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Dermal quercetin lipid nanocapsules: Influence of the formulation on antioxidant activity and cellular protection against hydrogen peroxide

T. Hatahet[a,*], M. Morille[a], A. Shamseddin[b], A. Aubert-Pouëssel[a], J.M. Devoisselle[a], S. Bégu[a]

[a] Institut Charles Gerhardt Montpellier, UMR 5253 CNRS-ENSCM-UM, Equipe Matériaux Avancés pour la Catalyse et la Santé, 8 rue de l'Ecole Normale, 34296 Montpellier Cedex 5, France
[b] Comparative Molecular Immuno-Physiopathology, IRD Laboratory, UMR-MD3, Faculty of Pharmacy, Montpellier University, 15 Avenue Charles Flahault, 34093 Montpellier Cedex 5, France

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ABSTRACT

Quercetin is a plant flavonoid with strong antioxidant and antiinflammatory properties interesting for skin protection. However, its poor water solubility limits its penetration and so its efficiency on skin. For this purpose, quercetin lipid nanocapsules were formulated implementing phase inversion technique wherein several modifications were introduced to enhance quercetin loading. Quercetin lipid nanocapsules were formulated with two particle size range, (50 nm and 20 nm) allowing a drug loading of 18.6 and 32 mM respectively. The successful encapsulation of quercetin within lipid nanocapsules increased its apparent water solubility by more than 5000 fold (from 0.5 μg/ml to about 5 mg/ml). The physicochemical properties of these formulations such as surface charge, stability and morphology were characterized. Lipid nanocapsules had spherical shape and were stable for 28 days at 25 °C. Quercetin release from lipid nanocapsules was studied and revealed a prolonged release kinetics during 24 h. Using DPPH assay, we demonstrated that the formulation process of lipid nanocapsules did not modify the antioxidant activity of quercetin *in vitro* (92.3%). With the goal of a future dermal application, quercetin lipid nanocapsules were applied to THP-1 monocytes and proved the cellular safety of the formulation up to 2 μg/ml of quercetin. Finally, formulated quercetin was as efficient as the crude form in the protection of THP-1 cells from oxidative stress by exogenous hydrogen peroxide. With its lipophilic nature and occlusive effect on skin, lipid nanocapsules present a promising strategy to deliver quercetin to skin tissue and can be of value for other poorly water soluble drug candidates.

1. Introduction

Flavonoids are plant pigments which possess physiological activities. They are found in fruits and vegetables such as apples (Awad et al., 2000), onions (Zille et al., 2009), strawberries (Shin et al., 2007), spinach (Dehkharghanian et al., 2010) and wine (Arranz et al., 2014). Their *2-phenyl-1, 4-benzopyrone C6-C3-C6* skeleton allows the classification of flavonoids into several groups of molecules regarding the presence of the *C4* ketone, *C3—C4* double bond, and the hydroxyl at *C3*. Because of an exceptional free radical scavenging (Pietta, 2000), antiinflammatory (Guardia et al., 2001) and immunomodulatory activities (Kumazawa et al., 2006) flavonoid are believed to be very promising drug candidates. As a result, flavonoids were tested for various inflammatory disorders like osteoporosis (Zhang et al., 2006), psoriasis (Vijayalakshmi and Madhira, 2014), arthritis (Guardia et al., 2001), and other cardiovascular diseases (Arranz et al., 2014). Their immunomodulatory functions were highlighted while investigating anti-cancer activity (Kandaswami et al., 2005; So et al., 1996). The main reason for the diversity of flavonoids physiological actions is their very strong antioxidative properties and the capability to scavenge free radical species and inhibit lipid peroxidation *in vitro* (Middleton et al., 2000).

Quercetin (3,3′,4′,5,7-pentahydroxyflavone) is one of the strongest antioxidants among flavonoids (Gordon and Roedig-Penman, 1998; Saija et al., 1995). In regards to systemic drug delivery, quercetin presented the highest inhibition of free radical-induced membrane lipid peroxidation, when compared to other flavonoids such as hesperetin, rutin, and naringenin (Saija et al., 1995). Quercetin was also extensively tested in cancer therapy

* Corresponding author.
E-mail address: taher.hatahet90@gmail.com (T. Hatahet).

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especially in trastuzumab-insensitive breast cancer (Wong and Chiu, 2011; Jain et al., 2014), prostate cancer (Sharmila et al., 2014), colon cancer (Zhang et al., 2012), gastric carcinoma (Borska et al., 2012), squamous-cell carcinoma of head and neck origin (Castillo et al., 1989) and chemosensitizing activity in multidrug resistance (Chen et al., 2010) with interesting results. This flavonol demonstrated metal chelating abilities (Sestili et al., 1998) and protected mice hepatic tissue from sodium fluoride induced hepatotoxicity (Nabavi et al., 2012; Kim et al., 2011), Quercetine also had positive effects on myocardial infracted rats by inhibiting mitochondrial lipid peroxidation and increasing mitochondrial marker enzymes and antioxidants (Prince and Sathya, 2012).

Quercetin also holds great promise for topical application, as it shows strong protective effect against UV-induced lipid peroxidation (Saija et al., 2003) and proved to be effective on human keratinocytes with anti-ageing activity and skin rejuvenation capability (Chondrogianni et al., 2010). Quercetin, dissolved in a mixture of ethanol, propylene glycol and water, was applied topically on hairless mice before the exposure to UV irradiation and showed wrinkle diminishing ability and an increase in collagen content with an increase in glutathione and a decrease in thiobarbituric acid reactive substances (Singh Joshan and Singh, 2013). However, because quercetin possesses a poor water solubility, instability and very low skin permeability in its crude form (Bonina et al., 1996), the development of adapted formulations should be investigated in order to deliver the effective dose of quercetin to skin tissue (epidermis). In this context, nanoformulations, such as nanostructured lipid carriers, nanoemulsions and liposomes have the potential to deliver poorly water-soluble drugs to skin tissue (Pardeike et al., 2009; Schäfer-Korting et al., 2007; Sonneville-Aubrun et al., 2004; El Maghraby et al., 2008). Among nanoformulations developed, lipid nanocapsules (LNC), prepared by a phase inversion dependent process, are spherical vesicles that can be formulated with selected size depending on excipients percentage with a high monodispersity (Heurtault et al., 2003a, 2002). Many hydrophobic drugs (taxane, etoposide, docetaxel, paclitaxel, tamoxifen), but also hydrophilic (nucleic acids, insulin, peptides . . .) and even amphiphilic compounds (amiodarone) were successfully incorporated into LNC (Lainé et al., 2013; Huynh et al., 2009; Morille et al., 2010, 2011; Lamprecht et al., 2004, 2002; Laine et al., 2014; Weyland et al., 2011; David et al., 2013; Vrignaud et al., 2013). The lipophilic composition of these particles along with their higher skin occlusive effect (Müller et al., 2002) highlight their applications for the improvement of topical delivery of drugs. In this way, quercetin lipid nanocapsules were previously prepared by Barras et al. (2009), but quercetin drug loading was limited, which is not sufficient for pharmacological application (Barras et al., 2009).

In this study, 20 nm and 50 nm LNC formulations were modified to improve quercetin drug loading by using novel excipient and a pre-solubilization step of quercetin in ethanol (Weyland et al., 2011). Physicochemical characterizations such as size, polydispersity index (PDI), surface charge, drug loading (DL), and encapsulation efficacy (EE) of quercetin were performed. This evidenced that 20 nm LNC efficiently encapsulated quercetin with a drug loading of 32.0 mM. X-ray diffractograms of crude quercetin and quercetin nanocapsules were recorded and compared to determine the influence of formulation on quercetin crystalline nature. Quercetin nanocapsules were then characterized to verify their size and spherical shape (TEM). Quercetin *in vitro* antioxidant activity was determined by DPPH assay to validate the preservation of quercetin activity after formulation. Finally, regarding a dermal application, the excessive immune response is a dominant feature of chronic inflammatory skin disorder such as psoriasis and in response to UV irradiation (Nestle et al., 2009a; Hruza and Pentland, 1993). As a consequence to chronic inflammation an additional group of dermal dendritic cells coming from monocytes and called monocytes-derived dendritic cells is activated (Auffray et al., 2009; Shortman and Naik, 2007). Therefore, quercetin interest against oxidative stress was tested on monocytic cell line (THP-1). First, the cellular toxicity of quercetin LNC formulations on THP-1 was determined via XTT assay. Second, the protective effect of these formulations against H_2O_2 induced oxidative stress was established on the same cellular model.

Lipid nanocapsules hold great promise for the topical delivery of quercetin as a UV sunscreen or even in the supportive treatment of inflammatory skin disorders such as psoriasis.

2. Materials and methods

Quercetin aglycone was purchased for Sigma-Aldrich (Sigma-Aldrich Chimie, France). Cremophor® EL (polyoxyl 35 castor oil) and Solutol® HS 15 (a mixture of free polyethylene glycol 660 and polyethylene glycol 660 hydroxystearate) were gift from BASF (Ludwigshafen, Germany). Lipophillic Labrafac® WL 1349 (caprylic acid triglycerides) and Lipoid® S75-3 (soybean lecithin at 69% of phosphatidylcholine) were kindly provided by Gattefosse® (Saint-Priest, France) and Lipoid® (Ludwigshafen, Germany) respectively. Because of the complex chemical composition of the mixtures, brand name will be used throughout the article and any amount indicated in the formulation model represents the whole mixture regardless of its constituents. NaCl was provided from Prolabo® (Fontenay-sous-Bois, France), MilliQ water was obtained by the Milli® RO System (Millipore, Paris, France). 2,3-Bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide salt (XTT) and 2′,7′ –dichlorofluorescin diacetate (DCFDA) were purchased for Sigma-Aldrich (Sigma-Aldrich Chimie, France). All HPLC chemicals and buffer components were purchased from Sigma-Aldrich (France).

2.1. Preparation of quercetin loaded lipid nanocapsules (que-LNC)

The phase inversion method reported by Heurtault et al. (2002) was improved for the preparation of quercetin lipid nanocapsules with the addition of Cremophor® EL to increase the solubility of quercetin within formulation (Heurtault et al., 2002) (Table 1). In brief, all the LNC excipients were mixed together along with quercetin. Magnetic agitation was kept at 300 rpm during the whole process. Temperature was recorded during the whole preparation process with HI98501 Checktemp® digital thermometer (Hanna Instruments, USA). A first homogenization step of the mixture was established by heating up to 85 °C. At the end of this

Table 1
Chemical composition of original lipid nanocapsules (Heurtault et al.) and quercetin modified lipid nanocapsules (w/w%).

Composition (w/w%)	Solutol® HS 15	Cremophor® EL	Labrafac® WL 1349	NaCl	Lipoïd® S75-3	Quercetin	MilliQ water
Original formula for 50 nm LNC	16.92		20.56	1.78	1.50		59.24
Modified formula for 50 nm LNC	5.00	15.00	20.56	1.78	1.50	2.85	56.16
Original formula for 20 nm LNC	38.68		17.36	1.78	1.50		40.68
Modified formula for 20 nm LNC	14.50	29.00	16.60	1.78	1.50	3.23	36.80