Influence of Topical, Systemic and Combined Application of Antioxidants on the Barrier Properties of the Human Skin

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Key Words

Penetration · Skin barrier · Skin physiology · Antioxidants · Free radicals · Epidermal thickness

Abstract

Background: The formation of free radicals in human skin by solar ultraviolet radiation is considered to be the main reason for extrinsic skin aging. The antioxidants in human tissue represent an efficient protection system against the destructive action of these reactive free radicals. In this study, the parameters of the skin, epidermal thickness, stratum corneum moisture, elasticity and wrinkle volume, were determined before and after the treatment with antioxidant- or placebo-containing tablets and creams. **Methods:** The study included 5 groups of 15 volunteers each, who were treated for 2 months with antioxidant-containing or placebo tablets, creams or a combination of antioxidant-containing tablets and cream. The skin parameters were measured at time point 0 and at week 8 utilizing ultrasound for the determination of epidermal thickness, a corneometer for stratum corneum moisture measurements, skin profilometry for quantifying the wrinkle volume and a cutometer for determining the elasticity. **Results:** The verum cream had a positive influ-

ence on epidermal thickness, elasticity and skin moisture, but the verum tablets improved the epidermal thickness only. The combined application of verum tablets and creams led to a significant improvement of all investigated skin parameters, whereas the application of placebo tablets or cream did not influence any parameters. **Conclusion:** The topical and oral supplementation of antioxidants can be an instrument to improve several skin parameters and potentially counteract or decelerate the process of extrinsic skin aging.

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Introduction

The skin is not only the largest organ of the human body but also the barrier between the organism and the environment. It protects the human body from water loss, the penetration of harmful agents and solar UV radiation [1]. Solar radiation represents the factor most responsible for the formation of free radicals in human skin [2, 3]. The spectral distribution of these free radicals has a maximum in the UV region [4]. These highly reactive molecules can destroy cells and cell compartments. The

Table 1. Initial values of the skin parameters of all groups

Cream group	–	–	AO	AO	P
Tablet group	AO	P	AO	–	–
Gender	female	female	female	female	female
Age, years	38±8	27.4±3	31.5±8	36.7±9	35.3±10
Skin moisture, arbitrary units	81±2	81.5±50	72.5±7	75.5±6	84.7±10
Wrinkle volume, mm ³	0.9±0.6	0.5±0.1	0.8±0.2	1±0.3	0.6±0.5
Epidermal thickness, µm	85.1±9	84.8±10	81±9	82.6±11	88.7±8
Skin elasticity, arbitrary units	0.5±0.2	0.5±0.1	0.6±0.1	0.6±0.1	0.5±0.1

Values are expressed as means ± SD.

radicals and reactive oxygen species (ROS) not only damage DNA but also negatively influence the generation of connective tissue [5, 6]. UV irradiation induces matrix metalloproteinases that are responsible for alterations in the collagenous extracellular matrix of connective tissue [7]. These alterations result in an impaired integrity [8]. Skin aging, immunosuppression and also skin cancer can be the consequences of all these processes [9–11]. The human organism has developed a protection system against the destructive action of the free radicals in the form of antioxidants [12, 13]. The antioxidants in human tissue are able to neutralize free radicals before they are in a position to damage the tissue. The most important antioxidants in human skin are carotenoids, vitamins and various enzymes [14, 15]. However, most of these substances cannot be produced by the human organism but must be supplemented by nutrition such as fruits and vegetables. Thus, the antioxidant status of every human subject crucially depends on his specific nutrition. Interestingly, carotenoids have been shown to serve as marker substances for the complete antioxidant status of the human epidermis [16]. A correlation of the antioxidant status and the skin status has been shown previously. Darvin et al. [17] demonstrated that individuals who maintained a healthy diet over decades had a younger-looking skin with less furrows and wrinkles than persons of the same age with lower antioxidant levels in their skin. An accumulation of topically and systemically applied antioxidants in the human skin has been demonstrated in several studies [18–20].

The aim of this study was to assess the influence of systemic, topical and combined applications of antioxidants on the skin barrier by examining 4 parameters (epidermal thickness, stratum corneum moisture, elasticity and wrinkle volume) over a period of 8 weeks.

Materials and Methods

Volunteers

In total, the investigations were performed on 75 female volunteers aged between 20 and 73 years with skin types II and III according to the Fitzpatrick classification [21]. Only volunteers with healthy skin conditions and without any known sensibilization to cosmetic products were included in the study. The study included 5 groups of 15 volunteers each, treated for 2 months with antioxidant-containing or placebo tablets, creams or a combination of antioxidant-containing tablets and cream. The skin parameters were measured at time point 0 and at week 8. The mean initial values of the skin parameters of all groups are presented in table 1.

The study was approved by the Ethics Committee of the Charité – Universitätsmedizin Berlin and was conducted in accordance with the standard ethical rules stated in the principles of the Declaration of Helsinki.

Applied Substances

The active substrate in the verum cream and tablets comprised a basic compound of antioxidants including active ingredients such as vitamin E acetate, plant-derived vitamin C acetate, plant extracts (green tea, green coffee, pongamia, pinnata seed and angelica) and 0.2% of the carotenoids β -carotene and lycopene at the same concentrations. The placebo cream and tablets did not contain any antioxidants. The cream basis was a soft water-in-oil emulsion containing the following ingredients: isopropyl myristate, aramol E, veegum, xanthem gum, carbopol, cycomethicon and shea butter. The placebo tablets were composed of an inert tablet carrier. The antioxidant-containing cream and tablets are a proprietary composition of Coty Lancaster SAM as specified and published in Patent EP 1877095 ('Verwendung von freien Radikalfängern zum Schutz und Behandlung von durch Chemotherapie verursachten Haut- und Haarschäden' dated May 27, 2009).

Study Design

The study design was double-blind. Each group of 15 volunteers was either treated with antioxidant-containing tablets (tablet-AO), placebo tablets (tablet-P), antioxidant-containing cream (cream-AO), placebo cream (cream-P) or a combination of antioxidant-containing tablets and cream (tablet-AO + cream-AO). During the study, the creams were applied to the face and the tablets were administered orally (1 tablet) twice daily. All study groups

Fig. 1. Epidermal thickness of the human skin in vivo prior to and after 8 weeks of treatment. * $p < 0.05$, ** $p < 0.01$.

applied the respective test products for 8 weeks. The following parameters were determined at time point 0 and at week 8.

Epidermal Thickness

The thickness of the epidermis was determined by ultrasound measurements. The measurements were performed using the 50-MHz system DermaScan® v3 (Cortex Technology, Denmark). Each skin area was measured 3 times and the mean value was determined. Absolute differences of approximately 3 µm were regarded as changes.

Classic Skin Parameters

The wrinkle volume was measured using the PRIMOS premium (GF Messtechnik GmbH, Teltow, Germany). The elasticity measurements were performed by suction (Cutometer® dual MPA 580; Courage + Khazaka electronic GmbH, Köln, Germany). The parameter R2 showing the resistance versus the ability of returning was utilized for comparison. The stratum corneum moisture was measured by a capacitance method using the corneometer CM 825 (Courage + Khazaka electronic).

Statistical Evaluation

For statistical evaluation, mean values and standard deviations were calculated. The mean values were compared by means of the nonparametric Mann-Whitney U test and the Kruskal-Wallis test at a significance of $p < 0.05$, using the software program IBM SPSS Statistics 20.0.

Results

The results of the different skin parameters are shown in the figures. In figure 1, the epidermal thickness is shown prior to and after 8 weeks of treatment for all 5

Fig. 2. Moisture in the stratum corneum prior to and after treatment. * $p < 0.05$.

groups. While placebo tablets and cream had no effect on the epidermal thickness, the application of antioxidants in the form of tablets and/or cream led to a significant increase in this parameter.

In figure 2, the moisture in the stratum corneum is presented prior to and after 8 weeks of treatment for all 5 groups. Volunteers who received the cream-AO always showed an increase in stratum corneum moisture, but the stratum corneum moisture in the tablet-AO and tablet-P groups remained stable.

The effect of the different treatments on skin elasticity is shown in figure 3. The elasticity of the skin was slightly but significantly improved in the cream-AO and tablet-AO + cream-AO groups. In the case of systemic application of the antioxidants, only an insignificant increase in the elasticity could be observed after 8 weeks. Likewise, no effect was observed in the cream-P group.

The volume of the wrinkles changed significantly only in the tablet-AO + cream-AO group as shown in figure 4.

Discussion

Free radicals produced in the human skin by solar radiation destroy cells and cell compartments [22, 23], collagen and elastin fibers and the lipid layers of the stratum corneum [24]. All this damages the skin barrier [25, 26]. Antioxidants in the skin can neutralize these reactive species [27, 28]. Usually, these antioxidants must be supplemented by nutrition as they cannot be synthesized by the

Fig. 3. Elasticity of the skin prior to and after 8 weeks of treatment. * $p < 0.05$.

Fig. 4. Wrinkle volume prior to and after 8 weeks of application. * $p < 0.05$.

organism itself. In this study, antioxidants were administered systemically in the form of tablets, topically in the form of a cream or as a combination of both over a period of 8 weeks. The verum cream had a positive influence on the epidermal thickness, elasticity and skin moisture; the verum tablets only improved the epidermal thickness. The combined application of verum tablets and creams led to a significant improvement of all skin parameters, whereas the application of placebo tablets or cream had no influence on any of the investigated skin parameters. This excludes that the observed effects were due to other ingredients of the creams or tablets.

Darvin et al. [29] showed that antioxidants administered in the form of tablets increased the antioxidant concentration in the skin. Their findings are the basis for explaining the results of our study. In the verum groups, the destructive action of the free radicals induced by solar radiation was reduced during the 8 weeks of investigation. UV exposure is thought to cause skin aging mainly by singlet oxygen-dependent pathways. This, in turn, leads to degradation, e.g. of the extracellular matrix proteins, by inducing matrix metalloproteases [30]. On the contrary, antioxidants such as β -carotene significantly reduce stress and degradation of the extracellular matrix, in addition to promoting the differentiation of keratinocytes [31], which might explain the improved skin parameters that we observed. Skin elasticity is particularly influenced by dermal collagen [32]. UV radiation, however, attacks keratinocytes and fibroblasts, resulting in

the activation of cell surface receptors, which initiate signal transduction cascades. This, in turn, leads to a variety of molecular changes, causing a breakdown of collagen in the extracellular matrix and a shutdown of new collagen synthesis [8]. Protection of the skin by the application of antioxidants inhibited the breakdown of collagen and promoted new synthesis, which resulted in increased elasticity in the verum groups when cream application was involved.

The parameters stratum corneum moisture, wrinkle volume and epidermal thickness might all have been affected by simple hydration with the creams. Interestingly, however, the application of P-cream only did not involve any significant improvements.

Our study revealed that the combined oral and topical application of antioxidants leads to a reduction of the wrinkle volume. Similar results were observed by other groups. Heinrich et al. [33] found a significant decrease of skin roughness after antioxidant supplementation, and Darvin et al. [17] found a significant correlation between skin roughness and lycopene concentration. The increased epidermal thickness might be induced by increased differentiation of keratinocytes caused by β -carotene application as described above. The oral supplementation or topical application of antioxidants seems to sometimes also influence the skin moisture as reported by diverse groups [34, 35] and revealed in this study.

The combined application of the verum cream and verum tablets did not result in increased values of skin

parameters compared to single verum cream and single verum tablet application. A saturation effect on the stratum corneum might be a potential explanation. If the stratum corneum is already saturated by topically applied antioxidants, further antioxidants which are delivered to the skin surface via the sweat cannot be absorbed additionally [36, 37].

In summary, it can be stated that topical application and systemic supplementation of antioxidants have a positive effect on several skin parameters. The advantage of systemic supplementation might be that the antioxidants remain in the skin for several weeks, even after discontinuance of the systemic treatment [29]. Cream application, however, has to be continuous, because otherwise the concentration in the skin quickly degrades, i.e. within only 1 or 2 days [29]. In any event, the antioxidants utilized in relevant products should be optimized in their composition and concentration to exclude quick oxidation [38, 39]. However, whereas topical application of cosmetic products containing antioxidants seems to not be critical, consumers of nutritional supplements should ensure that their products contain a broad mixture of antioxidants at physiological concentrations, because high concentrations of antioxidants that exceed a critical value can even act as producers of free radicals [39]. This criti-

cal concentration can hardly be achieved by eating fruit and vegetables, as saturation occurs. Although a wide range of food supplements are available, providing a possible alternative to the intake of fruit and vegetables, it is difficult for the consumers to classify these products by their physiological concentrations and compositions, with comprehensive labeling and other relevant regulations being insufficient. Consequently, a healthy diet remains the best alternative for the systemic intake of antioxidants.

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