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Original article

Age influences the skin reaction pattern to mechanical stress and its repair level through skin care products

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ABSTRACT

Skin aging is associated with alterations of surface texture, sebum composition and immune response. Mechanical stress induces repair mechanisms, which may be dependent on the age and quality of the skin. The response to mechanical stress in young and aged individuals, their subjective opinion and the objective effectiveness of skin care products were evaluated by biophysical skin quality parameters (stratum corneum hydration, transepidermal water loss, skin pH, pigmentation and erythema) at baseline, 1, 6, 24 h and 7 days at the forearms of 2 groups of healthy volunteers, younger than 35 years (n = 11) and older than 60 years (n = 13). In addition, casual surface lipid composition was studied under the same conditions at the baseline and day 7 after mechanical stress induction. Evaluations were also performed in stressed skin areas treated daily with skin care products and the subjective opinion of the volunteers was additionally documented. The tested groups exhibited age-associated baseline skin functions as well as casual surface lipid composition and different reaction patterns to mechanical stress. Skin care was more effective in normalizing skin reaction to stress in the young than in the aged group. The subjective volunteer opinion correlated with the objective measurements.

1. Introduction

Aging is strongly associated with alterations of skin quality and function parameters (Zouboulis and Makrantonaki, 2011; Farage et al., 2017). On the other hand, in own previous studies, aged skin was shown to be in an enhanced stress condition, represented by up-regulation of the corticotropin-releasing hormone system components (Elewa et al., 2012). We have also found differential expression of several molecules and receptors involved both in the lipogenesis and the inflammatory skin reaction between young and aged skin (Makrantonaki et al., 2012; Elewa et al., 2015). These alterations may – at least – partially lead to several skin disorders and diseases, which accumulate with age (Makrantonaki and Zouboulis, 2008; Makrantonaki et al., 2016b). Among them skin xerosis, skin roughness and changes of surface texture are results of reduced barrier integrity, clinically reflected by transepidermal water loss (TEWL), and a global reduction of sebum production (Zouboulis and Boschnakow, 2001). The latter were combined with changes in sebum composition (Pochi et al., 1979; Stewart et al., 1989), since cholesterol (CH) and squalene (SQ) synthesis decrease markedly in elderly individuals compared to younger ones (Elias and Ghadially, 2002). The hydration level of the stratum corneum (SC) is also modified in aging skin, due to the reduced epidermal cell renewal and decreased sweating (Elsner et al., 2011).

Possible contributors to these changes could be a chronic inflammatory process and/or hormone alterations (Makrantonaki and Zouboulis, 2009; Makrantonaki et al., 2010). Dysregulated immune and inflammatory responses have been documented both in humans and mice with increasing age (Kovaiou et al., 2007; Elewa et al., 2015), including changes in the skin, such as pH alterations and erythema.

Aged skin has also shown a defective and prolonged recovery after

Abbreviations: A, aged; CH, cholesterol; CSL, casual surface lipid(s); FA, fatty acid(s); FAME, fatty acid methyl esters; GC–MS, gas chromatography-mass spectrometry; SC, stratum corneum; SQ, squalene; TEWL, transepidermal water loss; Y, young

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irritation with sodium lauryl sulphate (Schwindt et al., 1998; Ye et al., 2002). Externally applied lipids can reduce the skin reaction to irritants (Lodén and Andersson, 1996; Zettersten et al., 1997). After removal of skin surface lipids by an irritant, a burst of lipid synthesis occurs to rapidly substitute the lipids for rebuilding the skin surface barrier. Skin care products are considered to accelerate disrupted skin barrier recovery (Byun et al., 2012). Lipid compositions imitating the normal casual surface lipid (CSL) composition normalized barrier recovery and a ceramides: cholesterol (CH): fatty acid (FA) ratio of 3:1:1 could even accelerate the recovery rate (Zettersten et al., 1997). Skin care products imitating the composition of vernix caseosa have also been proved beneficial (Visscher et al., 2011).

The present study was performed to evaluate the mechanical stress reaction of the skin in young and aged individuals and to assess the effectiveness of skin care on skin repair parameters.

2. Subjects and methods

2.1. Volunteers

Twenty-four healthy volunteers divided into 2 groups, namely a group below 35 years of age ("young group", n = 11, age = 30.1 ± 6.7) and older than 60 years ("aged group", n = 13, age = 68.1 ± 7.2) were included in the study after providing informed consent. The study protocol was approved by the Saxony–Anhalt Medical Association Ethics Committee (# 9/11). Recruited were individuals with apparently healthy skin, not using external skin care products in the last week before enrollment in the study and with reliability for long term compliance. Excluded were smokers, men with extremely hairy skin, individuals with chronic skin diseases (with the exception of epithelial tumours), type IV allergy, apparent skin abnormalities on the forearms, usage of external skin care products or therapy in the last week before initiation of the study and known hormonal disturbances. Four skin windows (each 3.5 × 3.5 cm, surface area 12.25 cm^2) on the volar sides of both forearms of each volunteer were treated or served as control.

2.2. Mechanical stress application

Tape stripping was performed as standardized by Breternitz et al. (2007). Shortly, the skin was stretched with 3 fingers, D-squame disc tapes (D 100, 22 mm diameter, 3.8 cm^2 surface area; Cuderm, Dalas, TX, USA) were applied on the skin with a 2 N stamp (Cuderm) for 2 s and removed with a forceps in one swift movement. For each strip a new tape was used and placed on exactly the same skin area. Eighteen strippings were performed in each of the four tested areas. The stripping procedure occurred at the four skin windows at the baseline (day 0) and on day 7 (168 h). The tapes were stored at −70 °C for gas chromatography analysis of the CSL composition.

2.3. External skin care product application

Creams 1 and 2 were anti-aging skin care products from LVMH (Saint Jean de Braye, France), cream 3 is a standard German pharmaceutical vehicle cream (DAC basis cream). The composition of creams 1 and 2 was similar, whereas cream 2 included C12-16 alcohols, hydrogenated lecithin, palmitic acid and commiphora mukul resin extract in addition to the composition of cream 1. The creams were packed in neutral exactly similar jars.

Three of the skin windows were treated daily each with one of the tested creams starting on day zero after the 6-h measurement. The fourth window (control area) remained untreated. The volunteers were instructed to apply a certain amount of each cream (2 mg/cm^2, i.e. 25 mg on each test area) on the right skin window. The volunteers were also instructed not to apply any other external care products, soap or hot water on the test areas. Room temperature water was allowed for washing, without violent rubbing or scratching. Exposure of the test areas to direct sunlight was also prohibited.

A randomization plan was used for the treated skin areas and the creams. The cream application and skin quality measurements were done in an examiner blinded manner.

2.4. Skin quality measurements

The measurements were performed in the same room under stable temperature, without air current. The average temperature during the measurements was 22 ± 4 °C, and average humidity was 32 ± 8%. The patients were allowed to acclimatize in the measurement room for at least 15 min before starting the measurements. The skin quality measurements were performed in the four skin windows on both forearms at baseline (day 0), 1 h, 6 h, 24 h and at day 7 (168 h) after stress application.

The skin quality measurements were performed on the volar side of both forearms. Hydration level of SC, TEWL, pH of the skin surface, melanin content and erythema were measured using the Corneometer CM 825 probe, Tewamater TM 300 probe, pH 905 probe and Mexameter MX 18 probe, respectively, attached to multi probe adaptor MPA 9 (Courage + Khazaka, Bonn, Germany) and the measurements were performed according to operation instructions provided by the manufacturer. The optimum conditions and procedures for the measurements were set according to the European group for Efficacy and Measurements on Cosmetics and Other topical products guidelines (Berardesca and EEMCO, 1997; Piérard, 1998; Rogiers and EEMCO Group, 2001; Parra et al., 2003). The probes were calibrated before the beginning of the study and weekly during the study according to the published instrument recommendations.

2.5. Assessment of the individual subjective opinion of the external care products tolerability

At the end of the study, the volunteers were asked to subjectively evaluate the three creams regarding general tolerability with a scale of 1–3 (irritating, neutral effect, pleasant) and consistency with a scale of 1–3 (too fluid, just the right consistency, too thick).

2.6. CSL

Lipids were extracted from the SC sampled with D-squame tapes with a procedure adapted from t'Kindt et al., 2012. The first two tapes were discarded in order to exclude surface dirt or cream traces. Briefly, the tapes were extracted twice with anhydrous ethanol containing 0.0025% butyl hydroxytoluene to prevent oxidation of oxygen sensitive compounds. The ethanol fractions were collected and evaporated under nitrogen and the bound fatty acids (FA), SQ and CH content of the CSL were analyzed. Lipid extracts were subjected to two-step analysis. First, saponification and methylation of the lipid extracts was performed to yield FA methyl esters (FAME) that were analyzed by gas chromatography-mass spectrometry (GC–MS; Thermo-Finnigan, Waltham, MA, USA) to establish the FA profiles in the lipid extracts. Subsequently, FAME extracts were dried under nitrogen stream and analyzed with GC–MS for the determination of SQ and CH following derivatization with bis(trimethylsilyl)-trifluoacetamide in pyridine to obtain trimethylsilyl derivatives, as previously reported (Capitanio et al., 2009).

2.7. Statistical evaluation

Significance at certain time points was calculated using Kruskal Wallis and Wilcoxon (Mann Whitney U) non parametric tests. For time-dependent parameters an analysis of variance (ANOVA) was used to compare means of each factor modalities. When there was a significant difference between the times of the kinetic, a Student Newman Keuls test was performed. P was considered significant when $p < 0.05$.