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Research paper

Ultra-small lipid nanoparticles promote the penetration of coenzyme Q10 in skin cells and counteract oxidative stress

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ABSTRACT

UV irradiation leads to the formation of reactive oxygen species (ROS). An imbalance between the antioxidant system and ROS can lead to cell damage, premature skin aging or skin cancer. To counteract these processes, antioxidants such as coenzyme Q10 (CoQ10) are contained in many cosmetics. To improve and optimize cell/tissue penetration properties of the lipophilic CoQ10, ultra-small lipid nanoparticles (usNLC) were developed. The antioxidant effectiveness of CoQ10-loaded usNLC compared to conventional nanocarriers was investigated in the human keratinocyte cell line HaCaT. Using confocal laser scanning microscopy investigations of the carriers additionally loaded with nile red showed a clear uptake into cells and their distribution within the cytoplasm. By use of the XTT cell viability test, CoQ10 concentrations of 10–50 μg/ml were shown to be non-toxic, and the antioxidant potential of 10 μg/ml CoQ10 loaded usNLC in the HaCaT cells was analyzed via electron paramagnetic resonance spectroscopy after cellular exposure to UVA (1 J/cm^2) and UVB (18 mJ/cm^2) irradiation. In comparison with the CoQ10-loaded conventional carriers, usNLC-CoQ10 demonstrated the strongest reduction of the radical formation; reaching up to 23% compared to control cells without nanocarrier treatment. Therefore, usNLC-CoQ10 are very suitable to increase the antioxidant potential of skin.

1. Introduction

The skin is the primary barrier of the body and protects it against harmful environmental factors, e.g. heat, mechanical stress, microbes, xenobiotics or radiation [1,2]. Normally, there is a balance between the antioxidant defense system and the amount of reactive metabolites of the body, which are produced during various physiological processes (e.g. enzymatic oxidation, cell respiration) [3]. However, exogenous factors such as xenobiotics and ultraviolet (UV)/infrared (IR) irradiation can promote excess production of free radicals and thus lead to an imbalance with the endogenous antioxidant system within the skin. This imbalance leads to oxidative stress, which is associated with harmful oxidation of proteins, lipid peroxidation and DNA mutations [4–7]. These changes may adversely affect chemical processes and signaling pathways so that clinical effects, such as premature skin aging, tissue damage, photocarcinogenesis or immunosuppression can develop [8,9] (Fig. 1).

The reactive metabolites induced by UV irradiation include reactive oxygen species (ROS) and reactive nitrogen species (RNS) [2]. The antioxidant system of the body scavenges or reduces free radicals and comprises endogenous enzymatic (e.g. catalase, glutathione peroxidase) and non-enzymatic components (e.g. uric acid, bilirubin, glutathione) as well as exogenous antioxidants, which have to be taken up with food [3,10].

Due to the fact that an increased concentration of free radicals (especially ROS) and a decrease in endogenous or exogenous

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antioxidants is associated with premature cell aging ("the free radical theory of aging") [11], antioxidants are used in many skin care and sun protection products [12]. One example of such an antioxidant is the coenzyme Q10 (CoQ10) [13,14]. CoQ10 is a part of the mitochondrial respiratory chain and operates as cofactor by transmitting free electrons of complex I and complex II to complex III during the process of oxidative phosphorylation and ATP synthesis [15,16]. Moreover, in its reduced form (ubiquinol), CoQ10 has a strong antioxidant potential. It inhibits lipid peroxidations and protects proteins of the inner mitochondrial membrane, and the DNA against oxidative stress [15,16]. Within the skin, its concentration in the epidermis is about 10-fold higher than in the dermis [17]. It has been shown that a six-monthly topical application of CoQ10 in ethanol as a vehicle caused a measurable reduction in wrinkle depths in aged skin [18]. The improved delivery of Q10 from lipid nanoparticles was already shown by some studies [19,20]. Recent studies also showed an increased penetration of CoQ10 in rat abdominal skin by the development of "solid lipid nanoparticle CoQ10 loaded hydrogels" [21]. Increased penetration of CoQ10 from solid lipid nanoparticles is caused by the formation of an invisible lipid film on the skin upon application of the particles, which leads to occlusion and thus to improved penetration of them [19,22–25]. Hence, lipid nanoparticles are of particular interest for the dermal delivery of actives [26,27]. CoQ10-loaded nanostructured lipid carriers (NLC), known as the second generation of lipid nanoparticles [28] with a size of about 220 nm were previously developed and their potential for the improved delivery of CoQ10 in comparison with traditional formulations was demonstrated [29]. To further improve the penetration characteristics of CoQ10, we recently developed ultrasmall lipid nano-carriers (usNLC) [30]. These particles have a size of only approximately 85 nm and consist of a solid lipid core and an outer sheath of liquid lipid, in which the active ingredient (CoQ10) is located [31,32]. An improved dermal delivery of CoQ10 by these particles could be shown by means of a Franz diffusion cell system with porcine skin and via tape stripping analysis [30]. The structure of these nanoparticles improves their solubility and bioavailability compared to other classical nanocarrier systems, such as nanostructured lipid carriers (NLC) or nanoemulsions (NE) [28,30,33].

In this study, usNLC-CoQ10 were investigated for their influence of viability, their uptake (laser scanning microscopy LSM) and antioxidant potential (electron paramagnetic resonance spectroscopy (EPR) measurements) in skin cells, and were compared to classical carrier systems (NLC, NE). The results were completed with investigations on the cell viability after incubation and/or irradiation with UV light. For these experiments, the HaCaT keratinocyte cell line was used. With about 90%, keratinocytes represent the most common cell type in the epidermis and are thus constantly exposed to UV radiation. HaCaT cells are frequently used as an epidermal model system and are well suited for studies of antioxidant capacity [34]. For the first time, this study has demonstrated the antioxidant potential of usNLC loaded with CoQ10 in UV-exposed epidermal cells.

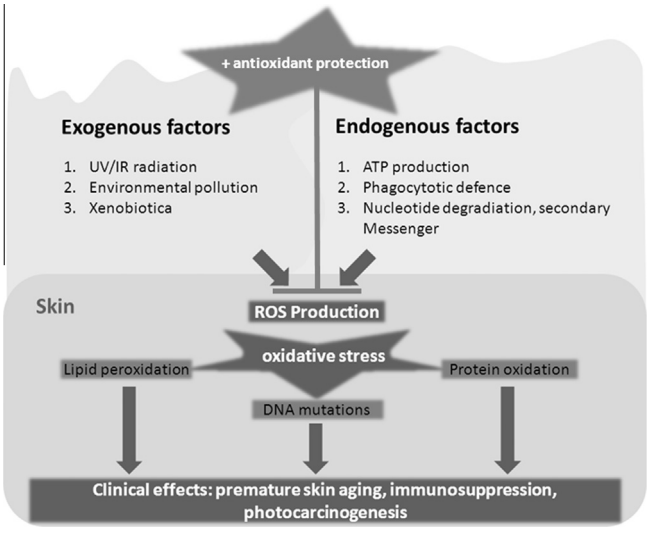

Fig. 1. Development of reactive oxygen species in skin. Overview of exogenous and endogenous factors that may promote oxidative stress in the skin and can lead to clinical effects and the mode of action of anti-oxidative agents.

2. Materials and methods

2.1. Material nanoparticle systems

The materials for the analyzed nanoparticles (usNLC-CoQ10, NLC-CoQ10 and NE-CoQ10 and usNLC w/o CoQ10) were obtained as described [30]. CoQ10 was obtained from BIK Internationaler Handel GmbH (Oberrieden, Switzerland), dioctylether (Cetiol® OE) and cetyl palmitate from Cognis (now BASF, Ludwigshafen, Germany), medium chain triglycerides (Miglyol® 812) from Gattefosse, (Nanterre, France), polyglyceryl-3 methylglucose distearate (Tegocare® 450) from Goldschmidt (now Evonik, Essen, Germany), polyoxyethylene (20) sorbitan monooleate (Tween® 80) from Uniqema Ltd. (Everberg, Belgium) and sorbitan monolaurate (Span® 20) from Casesar & Loretz (Hilden, Germany).

2.2. Nanocarrier preparation and particle size analysis

The different nanocarrier systems (Table 1) were produced as previously described [29,35]. All formulations were obtained by using the continental emulsification method, i.e. prior to homogenization a pre-emulsion was obtained by pouring the heated water phase (85 °C) into the melted oil phase. The obtained mixture was stirred for 30 s using an Ultra Turrax T25 (Janke and Kunkel, Staufen, Germany) and subsequently subjected to hot high pressure homogenization (HPH) using a Micron LAB 40 (APV Deutschland GmbH, UNNA, Germany) in discontinuous mode. HPH production conditions for NLC were three cycles at 500 bar and 85 °C, and three homogenization cycles at 800 bar and 75 °C for the usNLC and the nanoemulsion. The particle size was analyzed using dynamic light scattering (DLS) (Zetasizer Nano ZS, Malvern, Herrenberg, Germany) and light microscopy (Ortophlan, Leitz, Dürkheim, Germany). DLS measurements consisted of 10 single measurements and were analyzed using the standard mode. Light microscopy at 160× and 100× magnification was used to prove the absence of larger particles. The zeta potential of the particles was analyzed in water adjusted to a conductivity of 50 μS/cm by using a Zetasizer Nano ZS (Malvern, Herrenberg Germany). The compositions, sizes, polydispersity indices and zeta potentials of the nanocarriers are shown in Table 1.

2.3. Cell culture preparation, nanocarrier incubation and UV irradiation

Human keratinocytes (HaCaT cells, Deutsches Krebsforschungszentrum, Heidelberg Germany) were cultivated in 75 cm^2 flasks in RPMI 1640 medium (Life Technologies GmbH, Darmstadt, Germany) supplemented with 2% glutamine (Biochrom GmbH, Berlin, Germany), 10% fetal calf serum (PAA Laboratories GmbH, Wien Austria), and 1% streptomycin/penicillin (Biochrom GmbH, Berlin, Germany). Cells were cultivated at 37 °C, 100% humidity and 5% CO_2 and were seeded in new flasks every 2–3 days. For irradiation experiments, an UVA lamp (315–400 nm; dermalight 80 UV-A; Dr.