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Neuroprotective effects of quercetin 4'-*O*-β-D-diglucoside on human striatal precursor cells in nutrient deprivation condition

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ABSTRACT

Several investigations have demonstrated neuroprotective effects of quercetin, a polyphenol widely present in nature, against neurotoxic chemicals, as well as in neuronal injury/neurodegenerative disease models. Most of these studies have been performed with quercetin aglycone and its metabolites, while scanty data are available on its glycosides. This study is aimed at investigating the neuroprotective effects of quercetin 3,4'-*O*-β-D-diglucoside (Q3,4'dG), isolated from the bulbs of the white cultivar (*Allium cepa* L.), using an in vitro model of human striatal precursor cells (HSPs), a primary culture isolated from the striatal primordium and previously characterized. To study the effect of Q3,4'dG on cell survival, HSPs were exposed to nutrient deprivation created by replacing culture medium with phosphate buffer saline (PBS). Our findings showed that Q3,4'dG treatment significantly promoted cell survival and strongly decreased apoptosis induced by nutrient deprivation, as evaluated by cell proliferation/death analyses. In addition, since the adhesive capacities of cells are essential for cell survival, the expression of some adhesion molecules, such as pancadherin and focal adhesion kinase, was evaluated. Interestingly, PBS exposure significantly decreased the expression of both molecules, while in the presence of Q3,4'dG this effect was prevented.

This study provides evidence of a neuroprotective role exerted by Q3,4'dG and suggests its possible implication in sustaining neuronal survival for prevention and treatment of neurodegenerative disorders.

1. Introduction

Epidemiological studies have shown that a reduced risk of degenerative diseases is correlated with a regular consumption of fruits and vegetables, many of which are rich in polyphenols (Stanner et al., 2004). Flavonoids represent a class of phenolic metabolites with significant antioxidant and chelating properties, and several studies have demonstrated the beneficial effects of flavonoid-rich foods as anti-inflammatory and anticancer agents, as well as their protective role in degenerative diseases, including cardiovascular and neurodegenerative disorders and age related neuronal decline, both in humans and animal models (Dajas, 2012; Ferreres et al., 2013; Guerra-Araiza et al., 2013; Kling et al., 2014; Costa, 2014; Fernandes et al., 2017).

One of the most abundant sources of flavonoids are the onion bulbs (*Allium cepa* L.), particularly rich in quercetin and its glycosides, quercetin 4'-*O*-β-D-glucoside and quercetin 3,4'-*O*-β-D-diglucoside, which account for more than 85% of the total flavonoid content (Slimestad et al., 2007; Corzo-Martinez et al., 2007). Scientific reports indicate that onion has biological activities, such as general antioxidant, anti-inflammatory, antimicrobial, antiallergic and neuroprotective properties (Shri and Singh Bora, 2008; Sato et al., 2015; Singh and Goel, 2015). Thus, many antioxidants have been tested in various in vitro and in vivo neurodegenerative models. Among flavonoids, quercetin is the most studied but the mechanisms through which quercetin exerts its neuroprotective effects are not fully elucidated, although various hypotheses have been made. In addition to direct antioxidant effect, it has been demonstrated that quercetin modulates intracellular signaling and transcription factors, increasing the expression of antioxidant and pro-survival proteins and downregulating inflammation. Quercetin also regulates the activity of kinases, changing the phosphorylation state of target molecules, resulting in modulation of cellular function and gene expression (Dajas et al., 2015). Most of these studies has been performed on quercetin aglycone and its metabolites, while scanty data, but interesting, are available on its

Abbreviations: Q3,4'dG, quercetin 3,4'-*O*-β-D-diglucoside; HSPs, human striatal precursor cells; PBS, phosphate buffer saline; PD, Parkinson's disease; HD, Huntington's disease; 6-OHDA, 6-hydroxydopamine; TFA, trifluoroacetic acid; FBS, fetal bovine serum; SD, standard deviation; SEM, standard error of the mean; ANOVA, one-way analysis of variance; ECM, extracellular matrix; FAK, Focal Adhesion Kinase

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glycosides. Of note, glycoside forms of quercetin, as rutin (quercetin-3-*O*-rutinoside) and isoquercetin (quercetin 3-*O*-glucoside), abundant in many fruits and vegetables, have demonstrated a significant neuroprotective effect against neurotoxicity induced by 6-hydroxydopamine (6-OHDA) in PC12 neuronal cells, a good in vitro model of Parkinson's disease (PD) (Magalingam et al., 2013, 2014, 2015a,b, 2016). Other studies revealed that hyperoside (quercetin 3-*O*-β-D-galactoside), mainly extracted from *Hypericum perforatum* L., has protective effects against neuronal ischemia-reperfusion (Liu et al., 2012) and β-amyloid protein-induced impairment (Zeng et al., 2011) in cultured cortical neurons of rats. However, no data are present in literature on possible effects of quercetin 3,4'-*O*-β-D-diglucoside and quercetin 4'-*O*-β-D-diglucoside, the two most abundant glycosides in *Allium cepa*.

The striatum is a brain area localized in a non-neurogenic region of the basal forebrain crucially involved in the complex cortico-basal ganglia network governing planned and motivated behaviours through motor, cognitive, and limbic circuits (Obeso et al., 2008; O'Callaghan et al., 2014). Indeed, the impairment of these brain functions is hallmark of neurodegenerative disorders such as PD and Huntington's disease (HD), both associated to striatal dysfunction (O'Callaghan et al., 2014). The striatal primordium originates during development in the ganglionic eminence, a conspicuous domain of the telencephalic proliferative zone (Ulfig, 2000). The striatal neuronal precursors divide, migrate, and differentiate establishing both intrinsic and afferent/efferent connections responsible for the basal ganglia circuits (Evans et al., 2012).

In the present study, we aimed at investigating the neuroprotective effects of quercetin 3,4'-*O*-β-D-diglucoside (Q3,4'dG) (Fig. 1), isolated from the bulbs of the white cultivar (*Allium cepa* L.) grown in Molise region (Italy), using an in vitro model of human striatal precursor cells (HSPs). These cells are primary cultures recently established by our research group isolating the striatal primordium from the ganglionic eminence of 9–12 weeks old human fetuses (Sarchielli et al., 2014). HSPs were extensively characterized (Sarchielli et al., 2014) and recapitulate the cell composition of the striatum during development, being a mixed population of neural stem cells, neuronal-restricted progenitors and striatal neurons that express and are responsive to neurotrophins, such as brain derived neurotrophic factor and fibroblast growth factor-2 (Sarchielli et al., 2014). Moreover, HSPs showed an adaptive response to stress conditions, such as nutrient deprivation (Sarchielli et al., 2014) and hypoxia (Ambrosini et al., 2015), which characterize the neurodegenerative processes, when the loss of neurons is accompanied by a reduced astrocyte- and blood vessel-mediated trophic support (Cisbani et al., 2013). Hence, HSPs were exposed to nutrient deprivation by replacing the culture medium with phosphate buffer saline (PBS) in order to evaluate Q3,4'dG role in promoting cell survival.

2. Material and methods

2.1. Quercetin 3,4'-O-β-D-diglucoside extraction

Bulbs of *Allium cepa* L. were extracted with MeOH for 12 h at room temperature, concentrated and subjected to a modified Kupchan's partitioning procedure (Kupchan et al., 1973; De Marino et al., 2012). The *n*-BuOH fraction was submitted to DCCC (Droplet Counter-Current Chromatography) with *n*-BuOH/Me_2CO/H_2O (3:1:5) on a DCC-A apparatus (Tokyo Rikakikai Co., Tokyo, Japan) equipped with 250 glass-columns. Six fractions A-F were obtained and purified by HPLC using a Waters 510 pump equipped with a Rheodyne 7125 injector and a Waters 401 differential refractometer as detector, on a Nucleodur 100-5 C18 column (5 µm, 250 mm × 4.6 mm i.d.; Macherey-Nagel, GmbH & Co. KG), flow rate 1.0 ml/min. Fraction C was separated with 35% of aqueous MeOH + 0.1% trifluoroacetic acid (TFA) as eluent to give mainly quercetin 3,4'-*O*-β-D-diglucoside (Q3,4'dG) which was identified on the basis of spectroscopic data from 1D and 2D NMR experiments (Varian Inova 500 NMR spectrometer, ^1H at 500 MHz and ^{13}C at 125 MHz equipped with a Sun hardware) and ESI–MS spectral analyses (ESI–MS: Applied Biosystem API 2000 triple-quadrupole mass spectrometer). MeOH (methanol, RCI Labscan Ltd. HPLC grade); *n*-BuOH (*n*-buthanol, RCI Labscan Ltd., AR grade); Me2CO (acetone, RCI Labsca Ltd., AR grade); H2O (water, RCI Labsca Ltd., AR grade); TFA (trifluoroacetic acid, Sigma-Aldrich).

2.2. Cell cultures

HSP cells were prepared as previously described (Sarchielli et al., 2014). Briefly, striatal tissue from 9 to 12 weeks old legally aborted human fetuses were obtained according to Italian National Institute of Health ethical guidelines. The use of human fetal tissue for research purposes was approved by the National Ethics Committee and the Committee for investigation in Humans of the University of Florence (Protocol n° 678304) (Gallina et al., 2008, 2010, 2014). Striatal tissue was dissected from 3 human fetuses under sterile conditions, cut into fragments and enzymatically digested by 1 mg/ml collagenase type IV (Sigma-Aldrich Corp., St. Louis, MO, USA) incubation. The cell suspensions were mechanically dispersed by pipetting in Coon's modified Ham's F12 medium (catalog No. ECM0019L, Euroclone, Milan, Italy) supplemented with 10% fetal bovine serum (FBS, catalog No. CHA1115L, Hyclone, Logan, UT, USA) and cultured at 37 °C in 5% CO_2 atmosphere. Confluent cells were split to 1:2–1:4 ratio using EDTA-trypsin solution, and used within the 15th passage.

2.3. MTT assay

Cell viability was determined by MTT assay (catalog No. 96992, Sigma–Aldrich Corp.). Briefly, 8 × 10^3 HSP cells were seeded in 96-well plates in Coon's modified Ham's F12 medium supplemented with 10% FBS. After 24 h cells were maintained in serum-free medium for 8 h and

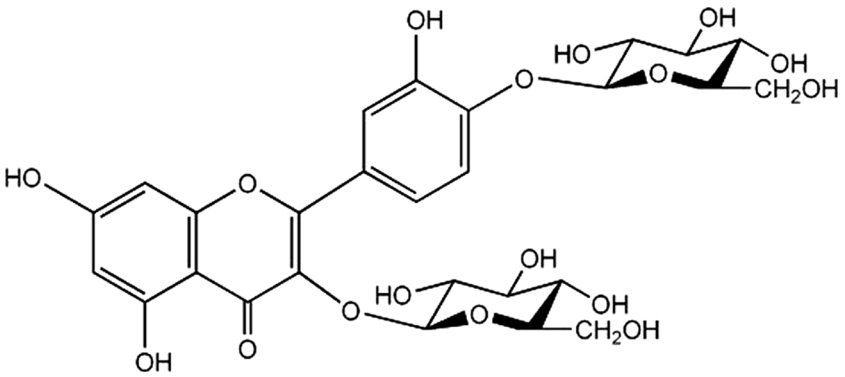

Fig. 1. Chemical structure of quercetin 3,4'-*O*-β-D-diglucoside.

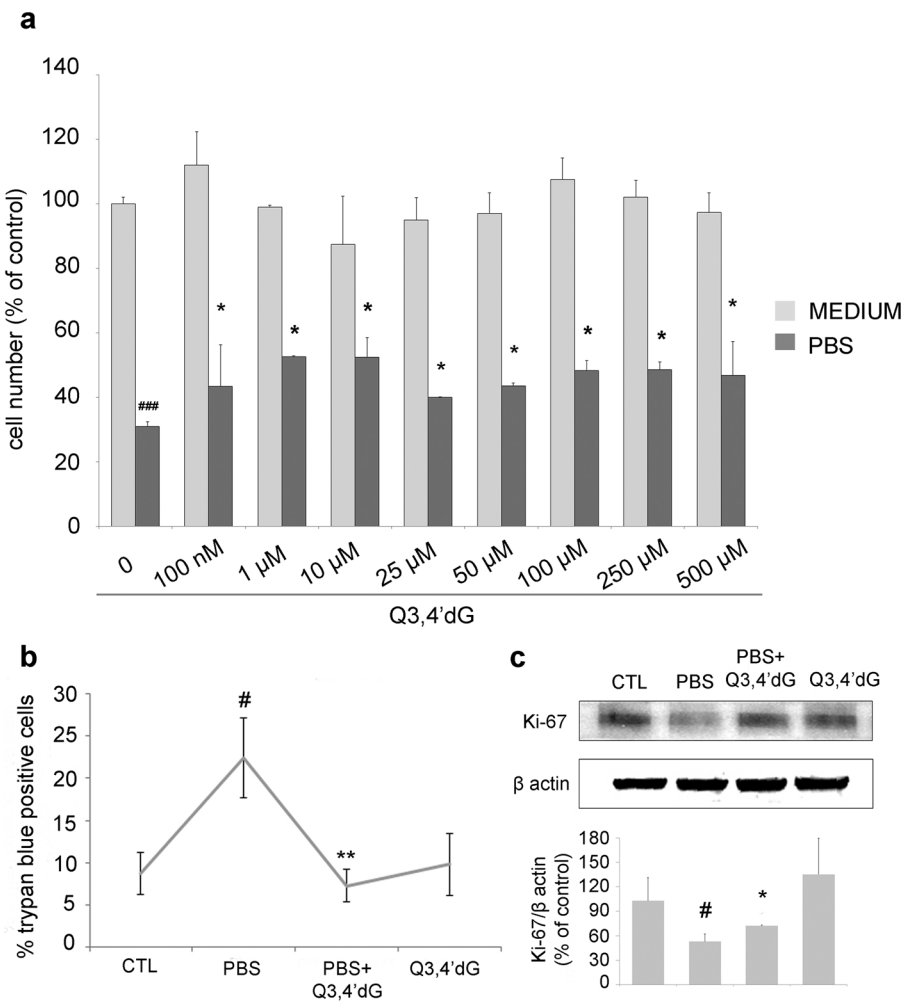

Fig. 2. Effect of Q3,4'dG on cell viability and proliferation. a) MTT assay of HSPs shows that the exposure to Q3,4'dG (100 nM–500 μM) for 20 h in standard medium does not affect cell survival at any concentration; the incubation of HSPs in PBS determines a remarkable reduction of viability compared to cells maintained in standard medium and the addition of Q3,4'dG to PBS significantly increases cell proliferation at all the tested concentrations; ###$p < 0.001$ PBS vs. standard medium; *$p < 0.05$ PBS + Q3,4'dG vs. PBS. Data are expressed as percentage of standard medium taken as 100%, and reported as mean ± SEM from six independent experiments performed in triplicate. b) Trypan Blue staining shows a noteworthy increased number of death (Trypan Blue positive) cells in nutrient deprivation condition respect to control (#$p < 0.05$ PBS vs. CTL); the addition of Q3,4'dG 100 μM to PBS is able to decrease the number of stained cells to the control level (**$p < 0.005$ PBS + Q3,4'dG vs. PBS). Data are expressed as percentage of Trypan Blue positive cells (mean ± SEM) from four separate experiments (10 counts for each experiment). c) Western blot analysis of Ki-67 expression shows an evident reduction of Ki-67 protein expression in cells maintained in PBS respect to standard medium (CTL); the addition of Q3,4'dG 100 μM to PBS prevents the Ki-67 reduction, keeping the protein at the control level. The bar graph shows the computer assisted quantification of Ki-67 band intensity from three separate experiments: data are normalized over β actin signal and are reported as mean ± SD, taking control as 100%; #$p < 0.05$ PBS vs. CTL; *$p < 0.05$ PBS + Q3,4'dG vs. PBS.

subsequently incubated for 20 h in serum-free medium or in PBS without Ca^{2+} and Mg^{2+} (Euroclone) containing or not increasing doses of Q3,4'dG (0–500 μM). Then the medium was changed and 10 μl of MTT solution was added for 3 h at 37 °C. The optical density was measured at 450 nm using a Multiskan FC spectrophotometer (Thermo Fisher Scientific, Milan, Italy). Cell viability was expressed as percentage of viable cells over control, taken as 100%, and is the result of six experiments performed in triplicate.

2.4. Trypan blue staining

5×10^4 cells were seeded in 24-well plates, starved for 8 h in serum-free medium and incubated in serum-free medium or PBS in the presence or absence of 100 μM Q3,4'dG for 20 h. Thereafter, cells were trypsinized and stained with 0,4% Trypan Blue before the hemocytometer count. Cell death results were expressed as percentage of Trypan Blue positive cells (mean ± SEM) from four independent experiments (10 counts for each experiment).

2.5. Western blotting

Western blot analysis was performed as previously described (Sarchielli et al., 2014). Briefly, HSP cells were grown in F12 Coon's Modification Medium supplemented with 10% FBS for 24 h, maintained in serum-free medium for 8 h and subsequently incubated for 20 h in serum-free medium or in PBS in the presence or absence 100 μM Q3,4'dG. Protein extracts were obtained in standard lysis buffer supplemented with protease and phosphatase inhibitor cocktails (Sigma-Aldrich Corp.); protein concentration was measured with Coomassie protein assay kit (catalog No. 500-0006, Bio-Rad Laboratories Inc., Hercules, CA, USA) and aliquots containing 20 μg of proteins were loaded on SDS-PAGE. Proteins were then transferred on polyvinylidene difluoride membranes (Amersham Biosciences, Little Chalfont, UK), blocked in 3% BSA and incubated with the following primary antibodies: rabbit polyclonal anti-Ki-67 (1:1000; catalog No. ab15580, RRID:AB_443209, Abcam, Cambridge, UK), mouse monoclonal anti-β actin (1:10000, catalog No. sc-47778, RRID:AB_626632, Santa Cruz Biotechnology, Santa Cruz, CA, USA), rabbit polyclonal anti-BAX (1:1000, catalog No. sc-526, RRID:AB_2064668, Santa Cruz Biotechnology), rabbit polyclonal anti-Bcl-2 (1:2000, catalog No. 2876, RRID:AB_2064177, Cell Signaling Technology, Danvers, MA, USA), mouse monoclonal anti-Pancadherin (1:1000, catalog No. C1821, RRID:AB_476826, Santa Cruz Biotechnology), rabbit polyclonal anti-FAK (1:2000, catalog No. sc-558, RRID:AB_2300502, Santa Cruz Biotechnology). The incubation with the primary antibodies was followed by peroxidase conjugated secondary IgG treatment (catalog No. sc-2004 and sc-2005, Santa Cruz Biotechnology) and the reacted proteins were revealed by the enhanced chemiluminescence system (catalog No. EMP013001, Euroclone). Image acquisition and densitometric analysis were performed with Quantity One software on a ChemiDoc XRS instrument (Bio-Rad Laboratories Inc.) and using β actin for normalization.

2.6. Statistical analysis

Results were expressed as mean ± standard deviation (SD) or

± standard error of the mean (SEM) for n experiments. Data were analyzed statistically by *t*-test and one-way analysis of variance (ANOVA) with post-hoc Bonferroni's correction for multiple comparisons and $p < 0.05$ was considered significant. Statistical analysis was performed with the Statistical Package for the Social Sciences (version 23.0; SPSS Inc., Chicago, IL, USA) for Windows.

3. Results

3.1. Effect of Q3,4'dG on HSPs proliferation and viability during nutrient deprivation

The effect of nutrient deprivation and Q3,4'dG treatment was first analyzed by MTT test, a colorimetric assay for assessing cell metabolic activity. The exposure to increasing concentrations of Q3,4'dG (100 nM–500 μM) for 20 h in standard medium did not affect cell proliferation/viability (Fig. 2a), demonstrating the lack of cytotoxic effects of the flavonoid even at higher concentrations. The incubation of HSP cells in PBS for 20 h determined a remarkable reduction of cell viability compared to control cells maintained in Ham's F12 medium ($p < 0.001$ PBS vs. control); the addition of Q3,4'dG significantly increased cell proliferation/metabolism at all the tested concentrations, when compared to PBS alone ($p < 0.05$ PBS + Q3,4'dG vs. PBS) (Fig. 2a). On the basis of the data present in literature concerning other quercetin glycosides, a dose of Q3,4'dG 100 μM was chosen to perform the subsequent experiments (Magalingam et al., 2013, 2015a, 2016).

MTT results were confirmed by the Trypan blue staining of dead cells; as shown in Fig. 2b the percentage of Trypan blue positive cells dramatically increased after PBS exposure (8,74% ± 2,46% control vs. 22,41% ± 4,72% PBS, $p < 0.05$). The addition of Q3,4'dG 100 μM to PBS was able to lower the percentage of stained cells at 7,29% ± 1,90% (PBS + Q3,4'dG vs. PBS, $p < 0.005$). We next analyzed the protein expression of Ki-67, a protein necessary for the cellular proliferation and present during all active phases of the cell cycle (G1, S, G2, and mitosis), but absent in resting cells (G0). Western blot analysis revealed a strong reduction of Ki-67 expression in PBS-exposed cells (20 h; $p < 0.05$ vs. control), an effect significantly prevented and normalized to the control level by the addition of 100 μM Q3,4'dG ($p < 0.05$ vs. PBS; $p = 0.1$ vs. control; Fig. 2c).

Thereafter, we investigated whether PBS affected apoptotic index, as evaluated by western blot analysis of Bax (apoptotic activator)/Bcl-2 (survival promoter) ratio. As shown in Fig. 3, PBS incubation significantly increased Bax/Bcl2 ratio, indicating the activation of apoptosis ($p < 0.05$ vs. control), while the addition of Q3,4'dG (100 μM for 20 h) significantly counteracted this phenomenon ($p < 0.05$ vs. PBS), although without bringing the Bax/Bcl-2 ratio to the control level ($p < 0.05$ vs. control).

3.2. Influence of Q3,4'dG administration on HSPs morphology and adhesion molecule's expression in nutrient deprivation condition

The morphological aspect of HSP cells, evaluated with phase contrast microscope, confirmed all the previous observations (Fig. 4a). In fact, cells maintained in medium supplemented or not with Q3,4'dG 100 μM appeared flattened and with a spindle shaped fibroblast-like morphology (Fig. 4a A, D); conversely PBS determined a massive detachment from the plate and the acquisition of a round shape, typical of dying cells (Fig. 4a B). Interestingly, the simultaneous treatment with PBS and Q3,4'dG (100 μM for 20 h) maintained the aspect of adherent cells with extended cytoplasmic processes, more similar to control cells (Fig. 4a C).

To further investigate the effect of PBS and Q3,4'dG on cell adhesive capacities, the expression of the intercellular adhesion molecule Pancadherin and the extracellular matrix (ECM) adhesion molecule Focal Adhesion Kinase (FAK) was evaluated by western blot analysis (Fig. 4b,c). The results showed that PBS exposure determined a strong

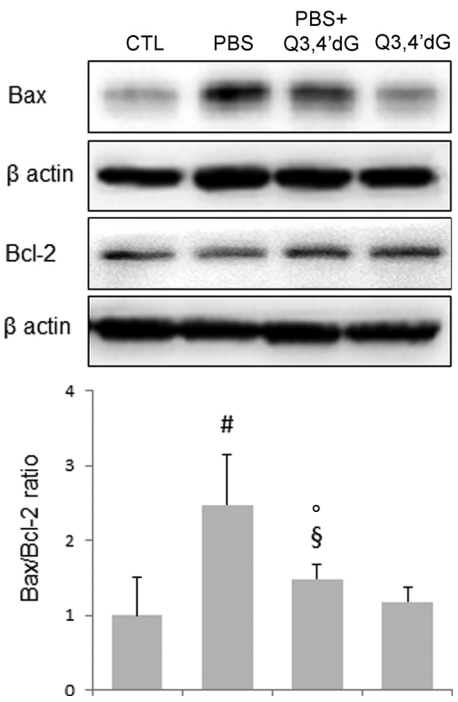

Fig. 3. Western blot analysis of Bax and Bcl-2 expression in HSP cells untreated (CTL) or treated with Q3,4'dG 100 μM for 20 h in Coon's modified Ham's F12 medium or PBS. PBS incubation increases the levels of Bax and decreases Bcl-2; the addition of Q3,4'dG 100 μM significantly reverts this phenomenon. The bar graph shows Bax/Bcl-2 ratio after the normalization of each signal with the respective β actin staining. The ratio increases in cells exposed to PBS and decreases after the addition of Q3,4'dG 100 μM, although not reaching the control level, but indicating an active role of Q3,4'dG to counter apoptosis. Data are reported as mean ± SD from three separate experiments, taking CTL as 1; #$p < 0.05$ PBS vs. CTL, §$p < 0.05$ PBS + Q3,4'dG vs. PBS, °$p < 0.05$ PBS + Q3,4'dG vs. control.

decrease in both the analysed adhesion molecules ($p < 0.05$ vs. control), while Q3,4'dG was able to increase significantly the two proteins ($p < 0.05$ vs. PBS). In particular, Pancadherin was brought back to the control level ($p = 0.23$ vs. control), while FAK remained significantly lower than control ($p < 0.001$ vs control).

4. Discussion

In the last 20 years, there has been an explosion of studies on the neuroprotective effects of various fruits and vegetables due to their bioactive components, particularly the flavonoids (Magalingam et al., 2015b). Among vegetables the onion, *Allium cepa*, is rich in flavonoids and has been demonstrated to have neuroprotective proprieties mainly attributed to its principal flavonoid quercetin (Shri and Singh Bora, 2008; Singh and Goel, 2015; Yang et al., 2013; Lee and Jung, 2016).

In the present study, we tested and demonstrated for the first time the benefic effect of Q3,4'dG, one of the most abundant quercetin glycosides of onion, on some fundamental survival features of human striatal precursor cells (HSPs) in nutrient deprivation environment, as stress condition. The findings showed a strong reduction of viability and cell proliferation of HSP cells and an increase in apoptosis in nutrient deprivation (PBS) comparing to control cells, while the addition of Q3,4'dG to PBS reverted this phenomenon promoting cell viability/proliferation.

No data are present in literature regarding the effect of quercetin and/or its derivatives on adult or fetal striatal cell cultures in different stress conditions. However, in vivo investigations in animal models of HD and PD showed some and different benefic effects of quercetin on adult striatum. In 3-Nitropropionic acid-induced rat model of HD quercetin showed to attenuate some behavioral (anxiety, motor coordination deficits, gait despair), neurochemical (reduction of serotine

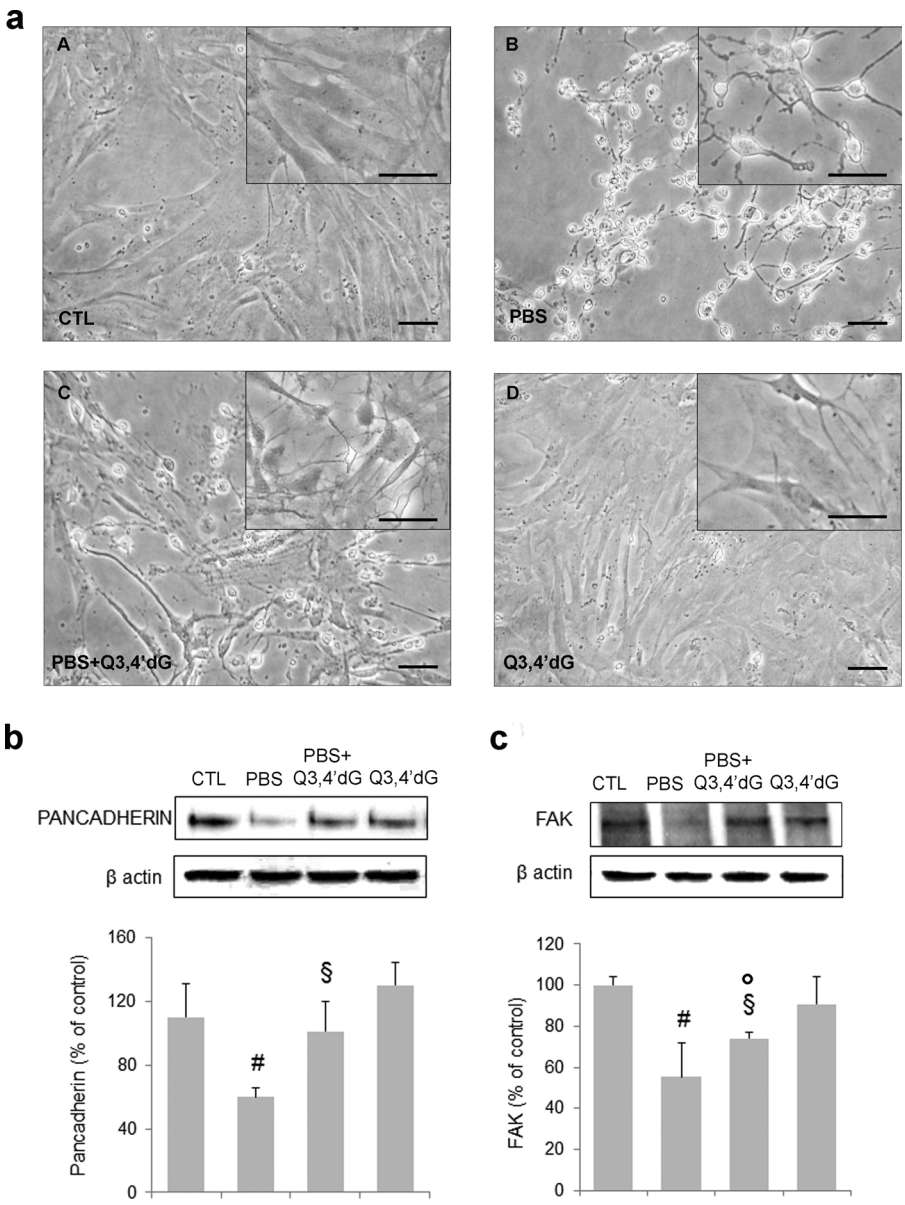

Fig. 4. Q3,4'dG effect on cell adhesion. a) Phase contrast microphotographs of cells maintained in medium supplemented or not with Q3,4'dG 100 μM show a fibroblast-like morphology and a flattened shape (panels A, D); cells incubated with PBS exhibit a marked detachment from the plate and the acquisition of a round shape (panel B). Conversely, the simultaneous treatment with PBS and Q3,4'dG 100 μM partially restores the aspect of untreated cells, showing several cytoplasmic projections and a mostly preserved cell body (panel C). Scale bar = 50 μM. b–c) Western blot analysis demonstrates a significant decrease in both Pancadherin (b) and FAK (c) protein expression in PBS treated cells; the addition of Q3,4'dG 100 μM to PBS reverts this phenomenon. The bar graph shows the computer assisted quantification of the band intensity from three separate experiments: data are normalized over the respective β actin signal and are reported as mean ± SD, taking control as 100%; #p < 0.05 PBS vs. CTL, §p < 0.05 PBS + Q3,4'dG vs. PBS, °p < 0.001 vs CTL.

metabolism) and striatal neuropathological features characteristic of this pathology. In particular, quercetin administration did not reduce striatal neuronal lesion, but increased astrocytes number and decreased microglial proliferation, confirming its anti-inflammatory effect (Chakraborty et al., 2014; D'Andrea, 2015). Moreover, investigations on striatum in 6-hydroxydopamine (6-OHDA)-induced rat model of PD demonstrated that quercetin treatment defended against oxidative stress and increased neuronal survivability and the level of striatal dopamine (Haleagrahara et al., 2011, 2013). In addition, several studies demonstrated the neuroprotective effects exerted by some quercetin glycosides such as rutin, isoquercetin and hyperoside in in vitro neuropathological models. As an instance, investigations on 6-OHDA-induced rat PC12 cells, an in vitro PD model, demonstrated that rutin and isoquercetin have not only a benefic effect on viability and on antioxidant machinery, but also have a direct antioxidant action (Magalingam et al., 2013, 2014, 2016) and modulate several genes to prevent neuronal cell death (Magalingam et al., 2015a). It is noteworthy that rutin has been demonstrated to have more neuroprotective effect with respect to isoquercetin, inducing higher cell viability and better functionality of some antioxidant enzymes. This seems to be correlated with their structure; rutin has two monosaccharide units (glucose 1 → 6 rhamnose) at C-3 position, while isoquercetin has only a glucose unit, so rutin seems to have higher solubility, stability and better interaction with antioxidant machinery (Magalingam et al., 2016). A study on an in vitro ischemic model of oxygen–glucose deprivation followed by reperfusion (OGD-R) provided evidence that hyperoside, which contains a galactose unit, has a protective effect on cortical neurons with a decrease of NO production via inhibition of NF-kB activation and ameliorating ERK, JNK and Bcl-2 family-related apoptotic signaling pathways (Liu et al., 2012). Another investigation on β-amyloid protein (Aβ)-treated primary rat cortical neurons showed that hyperoside seems to protect from cell death via PI3K/Akt/Bad/BclXL-regulated mitochondrial apoptotic pathway (Zeng et al., 2011). Moreover, this study suggests that hyperoside neuroprotective effect may mainly originate from its galactoside-derivative structure, not its basic aglycone structure. In fact, quercetin had no effect on Aβ-induced neurotoxicity in rat cortical neurons (Wang et al., 2001).

Our findings confirm the benefic effect of quercetin, particularly one of its glycosides, also in other stress conditions; in fact, Q3,4'dG can maintain neuron viability and proliferation and protect from apoptotic process in nutrient deprivation environment, probably through some of the same mechanisms disclosed in the above-mentioned studies. It is

worth remembering that HSP cells reflect the heterogeneous cell composition of the striatal primordium, including both neuronal and glial cells (Sarchielli et al., 2014). Therefore, it is to be hypothesized that protection by Q3,4'dG occurs both by acting directly on the neuronal component and regulating glial functions such as neuron mechanic and trophic support, development of nerve fibers and inhibition of an inflammatory condition.

Another interesting finding arising from this study is a strong decrease of adhesion molecules, such as Pancadherin and FAK, in nutrient deprivation, confirmed by the morphological data such as cell detachment and cytoplasmic projections loss. Of note, Q3,4'dG addition opposed to the loss of cell adhesion and interaction with ECM. Pancadherin has been identified as a protagonist of neuronal cell adhesion, axonal sprouting, axon-dendritic contacts, synaptic plasticity and rearrangement (Atalay et al., 2007, 2008). Studies of FAK in nervous system have demonstrated its importance both during development and in adult brain. In fact, several lines of evidence support FAK as key component in regulating axon guidance, particularly axon outgrowth by its interaction with different extracellular molecules and cytoskeleton. In addition, FAK expression in neurons is activated by multiple adhesion proteins (e.g., integrins and NCAM) (Xiong and Mei, 2003; Navarro and Rico, 2014). It is noteworthy, that neutrophic factors in HSP cells are able to induce the expression of some integrins and adhesion molecules, such as CD15, CD24, CD29 and CD56 (Sarchielli et al., 2014). Therefore, we can speculate that Q3,4'dG may play a neuroprotective role preserving directly the adhesion molecules or indirectly stimulating the expression of neurotrophic factors. Interestingly, our results showed also that the loss of cell adhesive abilities are related to increased apoptosis in nutrient deprivation. Then Q3,4'dG could protect against the common apoptosis, as previously reported, and/or against its peculiar form anoikis. In fact, this is a programmed cell death induced upon cell detachment from extracellular matrix and FAK, being a key component of cell-substratum adhesion, has a fundamental role in impede anoikis (Lu and Rounds, 2012; Paoli et al., 2013).

The identification of the molecular forms of quercetin circulating in the blood and their pharmacological effects has often been discussed in the light of its controversial results (Dajas, 2012; Graefe et al., 2001; Prasain and Barnes, 2007; Lee and Mitchell, 2012). Quercetin is mainly present in food and beverages as hydrophilic glycosides, that are scarcely absorbed and rapidly de-glycosidated by enterobacteria for the intestinal absorption. Plasma samples revealed that quercetin circulates in plasma only in conjugated form, as methylate, sulphate and glucuronide metabolites (Graefe et al., 2001). The issue of intestinal absorption is a key step and has previously been a point of discussion. In vitro studies suggest that blood barrier is traversed both by quercetin and probably by its glycosides (Zeng et al., 2011; Dajas, 2012; Faria et al., 2014; Ishisaka et al., 2014; Dajas et al., 2015; Costa et al., 2016; Sharma et al., 2016; Suganthy et al., 2016). Kelly (2011) reported that the oral administration of quercetin to humans (50–150 mg for 2 weeks) significantly increased plasma concentrations of quercetin. Its accumulation as metabolite forms was observed after oral administration of quercetin (one month), in the brain tissue of rats and resulted with antioxidant activity (Ishisaka et al., 2011). The study we addressed on striatal neural cells is an in vitro study, so when transferred in vivo, the various barriers such as the intestinal and blood brain barriers, that the glycoside encounters if introduced orally and/or by the bloodstream, should be considered. The positive results that we obtained with Q3',4dG in vitro are probably linked to the presence of two monosaccharide units associated with the absence/decrease of toxic effects as observed for other quercetin glycosides (Tenore et al., 2013; Magalingam et al., 2014), so some strategies for transporting these glycosylated flavonoids undisturbed in vivo should be considered. Different types of liposomes have been utilized with success in cases of experimental ischemia (Rivera et al., 2008) and recent studies demonstrated that several nanodelivery techniques, including solid lipid nanoparticles, nanostructured lipid carriers, nanoliposomes, and nanoniosomes can be used to obtain effective concentrations and controlled delivery of nanobioactive compounds to the brain (Kelly, 2011; Dajas et al., 2015, Caruana et al., 2016; Costa et al., 2016; Ganesan et al., 2015; Suganthy et al., 2016).

It is to keep in mind that this is the first investigation on protective effect of a single quercetin glycoside from *Allium cepa* on HSP cells survival. Further investigations are necessary to better clarify the underlying mechanisms. In addition, the effect of other abundant flavonoid components of Allium as Q4'dG and quercetin would be tested, comparing them to each other, and evaluating if their mix may have an enhanced action.

5. Conclusions

Our findings demonstrate that Q3,4'dG treatment has a beneficial effect on HSPs survival in nutrient deprivation condition, leading to reduction of apoptosis, and maintaining proliferation, morphology and the adhesive capacities. Therefore, this study highlights a fundamental neuroprotective role by Q3,4'dG, suggesting its possible implications in sustaining striatal neural cells to prevent neurodegeneration (Cisbani et al., 2013; Caruana et al., 2016). In addition, these findings may aid to develop new pharmaceutical formulations, as there are not yet effective therapies for neurodegenerative pathologies such as HD and PD.

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Conflict of interest

The authors declare no conflict of interests.

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