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Development of a Quercetin-loaded nanostructured lipid carrier formulation for topical delivery

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ABSTRACT

The main objective of this study was to evaluate the potential of Quercetin-loaded nanostructured lipid carriers (QT-NLCs) as a topical delivery system. QT-NLCs were prepared by the method of emulsion evaporation–solidification at low temperature. The average entrapment efficiency and drug loading of the optimized QT-NLCs were 89.95 ± 0.16% and 3.05 ± 0.01%, respectively. Under the transmission electron microscope, the nanoparticles were spherically shaped. The average particle size was 215.2 nm, the zeta potential was −20.10 ± 1.22 mV and pH value of QT-NLCs system was 4.65. Topical delivery of QT in the form of NLCs was investigated in vitro and in vivo. The results showed that QT-NLCs could promote the permeation of QT, increase the amount of QT retention in epidermis and dermis, and enhance the effect of anti-oxidation and anti-inflammation exerted by QT. Then the mechanism of NLCs for facilitating drug penetration was further investigated through histological sections. In conclusion, NLCs could be a promising vehicle for topical delivery of QT.

1. Introduction

In modern life, the skin is often subjected to environmental insults including excess sun exposure and increased air pollution, which commonly leads to oxidative stress (OS). Under these conditions, excess reactive oxygen species (ROS) can form, overwhelming the diminishing capability of skin, and induce skin disorders (Wheeler et al., 1986; Fuchs et al., 1989).

Quercetin (QT, 3, 3′, 4′, 5, 7-pentahydroxyflavone) is a natural flavonoid (Fig. 1) which has a variety of biological activities and pharmacological actions, such as anti-cancer, anti-oxidation, anti-inflammation, decreasing blood lipid, dilating coronary arteries, anti-platelet aggregation, anti-anemic action, and anti-anaphylaxis effects (Hollman and Kata, 1999). Recently, studies have shown that natural flavonoids possess the potential against OS-induced skin damage (Fuchs, 1998; Aquino et al., 2002). QT, the most commonly investigated flavonoid, presents the highest antiradical property compared to other flavonoids. It has been verified that QT can scavenge free radicals and inhibit lipid peroxidation (Formica and Regelson, 1995; Skaper et al., 1997). Therefore, topical application of QT has received considerable attention for the ability of against ROS-mediated damage in the skin (Casagrande et al., 2006, 2007; Vicentini et al., 2008). However, QT has a low solubility (7.7 μg/mL in water, 5.5 μg/mL in simulated gastric fluid and 28.9 μg/mL in simulated intestinal fluid), which contributes to a low absorption in vivo (Li et al., 2009; Khaled et al., 2003; Gugler et al., 1975). As a result, clinical application of QT is greatly restricted, and developing a novel vehicle that can transport sufficient QT into skin and exert its bioactivity is urgently needed.

Nanostructured lipid carriers (NLCs) are second generation lipid-based nanoparticles which are developed based on solid lipid nanoparticles (SLN) (Müller et al., 2002). In order to overcome the drawbacks of SLN including relatively low drug payloads and potential drug expulsion, liquid lipid which can disturb the highly regular lattice structure and form an imperfect matrix structure and further increase the space for accommodating drugs is introduced into NLCs (Saupe et al., 2006). In recent years, many studies have been focus on topical application of NLCs for their unique properties (Pardeike et al., 2010; Mitri et al., 2011; Nikolićć et al., 2011). Further, NLCs can enhance the apparent solubility of embedded drugs, which can form high concentration gradient on skin to facilitate drug permeation. The nano-sized particles can tightly adhere to the skin surface and transport the drugs in a more controlled

Abbreviations: QT, Quercetin; QT-NLCs, Quercetin-loaded nanostructured lipid carriers.

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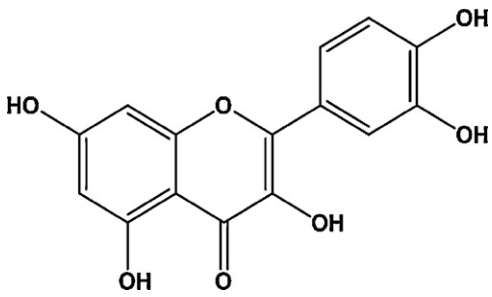

Fig. 1. The chemical structure of QT.

fashion. The occlusive effect exerted by NLCs can improve skin hydration and promote the penetration of drugs. Additionally, the components of NLCs including lipid and surfactants can be acted as permeation enhancers to loosen or fluidize the lipid bilayers of stratum corneum (SC). For example, Fang et al. (2008) prepared psoralen-loaded NLCs and evaluated the potential use in topical application. Results indicated that NLCs could significantly promote the permeation of psoralen compared to the drug suspension and lipid emulsion, showing the usefulness of NLCs as carriers for topical administration. Additionally, NLC has been considered as a novel and safe carrier for skin application of antioxidant. Ruktanonchai et al. (2009) prepared the NLCs for topical administration of alpha-lipoic acid (LA) as an effective antioxidant, found that the NLCs formulation showed lower cytotoxicity (more than 80% of cell survivals were found up to 1 μM of LA concentrations) and the similar antioxidant activity to pure LA. Coenzyme Q10 (CoQ10) as a powerful antioxidant can protect skin from ROS, recent study showed that CoQ10 loaded NLC had greater antioxidant properties and topical skin penetration than the CoQ10 loaded emulsion (Yue et al., 2010).

The aim of the present study was to design and characterize Quercetin-loaded nanostructured lipid carriers (QT-NLCs). In vitro drug permeation through excised mouse skin and in vivo drug distribution in epidermis and dermis of mice were evaluated. The effect of QT-NLCs application on the skin surface was also discussed based on the observation under light microscope. In addition, anti-inflammatory and anti-oxidation effects were investigated to estimate the potential use of NLCs for topical delivery of QT.

2. Materials and methods

2.1. Materials

QT was supplied by Xi'an Senmu Biological Technology Co. Ltd. (Xi'an, China). TPGS was purchased from Wuhan Yuancheng Co. Ltd. (Wuhan, China). Soya lecithin (SL) was obtained from Shanghai Taiwei Pharmaceutical Co. Ltd. (Shanghai, China). Glyceryl monostearate (GMS) was provided by Shanghai Chemical Reagents Co. Ltd. (Shanghai, China) and stearic acid (SA) by Beijing Chemical Reagents Co. Ltd. (Beijing, China). Media chain triglyceride (MCT) was purchased from Tieling Beiya Medicinal Oil Co. (Tieling, China). All other chemicals and solvents used in the study were of analytical reagent grade.

2.2. Preparation of QT-NLC

QT-NLCs were prepared by the method of emulsion evaporation–solidification at low temperature according to the previous report (Jia et al., 2010; Li et al., 2009). In brief, based on the formulation compositions, certain amount of QT, GMS, SA and MCT were dissolved in chloroform, and SL was dissolved in acetone, and then the two organic phases were mixed and kept in a water bath at 70 °C. The aqueous phase was prepared by dissolving TPGS in distilled water at 70 °C. Subsequently, the organic phase was slowly injected into the aqueous phase with magnetic stirring (800 rpm) and the resulting solution was emulsified for 4 h at 70 °C. After removing the organic solvent, semi-transparent nanoemulsion was obtained, and then it was transferred into cold distilled water (0–2 °C) under stirring for 2 h in order to acquire QT-loaded NLCs.

2.3. Physicochemical characterization of QT-NLCs

2.3.1. Morphology

The microstructure of QT-NLCs was observed using transmission electron microscope (TEM, JEM-1200EX, JEOL, Tokyo, Japan). Initially, samples diluted with double-distilled water were deposited on a film-coated copper grid, following by stained with 1% aqueous solution of phosphotungstic acid, ultimately the superfluous phosphotungstic acid on the samples was wiped off by filer paper and the sample was allowed to dry before examined under the TEM.

2.3.2. Particle size, zeta potential and pH value

The mean diameter of QT-NLCs was measured by photon correlation spectroscopy using a particle sizer (Zetasizer 3000 HAS; Malvern Instruments Ltd., Worcestershire, UK) at a fixed angle of 90° with a He–Ne laser of 633 nm at 25 °C. Particle size was evaluated using volume distribution.

The zeta potential was determined using a microscopic electrophoresis system (DXD-II; Jiangsu Optics Co. Ltd., Jiangsu, China) at 25 °C.

The pH values were evaluated at 25 °C using a pHS-25 digital acidimeter (Shanghai Rex Instrument Factory, Shanghai, China).

2.4. Entrapment efficiency (EE) and drug loading (DL)

Based on the pre-experiment, mini column centrifugation technique was adopted to separated free drug from prepared NLCs (Fry et al., 1978). In brief, 0.5 mL of the prepared QT-NLCs suspension was placed in a minicolumn of Sephadex G-50 made from the barrel of 5 mL syringe (pre-saturated with drug-free NLCs prepared with the same composition and methods) and centrifuged at 500 rpm for 1 min and the column was subsequently eluted six times with 0.5 mL of distilled water under the same condition. Then a certain volume of ethanol was added to the collected elutes containing drug-loaded NLCs, and the resultant was vortexed for 3 min to ensure QT completely dissolve. After centrifugation at 10,000 rpm for 10 min, QT dissolving in the supernatant was measured using an ultraviolet–visible spectrophotometer (UV-2102, Shanghai Instrument Ltd., China) at a wavelength of 373 nm. EE and DL of QT-NLCs were calculated according to the following equations (Li et al., 2009):

$$\mathrm{EE}(\%) = \frac{W_{\mathrm{entrapped}}}{W_{\mathrm{total}}} \times 100\% \quad (1)$$

$$\mathrm{DL}(\%) = \frac{W_{\mathrm{entrapped}}}{W_{\mathrm{NLCs}}} \times 100\% \quad (2)$$

In above equations, $W_{\mathrm{entrapped}}$ showed the amount of QT entrapped in QT-NLCs, W_{total} was the total amount of QT in QT-NLCs, and W_{NLCs} presented the weight of QT-NLCs.

2.5. Superoxide radical scavenging activity in vitro

The autoxidation of pyrogallol could occur in weak alkaline environment, and the autoxidation products had UV absorption at a wavelength of 325 nm. When the antioxidant was added into this system, the autoxidation rate of pyrogallol was reduced. Based on the previous report (Huang et al., 2011), the anti-oxidation effects of