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ABSTRACT

The cosmetic industry requires more and more expensive actives and ingredients such as retinol, coenzyme Q10, proteins, peptides and biotechnologically produced molecules. In this study, we demonstrate the development of a cost effective formulation of a nanostructured lipid carrier (NLC) or solid lipid nanoparticles (SLN) improving peptide delivery into skin. NLC or SLN are very suitable vehicles for the delivery of active ingredients into skin. The SLN, produced by using hot high pressure homogenization method combine advantages such as physical stability, protection of incorporated labile actives and controlled release. By the used method we dispersed the amorphous heptapetide DEETGEF in shea butter and homogenized this pre-dispersion at 60°C together with the water phase using a Microfluidizer at 1000 bar. The analysis of the obtained SLN-P7 showed a particle size of 173 nm, incorporated peptide of 0.014%, entrapment efficiency of 90.8%, melting peak (DSC) of the core lipid of 27°C and a zeta potential of -54 mV. By an ex vivo study with skin explants we could stimulate NQO1 (NAD(P)H quinone oxidoreductase), HMOX1 (Heme oxygenase-1) and PRDX1 (Peroxiredoxin-1) genes all of which are cell protecting enzymes. In a multicellular protection against UV induced stress study with skin explants we detected the formation of sun burn cells and the number and morphology of Langerhans cells. The application of our SLN-P7 formulation on skin explants led to a significant and dose dependent protection against UV irradiation. In the clinical suction blister study, irradiation with UVA light for two hours after final product application led to a statistically significant increase of the 8-OhdG (8-hydroxy-2´-deoxyguanosine) concentration in the human epidermis. The skin treated with our verum formulation showed a statistically significant 20% decrease in DNA damage compared to placebo. In conclusion, it was demonstrated that SLN technology enabled peptide delivery into skin allowing it to perform protective functions.

1. Introduction

It is well known that lifestyle factors such as sun bathing or smoking and environmental factors such as pollution accelerate skin aging. The common mechanism involved is based on the formation of reactive chemical species. These reactive chemical species finally lead to DNA damage and the formation of oxidized lipids and proteins. The cells in our tissues normally react with antioxidant molecules by an up-regulated expression of antioxidant and detoxification enzymes. The expression of these proteins is regulated by the transcription factor Nrf2 which binds at the ARE site in the promoter regulatory sequence to induce gene expression (Figure 1) [1]. Under basal conditions, Nrf2 is repressed in the cytoplasm by binding to Keap1. Disruption of the Nrf2 - Keap1 complex would be a way to enhance protection against reactive chemical species.

Figure 2 shows the interaction between Nrf2 and Keap1 at the molecular level. To activate Nrf2, a peptide was designed to compete with Nrf2 for binding to Keap1. The sequence acetyl-DEETGEF was chosen, which contains the Keap1 binding motif of Nrf2 [2]. The challenge was to deliver this hydrophilic peptide in a stabilized and encapsulated form to the target cytosol in skin cells.

In the present study, SLN technology was used to overcome these problems. SLN were developed at the beginning of 1990s based on the concept of solid particles, emulsions and liposomes [3-7]. This method possesses several advantages: delivery system enables the use of physiologically acceptable lipids, the avoidance of organic solvents in the preparation process, protection of sensitive molecules from the environment and controlled release characteristics. The most important disadvantages are the polymorphic transitions and inherently low incorporation capacities due to the crystalline structure of the solids. In this study the SLN-P7 was produced by hot high pressure homogenization method using hydrogenated lecithin as emulsifying agent and shea butter as molten lipids and carrier for the hydrophilic peptide. The physicochemical properties of the resulted carriers, such as particle size, entrapment efficiency and *in vitro* release were determined. The formulation was also tested *ex vivo* on human skin regarding its ability to influence gene expression, and to protect skin cells from UV damage.