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Encapsulation of sesamol in phosphatidyl choline micelles: Enhanced bioavailability and anti-inflammatory activity

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ABSTRACT

Sesamol, the phenolic degradation product of sesamolin, although recognised for its anti-inflammatory effects, has low bioavailability. In this manuscript, we attempted to improve its bioavailability by encapsulation in mixed phosphatidylcholine micelles. Sesamol could be solubilised and entrapped in phosphatidylcholine mixed micelles (PCS) with 96.8% efficiency (particle size 3.0 ± 0.06 nm). Fluorescence spectra of PCS revealed lower relative fluorescence intensity (RFI 112) compared to 'free' sesamol (FS) (RFI 271). The bioaccessibility, transport across a monolayer of cells and cellular uptake of PCS was 8.58%, 1.5-fold and 1.2-fold better, respectively, compared to FS. The anti-inflammatory effects of FS and PCS were compared using LPS treated RAW 264.7 cell line and lipoxygenase inhibition. PCS effected downregulation of iNOS protein expression (27%), NO production (20%), ROS (32%) and lipoxygenase inhibition (IC_{50} = 31.24 μM) compared to FS.

1. Introduction

Sesame (*Sesamum indicum*, L.), one of the oldest oilseed crops known to mankind, is valued for its health benefits (Namiki, 2007). Health benefits of sesame are attributed to lignans, sesamin and sesamolin, which are stereospecific dimers of cinnamic alcohols bonded at carbon 8 and extracted into sesame oil (about 0.4% and 0.3%, respectively) (Peterson et al., 2010; Yashaswini, Sadashivaiah, Ramaprasad & Singh, 2017). Roasting of sesame seeds, before oil extraction, hydrolyze sesamolin to sesamol, which is a thermo stable antioxidant (Namiki, 2007). Sesamin and sesamol are reported to lower serum lipids, blood pressure, 5-delta- and desaturase activity (Chu, Hsu, Hsu, & Liu, 2010; Jeng & Hou, 2005; Kanu, Bahsoon, Kanu, & Kandeh, 2010). Sesame lignans are metabolized to mammalian lignans, viz. enterolactone and enterodiol, in the intestine by the gut microflora (Liu, Saarinen, & Thompson, 2006; Peterson et al., 2010).

Health benefits of a nutraceutical molecule can be harnessed by the human body provided it is bioaccessible and bioavailable. Bioaccessibility is defined as that amount of a bioactive molecule/nutraceutical released from the food matrix in the digestion tract for absorption, while, bioavailability is the fraction that is absorbed by the enterocyte and further transported to the blood steam (Bouayed, Deußer, Hoffmann, & Bohn, 2012). The oral bioavailability of pure sesamol in Sprague Dawley rats and tissue distribution of its metabolites (sesamol sulphate and sesamol glucuronide) has been reported (Jan, Ho, & Hwang, 2008). Sesamol had poor bioavailability and cleared rapidly with the appearance of its metabolites (within 0–2 h) – sesamol sulphate/glucuronide – in the blood serum (Hou, Tsai, Liu, Yu, & Chao, 2008; Jan, Ho, & Hwang, 2009). The metabolites have lower bioactivity compared to the parent molecule accentuating the need to prevent rapid metabolism of sesamol. Enhancement of the bioavailability is of great importance, since sesamol is a powerful antioxidant and anti-inflammatory molecule. Poor bioavailability, circulatory life and chemical/physical/biological stability of a nutraceutical molecule can be improved by encapsulation in micelles, liposomes or solid lipid nanoparticle emulsions (Augustin & Sanguansri, 2012).

The desirable qualities of nutraceutical carriers include small size, biodegradability, good drug loading capacity and high content of the bioactive ingredient in the final preparation, prolonged circulation and ability to accumulate in the target areas. These qualities are reasonably met by encapsulating nutraceuticals in microcapsules or liposomes. However, these are only used extensively for water soluble bioactive molecules (Augustin & Sanguansri, 2012; Torchilin, Lukyanov, Gao, & Papahadjopoulos-Sternberg, 2003). Mixed micelles, used successfully to encapsulate hydrophobic nutraceuticals, are prepared by combining water insoluble molecules with a surfactant like bile salts (Li et al.,

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2011). Encapsulation of nutraceuticals in mixed micelles can improve their bioavailability by enhancing their absorption and reducing the clearance time during circulation. Phospholipids, having surfactant-like properties and excellent biocompatibility (by virtue of their being main components of cellular membranes), are exceptional nutraceutical carriers and can be used for the preparation of mixed micelles. Caco-2 cell line in conjunction with stimulated *in vitro* gastric digestion offers a low cost, rapid and effective method to analyse bioavailability of nutrients.

The human colon adenocarcinoma cell line – Caco-2 cell line has been used extensively for studying permeability, absorption and bioavailability of food and beverages (Glahn, Lee, Yeung, Goldman, & Miller, 1998; Pullakhandam, Nair, Pamini, & Punjal, 2011). The cell line has been used to obtain a clear mechanistic insight into the metabolism, transport and uptake of nutrients (Maznah, 1999). The cells grow and spontaneously differentiate into a polarized monolayer of cells with tight junctions and microvilli structures. Many enzymes are produced by the differentiated cells have similar characteristics to the brush border enzymes produced by the small intestine (Maznah, 1999).

In the current report, we present the encapsulation of sesamol in phosphatidylcholine mixed micelles. Experimental data on the effect of encapsulation on bioavailability and anti-inflammatory activity of sesamol is also presented.

2. Materials and methods

Sesamol, phosphatidylcholine from soy bean, deoxysodium cholate, 2,2-diphenyl-1-picrylhydrazyl, pepsin, pancreatin, bile extract porcine, lucifer yellow dipotassium salt, 2′,7′-dichlorodihydrofluorescein-diacetate, lipopolysaccharide (LPS) and lipoxygenase were purchased from Sigma Aldrich (St. Louis, Missouri, USA). Linoleic acid was purchased from Nu-Chek-Prep (Elysian, USA). PBS, RPMI 1640, FBS, MEM(E) and antibiotics were purchased from HiMedia chemicals (HiMedia Laboratories Pvt. Ltd., Mumbai, India). Plastic-ware for cell culture experiments were purchased from SPL, India. HPLC grade methanol was purchased from Merck chemicals, India. All other reagents were of analytical grade.

2.1. Preparation of phosphatidylcholine mixed micelles and insertion of sesamol into micelles

Mixed micelles were prepared as described previously (Began, Sudharshan, & Rao, 1998) using phosphatidylcholine (PC) and deoxycholate (DOC) in the ratio of 1:2, respectively. PC and DOC were solubilized in chloroform/methanol (2:1). Sesamol (in water/Tris-HCl buffer pH 7.4, 50 mM) was added drop-wise to the prepared PC micelles solution with slow stirring to avoid foaming.

2.2. Bioaccessibility of sesame lignans: Sample preparation

Sesamol solubilized in water/Tris HCl buffer, pH 7.4 or encapsulated in 10 μM PC micelles was termed as 'free' sesamol (FS) or PC-sesamol (PCS), respectively. For analysis of the bioaccessibility of FS or PCS, 0.1 mg/ml of sample was used. For sesamin and sesamolin bioaccessibility, unroasted sesame seeds (2 g of crushed seeds in 20 ml of water) were used. For bioaccessibility of sesamol, roasted sesame seeds (2 g of crushed infrared (at 220 °C) roasted seeds in 20 ml of water) were used. Samples were subjected to pepsin (5000 units, incubation for 2 h at 37 °C) and pancreatin digestion (4 mg/ml pancreatin and 25 mg/ml of bile salt mixture, incubation for 2 h at 37 °C) as described elsewhere (McDougall, Dobson, Smith, Blake, & Stewart, 2005). Hexane (50 ml) was added to 'In' and 'Out' fractions of whole seeds samples and kept overnight (under shaking) for oil extraction. Oil obtained was subjected to saponification with 5% alcoholic KOH and unsaponified matter (containing sesamin, sesamolin and sesamol) was recovered using petroleum ether. Sesamin, sesamolin and sesamol were redissolved in methanol, after evaporating the petroleum ether, and their quantities were estimated to determine their bioaccessibility. 'In' and 'Out' fraction of FS and PCS were treated with 0.5% TFA to precipitate proteins, centrifuged and supernatants used to estimate bioaccessibility of both FS and PCS.

2.3. Estimation of sesamin, sesamolin and sesamol

Bioaccessibility of sesamin, sesamolin and sesamol was quantified using Shimadzu UFLC (model LC-20AD) equipped with PDA detector as described by Yashaswini, Rao, and Singh (2017).

2.4. Physico-chemical characterisation of PCS

2.4.1. Morphology, particle size analysis and entrapment efficiency

Morphology of 10 mM PCS was analysed using a phase contrast microscope (model BX 40, Olympus, Japan). Particle size of the prepared micelles was determined using a particle size analyser (Nanotrac wave, Microtrac®, USA). The micelles were filtered through 0.22 μ filter and the filtrate was used for particle-size determination. The entrapment efficiency (EE) of sesamol was analysed as described previously (Liu et al., 2013). Lyophilised PCS was solubilized in methanol and amount of sesamol was determined using UFLC as described in Section 2.3.

EE was calculated as:

$$\text{EE } (\%) = [\text{Experimental sesamol loading}/\text{Theoretical sesamol loading}] \times 100$$

2.4.2. In vitro release of sesamol

In vitro release of sesamol was analysed as described previously (Li et al., 2011). Briefly, 1 ml of 10 mM PC with 1 mg of sesamol or 1 ml of 1 mg sesamol in 50 mM Tris HCl buffer, pH 7.4 were placed in two separate cellulose dialysis tubings (Spectrochem) of 3.5 KDa cut off. The dialysis tubings were immersed in a beaker containing release medium (30 ml of 20 mM PBS pH 7.4 containing 30% ethanol) and kept in orbital shaking incubator at 37 °C and 100 rpm/min. At regular intervals (up to 168 h), aliquots of release medium were removed and replaced with fresh release medium. Sesamol content in the release medium were determined using UFLC as described in section 2.3.

2.4.3. Fluorescence spectroscopy

Fluorescence spectra of freshly prepared PC micelles, PCS and FS were read using a Cary Eclipse spectrofluorimeter (Agilent, USA) equipped with a peltier attachment at 25 ± 1 °C. Samples were excited at 296 nm and emission was followed from 300 to 500 nm at 25 ± 1 °C. Emission and excitation slits were 5 nm each. Concentration of sesamol and PC micelles were 10 μg and 10 mM, respectively.

2.5. Cell culture experiments

2.5.1. Cell line and maintenance

Colon adenocarcinoma (Caco-2) cells and murine macrophage-RAW 264.7 cells were obtained from the National Centre for Cell Science, Pune, India. Caco-2 cells were maintained on Eagle's Minimum Essential Media (MEM (E)) with 10% FBS and RAW 264.7 cells were maintained on Roswell Park Memorial Institute (RPMI) 1640 medium with 10% FBS at 37 °C, 95% humidity and 5% CO_2 in a CO_2 incubator (Eppendoff, model- Galaxy 170 S).