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Complexation of hydroxytyrosol and 3,4-dihydroxyphenylglycol with pectin and their potential use for colon targeting

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ABSTRACT

Hydroxytyrosol (HT) and 3,4-dihydroxyphenylglycol (DHPG) are two phenolic antioxidants naturally found in olive fruit with anti-inflammatory properties. This study explored the interaction of pectin with HT and DHPG via their encapsulation into pectinate beads. Purification by size exclusion chromatography, changes in the fluorescence spectrum of the HT and pectin, and MALDI TOF–TOF analysis suggested the existence of the phenol-pectin complexes. The entrapment efficiency, swelling properties, and *in vitro* release of HT and DHPG of the beads were studied.

The results show that the beads can entrap the water soluble compounds HT and DHPG in sufficient amounts to reach the colon. The beads consisted of an important amount of pectin-bound HT or DHPG after two hours at gastric pH. This study highlights the potential use of HT-and DHPG-loaded pectinate gel beads for the colon-targeted delivery of these bioactive compounds to help prevent or relieve chronic inflammatory bowel disease.

1. Introduction

Chronic inflammatory bowel disease (IBD) includes ulcerative colitis and Crohn's disease, two autoimmune disorders exacerbated by inflammatory mediators (Marks et al., 2006).

Evidence shows that virgin olive oil might exert beneficial effects on markers of inflammation (De la Puerta, Martínez-Domínguez, & Ruiz-Gutiérrez, 2000). The oil's high monounsaturated fatty acid content and the presence of minor components such as erythrodiol, β-sitosterol, squalene, tocopherols, carotenoids, and phenolic compounds, exert the anti-inflammatory effect (Lyons et al., 2016).

Considering the involvement of oxidative stress in inflammation, antioxidants might bring benefits in inflammatory diseases. In fact, virgin olive oil contains numerous antioxidant phenolic compounds that exert potent anti-inflammatory actions (Muto et al., 2015). In the last few years, several studies demonstrated that diets supplemented with olive oil and/or olive oil phenolics compounds exert a protective effect in experimental colitis in rodents, which may be mediated by their strongly anti-oxidative potential (Sánchez-Fidalgo et al., 2013; Takashima et al., 2014).

Hydroxytyrosol (HT) is a simple phenol and considered to be the main olive oil antioxidant with a potent anti-inflammatory activity (Bitler, Viale, Damaj, & Crea, 2005; Ciriminna, Meneguzzo, Fidalgo, Ilharco, & Pagliaro, 2016). The European Food Safety Authority (EFSA) allows the health claim that ≥5 mg HT/day prevents low-density lipoprotein (LDL) oxidation and, therefore, reduces the risk of atherosclerosis (EFSA NDA Panel, 2012). Thus, it is an indirect recognition of the anti-inflammatory effect of HT (Pontoniere & Martiradonna, 2012). 3,4-dihydroxyphenylglycol (DHPG) is another simple phenol present in the olive fruit with the same *ortho*-diphenolic structure as HT but with an additional hydroxyl group in the β position. DHPG also has powerful antioxidant properties and potentially has anti-inflammatory properties (Rodríguez, Rodríguez, Fernández-Bolaños, Guillén, & Jiménez, 2007).

In this paper, we propose the delivery of HT, DHPG, or olive oil to the colon as agents capable of preventing or improving IBD. The encapsulation of HT or DHPG with calcium-pectinate beads as a colon-targeted delivery system will be assessed for the first time. Moreover, beads will be prepared by the emulsion method using olive oil to form an oil-in water emulsion with citrus pectin act-

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ing as the emulsifier. Pectin is naturally present in plant cell walls and possesses an interesting potential for the delivery of drugs to the colon (Das & Ng, 2010). It is specifically biodegraded by colonic bacteria and has been found to inhibit both local and systemic inflammation and prevent intestinal inflammation (Markov, Popov, Nikitina, Ovodova, & Ovodov, 2011; Popov et al., 2013). To our knowledge, there has been no study to date on the encapsulation of HT, combined with olive oil and pectin – all of which have anti-inflammatory properties – for the local treatment of bowel diseases.

The preventive and therapeutic efficacy of HT for IBD is well documented (Sánchez-Fidalgo, Sánchez de Ibarguen, Cardeno, & Alarcón de la Lastra, 2012; Takashima et al., 2014). However, due to its rapid absorption and metabolism – reaching the maxima plasma concentration after oral administration in 5–10 min followed by rapid decline (D'Angelo et al., 2001) and with a very short elimination half-life of 2.43 h (Miró-Casas et al., 2003) – an insufficient amount of HT will reach the colonic region.

2. Hypotheses

Our hypothesis for the current study was a delayed release of HT or DHPG with the delivery system in the colonic region, thus providing a novel possibility for the treatment of oxidative-stress mediated inflammatory disease.

The objective of the present investigation was to compare the entrapment efficiency and release profiles of HT and DHPG using three different types of beads based on the natural polysaccharides pectin and alginate in combination with and without virgin olive oil and amidated pectin. The beads were treated *in vitro* through dissolution conditions that mimic the gastric to colonic transit. The interactions between pectin and HT were also studied in order to check their efficacy for colon targeting.

3. Material and methods

3.1. Materials

HT and DHPG were extracted and purified from olive by-products using a chromatographic system following the processes described by Fernández-Bolaños et al. (2011, 2014). Citrus pectin with a high degree of esterification (53%), and sodium alginate were purchased from Sigma-Aldrich (St Louis, MO, USA). Amidated pectin, with a low degree of esterification (30%) and a 21% degree of amidation was a generous gift by Herbstreith & Fox KG (Neuenbürg, Germany). Virgin olive oil was purchased from a local supermarket. Calcium chloride dihydrate ($CaCl_2{\cdot}2H_2O$) and a mixture of pectinolytic enzymes from Novozyme Corp. were purchased from Sigma-Aldrich (St. Louis, MO, USA).

3.2. Preparation of formulations

Calcium pectin-alginate beads and amidated pectin beads were instantaneously produced by the ionotropic gelation method. Citrus pectin (0.8 g) and sodium alginate (0.2 g), or 1.0 g of amidated pectin were homogeneously dispersed in 30 mL of aqueous solution containing HT (65%) or DHPG (95%) using a homogenizer (Ultra-Turrax® T50 Basic, IKA, Germany) at a speed of 24,000 rpm for 5 min in an ice-bath to avoid overheating. The pectin dispersions containing HT or DHPG were dropped into 30 mL of 10% w/v $CaCl_2$ solution using a nozzle with an inner diameter of 1.2 mm. The beads formed were allowed to stand in the $CaCl_2$ solution for different cross-linking times (from 2 to 30 min) until an optimized curing time. HT/DHPG loaded beads gels were separated from the $CaCl_2$ solution by filtration and washed with distilled water. In the filtrated and washed liquid were measured the free HT/DHPG that was not entrapped within the beads. The separated gel beads were dried at 37 °C for 48 h in an air-circulated oven until a constant weight was obtained.

Pectin-alginate beads containing olive oil were prepared by the emulsion-gelation method. 7.5 g of virgin olive oil was added to the polymers and HT/DHPG solution. The mixture was emulsified using the same homogenization conditions and the olive oil-incorporated into emulsion gel beads were treated in the same manner as the calcium pectin-alginate beads.

3.3. Extraction and analysis of HT and DHPG from beads and emulsions

Extraction of HT/DHPG from the hydrocolloids (beads and emulsions) was evaluated in aqueous solutions and various organic solvents. The unbound phenolics were extracted as follows: 0.1 g of dry beads or emulsion was dispersed in 100 mL of acidified water for 2 h at 37 °C in a shaking water bath. The quantities of HT or DHPG were determined by HPLC according to a previously published method (Rodríguez et al., 2007). The extraction of strongly bound HT/DHPG was also assayed according to the method of Nordkvist, Salomonsson, & Åman, (1984), using 1 M NaOH containing 0.5% sodium borohydride for 20 min. The hydrocolloids were then subjected to acid hydrolysis according to the method of Graciani & Vázquez (1980). 0.1 g of dry beads/emulsion were treated with 10 mL of 3 N HCl in a homogenizer Ultra-Turrax (24,000 rpm for 5 min) and then heated to 100 °C for 10 min and filtered.

Also 0.1 g of the beads or emulsion were extracted with 25 mL of organic solvent by stirring (30 min), sonication (15 min), and Ultra-Turrax (24,000 rpm for 5 min), using methanol:water (20, 40, 80%) and dimethyl sulfoxide (DMSO): water (10, 30, 60, 90%). All the dispersions were filtered. In any case it was possible to quantify the strongly bound HT or DHPG. Also, due to the difficulty of dissolving the dry beads, the HT/DHPG loaded dried hydrogel beads were broken down by immersion in sodium phosphate buffer (50 mM, pH 6.8) containing 5 mM of EDTA (Ethylenediaminetetraacetic acid). A preliminary acid treatment was necessary to help the erosion of the matrix (Nguyen, Winckler, Loison, & Wache, 2014): Beads (0.1 g) were dispersed in 100 mL of 0.1 M HCl for 2 h at 37 °C in a shaking water bath. After filtration, the beads were further treated by sodium phosphate buffer with EDTA at 37 °C until dissolution. An alkali extraction ($NaHCO_3$, pH 10) at 100 °C for 30 min was also tested.

3.4. Entrapment efficiency (EE) and bioactive compounds loading (BCL)

The EE of the bioactive compounds (HT/DHPG) was calculated indirectly by determination of the free HT/DHPG content in the aqueous solution after filtration and washes, according to the following equation: EE (%) = [(Qt-Qr)/Qt] x 100, where Qt is the bioactives content initially added during the bead loading, and Qr is the sum of content recovered in the aqueous solution after separating and washing the beads.

The amount of bioactive compounds (HT/DHPG) present in the beads (bioactive compounds loading, BCL) was also determined by an indirect method according to the following equation: BCL (%) = [(Qt-Qr)/Wp] x 100, where Qt is the bioactives content initially added during the bead loading, Qr is the sum of content recovered in the aqueous solution after separating and washing the beads, and Wp is the total weight of dry beads recovered per batch. All experiments were performed in triplicate.