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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

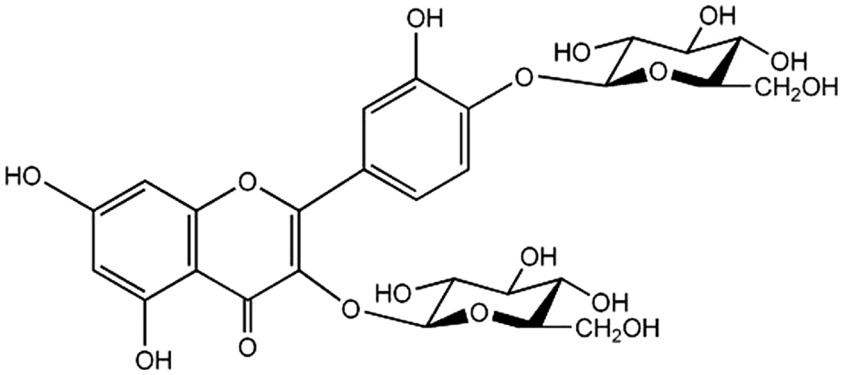

Fig. 1. Chemical structure of quercetin 3,4'-*O*-β-D-diglucoside.