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Quercetin solid lipid microparticles: A flavonoid for inhalation lung delivery

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ABSTRACT

Purpose: The aim of the present work was to develop solid lipid microparticles (SLMs), as dry powders containing quercetin for direct administration to the lung.
Methods: Quercetin microparticles were prepared by o/w emulsification via a phase inversion technique, using tristearin as the lipid component and phosphatidylcholine as an emulsifier. The quercetin SLMs were characterised for morphology, drug loading (15.5% ± 0.6, which corresponded to an encapsulation efficiency of 71.4%), particle size distribution, response to humidity, crystallinity, thermal behaviour and *in vitro* respirable fraction. Furthermore, the toxicity and the *in vitro* transport of the SLMs on an air liquid interface model of the Calu-3 cell line were also investigated using a modified twin-stage impinger apparatus.
Results: Results showed that quercetin SLMs could be formulated as dry powder suitable for inhalation drug delivery (20.5 ± 3.3% fine particle fraction ⩽4.46 μm) that was absorbed, via a linear kinetic model across the Calu-3 monolayer (22.32 ± 1.51% over 4 h). In addition, quercetin SLMs were shown to be non-toxic at the concentrations investigated. Interestingly, no apical to basolateral transport of the micronised quercetin was observed over the period of study.
Conclusions: These observations suggest quercetin diffusion was enhanced by the presence of the lipid/emulsifying excipients in the SLMs; however further studies are necessary to elucidate the exact mechanisms.

1. Introduction

Quercetin is a flavonoid, a natural substance with a phenolic structure. Flavonoids can be divided into various classes on the basis of their molecular structure with quercetin belonging to the flavone class, which has a planar structure due to the double bond in the central ring of the 2-phenylchromen-4-one (2-phenyl-1-benzopyran-4-one) backbone. This flavonoid group can be found in abundance in onions, apples, broccoli, and berries (Nijveldt et al., 2001; Formica and Regelson, 1995). Quercetin pharmacokinetics and bioavailability in humans has previously been studied (Moon et al., 2008; Graefe et al., 2001). An important effect of quercetin is its ability to scavenge for oxygen-derived free radicals (Heijnen et al., 2001), given its antioxidant properties at low concentrations (Robaszkiewicz et al., 2007). Furthermore, *in vitro and in vivo* experiments have also shown that flavonoids possess potential anti-inflammatory, anti-allergic, antiviral, anti-carcinogenic and anti-asthmatic properties (Pettinari et al., 2006; Wu et al., 2004; Li et al., 2001; Davis et al., 2008; Gang et al., 2012; Moon et al., 2008).

Specifically, for the lungs, cell culture studies have shown that quercetin can reduce infectivity of target cells and replication against a wide variety of respiratory viruses, including herpes simplex virus and adenovirus (Chiang et al., 2003), coronavirus (Debiaggi et al., 1990), parainfluenza and respiratory syncytial virus (Kaul et al., 1985), rhinovirus (Dimova et al., 2003), and severe acute respiratory syndrome (Chen et al., 2006). Furthermore, there is evidence that quercetin plays a critical role in the amelioration of the pathogenic process of asthma in a murine model (mice) (Park et al., 2009), due to the alteration of specific cytokine production (Th1/Th2) and transcriptor factors (T-bet and GATA-3) gene expression in OVA-induced asthma model mice, suggesting quercetin could be used as a new therapeutic approach to allergic airway diseases. More recently, when given orally to guinea pigs, quercetin has been found to reduce hyper-reactivity of airways, one of the main attributes of allergic asthma (Joskova et al., 2011); causing significant broncodilation.

Furthermore, a report suggests that flavonoids such as quercetin and luteolin could stimulate Cl^- secretion by activating an entry step of Cl^- across the basolateral membrane through $Na^+/K^+/2Cl^-$ co-transporter; contributing to maintenance and/or production of

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airway surface liquid by regulating Cl^- secretion in airway epithelial cells (Asano et al., 2009), very important in chronic obstructive pulmonary disease.

Quercetin is sparingly soluble in water and has poor bioavailability. As a result, the clinical application of the drug is greatly restricted. Previous studies have tried to resolve this issue by producing nanocrystals with enhanced dissolution (Kakran et al., 2012; Li et al., 2009). An alternative approach would be to incorporate the drug into a lipid matrix (Li et al., 2009). The production of solid lipid microparticles (SLMs) has been previously studied as a respiratory drug delivery vehicle for both poorly water-soluble drugs, such as budesonide (Mezzena et al., 2009) and for more water-soluble drugs, such as salbutamol (Scalia et al., 2012). There are many advantages in using SLMs (Jaspart et al., 2005), with the most significant being the ability to control release after deposition. Furthermore, SLMs should be well tolerated *in vivo* since they are made of physiological compounds. Of course, the toxicity of the surfactants and other excipients, used for their manufacture, needs to be considered.

While solid lipid nanoparticles are currently attracting a lot of attention in the research community, solid lipid micropatrticles have been rather unexploited, especially for inhalation drug delivery (Mezzena et al., 2009; Scalia et al., 2012; Jaspart et al., 2005), where particles should have an optimum aerodynamic diameter between 1 and 6 μm (Patton, 1999). Mezzena et al. (2009) and Scalia et al. (2012) used this concept to deliver budesonide and salbutamol, two anti-asthma drugs, respectively, using glycerol behenate as the solid lipid component. In other studies, Dellamary et al. (2004), used dipalmitoylphosphatidylcholine, a biocompatible lipid and component of normal lung surfactant, to modulate immunoglobulin release while Sanna et al. (2004) applied the same concept for the formulation of SLMs to be used as carrier for lung delivery, using Compritol 888 as lipophilic component and Poloxamer as emulsifying agent.

The aim of this study was to investigate the potential use of solid lipid microparticles containing quercetin delivered as a dry powder for inhalation. The physicochemical characteristics of this formulation was investigated together with aerosol performance, *in vitro* cell toxicity and transport studies, using a Calu-3 adenocarcinoma cell line grown using an air-interface model.

2. Materials and methods

2.1. Materials

Micronised quercetin hydrate (referred to as quercetin hereafter) and tristearin were supplied by Sigma Aldrich (Steinheim, Germany). Phosphatidylcholine was supplied by Cargill (Hamburg, Germany). Ammonium acetate was supplied by Ajax Finechem Pty Ltd. (Sydney, Australia). Acetic acid was purchased from AnalaR VWR International (Poole, England) and EDTA di-sodium salt was supplied by APS Finechem (Sydney, Australia).

The Calu-3 cell line (HTB-55) was purchased from the American Type Cell Culture Collection (ATTC, Rockville, USA). Dulbecco's modified Eagle's medium, fetal bovine serum, Hanks balanced salt solution, phosphate buffer saline, HEPES, trypsin–EDTA solution (2.5 g/L trypsin, 0.5 g/L EDTA), L-glutamine solution (200 mM), nonessential amino acids solution and CelLytic M Cell Lysis (50 mM Tris–HCl, pH 8, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS) were supplied by Invitrogen (Sydney, Australia). Transwell cell culture supports (0.33 cm^2 polyester, 0.4 μm pore size) were obtained from Corning Costar (Lowell, MA, USA).

All solvents were analytical grade and were obtained from Sigma (Sydney, Australia).

Water was purified by Milli-Q reverse Osmosis (Molsheim, France).

2.2. Methods

2.2.1. Preparation of the solid lipid microparticles

For the development of SLMs loaded with quercetin, melt o/w emulsification technique was employed, since it circumvents the use of organic solvents (Mezzena et al., 2009; Jaspart et al., 2005). This approach is environmentally friendly, but also eliminates the potential for residual solvent in the final dosage form. Quercetin (1 g) was dissolved into a melted (70–75 °C) lipid phase (3.6 g tristearin) and the phase inversion was obtained adding hot (70–75 °C) MilliQ water (50 mL), containing 0.7 g of phosphatidylcholine as surfactant. The mixture was maintained at 75 °C and was subjected to high-shear mixing (21,500 rpm for 2 min, using a T-25 Ultra-Turrax; IKA-Werk, Staufen, Germany). Additionally, the o/w emulsion was sonicated at a constant duty cycle (22 kHz) for 2 min, using a probe (Microson XL2000 Ultrasonic Cell Disruptor, Misonix, Farmingdale, NY) at a power input of 80 W. The emulsion was quickly cooled to a room temperature, using an ice bath and the formed microparticles recovered by freeze-drying at −50 °C (Hetosicc, Heto Lab Equipment, Saint-Julie, Canada).

The amount of quercetin entrapped in the SLMs was determined by heating (75°C for 2 min) and sonication (10 min) of the microparticles (20–25 mg) in ethanol in sealed glass vials. The obtained sample was diluted to volume (50 mL) with methanol, filtered and assayed by high performance liquid chromatography (HPLC).

2.2.2. Physical and chemical characterisation

2.2.2.1. Particle size analysis. The particle size distribution of the SLMs was analysed using laser diffraction (Malvern Mastersizer 2000, Malvern Instruments Ltd., UK). Samples of powder (ca. 10 mg) were dispersed using the Scirocco dry dispersion unit (Malvern, UK) with a feed pressure of 4 Bar and feed rate of 50%. Samples were analysed in triplicate, with an obscuration value between 0.3% and 10% and a reference refractive index of 1.553.

2.2.2.2. Scanning electron microscopy. The morphology of SLMs particles was studied using a field emission scanning electron microscope (Zeiss Ultra plus, Carl Zeiss Pty Ltd., Sydney, Australia). The samples were sputter coated with gold to a thickness of 15 nm, under an argon atmosphere prior to analysis. Samples were dispersed onto carbon sticky tabs and images were taken at random locations.

2.2.2.3. Differential scanning calorimetry. The thermal response of quercetin raw material and SLM powders were studied using differential scanning calorimetry (DSC 823e; Mettler-Toledo, Schwerzenbach, Switzerland).

Samples (3–5 mg) were crimp-sealed in DSC sample pans and exposed to a 10 °C min^{-1} temperature ramp between 40 and 400 °C. Exothermal and endothermic peak temperatures, onset temperature and heat of enthalpy (ΔH) for each peak were determined using STARe software V.9.0x (Mettler Toledo, Greifensee, Switzerland).

2.2.2.4. X-ray powder diffraction. The X-ray powder diffraction (XRPD) pattern for the SLMs was analysed using a D5000 X-ray powder diffractometer (Siemens, Munich, Germany). Measurements were conducted at 25 °C, using Cu Kα radiation at 30 mA and 40 kV, with angular increment of 0.04° s^{-1}.

2.2.2.5. Dynamic Vapour Sorption. Dynamic Vapour Sorption (DVS) was used to investigate the relative moisture sorption and stability