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Preparation, physicochemical characterization, and antioxidant effects of quercetin nanoparticles

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Abstract

The purpose of this study was to develop quercetin-loaded nanoparticles (QUEN) by a nanoprecipitation technique with Eudragit® E (EE) and polyvinyl alcohol (PVA) as carriers, and to evaluate the antioxidant effects of quercetin (QU) and of its nanoparticles. The novel QUEN systems were characterized by particle size and morphology, yield and encapsulation efficiency, differential scanning calorimetry (DSC), powder X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FT-IR), ^{1}H nuclear magnetic resonance (^{1}H NMR), and dissolution study. It was observed that the weight ratio of QU:EE:PVA at 1:10:10 carried a particle size of <85 nm, a particle distribution with polydispersity index <0.3, and its yield and encapsulation efficiency were over 99%. The results from XRD and DSC of the QUEN showed that the crystal of the drug might be converted to an amorphous state. The FT-IR and ^{1}H NMR demonstrated that QU formed intermolecular hydrogen bonding with carriers. The release of the drug from the QUEN was 74-fold higher compared with the pure drug. In addition, the antioxidant activity of the QUEN was more effective than pure QU on DPPH scavenging, anti-superoxide formation, superoxide anion scavenging, and anti-lipid peroxidation.

Keywords: Quercetin; Nanoparticles; ^{1}H nuclear magnetic resonance; Free radical-scavenging activity; Anti-lipid peroxidation

1. Introduction

Reactive oxygen species (ROS), such as superoxide anion, hydroxyl radical, and hydrogen peroxide, have a causal relationship with oxidative stress. Many studies have demonstrated that overproduction of ROS can further aggravate the oxidative stress and the result is a unifying mechanism of injury in many developments of clinical disease processes, such as heart disease (Giordano, 2005), diabetes (Rolo and Palmeira, 2006), liver injury (Jaeschke, 2000), cancer (Klaunig and Kamendulis, 2004), aging (Bokov et al., 2004), etc. The balance of ROS and antioxidant is a major mechanism in preventing damage by oxidative stress. Therefore, the dietary supplement of antioxidants such as vitamins (Fairfield and Fletcher, 2002), flavonoids (Peluso, 2006), etc., has been used to prevent the occurrence of many chronic diseases.

Quercetin (QU) (Fig. 1), is a well-known flavonoid distributed ubiquitously in fruits, vegetables, and herbs or related products, e.g. apples, onions (Hertog et al., 1992), *Ginkgo biloba* (Watson and Oliveira, 1999), and red wine (Kerem et al., 2004), respectively. QU has been extensively investigated for its pharmacological effects that include anti-tumor (Kanadaswami et al., 2005), anti-inflammatory (Comalada et al., 2005), antioxidant (Inal and Kahraman, 2000), and hepatoprotective (Lee et al., 2003) activities.

Clinical studies investigating different programs of administration of QU have been limited by its poor water solubility. Many researchers have attempted to improve its solubility by adding dimethylsulfoxide (DMSO) (Ader et al., 2000). How-

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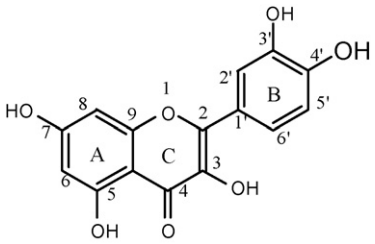

Fig. 1. Chemical structure of quercetin (3,3′,4′,5,7-pentahydroxylflavone).

ever, the safety of the higher DMSO is questionable due to risk of vasoconstrictor effect and neurological toxicity (Windrum et al., 2005). Additionally, a water-soluble derivative of QU has been synthesized but its bioavailability was only 20% (Mulholland et al., 2001). Various techniques have also been used to increase the solubility of QU including the complexation with cyclodextrin and liposome (Pralhad and Rajendrakumar, 2004; Yuan et al., 2006). Nevertheless, the use of cyclodextrin is associated with a risk of nephrotoxicity (Frijlink et al., 1991) and employing liposome might incur stability problems during storage (Mu and Zhong, 2006). It is therefore clear that a safe, stable, and efficient delivery method in increasing the solubility of QU is warranted.

Nanoparticles are particularly useful in drug delivery for water-insoluble compounds such as cyclosporine A (Dai et al., 2004), ellagic acid (Bala et al., 2006) and coenzymeQ10 (Hsu et al., 2003) because their size (less than 1000 nm) can increase the absorption and the bioavailability of the delivered drug. A novel quercetin nanoparticles system (QUEN) was therefore prepared by a simple nanoprecipitation technology with Eudragit® E (EE) and polyvinyl alcohol (PVA) as carriers. The physicochemical characterization of the QUEN was inspected by transmission electron microscopy (TEM), differential scanning calorimetry (DSC), powder X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FT-IR), ^{1}H nuclear magnetic resonance (^{1}H NMR), and dissolution study. Furthermore, the antioxidant effects of pure QU and of its nanoparticles were also determined by free radical scavenging, anti-lipid peroxidation, anti-superoxide formation, and scavenging superoxide studies.

2. Materials and methods

2.1. Materials

Quercetin (QU), polyvinyl alcohol (PVA), Tris–HCl, thiobarbituric acid (TBA), ferrous chloride, ascorbate, xanthine, xanthine oxidase, cytochrome *c*, 2,2-diphenyl-1-picrylhydrazyl (DPPH), dimethyl sulfoxide (DMSO), and ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma–Aldrich Chemicals Co. (St. Louis, MO, USA). Aminoalkyl methacrylate copolymers (Eudragit® E; EE) were obtained from Röhm Pharma (Dramstadt, Germany). All other chemical regents were of analytical grade.

2.2. HPLC analysis of quercetin

The chromatographic system was composed of a pump P-680, autosampler ASI-100, and UVD-170U detector. The analytical column used was the LichroCART® Purospher® STAR (250 mm × 4.6 mm i.d., 5 μm) and the temperature must be maintained at 37 °C. The mobile phase was composed of 25 mM phosphate buffer and acetonitrile (50:50), and pH value was adjusted to 2.3 with hydrochloride acid. The flow rate was set at 0.5 ml/min. The wavelength of UV detector was kept at 258 nm. The calibration curve of quercetin was linear ($r = 0.9999$) within range 1–100 μg/ml. The relative standard deviations of the intra-day and interday were less than 5% ($n = 5$).

2.3. Preparations of physical mixtures and nanoparticles systems

2.3.1. Physical mixtures

The physical mixtures were pulverized and mixed with different ratios of QU:EE:PVA (1:1:1, 1:5:5, and 1:10:10; w/w/w) in a mortar.

2.3.2. Nanoparticles systems

Nanoparticles systems were prepared with various ratios of QU:EE:PVA (1:1:1, 1:5:5, and 1:10:10; w/w/w) by the nanoprecipitation technique (Bilati et al., 2005; Zili et al., 2005). An amount of 100 mg of quercetin and the appropriate amount of EE were dissolved in 50 ml of ethanol. The internal organic phase solutions were quickly injected into the 150 ml external aqueous solution containing the appropriate amount of PVA, and then the solutions were homogenized at 22,000 rpm for 25 min. The ethanol was completely removed by rotary vacuum evaporation at 40 °C water bath and then lyophilized with a freeze dryer. The lyophilized powders were collected and stored in the moisture-proof instrument until use.

2.4. Particle size analysis

The mean particle size and polydispersity index (PI) of the QUEN were determined by a N5 submicron particles size analyzer (Beckman Coulter, USA). The samples were diluted 10-times with distilled water for analysis. Each value was measured in triplicate determination. The results are showed as mean ± standard distribution.

2.5. Yield and encapsulation efficiency

Regarding the yield of the QUEN, the appropriate volume of each sample was dissolved in methanol and the drug concentration was measured by the above-mentioned HPLC method. Additionally, the encapsulation efficiency of the QUEN was conducted according to the modified procedures described previously (Venkateswarlu and Manjunath, 2004). The encapsulated and unencapsulated portions of QU from the QUEN were separated using the centrifugal filter devices (Microcon YM-10, Millipore®) with centrifuged at 10,000 rpm for 30 min. The yield and encapsulation efficiency of QU can be calculated by the following equations (1) and (2):

$$\text{yield } (\%) = C_Q \times \frac{V_Q}{W_Q} \times 100 \quad (1)$$