 26−28

[2] Goedert M, Spillantini M G. A century of Alzheimer′s disease. Science, 2006, **314**(5800): 777−781

[3] Roher A, Wolfe D, Palutke M, *et al*. Purification, ultrastructure, and chemical analysis of Alzheimer′s disease amyloid plaque core protein. Proc Natl Acad Sci USA, 1986, **83**(8): 2662−2666

[4] Hardy J, Selkoe D J. The amyloid hypothesis of Alzheimer′s disease: progress and problems on the road to therapeutics. Science, 2002, **297**(5580): 353−356

[5] Lin H, Bhatia R, Lal R. Amyloid beta protein forms ion channels: implications for Alzheimer′s disease pathophysiology. FASEB J, 2001, **15**(13): 2433−2444

[6] Ma Q L, Yang F, Rosario E R, *et al*. Beta-amyloid oligomers induce phosphorylation of tau and inactivation of insulin receptor substrate *via* c-Jun N-terminal kinase signaling: suppression by omega-3 fatty acids and curcumin. J Neurosci, 2009, **29**(28): 9078−9089

[7] Tseng B P, Kitazawa M, LaFerla F M. Amyloid beta-peptide: the inside story. Curr Alzheimer Res, 2004, **1**(4): 231−239

[8] Butterfield D A, Drake J, Pocernich C, *et al*. Evidence of oxidative damage in Alzheimer's disease brain: central role for amyloid β-peptide. Trends Mol Med, 2001, **7**(12): 548−554

[9] Butterfield D A, Lauderback C M. Lipid peroxidation and protein oxidation in Alzheimer′s disease brain: potential causes and consequences involving amyloid beta-peptide-associated free radical oxidative stress. Free Radic Biol Med, 2002, **32**(11): 1050−1060

[10] Butterfield D A. Amyloid beta-peptide (1-42)-induced oxidative stress and neurotoxicity: implications for neurodegeneration in Alzheimer′s disease brain. Free Radic Res, 2002, **36**(12): 1307−1313

[11] Hensley K, Carney J M, Mattson M P, *et al*. A model for beta-amyloid aggregation and neurotoxicity based on free radical generation by the peptide: relevance to Alzheimer disease. Proc Natl Acad Sci USA, 1994, **91**(8): 3270−3274

[12] Chauhan V, Chauhan A. Oxidative stress in Alzheimer's disease. Pathophysiology, 2006, **13**(3): 195−208

[13] Resende R, Moreira P I, Proenca T, *et al*. Brain oxidative stress in a triple-transgenic mouse model of Alzheimer disease. Free Radic Biol Med, 2008, **44**(12): 2051−2057

[14] Markesbery W R. The role of oxidative stress in Alzheimer disease. Arch Neurol, 1999, **56**(12): 1449−1452

[15] Sheng B, Gong K, Niu Y, *et al*. Inhibition of γ-secretase activity reduces Aβ production, reduces oxidative stress, increases mitochondrial activity and leads to reduced vulnerability to apoptosis: Implications for the treatment of Alzheimer′s disease. Free Radic Biol Med, 2009, **46**(10): 1362−1375

[16] Butterfield D A, Reed T, Newman S F, *et al*. Roles of amyloid β-peptideassociated oxidative stress and brain protein modifications in the pathogenesis of Alzheimer′s disease and mild cognitive impairment. Free Radic Biol Med, 2007, **43**(5): 658−677

[17] Pensalfini A, Cecchi C, Zampagni M, *et al*. Protective effect of new S-acylglutathione derivatives against amyloid-induced oxidative stress. Free Radic Biol Med, 2008, **44**(8): 1624−1636

[18] Zhang H Y, Liu Y H, Wang H Q, *et al*. Puerarin protects PC12 cells against b-amyloid-induced cell injury. Cell Biol Int, 2008, **32**(10): 1230−1237

[19] Wadsworth T L, Bishop J A, Pappu A S, *et al*. Evaluation of coenzyme Q as an antioxidant strategy for Alzheimer's disease. J Alzheimers Dis, 2008, **14**(2): 225−234

[20] Rahman M M, Ichiyanagi T, Komiyama T, *et al*. Effects of anthocyanins on psychological stress-induced oxidative stress and neurotransmitter status. J Agric Food Chem, 2008, **56**(16): 7545−7550

[21] Mazza G, Miniati E. Anthocyanins in Fruits, Vegetables and Grains.

USA: CRC Press Inc, 1993: 362−364
[22] Wang H, Cao G, Prior R L. Oxygen radical absorbing capacity of anthocyanins. J Agric Food Chem, 1997, **45**(2): 304−309
[23] Narayan M S, Naidu K A, Ravishankar G A, *et al*. Antioxidant effect of anthocyanin on enzymatic and non-enzymatic lipid peroxidation. Prostaglandins Leukot Essent Fatty Acids, 1999, **60**(1): 1−4
[24] Galli R L, Shukitt-Hale B, Youdim K A, *et al*. Fruit polyphenolics and brain aging: nutritional interventions targeting age-related neuronal and behavioral deficits. Ann N Y Acad Sci, 2002, **959**: 128−132
[25] Wang J, Mazza G. Inhibitory effects of anthocyanins and other phenolic compounds on nitric oxide production in LPS/IFN gamma activated RAW 264.7 macrophages. J Agric Food Chem, 2002, **50**(4): 850−857
[26] Mazza G J. Anthocyanins and heart health. Ann Ist Super Sanita, 2007, **43**(4): 369−374
[27] Davis R J. Signal transduction by the JNK group of MAP kinases. Cell, 2000, **103**(2): 239−252
[28] Shen H M, Liu Z G. JNK signaling pathway is a key modulator in cell death mediated by reactive oxygen and nitrogen species. Free Radic Biol Med, 2006, **40**(6): 928−939
[29] Cai D, Leem J Y, Greenfield J P, *et al*. Presenilin-1 regulates intracellular trafficking and cell surface delivery of β-amyloid precursor protein. J Biol Chem, 2003, **278**(5): 3446−3454
[30] Liao X, Liu J M, Du L, *et al*. Nitric oxide signaling in stretch-induced apoptosis of neonatal rat cardiomyocytes. FASEB J, 2006, **20**(11): 1883−1885
[31] Aticò D. Evidence of Oxidative stress in Alzheimer′s disease brain and antioxidant therapy. Ann N Y Acad Sci, 2008, **1147**: 70−78
[32] Selkoe D J. Alzheimer's disease: genes, proteins, and therapy. Physiol Rev, 2001, **81**(2): 741−766
[33] McCubrey J A, Lahair M M, Franklin R A. Reactive oxygen species-induced activation of the MAP kinase signaling pathways. Antioxid Redox Signal, 2006, **8**(9−10): 1775−1789
[34] Laloi C, Apel K, Danon A. Reactive oxygen signalling: the latest news. Curr Opin Plant Biol, 2004, **7**(3): 323−328

花青素能减轻阿尔茨海默症模型细胞的氧化损伤 *

刘玲玲 盛柏杨 闫玉芳 龚 锴 马 拓 赵南明 张秀芳 公衍道 **
(清华大学生命科学学院，生物膜与膜生物工程国家重点实验室，北京 100084)

摘要 氧化胁迫在阿尔茨海默症(AD)的发病过程中起重要作用．花青素是一种广泛存在于植物中的黄酮类物质．实验结果表明，100 μmol/L 的花青素可以有效地缓解 AD 模型细胞氧化胁迫，具体表现为，降低 AD 模型细胞的 H_2O_2 易感性，减少胞内 ROS 和$[NO]_i$．c-Jun 氨基端激酶(JNK)是氧化胁迫导致细胞损伤的重要信号途径．实验结果显示花青素可以有效地抑制氧化胁迫对 JNK 的激活．提示花青素的抗氧化胁迫作用与 JNK 信号途径有关．因此，花青素可以作为一种氧化胁迫因子的清除剂来保护 AD 模型细胞，有望用于 AD 的辅助治疗．

关键词 阿尔茨海默症，氧化胁迫，花青素，c-Jun 氨基端激酶(JNK)
学科分类号 Q6，R338，Q189 **DOI:** 10.3724/SP.J.1206.2009.00773

* 清华 - 裕元医学科学基金(20240000514)和生物膜与膜生物工程国家重点实验室资助项目．
** 通讯联系人．
Tel: 010-62785049, E-mail: gongyd@tsinghua.edu.cn
收稿日期：2009-12-31，接受日期：2010-03-18