



Neuroprotective effects of cyanidin 3-O-glucopyranoside on amyloid beta (25–35) oligomer-induced toxicity

Andrea Tarozzi^{a,*}, Fabiana Morroni^a, Adriana Merlicco^a, Cecilia Bolondi^a, Gabriella Teti^b, Mirella Falconi^b, Giorgio Cantelli-Forti^a, Patrizia Hrelia^a

^a Department of Pharmacology, Alma Mater Studiorum, University of Bologna, Via Irnerio 48, 40126 Bologna, Italy

^b Department of Anatomical Sciences, Alma Mater Studiorum, University of Bologna, 40126 Bologna, Italy

ARTICLE INFO

Article history:

Received 10 December 2009

Received in revised form 19 January 2010

Accepted 2 February 2010

Keywords:

Amyloid beta

Oligomers

Plasma membrane damage

Redox status impairment

Cyanidin 3-O-glucopyranoside

Neuroprotection

ABSTRACT

Recent studies suggest that the oligomers of short amyloid beta (A β) peptides such as A β _{25–35} as well as full-length A β peptides (i.e. A β _{1–40} and A β _{1–42} peptides) are responsible for synaptic dysfunction and/or neuronal loss in Alzheimer's disease (AD). Among antioxidant phytochemicals derived from fruit and vegetables, cyanidin 3-O-glucoside (Cy-3G) has recently gained attention for its neuroprotective properties. In this *in vitro* study, we demonstrated that Cy-3G can inhibit A β _{25–35} spontaneous aggregation into oligomers and their neurotoxicity in human neuronal SH-SY5Y cells. In particular, the pre- and co-treatment of SH-SY5Y cells with Cy-3G reduced the neuronal death, in terms of apoptosis and necrosis, elicited by A β _{25–35} oligomers. Cy-3G also shows the interesting ability to prevent the early events leading to neuronal death such as the A β _{25–35} oligomer binding to plasma membrane and the subsequent membrane integrity loss. Taken together, these findings suggest that Cy-3G may be considered a phytochemical with neuroprotective properties useful in finding potential drug or food supplements for the therapy of AD.

© 2010 Elsevier Ireland Ltd. All rights reserved.

The extra- and intra-neuronal aggregation and deposition of amyloid beta (A β) peptides play a causal role in the pathogenetic cascade leading to Alzheimer's disease (AD) [11,14]. It is accepted that the A β _{1–40} and A β _{1–42} peptides, produced by the cleavage of the amyloid precursor protein, can exist in multiple aggregation forms, including soluble oligomers or protofibrils and insoluble fibrils, which are responsible for various pathological effects [12]. Several studies suggest that the A β oligomers diffuse readily through the brain parenchyma and cause a selective synaptic dysfunction and/or neuronal loss in cortex and hippocampus, two stricken brain regions in AD [7]. A β oligomers adhere to the plasma membrane of neurons and cause lesions by a combination of radical species-initiated lipid peroxidation and formation of ion-permeable pores which initiate a cascade of pathological processes that end with neuronal death [18]. Among produced A β peptides, A β _{1–42} is more hydrophobic and more prone to oligomer and fibril formation than A β _{1–40}, and it is the predominant peptide found in extra-neuronal deposition [4]. However, the accumulation in AD brains of other A β peptides inclined to form soluble oligomers such as A β _{17–40/42} and A β _{25–35} has also been recorded [6,10]. In particular, A β _{25–35} is a short peptide generated by proteolysis of A β _{1–40} [10], showing neurotoxic and aggregation properties similar to full-length pep-

tides (i.e. A β _{1–40} and A β _{1–42} peptides). Recent studies show that the soluble oligomeric forms of A β _{25–35} may exert neurotoxicity either directly or indirectly through promoting the formation of neurotoxic oligomeric forms of A β _{1–40} [13] and suggest their potential critical role as well as A β _{1–42} oligomers for AD pathology. In this regard, a particularly attractive AD-modifying therapeutic strategy is to selectively counteract soluble A β oligomers that have recently been shown to mediate neurotoxicity [22].

Recent studies have highlighted that a diet rich in antioxidant components such as anthocyanins may lower age-related cognitive decline and the risk of developing neurodegenerative diseases including AD [9]. Among anthocyanins, cyanidin 3-O-glucoside (Cy-3G), present in colored fruit and vegetables, has recently gained attention as a neuroprotective phytochemical. Our previous *in vitro* studies have shown the ability of Cy-3G to counteract the neurotoxicity induced by H₂O₂ and A β _{1–42} peptides in SH-SY5Y cells [20,21]. In addition, Cy-3G also reduced the cerebral ischemia and age-related neuronal deficits in rats, suggesting the ability of Cy-3G to cross the blood brain barrier and to deliver its neuroprotective capabilities centrally [1,19]. Up to now, there are no data available about the protective effects of Cy-3G against soluble A β _{25–35} oligomer neurotoxicity.

In this study we demonstrate the ability of Cy-3G to prevent the formation of soluble A β _{25–35} oligomers and their neurotoxicity in terms of membrane integrity loss, redox status impairment and cell death in a human neuronal cell line (SH-SY5Y).

* Corresponding author. Tel.: +39 051 2091795; fax: +39 051 248862.

E-mail address: andrea.tarozzi@unibo.it (A. Tarozzi).

Cy-3G was purchased from Polyphenols AS Laboratories (Sandnes, Norway). A β_{25-35} peptide, Congo Red (CR), 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) and Thioflavin T (Th-T) were obtained from Sigma Chemical Co. (St. Louis, MO). All other reagents were of the highest grade of purity commercially available.

A β_{25-35} peptides were first dissolved in hexafluoroisopropanol to 1 mg/ml, sonicated, incubated at room temperature for 24 h and lyophilized. The resulting unaggregated A β_{25-35} film was dissolved with dimethylsulfoxide (DMSO) to a final concentration of 1 mM and stored at -20°C until use. To make oligomers, A β_{25-35} stock in DMSO was diluted into phosphate buffer saline (PBS) at 40 μM and incubated at 4°C for 48 h [8,15]. To generate fibrils, A β_{25-35} stock was diluted into Dulbecco's modified Eagle's medium (DMEM) at 40 μM and incubated at 37°C for 120 h [20].

To analyze the morphology of the oligomeric and fibrillar A β_{25-35} forms, Transmission Electron Microscopy (TEM) was used as previously reported [20]. Briefly, aggregated A β_{25-35} solution in the absence or presence of Cy-3G was absorbed onto formvar-carbon coated grids (200 mesh size) for 40 min and stained with 2% aqueous phosphotungstic acid solution before viewing with a Philips CM10 transmission electron microscope at 80 kV.

To determine the spontaneous aggregation of A β_{25-35} peptide, the thioflavin T (Th-T) fluorescence method was performed as previously described [20]. Briefly, unaggregated A β_{25-35} peptide (40 μM) was incubated for 48 (at 4°C in PBS) and 120 h (at 37°C in DMEM), in the absence or presence of Cy-3G. After incubation, 50 μl of solution was added to Th-T 5 mM in a final volume of 1 ml of 50 mM glycine–NaOH buffer (pH 8.5). Fluorescence was monitored at $\lambda_{\text{excitation}} = 450 \text{ nm}$ and $\lambda_{\text{emission}} = 482 \text{ nm}$, respectively.

Human neuronal cell line (SH-SY5Y) was routinely grown at 37°C in a humidified incubator with 5% CO_2 in DMEM supplemented with 10% fetal bovine serum, 2 mM glutamine, 50 U/ml penicillin and 50 $\mu\text{g}/\text{ml}$ streptomycin. To determine neuronal viability, SH-SY5Y cells were seeded in 96-well plates at 3×10^4 cells/well, whereas to evaluate apoptosis and necrosis, the cells were seeded in 60-mm cultures dishes at 4×10^6 cells/dish. All experiments were performed after 24 h of incubation at 37°C in 5% CO_2 . To estimate the neuroprotective effects, the SH-SY5Y cells were treated with various concentrations of Cy-3G prior to (3 h) or during the different times of A β_{25-35} oligomer-treatment. Neuronal viability in terms of mitochondrial activity was evaluated with the colorimetric tetrazolium salt (MTT) assay, as previously described [21]. Briefly, after 3 h treatment with A β_{25-35} oligomers (5 μM), the SH-SY5Y cells were washed with PBS and then incubated with MTT (5 mg/ml) in PBS for 2 h. After removal of MTT and further washing, the formazan crystals were dissolved with isopropanol. The amount of formazan was measured ($\lambda = 570 \text{ nm}$; ref. $\lambda = 690 \text{ nm}$) with a spectrophotometer (Spectra model Classic, TECAN®, Männedorf, Switzerland). The mitochondrial activity is expressed as a percentage of control cells and calculated by the formula: (absorbance of treated neurons/absorbance of untreated neurons) $\times 100$. Apoptosis and necrosis, in terms of membrane phosphatidylserine exposure and loss of membrane integrity, respectively, were evaluated using the Annexin-V-FLUOS Staining Kit (Roche Diagnostics, Mannheim, Germany), according to the manufacturer's instructions. Briefly, after 24 h of treatment with A β_{25-35} oligomers (2 μM), SH-SY5Y cells were scraped, suspended at $2.5 \times 10^5/\text{ml}$, and washed with PBS. The cells were incubated with Annexin-V-FLUOS labeling solution at 24°C in the dark for 15 min. To determine the percentage of stained cells, four randomly selected areas with 50–100 cells in each were examined under a fluorescence microscope (Zeiss Axio Imager M1, Oberkochen, Germany) at $\lambda_{\text{excitation}} = 488 \text{ nm}$ and $\lambda_{\text{emission}} = 518$ and 617 nm for Annexin-V and propidium iodide (PI), respectively. The values are expressed as a percentage of apoptotic and necrotic cells and cal-

culated by the formula: (Annexin-V or PI-positive cells/ n total cells) $\times 100$.

A β binding to the cell surface was analyzed as previously described [3]. SH5Y5Y cells were seeded in 96-well plates at 5×10^3 cells/well for 24 h. After a 3 h treatment with Cy-3G, the medium was changed with a fresh one with A β_{25-35} (10 μM) and incubated for 10, 20 and 30 min and then washed twice with PBS. The residual A β_{25-35} oligomer–cell complex was stained with 1 μM CR in PBS for 20 min and measured with a spectrophotometer (Spectra model Classic, TECAN®) at 550 nm (bound CR). CR values were reported as percent increases in treated cells vs. untreated cells (taken as 100%).

To analyze the morphology of SH5Y5Y cells, Scanning Electron Microscopes (SEM) was used. SH5Y5Y cells were seeded on silicon chips in 6-well plates at $1.5 \times 10^3/\text{well}$ for 24 h. After 3 h treatment with Cy-3G, the medium was changed with a fresh one with A β_{25-35} oligomers (5 μM) and incubated for 3 h. Following each treatment, silicon chips were washed with 0.15 M phosphate buffer and fixed with 1% glutaraldehyde in 0.1 M phosphate buffer for 2 h at 4°C . After being washed in phosphate buffer, specimens were post fixed with a solution of 1% osmium tetroxide in 0.1 M phosphate buffer for 1 h at room temperature, dehydrated in an increasing ethanol series and critical-point-dried (Critical point dryer CPD 030, Bal-Tec AG, Lichtenstein). Samples were platinum metal-coated using a sputter (MED 010 Balzers, Lichtenstein). The analysis was carried out with a SEM Jeol JSM 890 (Jeol Ltd., Tokyo, Japan) at 7 kV accelerating voltage and $1 \times 10^{-11} \text{ A}$ probe current.

The cellular redox status in terms of intracellular ROS formation was determined using the fluorescent probe DCFH-DA. SH-SY5Y cells were seeded in 8-well culture slides at 1×10^4 cells/well for 24 h. After a 3 h treatment with Cy-3G, the medium was changed with a fresh one with A β_{25-35} oligomers (2 μM) and incubated for 3 h. The SH-SY5Y cells were then washed and incubated with DCFH-DA (5 μM) for 15 min in the dark. After removal of the probe, the cells were washed and incubated with serum-free DMEM for 1 h at 37°C . Intracellular ROS formation was performed under an Axio Imager M1 Zeiss motorized microscope with an AxioVision image recording system computer. Fluorescence images were captured through a FITC filter. Four randomly selected areas with 50–100 cells in each one were analyzed and the values obtained are expressed as densitometry/cell.

Data are reported as mean \pm SEM of at least 3 independent experiments. Statistical analysis was performed using one-way ANOVA with Dunnett post hoc test and Student's t -test, as appropriate. Differences were considered significant at $p < 0.05$. Analyses were performed using PRISM 3 software on a Windows platform.

We first determined whether Cy-3G can inhibit the spontaneous aggregation of A β_{25-35} by using the Th-T assay and TEM. As shown in Fig. 1a and b, the increases of Th-T fluorescence emission recorded after 48 and 120 h spontaneous aggregation of the A β_{25-35} peptide stand for the circular bodies and ribbon-like fibrils formations, respectively. In particular, A β oligomers showed a diameter of around 20 nm corresponding to high molecular weight oligomers [8]. In same experimental conditions, the addition of 50 μM Cy-3G at the beginning of the aggregation process strongly reduced both the Th-T fluorescence increase and the high number of A β_{25-35} oligomers and fibrils (Fig. 1a and b). These results show the ability of Cy-3G to prevent the formation of A β_{25-35} oligomers and their shift into fibrillar structures. Regarding these findings, it is plausible that Cy-3G interacts directly with A β_{25-35} peptide. Cy-3G has a polyphenol structure with a high number of hydroxyl groups which could form hydrogen bonds with donor/acceptor groups of amino acid residues present in A β_{25-35} peptide (i.e. Ile31, Ile32 and Met35) that are critical for its aggregation and subsequent neurotoxicity [17].

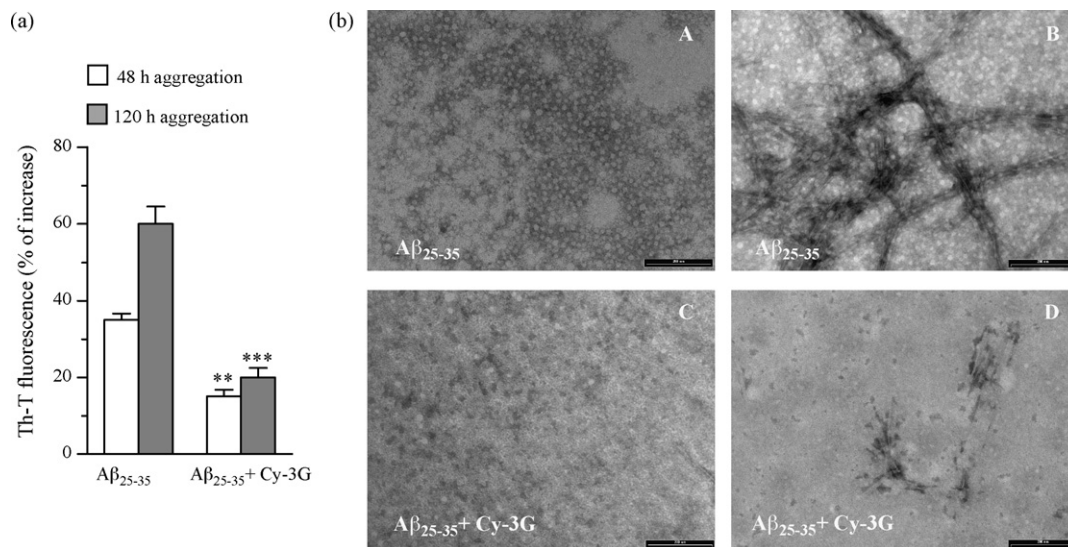


Fig. 1. Cy-3G inhibits Aβ₂₅₋₃₅ peptide spontaneous aggregation into oligomers and fibrils. (a) Unaggregated Aβ₂₅₋₃₅ peptide (40 μM) was incubated for 48 (at 4 °C in PBS) or 120 h (at 37 °C in DMEM), in the absence or presence of Cy-3G (50 μM). After incubation, the solutions were added to Th-T and the fluorescence was monitored. Values are shown as mean ± SEM of three independent experiments (**P* < 0.01, ****P* < 0.001 vs. untreated cells at Student's *t*-test). (b) TEM micrographs of Aβ₂₅₋₃₅ oligomers (A and C) and fibrils (B and D) obtained after 48 and 120 h of Aβ₂₅₋₃₅ spontaneous aggregation, respectively, in the absence or presence of Cy-3G (50 μM). Total magnification: ×92,000; scale bar = 200 nm.

We thus examined the protective effects of Cy-3G against Aβ₂₅₋₃₅ oligomer-induced neurotoxicity in terms of neuronal viability loss in SH-SY5Y cells by using the MTT assay. The SH-SY5Y cells were treated with Cy-3G (25–100 μM) either prior to (3 h) or during the Aβ-treatment for 3 h (5 μM). Preliminary experiments showed that the SH-SY5Y cell treatment with Cy-3G concentrations up to 100 μM for 24 h, in the absence of Aβ₂₅₋₃₅ oligomers, did not affect the neuronal viability (data not shown). As reported in Fig. 2a, both the pre- and co-treatment of SH-SY5Y cells with Cy-3G led to a significant decrease of Aβ₂₅₋₃₅ oligomer toxicity at 50 and 100 μM. The cytoprotective effects of Cy-3G 100 μM were also confirmed by morphological analysis with SEM. As shown in Supplementary Fig. a, the co-treatment of SH-SY5Y cells with Cy-3G partly sustained their original cell integrity. To support the observed protective effects of Cy-3G in SH-SY5Y cells, we also evaluated the neuronal death, in terms of apoptosis and necrosis, induced by 24 h treatment with Aβ₂₅₋₃₅ oligomers (2 μM) using Annexin-V/PI double staining. The pre-treatment of SH-SY5Y cells with Cy-3G 100 μM significantly reduced both the apoptotic (~38%) and necrotic cell (~44%) formation elicited by Aβ₂₅₋₃₅ oligomers (Fig. 2b). Comparable results were obtained after co-treatment with Cy-3G 100 μM (data not shown). Taken together, these results demonstrate the ability of Cy-3G directly to counteract the neurotoxicity of Aβ₂₅₋₃₅ oligomers and strengthen the hypothesis that chemical interactions between Cy-3G and Aβ₂₅₋₃₅ peptide block the access of Aβ₂₅₋₃₅ peptide within the neuronal plasma membrane. In this regard, recent studies have suggested that soluble forms of Aβ₂₅₋₃₅ and Aβ₃₁₋₃₅ peptides could initiate a cascade of events leading to neurotoxicity only after their internalization within the neuronal cells [2]. In this context, it is also plausible that the neuroprotective effects observed after pre-treatment of SH-SY5Y cells with Cy-3G could be ascribed to its predominantly membrane incorporation [21]. In particular, the more hydrophilic polyphenols, such as Cy-3G, could create a barrier on the membrane through the formation of hydrogen bonds with the polar head groups at the lipid–water interface of the membrane and protect it from external aggressors including Aβ [16].

These findings prompted us to evaluate the ability of Cy-3G to prevent the binding between β₂₅₋₃₅ oligomers and plasma membrane surface, an action that triggers irreversible membrane

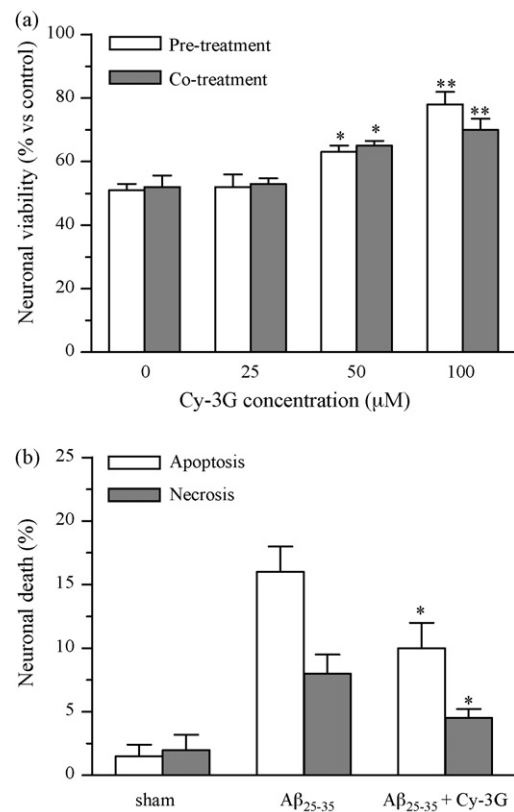


Fig. 2. Cy-3G protects SH-SY5Y cells against Aβ₂₅₋₃₅ oligomer-induced toxicity. (a) SH-SY5Y cells were treated with various concentrations of Cy-3G (3 h) prior to or during Aβ₂₅₋₃₅ oligomer-treatment (5 μM, 3 h). At the end of incubation the neuronal viability was determined by MTT assay and the results are expressed as a percentage of control cells. (b) SH-SY5Y cells were treated with Cy-3G (100 μM, 3 h) prior to the Aβ₂₅₋₃₅ oligomer-treatment (2 μM, 24 h). At the end of incubation the neuronal death was determined with Annexin-V and PI and the results are expressed as a percentage of apoptotic and necrotic cells. The values of (a) and (b) are shown as mean ± SEM of three/four independent experiments (**P* < 0.05, ***P* < 0.01 vs. untreated cells at ANOVA with Dunnett post hoc test and Student's *t*-test, as appropriate).

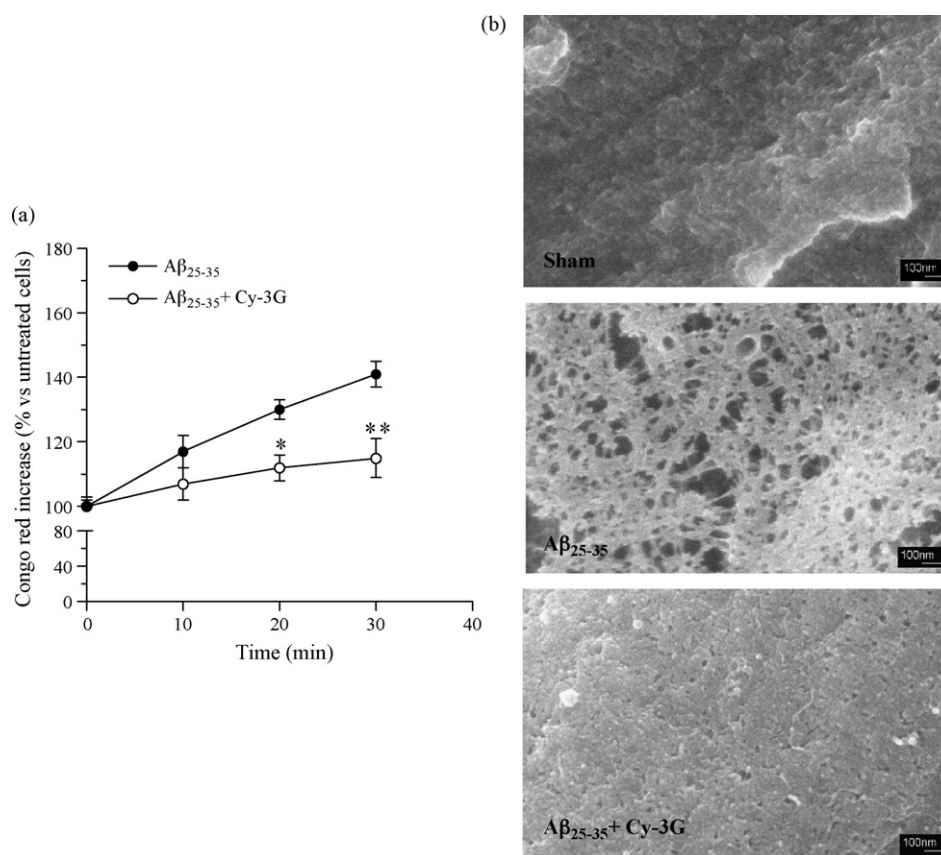


Fig. 3. Cy-3G inhibits the binding of the Aβ₂₅₋₃₅ oligomers with SH-SY5Y cells and preserves the membrane integrity. (a) SH-SY5Y cells were treated with Cy-3G (100 μM, 3 h) prior to the Aβ₂₅₋₃₅ oligomer-treatment for the indicated times (10 μM). At the end of incubation, the Aβ₂₅₋₃₅ oligomer binding to plasma membrane was determined by CR assay and the results are expressed as percentage of control cells. The values are shown as mean ± SEM of three independent experiments (* $P < 0.05$, ** $P < 0.01$ vs. untreated cells at Student's *t*-test). (b) SEM micrographs of plasma membrane of SH-SY5Y cells treated with Cy-3G (100 μM) prior to the Aβ₂₅₋₃₅ oligomer-treatment (5 μM, 3 h). Total magnification: ×70,000; scale bar = 100 nm.

alterations and initiates a sequence of pathological events leading to neuronal dysfunction and death [5]. The binding of Aβ₂₅₋₃₅ oligomers (10 μM) with SH-SY5Y cells reached a maximum in 30 min and was significantly reduced by pre-treatment of Cy-3G 100 μM, as determined by the CR assay (Fig. 3a). We also evaluated the morphological alteration of SH-SY5Y cell membranes elicited by β₂₅₋₃₅ oligomer binding (Fig. 3b). TEM micrographs showed that after 3 h of treatment with Aβ₂₅₋₃₅ oligomers, the plasma membrane architecture of the SH-SY5Y cells was markedly disrupted. In the same experimental conditions, the pre-treatment with Cy-3G preserved the membrane integrity of SH-SY5Y cells. In parallel, the impairment of SH-SY5Y cell redox status in terms of intracellular ROS formation evoked by β₂₅₋₃₅ oligomers (2 μM) was also investigated using DCFH-DA, a fluorescent probe. ROS formation has been directly related to membrane perturbation by soluble oligomers [5,18]. Remarkably, Cy-3G at 100 μM totally inhibited the Aβ₂₅₋₃₅ oligomer-induced ROS formation (Fig. 4).

These results provide the first evidence of the ability of Cy-3G to hinder the adsorption of Aβ₂₅₋₃₅ oligomers to plasma membrane as well as to prevent cellular membrane and redox status impairment. These findings could at least partly explain the observed antiapoptotic and antinecrotic effects of Cy-3G. In particular, Cy-3G could change the chemical and physical properties of SH-SY5Y cell membrane affecting Aβ binding and the pore formation with a concomitant increase in intracellular calcium. It is known that calcium overload is a proximate initiator of pathogenic pathways including ROS formation, altered signaling pathways, mitochondria dysfunction and following activation of neuronal death [5]. In this regard, the intrinsic antioxidant properties of Cy-3G may play a

marginal role for its membrane protective effects against the neuronal death induced by Aβ oligomers. The Cy-3G antioxidant effects observed could mainly be the consequence of its ability to prevent the interaction between Aβ oligomers and the lipid bilayer membrane, responsible for lipid peroxidation as well as activation of ROS cascade at cytosol level. These considerations are consistent with

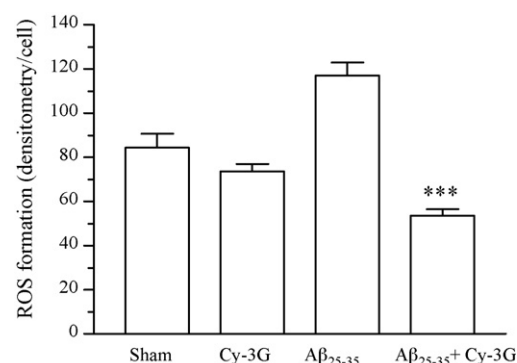


Fig. 4. Cy-3G counteracts Aβ₂₅₋₃₅ oligomer-induced intracellular ROS formation in SH-SY5Y cells. SH-SY5Y cells were treated with Cy-3G (100 μM, 3 h) prior to the Aβ₂₅₋₃₅ oligomer-treatment (2 μM, 3 h). At the end of incubation, ROS formation was determined using a fluorescent probe, DCFH-DA. Four randomly selected areas with 50–100 cells in each were analyzed under a fluorescence microscope and the values obtained are expressed as densitometry/cell. Values are shown as mean ± SEM of four independent experiments (***) $P < 0.001$ vs. untreated cells at Student's *t*-test). For representative images of ROS formation in SH-SY5Y cells see [Supplementary Fig. b](#).

our previously study that showed: (i) the absence of Cy-3G antioxidant activity at cytosol level suggesting a low uptake of Cy-3G into SH-SY5Y cells; (ii) the lack protective effects of Cy-3G against neuronal death induced by H₂O₂ an oxidant that easily crosses the membrane and induces oxidative damage at cytosol level [21].

Taken together, these findings show the ability of Cy-3G to counteract the neurotoxicity of A β _{25–35} oligomers, as well as that of A β _{1–42} oligomers already demonstrated in our previous study [20], suggesting its peculiar neuroprotective action against soluble oligomers regardless of the length of the A β peptides.

Acknowledgments

This work was supported by MIUR–COFIN 2007 (project: 2007AYMLFZ), –FIRB Piattaforme/Reti 2006 (project: RBPR05NWWC.002) and Fondazione del Monte di Bologna e Ravenna.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.neulet.2010.02.006.

References

- [1] C. Andres-Lacueva, B. Shukitt-Hale, R.L. Galli, O. Jauregui, R.M. Lamuela-Raventos, J.A. Joseph, Anthocyanins in aged blueberry-fed rats are found centrally and may enhance memory, *Nutr. Neurosci.* 8 (2005) 111–120.
- [2] M.E. Clementi, S. Marini, M. Coletta, F. Orsini, B. Giardina, F. Misiti, A β _{25–35} and A β _{25–35} fragments of amyloid beta-protein induce cellular death through apoptotic signals: role of the redox state of methionine-35, *FEBS Lett.* 579 (2005) 2913–2918.
- [3] Z. Datki, R. Papp, D. Zádori, K. Soós, L. Fülöp, A. Juhász, G. Laskay, C. Hetényi, E. Mihalik, M. Zarándi, B. Penke, In vitro model of neurotoxicity of A β _{1–42} and neuroprotection by a pentapeptide: irreversible events during the first hour, *Neurobiol. Dis.* 17 (2004) 507–515.
- [4] M.A. Findeis, The role of amyloid beta peptide 42 in Alzheimer's disease, *Pharmacol. Ther.* 116 (2007) 266–286.
- [5] C.G. Glabe, Common mechanisms of amyloid oligomer pathogenesis in degenerative disease, *Neurobiol. Aging* 27 (2006) 570–575.
- [6] E. Gowing, A.E. Roher, A.S. Woods, R.J. Cotter, M. Chaney, S.P. Little, M.J. Ball, Chemical characterization of A β _{17–42} peptide, a component of diffuse amyloid deposits of Alzheimer disease, *J. Biol. Chem.* 269 (1994) 10987–10990.
- [7] C. Haass, D.J. Selkoe, Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid beta-peptide, *Nat. Rev. Mol. Cell. Biol.* 8 (2007) 101–112.
- [8] H.S. Hong, I. Maezawa, N. Yao, B. Xu, R. Diaz-Avalos, S. Rana, D.H. Hua, R.H. Cheng, K.S. Lam, L.W. Jin, Combining the rapid MTT formazan exocytosis assay and the MC65 protection assay led to the discovery of carbazole analogs as small molecule inhibitors of A β oligomer-induced cytotoxicity, *Brain Res.* 1130 (2007) 223–234.
- [9] J. Joseph, G. Cole, E. Head, D. Ingram, Nutrition, brain aging, and neurodegeneration, *J. Neurosci.* 29 (2009) 12795–12801.
- [10] T. Kubo, S. Nishimura, Y. Kumagai, I. Kaneko, In vivo conversion of racemized beta-amyloid ([D-Ser 26]A β _{1–40}) to truncated and toxic fragments ([D-Ser 26]A β _{25–35/40}) and fragment presence in the brains of Alzheimer's patients, *J. Neurosci. Res.* 70 (2002) 474–483.
- [11] F.M. LaFerla, K.N. Green, S. Oddo, Intracellular amyloid-beta in Alzheimer's disease, *Nat. Rev. Neurosci.* 8 (2007) 499–509.
- [12] Y. Liu, R. Dargusch, C. Banh, C.A. Miller, D. Schubert, Detecting bioactive amyloid beta peptide species in Alzheimer's disease, *J. Neurochem.* 91 (2004) 648–656.
- [13] R. Liu, C. McAllister, Y. Lyubchenko, M.R. Sierks, Residues 17–20 and 30–35 of beta-amyloid play critical roles in aggregation, *J. Neurosci. Res.* 75 (2004) 162–171.
- [14] M.P. Mattson, Pathways towards and away from Alzheimer's disease, *Nature* 430 (2004) 631–639.
- [15] I. Maezawa, H.S. Hong, R. Liu, C.Y. Wu, R.H. Cheng, M.P. Kung, H.F. Kung, K.S. Lam, S. Oddo, F.M. LaFerla, L.W. Jin, Congo red and thioflavin-T analogs detect A β oligomers, *J. Neurochem.* 104 (2008) 457–468.
- [16] P.I. Oteiza, A.G. Erlejan, S.V. Verstraeten, C.L. Keen, C.G. Fraga, Flavonoid-membrane interactions: a protective role of flavonoids at the membrane surface? *Clin. Dev. Immunol.* 12 (2005) 19–25.
- [17] C.J. Pike, A.J. Walencewicz-Wasserman, J. Kosmoski, D.H. Cribbs, C.G. Glabe, C.W. Cotman, Structure-activity analyses of beta-amyloid peptides: contributions of the beta 25–35 region to aggregation and neurotoxicity, *J. Neurochem.* 64 (1995) 253–265.
- [18] A. Rauk, Why is the amyloid beta peptide of Alzheimer's disease neurotoxic? *Dalton Trans.* 14 (2008) 1273–1282.
- [19] W.H. Shin, S.J. Park, E.J. Kim, Protective effect of anthocyanins in middle cerebral artery occlusion and reperfusion model of cerebral ischemia in rats, *Life Sci.* 79 (2006) 130–137.
- [20] A. Tarozzi, A. Merlicco, F. Morroni, F. Franco, G. Cantelli-Forti, G. Teti, M. Falconi, P. Hrelia, Cyanidin 3-O-glucopyranoside protects and rescues SH-SY5Y cells against amyloid-beta peptide-induced toxicity, *Neuroreport* 19 (2008) 1483–1486.
- [21] A. Tarozzi, F. Morroni, S. Hrelia, C. Angeloni, A. Marchesi, G. Cantelli-Forti, P. Hrelia, Neuroprotective effects of anthocyanins and their in vivo metabolites in SH-SY5Y cells, *Neurosci. Lett.* 424 (2007) 36–40.
- [22] M. Townsend, J.P. Cleary, T. Mehta, J. Hofmeister, S. Lesne, E. O'Hare, D.M. Walsh, D.J. Selkoe, Orally available compound prevents deficits in memory caused by the Alzheimer amyloid-beta oligomers, *Ann. Neurol.* 60 (2006) 668–676.