EXHIBIT C

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Evaluation of anti-wrinkle efficacy of adenosine-containing products using the FOITS technique

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Synopsis

The objective of this study was to evaluate formulations containing adenosine to reduce periorbital lines and glabellar frowns in a blind, randomised, placebo-controlled study. One hundred twenty-six female volunteers between 45 and 65 years of age fulfilled inclusion criteria for periorbital lines. They were provided with two of three products (cream with adenosine, dissolvable film with adenosine or placebo), to be applied to the periorbital area on each side of the face (84 subjects per product). Eighty-four of these subjects also fulfilled the inclusion criteria for glabellar frowns, and received placebo or cream with adenosine to be applied to the glabellar area. Products were applied twice daily for 2 months, and evaluation was performed under dermatological supervision at 0, 3 and 8 weeks using Fast Optical *in vivo* Topometry of human Skin (FOITS) analysis to describe skin profile. Both adenosine-containing products led to significant improvements in skin smoothness in the periorbital area. Improvements were evidenced after 3 weeks of product application as measured by *Ra* and *Rz* parameters using the FOITS technique, and were steadily confirmed after 2 months, despite severe climatic conditions and independently of the analysis technique that was used with the FOITS data. Adenosine-containing cream also significantly improved glabellar frowns. This study demonstrates the potential beneficial effects of adenosine-containing products on crow's feet and glabellar facial wrinkles.

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Résumé

Le but de cette étude est d'évaluer dans une étude randomisée controlée contre placebo des formules contenant l'adénosine pour réduire les rides périorbitales et les sillons glabellaires. 126 femmes de 45 à 65 ans répondant aux critères d'inclusion pour les rides périorbitales ont reçu 2 des 3 produits étudiés (crème à l'adénosine, film solubilisable à l'adénosine ou placebo) pour application sur la zone périorbitale de chaque coté du visage (84 sujets par produit). 84 de ces sujets remplissaient également les critères d'inclusion pour les sillons glabellaires, et ont reçu soit la crème à l'adénosine soit le placebo pour application sur la zone glabellaire. Application des produits 2 fois par jour pendant 2 mois, et évaluation sous contrôle dermatologique à 0, 3 et 8 semaines à l'aide de la méthode FOITS (Fast Optical *in vivo* Topometry of human Skin) d'analyse du profil cutané. Les deux produits contenant l'adénosine ont conduit à une amélioration significative du relief cutané de la zone périorbitale. L'amélioration est mesurée dès 3 semaines pour les paramètres R*a* et R*z* de la méthode FOITS, et se maintient après 2 mois, malgré des conditions climatiques sévères. La crème contenant l'adénosine a également amélioré de façon significative les sillons glabellaires. Cette étude met en évidence les effets bénéfiques potentiels des produits contenant l'adénosine sur les rides de la patte d'oie et de la glabelle.

Introduction

Wrinkle development, as a direct consequence of skin ageing, has important social and psychological impacts which have become exaggerated with

longer life expectancy and changing social values. The skin ageing process can be separated into two simultaneous processes – chronological (or intrinsic) ageing and extrinsic ageing (induced by cumulative exposures to environmental factors, especially UV light).

Wrinkle development in intrinsically aged skin has been associated with decreased elastin [1] and laminin 5 [2], leading to the breakdown of the extracellular matrix and in particular disruption and flattening of the dermal–epidermal junction. This degradation of elastic fibres constituting the elastin network, in conjunction with marked collagen reduction, appears to be a major culprit in wrinkle formation, as it reduces the capacity of aged skin to resist gravitational effects and return to its native state after expressional changes. While expression lines such as periorbital wrinkles and glabellar frowns can be perceived as manifestations of personality, they are nevertheless early indicators of the future of the face, and will inevitably develop into ageing related wrinkles. These 'dynamic' wrinkles are perpendicular to the direction of the underlying facial muscles. Tensile forces are generated by these muscles and with time and repetition they can eventually lead to modifications of the contractile properties of fibroblasts.

The demonstration nearly 20 years ago in a controlled study that topical tretinoin could reduce both fine and coarse wrinkles in photoaged skin [3], provided the impetus for considerable research into the possibility of cosmetic correction of skin ageing. Products developed to date range from basic moisturisers (which offer only transient rehydrating effects on wrinkles) to sophisticated cosmetic formulations which exert a much more complex activity by protecting the skin from further damage, nourishing it, stimulating renewal of superficial layers, or even restoring skin structure, and contain functional agents with reported beneficial effects including vitamins, retinoids, hydroxy acids, fish and plant polysaccharides, enzyme inhibitors or antioxidant modulators [4]. In this respect, products containing agents such as adenosine which have been elaborated to limit the calcium-induced cellular contractions, are worthy of study for their possible beneficial effects on 'dynamic' wrinkles. This paper presents the results of a placebo-controlled study on the effects of cosmetic preparations containing adenosine to reduce crow's feet and glabellar facial wrinkles.

Subjects and methods

The original feature of this study is its double design. Two different methodologies have been used on the same subject, an incomplete block design on the crow's feet area and a parallel design on the glabellar area.

A placebo cream known from previous studies to have no effect on wrinkles was used as control.

Periorbital lines

Inclusion criteria

One hundred twenty-six women, aged between 45 and 65 years and with wrinkles greater than 2 cm in length without criss-crossing lines were enrolled. Wrinkle characteristics for inclusion in the study were assessed according to the L'Oreal crow's feet wrinkle photographic Chart, and criteria for inclusion was set as follows: wrinkles graded greater than 4 (if only one wrinkle), greater than depth grade 3 (for two wrinkles) or greater than depth grade 2 (for three or four wrinkles).

Methodology

The study was carried out as an incomplete block design where not every subject got all treatments. According to randomization, subjects were given two of three blinded products – (C) cream with 0.1% adenosine, (A) dissolvable film with 1% adenosine or (B) placebo cream (without adenosine), and requested to apply one product on the right crow's feet only and the other one on the left crow's feet only. Each product was used 84 times and all three combinations (A vs. B, C vs. A and B vs. C) 42 times.

Glabellar frowns

Inclusion criteria

Eighty-four of the 126 total subjects also fulfilled criteria for inclusion in the glabellar frown arm of this study: glabellar frowns were assessed according to a photographic scoring method (L'Oréal glabellar wrinkles Chart) in the same way as for the periorbital wrinkles.

Methodology

The study was carried out as a parallel group design. Subjects were divided into two groups of 42 each and given one of two blinded products (C)

cream or (B) placebo, to be applied to the glabellar area. Product A was not used on this location.

Exclusion criteria

Subjects who did not or could not sign an informed consent form, those unable to comply with the requirements of the protocol and those who were participating in any other clinical study were excluded.

Test substance application protocol

The study took place between mid September and mid December in the central part of Germany. Application of any cosmetic products was prohibited 3 days prior to the start of the study. During the study, facial cleansing was allowed in the morning using pure water, and in the evening using only the cleansing milk provided. Subjects were instructed to apply the test product twice daily (once in the morning, once in the evening). For periorbital lines, the right hand index was used to apply one product to the left periorbital region, and the left hand index to apply the other product on the right periorbital region. For those subjects also enrolled in the glabellar frown study, test product (C or B) was applied after periorbital applications, using the right-hand middle finger. For both studies, cream (products C and B) was applied by gentle circular massaging motion, and for those subjects applying dissolvable film (product A), the treated area was first moistened, and the dissolvable film applied in a similar way by gentle circular massage.

Assessment calendar, methodologies and statistical analyses

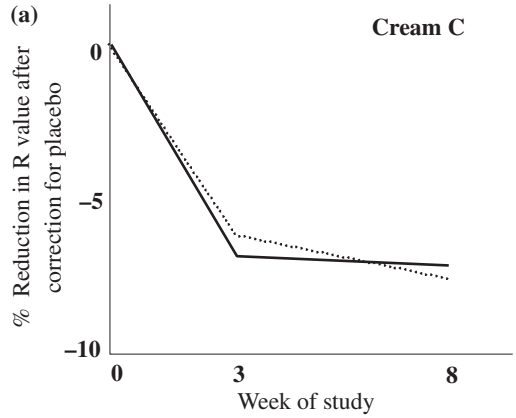
The study protocol included evaluation at day 0, and after 3 and 8 weeks of treatment. Subjects were requested to apply test products at least 12 h prior to assessment. Measurements of skin roughness were performed using the FOITS technique [5, 6], followed by analysis of the skin profile with the 'classical' Din parameters method and by the Frequency of distribution of Heights method (FDH) which gives additional information on effects on wrinkles of different depth. Measurements were taken after an acclimatization period of 45 min in a strictly controlled environment. Hygrometry and ambient temperature were recorded each day throughout the study period.

FOITS classical analysis was used to record the two roughness parameters *Ra* (average roughness) and *Rz* (average of the heights measured between the peaks and troughs of each relief feature) as described previously [5]. FOITS is an optical measurement procedure which uses a combination of grey code and phase shift technique. The system comprises a projection unit and a CCD camera. As for PRIMOS®, both methods use fringe projection, they differ in the way stripes are generated on the skin. *Ra* and *Rz* were determined on 50 parallel lines perpendicular to the axis of the main wrinkle and starting from the corner of the eye (for periorbital lines), adjacent lines being separated by 250 μm.

FDH measured the frequency of distribution of the heights in the field of [minus]600 to +600 μm with an interval step of 5 μm. So, three types of relief were evaluated according to time: micro-relief (0–50 μm), fine lines (55–110 μm) and coarse wrinkles (115–600 μm). As an average profile comprises approximately 65% micro-relief, 25% fine lines and only 10% coarse wrinkles, an increase in skin smoothness as detected by a reduction in coarse wrinkles will simultaneously affect fine lines or micro-relief. This additional analysis was performed for the periorbital area.

For *Ra* and *Rz* parameters, the statistical significance of measured parameters was evaluated using the ANOVA test. Logarithms of the true *Ra* and *Rz* values were used as the values do not follow the law of normal distribution. For measurements which were statistically significant after variance analysis, three types of comparison were performed: initial vs. final value; and for the crow's feet area study, comparison of effects of product C (cream) or A (dissolvable film) vs. placebo (Dunnett test) and for the glabellar study, comparison of product C (cream) vs. placebo (Dunnett test).

For FDH measurements, Student's *t*-tests were performed separately for each class of periorbital wrinkles. Climatic conditions can dramatically affect facial skin smoothness, and during the 2 months duration of the study in the winter season, climatic variations were unavoidable. To correct for this effect, the values obtained with adenosine-containing products were corrected for the placebo values.



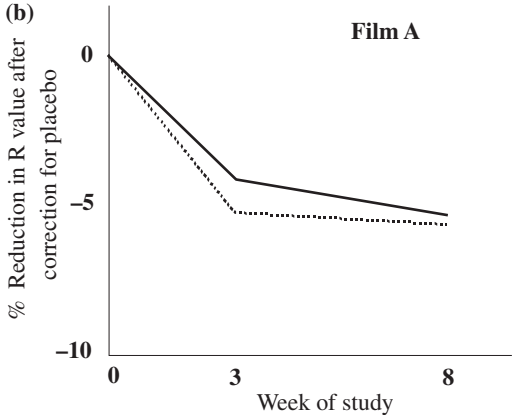
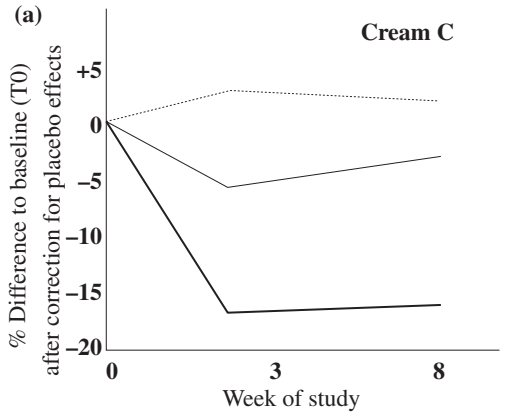


Figure 1 FOITS analysis of skin smoothness parameters in the periorbital region after application of cream C (a) or dissolvable film A (b) containing adenosine. The roughness parameters *Rz* and *Ra* were measured by FOITS technique. *R* values are plotted as percentage reduction from baseline values at the start of the study after correction for evolution of values obtained with placebo, as a function of time. Solid lines, *Rz*; dashed lines, *Ra*.



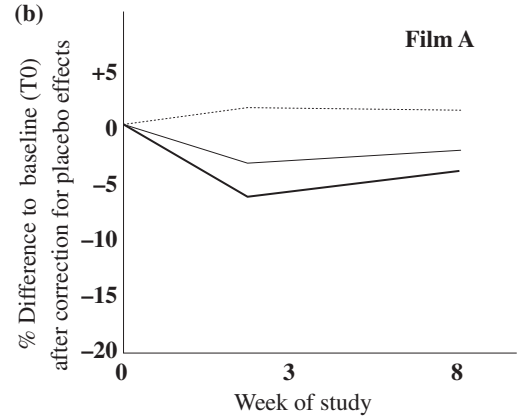


Figure 2 FDH analysis of periorbital regions after application of cream C (a) or dissolvable film A (b) containing adenosine. FDH measurements of micro-relief (0–50 μm, dashed lines), fine wrinkles (55–110 μm, solid lines), and coarse wrinkles (115–600 μm, bold lines) are plotted against time, and expressed as per cent difference to baseline values after correction for values obtained with placebo.

Results

Periorbital lines

All of the included 126 subjects completed this randomized trial. As measured by FOITS, both the *Ra* and *Rz* skin roughness parameters diminished dramatically following applications of both the C cream and A dissolvable film containing adenosine (Fig. 1a,b). Significant differences in evolution of skin smoothness were evidenced between both cream and film with adenosine and placebo at 3 and 8 weeks ($P \leq 0.05$ for both *Ra* and *Rz*, Tukey test). Similar results were obtained using the FDH method (Fig. 2). With both adenosine-containing products, C cream and A dissolvable film, significant improvements in skin smoothness were detected as early as 3 weeks after the start of the study (as evidenced by a decrease in the frequency of coarse wrinkles and fine lines, with a corresponding increase in micro-relief) and were maintained at week 8.

Glabellar frowns

All 84 subjects completed the study. As assessed by FOITS analysis, application of cream with adenosine (C) produced considerable reductions in both the *Rz* and *Ra* parameters in the glabellar region at 3 and 8 weeks into the study (Fig. 3),

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