Short Communication

Transdermal Delivery of Small-Sized Resveratrol Nanoparticles to Epidermis Using Anionic Phospholipids

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Abstract

Composite nanoparticles composed of anionic phospholipid of 1,2-dipalmitoyl-sn-glycero-3-phosphorylglycerol (DPPG) and resveratrol (Res) were successfully prepared by mixing them in water and a subsequent heating/cooling process. Small-sized DPPG-Res nanoparticles (<60 nm) could be prepared by ultrasonic fragmentation. Upon addition of size-controlled fluorescently labeled Res (FLRes) nanoparticles stabilized with DPPG (DPPG-FLRes) to rat skin tissue, FLRes molecules infiltrated into the epidermis layer permeating stratum corneum.

Keywords

resveratrol, DPPG, skin, epidermis, nanoparticle

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Resveratrol (Res; Figure 1) is a natural polyphenol containing a stilbene functional group in its molecular framework and it is found in fruits, such as grapes, berries, peanuts, and some medicinal plants. Res can inhibit formations of reactive oxygen species to control intracellular redox balance. As a result, Res exhibits relevant biological activities such as anticarcinogenic, anti-inflammatory, antiaging, antiamyloid, and antimicrobial activities.1–4 Especially, applications of Res to skin-care materials are promising. Since skin is frequently exposed to oxidative stress from ultraviolet radiation, which is a risk of cancers in worse cases such as melanoma, basal cell carcinoma, and squamous cell carcinoma, efficient transdermal delivery of Res would be useful to prevent these serious skin cancers. Also important is that Res is a natural abundant biocompatible oxidant, and thus it is practically advantageous. However, 10–40 µm thick stratum corneum layer consisting of densely packed cells provides a barrier to protect the underlying tissue from infection, chemicals, dehydration, and mechanical stress, which prevents permeation of large nanoparticles across the skin tissue. Because of this, transdermal delivery of Res to epidermis deeper than stratum corneum is particularly difficult. Although strategies for transdermal delivery of Res utilizing chemical penetration enhancers such as surfactants have been investigated, these chemical reagents sometimes destroy skin tissue to cause irritations. We have developed water-soluble Res nanoparticles stabilized by 1,2-dipalmitoyl-sn-glycero-3-phosphorylcholine (DPPC; Figure 1), a component of biological membranes, have been reported.5–10 Nevertheless, creations of phospholipid-based small-sized Res nanoparticles are still challenging because neutral phospholipids tend to form large-sized vesicles due to membrane fusions of the self-assemblies, which sometimes resulted in insufficient skin penetrations.

Here we report Res nanoparticles stabilized with anionic phospholipids of 1,2-dipalmitoyl-sn-glycero-3-phosphorylglycerol (DPPG; Figure 1). The size of DPPG-Res nanoparticles can be easily tuned by an ultrasonic fragmentation for the preparation of small-sized nanoparticles. Upon addition of size-controlled nanoparticles composed of fluorescent NBD-labeled Res (FLRes; Figure 1) stabilized with DPPG (DPPG-FLRes) to rat skin tissue, FLRes molecules infiltrated into epidermis layer permeating stratum corneum.

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stratum corneum. Since charged particles are hard to approach each other due to electrostatic repulsion, we expected that anionic phospholipids would prevent problems of fusion events in the case of conventional neutral phospholipids. DPPG is an anionic phospholipid synthesized in physiological conditions and molecularly similar to DPPC. Anionically charged DPPG is known to control lung pressure and functions of mitochondria because of repulsive force. In addition, DPPG molecules are expected to form kinetically stable nanoparticles maintaining the assemblies in physiological conditions because bilayer melting temperature ($T_m$) of DPPG is higher than body temperature (41 °C).

We utilized a neutral DPPC and an anionic DPPG to hybridize with Res and compared fundamental differences (Figure 2(A)). First of all, we dispersed DPPC powder (5.0 wt%) and Res powder (Figure 2(B) 1 mM) and heated for 15 minutes at 60 °C above the $T_m$ of DPPG (41 °C), and then cool down to room temperature. Consequently, we observed that the solution became a cloudy dispersion after mixing Res and DPPG (Figure 2(C)). Particle size analysis by laser diffraction revealed that the size of the Res hardly decreased (Figure 2(E), gray and blue), indicative of the formation of DPPC-Res particles although the size was still large. In sharp contrast, upon mixing Res with DPPG instead of DPPC, a highly transparent dispersion was observed after the preparation (Figure 2(D)). In line with the necked-eye observation, particle size analysis also showed a size of 100 nm, which was clearly smaller than DPPC-Res (Figure 2(E), red). We confirmed the encapsulation of Res into DPPG by absorption spectra, in which the absorption peak of Res around 300 nm was clearly observed from the DPPG-Res dispersion (Figure 2(F), red). This spectral profile is because of high dispersibility of DPPG-Res nanoparticles because Res powder hardly showed the peak due to the precipitation (Figure 2(F), gray).

Tuning particle size is important for designing drug delivery systems. Especially, small-sized nanoparticles are preferable for the transdermal drug delivery system because they need to penetrate narrow space between skin cells. For this purpose, we next tried to create small-sized DPPG-Res nanoparticles. When we performed an ultrasonication treatment to the sample for 3 hours, the DPPG-Res nanoparticles were fractionated to 54 nm-sized nanoparticles as confirmed by a particle size distribution analysis (Figure 3(A)). Transition electron
microscopy (TEM) also confirmed nanoparticles with around 50 nm size (Figure 3(B)). As can be seen from representative examples of previously reported composite nanoparticles composed of phospholipids and Res (Figure 3(C)),6–10 the size of DPPG-Res nanoparticles in this work was remarkably small, which have been hard to achieve so far. We consider that repulsive force between nanoparticles derived from anionic phospholipid of DPPG prevented fusion events between the nanoparticles.

Next, we investigated the skin permeability of DPPG-Res nanoparticles (Figure 4(A)). For the evaluation of skin permeation capability, we prepared small-sized DPPG-FLRes nanoparticles (Figure 4(B)) in the same method as DPPG and incubated them with rat skin tissue placed on Franz diffusion cells. We prepared a histological section of the skin sample after 24 hours of incubation and performed fluorescent microscopic observation. Surprisingly, strong fluorescence was successfully observed due to the penetration of FLRes molecules not only to the stratum corneum but also to the epidermis layer (Figure 4(D) and (F)), as compared with the sample without DPPG-FLRes (Figure 4(C) and (E)). Although the molecular structure of FLRes is not exactly as same as that of Res, we expected that DPPG-Res nanoparticles would have rather high skin permeation capability because the molecular structure of Res is much smaller than that of FLRes.

In this work, we have reported Res nanoparticles stabilized by anionic phospholipids of DPPG. The DPPG-Res nanoparticles can be easily prepared by heating followed by a cooling treatment to aqueous mixtures of DPPG and Res, and the nanoparticles can be fractionated by an ultrasonication treatment to prepare small-sized nanoparticles. Upon addition to rat skin tissue, the small-sized DPPG-Res nanoparticles were penetrated into the skin barrier of stratum corneum. Although Res is a well-known anti-oxygen agent, applications of Res to...
skin-care materials have been still challenging because of difficulty in transdermal delivery. The DPPG-Res nanoparticles having skin permeability demonstrated in this study would be a new candidate as skincare materials with antioxygen high effects.

**Experimental**

**General**

Ultrasonication was performed by using a QSonica model ultrasonic homogenizer. Particle sizes were measured by using a Horiba model LA-960 laser diffraction particle size analyzer (SALD) or a Malvern model Zetasizer Nano ZSP zeta potential analyzer (DLS). TEM was performed using a JEOL model JEM-1230 transmission electron microscope operating at an anode voltage of 80 kV, and the sample was stained with gadolinium acetate. DPPG and DPPC were purchased from Avanti Polar Lipids. FLRes was obtained as reported previously.11

**Preparation of DPPG-Res, DPPC-Res, and DPPG-FLRes Particles**

Res (1.0 mM) was mixed with DPPG powder (5.0 wt%) in water and sonicated for 2 minutes to disperse homogeneously, and then heated at 60 °C for 15 minutes where the solution turned clear. The resulting mixture was kept stand at room temperature for 1 hour before use. DPPC-Res was prepared in the same method except for using DPPC powder (5.0 wt%) instead of DPPG powder (5.0 wt%). DPPG-FL Res was prepared in the same method as DPPG-Res except for using FLRes (2.3 mM) instead of Res (1.0 mM). To prepare small-sized DPPG-piceid and DPPG-FL Res nanoparticles, the samples were ultrasonicated at 50 W for 3 hours with keeping the temperature at 4 °C.

**Transdermal Delivery**

In vitro skin permeation tests were performed using a vertical Franz diffusion cell with an effective diffusion area of 0.95 cm². Skin tissues were obtained from the abdominal hair of rats according to that reported previously.12 The subcutaneous fat and other extraneous tissues of rat skin were trimmed and removed. A piece of excised skin (area 3.14 cm², diameter 20 mm) was mounted on the Franz diffusion cell with the stratum corneum facing the donor compartment. One circular SS Nikasol or SS HGA patch (area 0.785 cm², diameter 10 mm) was applied to the stratum corneum side of the skin. The receptor compartment was filled with 3 mL of water and maintained at 32 °C using a circulating water bath stirred with magnetic bars. For microscopic observations, skin tissue was embedded into OCT compound, frozen, and cryosectioned.

**Declaration of Conflicting Interests**

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