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Research paper

Enhancement of skin radical scavenging activity and *stratum corneum* lipids after the application of a hyperforin-rich cream

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ABSTRACT

Hyperforin is well-known for its anti-inflammatory, anti-tumor, anti-bacterial, and antioxidant properties. The application of a hyperforin-rich verum cream could strengthen the skin barrier function by reducing radical formation and stabilizing *stratum corneum* lipids. Here, it was investigated whether topical treatment with a hyperforin-rich cream increases the radical protection of the skin during VIS/NIR irradiation. Skin lipid profile was investigated applying HPTLC on skin lipid extracts. Furthermore, the absorption- and scattering coefficients, which influence radical formation, were determined. 11 volunteers were included in this study. After a single cream application, VIS/NIR-induced radical formation could be completely inhibited by both verum and placebo showing an immediate protection. After an application period of 4 weeks, radical formation could be significantly reduced by 45% following placebo application and 78% after verum application showing a long-term protection. Furthermore, the skin lipids in both verum and placebo groups increased directly after a single cream application but only significantly for ceramide [AP], [NP1], and squalene. After long-term cream application, concentration of cholesterol and the ceramides increased, but no significance was observed. These results indicate that regular application of the hyperforin-rich cream can reduce radical formation and can stabilize skin lipids, which are responsible for the barrier function.

1. Introduction

The main task of the skin, being the primary barrier against exogenous influences, is to prevent extensive water loss of the body. Moreover, it also protects the human body from invading hazards, e.g., chemicals, microorganisms, environmental pollutants and allergens. This barrier is located in the *stratum corneum*, consisting of cross-linked corneocytes that are surrounded by a continuous lipid matrix, mainly composed of ceramides, cholesterol, and free fatty acids. Changes in the skin lipid profile lead to an impaired barrier function of the skin [1] and are associated with skin diseases, e.g., xerosis [2]. Patients suffering from atopic dermatitis exhibit decreased levels of *stratum corneum* ceramides [3].

Furthermore, the skin as the outermost organ of the human body is permanently exposed to a pro-oxidative environment, e.g., irradiation, nitrogen oxides, and ozone [4,5]. Free radical formation in the skin is mainly due to sun irradiation, which is one of the most important environmental hazards leading to a higher risk of skin cancer, accelerated skin aging, and inflammation [6–8]. Approximately 50% of the free radicals produced in the skin due to sun light irradiation originate from UV-A and UV-B spectral wavelengths. Recently, it was found that free radicals are also produced when the skin is irradiated within the visible and infrared spectral range of the sun [9–11]. The antagonist of free radicals is the antioxidant network of the skin, providing enzymatic as well as non-enzymatic antioxidants [12,13]. This barrier against oxidative stress is mainly located in the epidermis with higher concentrations of antioxidants than in the dermis and higher concentrations in the *stratum corneum* close to the viable epidermis than close to the skin surface [14].

Both parameters of the skin barrier could be strengthened by the application of special skin care products rich in antioxidants

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[15]. One promising, natural antioxidant is the substance hyperforin, a major constituent of the plant St. John's Wort (*Hypericum perforatum*, HP) being well-known for the treatment of atopic skin and providing anti-inflammatory, anti-tumor, anti-bacterial, and antioxidant properties [16–19]. Moreover, hyperforin affects the differentiation and proliferation of the keratinocytes and might therefore be a promising treatment for regulating a disturbed differentiation in skin diseases such as atopic dermatitis (AD) or psoriasis [20]. This results in hyperforin being an excellent substance to investigate the effects on the antioxidant properties of the skin with its lipid profile.

Therefore, in a placebo controlled double blind study with 11 healthy volunteers, the influence of a hyperforin-rich cream on the skin barrier function was investigated.

2. Materials and methods

2.1. Skin creams

The verum cream, provided by Klosterfrau Healthcare Group, contains the *H. perforatum* extract (1.5%), rich in hyperforin with their following INCI ingredients and functions in parenthesis: Aqua (solvent), Petrolatum (emollient), Propylene Glycol (film forming), Caprylic/Capric Triglyceride (emollient), PEG-20 Glyceryl Stearate (emulsifying), Cetyl Alcohol (emollient, emulsifying, opacifying, viscosity controlling), Glyceryl Stearate (emollient, emulsifying), *H. perforatum* extract (anti-bacterial, anti-inflammatory, antioxidant properties), Panthenol (emollient, moisturizer, skin regeneration), Phenoxyethanol (preservative), Tocopherol (antioxidant properties), Tocopheryl Acetate (antioxidant properties), and Allantoin (soothing).

The basic cream without the *H. perforatum* extract and without Panthenol, Tocopherol, Tocopheryl Acetate, and Allantoin was used as placebo.

In a previous study, it could be shown that the radical protection factor (RPF) of the verum cream was $425 \times [10^{14}$ radicals/mg] and the verum cream without HP-extract but with panthenol, tocopherol, tocopheryl acetate, and allantoin was $39 \times [10^{14}$ radicals/mg] [19].

2.2. Volunteers

Eleven healthy volunteers (6 females, 5 males) aged between 21 and 42 years, suffering from neither anamnestic allergies nor food intolerances, were included in the study and requested not to change their lifestyle and dietary habits while participating in the study. The study design was checked and approved by the local Ethics Committee of the Charité – Universitätsmedizin Berlin in accordance with the Declaration of Helsinki as revised in 1983. All volunteers had given their written informed consent. All volunteers completed a questionnaire before and after the cream application disclosing information about their lifestyle and nutrition in order to ensure that no changes occurred during the 4-week study period.

2.3. Electron paramagnetic resonance spectroscopy

Non-invasive electron paramagnetic resonance (EPR) spectroscopy is based on resonant microwave absorption of a sample with unpaired electrons in an external magnetic field [21]. The in vivo and in vitro measurements were undertaken using an L-Band-EPR spectrometer LBM MT 03 (Magnettech, Berlin, Germany) with the following settings: microwave frequency (1.3 GHz), central magnetic field (46 mT), sweep width (8 mT), sweep time (10 s), and modulation amplitude (0.15 mT).

The spin probe used was the nitroxide PCA (3-carboxy-2,3,5,5-tetramethyl-1-pyrrolidinyloxyl; Sigma–Aldrich, Steinheim, Germany). Visible in electron paramagnetic resonance spectroscopy, it responds sensitively to free radicals and subsequently becomes invisible.

2.4. In vivo EPR measurements

For preparing the measurements, the skin was cleaned using an ethanol-soaked paper towel in order to have similar skin conditions of the volunteers because the volunteers had a different oily skin. Hairs, if any, were removed using scissors with curved edges in order to avoid skin injury. Thereafter, the measuring areas were marked on both forearms. 100 μL of a 0.8% PCA solution (solved in ethanol/water at a 1:1 ratio) was pipetted onto 2 filter disks, which were covered by an epicutaneous test patch. The 0.8% PCA solution penetrated into the skin for a period of 40 min. Between the skin and the microwave surface coil, a thin glass slide cover was positioned in order to avoid contamination of the surface coil. At visit 1, PCA was applied first and allowed to penetrate for 40 min, then the cream (2 mg/cm^2) was applied and allowed to penetrate for 60 min followed by EPR measurements. At visit 2, the volunteers were given the last cream application approx. 8.5 h before the measurements; EPR measurements followed directly after PCA application and a 40-min penetration.

The radical formation, which is manifested by the decay of the PCA signal due to PCA reaction with free radicals, was initially measured for 12 min without irradiation, and subsequently for 12 min under exposure to VIS–NIR radiation at 120 mW/cm^2 (72 J/cm^2), on the same measuring area (at visit 1, irradiation time was 20 min). For *in situ* irradiation, a fiber-coupled solar simulator (Low Cost Solar Simulator LS 01104, wavelength 450–2000 nm, LOT Oriel) was used. All measurements were performed in duplicate on adjacent skin areas.

Four spectra were recorded per minute. To optimize the signal-to-noise ratio, eight spectra were subsequently accumulated to one single spectrum, which served for evaluation using the software Multiplot (Magnettech). The data were normalized to the first measurement and set to time zero. To determine the radical production due to irradiation, mean values of the double measurements were calculated and the irradiated data were subtracted from the non-irradiated data.

2.5. Determination of optical properties

To determine the optical parameters of turbid media double integrating sphere measurements followed by inverse Monte Carlo simulation (iMCS) were performed as described previously [22].

The total reflectance (R_t) and the total transmission (T_t) of 100 μm of the samples were measured in the wavelength range 400–2000 nm using an integrating sphere spectrometer (Lambda 1050; PerkinElmer, Rodgau-Jügesheim, Germany). Therefore, the cuvette can be fixed in front of or behind the integrating sphere. For the measurement of T_t, the reflectance port was closed with a diffuse reflecting Spectralon® standard. R_t was measured relative to the reflectance standard by replacing the special Spectralon® standard by the sample.

The optical parameters absorption coefficient μ_a and effective scattering coefficient μ_s' were calculated by inverse Monte Carlo simulation (iMCS). The iMCS uses forward Monte Carlo simulations iteratively to calculate the optical parameters μ_a and μ_s' on the basis of a given phase function and the experimentally measured values for reflection and transmission (R_t^M and T_t^M). The iMCS uses a start set of μ_a and μ_s' to calculate the resulting simulated reflectance and transmission values R_t^S and T_t^S. These values are then compared to the R_t^M and T_t^M values, measured experimentally. By