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Effect of the oral administration of nanoencapsulated quercetin on a mouse model of Alzheimer's disease

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ABSTRACT

Quercetin has been identified as a promising compound with a neuroprotective potential against age-related neurodegenerative diseases such as Alzheimer's disease (AD). Nevertheless, the clinical application of quercetin is hampered by its low oral bioavailability. The aim of this work was to evaluate the capability of nanoencapsulated quercetin in zein nanoparticles (NPQ), that significantly improves the oral absorption and bioavailability of the flavonoid, as potential oral treatment for AD. For this purpose, SAMP8 mice were orally treated for two months with either NPQ (25 mg/kg every 48 h) or a solution of quercetin (Q; 25 mg/kg daily). NPQ displayed a size of 260 nm and a payload of about 70 μg/mg. For Q, no significant effects were observed in animals. On the contrary, the oral administration of NPQ improved the cognition and memory impairments characteristics of SAMP8 mice. These observations appeared to be related with a decreased expression of the hippocampal astrocyte marker GFAP. Furthermore, significant levels of quercetin were quantified in the brain of mice treated with nanoparticles. These findings highlight the potential of zein nanoparticles to promote the oral absorption of quercetin as well as the therapeutic potential of this flavonoid in AD pathogenesis.

1. Introduction

Alzheimer's disease (AD) is a deadly neurodegenerative brain disorder characterized by the progressive cognitive impairment and memory loss, which is considered the most common cause of dementia in elderly (Najafi et al., 2016). The onset is gradual, with continuing cognitive decline that significantly impairs social or occupational behaving and represents a significant decrease from a previous level of functioning (Boada et al., 2014).

Due to the aging of the human population, the number of patients affected by AD is increasing rapidly and, in the last decades, the prevalence is doubling every 20 years (Khunnawutmanotham et al., 2016). Thus, worldwide, 36 million people with dementia were estimated 2010, nearly doubling every 20 years to 115 million by 2050 (Luo et al., 2016). In addition, AD is a life-shortening illness with an important impact on the patient quality of life (Larson et al., 2004). According to recent estimates, the costs of AD worldwide would be of approximately US$604 billion annually and only in Europe the direct costs of AD would represent close to €105 billion for the community per year (Dodel et al., 2015).

AD is characterized by a destruction of the functional activity of neurons in different areas of the brain, including those accounted for memory, learning, emotional reactions and behavior (Nelson et al., 2012). The mechanisms responsible for AD onset and the secondary development thereafter include amyloid plaques and neurofibrillary tangles, which are caused by deposits of β-amyloid fragments (Aβ) and hyperphosphorylated Tau-proteins (Qi et al., 2016). The small clusters of β-amyloid plaque may block the signaling between cells at synapses and, then, activate the immune cells that cause inflammation and trigger the destruction of neurons (López et al., 2012).

In the last years, some responsible factors on the onset of this pathology have been identified, including the low levels of acetylcholine (García-Ayllón et al., 2011), oxidative stress (Ishisaka et al., 2014) and neuroinflammation (Helmfors et al., 2015). Neuroinflammation is directly related to the decrease in synapses

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which causes cognitive decline (Hong et al., 2016). From this perspective, an active compound against one or more of these triggering factors may be a promising strategy for the treatment of this dementia.

Flavonoids have been identified as compounds with a neuroprotective potential by preventing the onset or by slowing the progression of age-related neurodegenerative diseases (Williams and Spencer, 2012). These capabilities of flavonoids seems to be related to their ability to interact with intracellular neuronal and glial signaling pathways, thus influencing the peripheral and cerebral vascular system, protecting vulnerable neurons, enhancing existing neuronal function, or stimulating neuronal regeneration (Spencer, 2009).

In this context, quercetin (3,5,7,30,40-penta hydroxyflavone) is a flavonoid with important antioxidant and anti-inflammatory properties (Oliveira et al., 2016). Furthermore, quercetin can cross the blood brain barrier (Youdim et al., 2004) and would exert a neuroprotective effect, increasing the resistance of neurons to oxidative stress and excitotoxicity by modulating the mechanisms of cell death (Choi et al., 2014; Liu et al., 2013). Very recently, Sabogal-Guaqueta and coworkers have been demonstrated that quercetin, intraperitoneally administered every two days during three months, reversed the histopathological hallmarks of AD and ameliorated cognitive and emotional impairments in a mice model of this disease (Sabogal-Guáqueta et al., 2015).

Nevertheless, the use of quercetin for clinical application is highly hampered by its low oral bioavailability (Patel et al., 2012). In fact, apart its low aqueous solubility, quercetin is a substrate of both the intestinal efflux pumps (e.g., P-glycoprotein and MRP2) (Chabane et al., 2009) and the cytochrome P450 enzymes (CYP3A) (Choi et al., 2011). As a consequence, the oral bioavailability of this flavonoid in humans has been calculated to be close to 2% (Suna et al., 2016).

In order to overcome these problems, one possibility may be the co-encapsulation of quercetin and 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) in zein nanoparticles as oral carriers for this flavonoid. In a very recent work, this combination between zein nanoparticles and HP-β-CD has demonstrated to be adequate to significantly improve the oral bioavailability of quercetin (close to 60%) and, more important, provide high and sustained plasma levels of the flavonoid for at least 2 days (Penalva et al., 2016). On one side, zein nanoparticles would offer a prolonged residence in close contact with the gut mucosa due to their mucoadhesive properties (Peñalva et al., 2015). On the other hand, the simultaneous release of HP-β-CD with quercetin would facilitate the inhibitory effect of the cyclodextrin in the activity of both the intestinal efflux pumps (Zhang et al., 2011) and the cytochrome P450 (Ishikawa et al., 2005).

In this context, the aim of this work was to evaluate quercetin-loaded in zein nanoparticles containing HP-β-CD as potential treatment for AD. For this purpose, free quercetin and quercetin nanoparticles were administrated orally during 2 months in SAMP8 mice and motor activity and memory tests were conducted.

2. Material and methods

2.1. Materials

Zein, quercetin, lysine, 2-hydroxypropyl-β-cyclodextrin (HP-β-CD), mannitol, poly(ethylene glycol) 400 (PEG400), Tween 20, Tris buffer saline, sodium chloride and rabbit polyclonal anti-β-actin were purchased from Sigma-Aldrich (Steinheim, Germany). Ethanol, methanol and acetic acid (HPLC grade) were obtained from Merck (Darmstadt, Germany). PBS (Phosphate-Buffered saline) was from Gibco by Life Technologies Corp. (New York, USA). Tris hydrocloride, ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*',*N*'-tetraacetic acid (EGTA), Nonidet P-40, phosphatase and protease inhibitor cocktail set II were from Calbiochem, (Darmstadt, Germany). Bradford protein assay was from Bio-Rad (Hercules, CA, USA). Sodium dodecyl sulphate-polyacrylamide gel nitrocellulose membrane was from Hybond-ECL; Amersham Bioscience (Barcelona, Spain). Mouse monoclonal anti-glial fibrillary acidic protein was from Cell Signaling Technology (Beverly, USA). Rabbit polyclonal (CD11b) was from Thermo Scientific (Rockford, USA). Secondary antibodies conjugated to IRDye 800CW or IRDye 680CW were LI-COR Biosciences (Lincoln, USA). All reagents and chemicals used were from analytical grade.

2.2. Preparation of quercetin-loaded nanoparticles

Zein nanoparticles were prepared by a controlled desolvation procedure followed by a purification step by ultrafiltration and subsequent drying in a Spray-drying apparatus (Penalva et al., 2016). This formulation of nanoparticles was identified as NPQ.

For the preparation of nanoparticles, 600 mg zein and 60 mg lysine were dissolved in 88 mL ethanol 60%. In parallel, 60 mg quercetin and 302.5 mg HP-β-CD were dissolved in 10 mL absolute ethanol and added under magnetic stirring to the zein solution. After incubation, nanoparticles were formed by the addition of 88 mL purified water. The result ing suspension of nanoparticles was purified and concentrated by ultrafiltration through a polysulfone membrane cartridge of 50 kDa pore size (Medica SPA, Italy). Then, 12 mL of an aqueous solution of mannitol (100 mg/mL) were added. Finally, the suspension of zein nanoparticles was dried in a Buchi Mini Spray Drier B-290 apparatus (Buchi Labortechnik AG, Switzerland) under the following experimental conditions: (i) inlet temperature, 90 °C; (ii) outlet temperature, 45–50 °C; (iii) air pressure, 2–5 bar; (iv) pumping rate, 5 mL/min; (v) aspirator, 100%; and (vi) air flow rate, 900 L/h.

2.3. Preparation of quercetin solution

A quercetin solution (Q) was used as control. For this purpose, 50 mg of quercetin were dissolved in 6 mL PEG 400 under magnetic stirring. Then 4 mL purified water were added and the final mixture was agitated in the dark for 10 min.

2.4. Characterization of nanoparticles

2.4.1. Size, zeta potential and morphology

The particle size and the zeta potential of spray-dried nanoparticles were determined by photon correlation spectroscopy (PCS) and electrophoretic laser Doppler anemometry, respectively, using a Zetaplus apparatus (Brookhaven Instruments Corporation). In all cases, the size was measured after dispersion of nanoparticles in ultrapure water whereas the zeta potential was quantified in KCl 0.1 M. The yield of the process (amount of protein transformed into nanoparticles) was calculated as described previously (Peñalva et al., 2015).

2.4.2. Quercetin quantification

The amount of quercetin loaded into the nanoparticles was quantified by HPLC-UV following a method previously described (Penalva et al., 2016). The analysis was carried out in an Agilent model 1100 series LC and a diode-array detector set at 370 nm. The chromatographic system was equipped with a reversed-phase C18 Alltima column (150 mm × 2.1 mm, particle size = 5 μm; Altech, USA) and a Gemini AJO-7596 C18 precolumn (Phenomenex, CA, USA). The mobile phase, pumped at 0.25 mL/min, consisted of a mixture of methanol, water and acetic acid under gradient conditions. The column was placed at 40 °C and the injection volume was 10 μL.