

Topical antioxidants protect the skin from chemical-induced irritation in the repetitive washing test: a placebo-controlled, double-blind study

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Summary

Background. There is increasing evidence that reactive oxygen species play an important role in the development of both irritant and allergic contact dermatitis.

Objectives. To assess the potential of topical antioxidants to prevent the development of experimentally induced irritant contact dermatitis.

Methods. We evaluated the effect of a cream containing a combination of antioxidants on sodium lauryl sulfate-induced irritant contact dermatitis in the repetitive washing test. As readout parameters for skin barrier function and cutaneous inflammation stratum corneum hydration, cutaneous blood flow and transepidermal water loss were assessed in 25 volunteers with bioengineering methods.

Results. In comparison with the cream base and a frequently used barrier cream, the antioxidant cream had high radical scavenging activity and effectively protected the skin from chemical-induced irritation.

Conclusions. The superiority of the cream with antioxidants to the cream base suggests that reactive oxygen species, at least in part, play a role in the development of irritant contact dermatitis.

Key words: irritant contact dermatitis; luteolin; radical protection factor; reactive oxygen species; skin barrier.

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Conflicts of interest: C.M.S. holds a patent on the dermatological use of luteolin-rich extracts from *Reseda luteola*. The other authors declare that they have no conflict of interest. **Contributions and Funding:** C.M.S. designed the study, coordinated the experiments and wrote the paper; M.C.M. and J.L. determined the radical protection factor of the test preparations; Y.F. and T.B. developed the cream base and the cream with antioxidants; W.G. performed the washing test. C.M.S., Y.F. and T.B. received a grant from the German Federal Ministry of Economics and Technology (AiF grant no. KF2556501SK0).

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There is increasing evidence that reactive oxygen species (ROS) play an important role in the development of allergic contact dermatitis (1). It has been shown in animal models of cutaneous inflammation that antioxidants such as *N*-acetylcysteine (NAC) and vitamin E (tocopherol) may antagonize the development of both irritant and specific contact hypersensitivity reactions to haptens (2–4). However, with the exception of one study that did not show a beneficial effect of an ointment with 7.5% quercetin (5) in sodium lauryl sulfate (SLS)-induced irritant contact dermatitis, controlled studies in humans are lacking so far. In the present study, we were interested in evaluating the effect of a cream containing a combination of antioxidants on SLS-induced irritant contact dermatitis in comparison with the vehicle without active principles and a frequently used barrier cream.

Materials and Methods

The randomized double-blind vehicle-controlled study (protocol no. 06911) was approved by the ethics committee of the University Medical Centre Freiburg. All volunteers gave their written informed consent before entering the study.

To induce a standardized irritant contact dermatitis, the repetitive washing test was performed on 25 healthy volunteers, as previously reported (6). In brief, standardized washings with 0.01 M SLS were performed three times daily on 7 consecutive days, on four test areas on the volar forearms of the volunteers. Fifteen minutes prior to the washings, 200 μ l of the test creams were applied topically on three of the four test areas. One of the test areas remained untreated before washing.

As readout parameters for skin barrier function and cutaneous inflammation, stratum corneum hydration (Corneometer CM 820; Courage and Khazaka, Cologne, Germany), transepidermal water loss (TEWL) (Tewameter TM 210; Courage and Khazaka) and cutaneous blood flow (Periflux PF2 laser Doppler flowmeter; Servomed, Berlin, Germany) were assessed with bioengineering methods, as previously described (6). Instrumental measurements were performed prior to the first treatment (T0), after 3 days (T3), and after 7 days (T7).

The antioxidant cream was compared with the corresponding cream base without active principles and an established barrier cream (Excipial Protect®; Spirig Pharma, Egerkingen, Switzerland) (7). The INCI declaration of all formulations is indicated in Table S1. As active ingredients, the antioxidant cream contains, at the expense of water, 0.1% wt/vol *Reseda luteola* extract (8), 0.1% tocopherol, and 0.05% ubiquinone. The active principle of the barrier cream is aluminium chlorohydrate (7).

Data from the instrumental measurements were analysed by ANOVA (PC-Statistik; Heise, Hannover, Germany), the Friedman test, and the Wilcoxon test for pairwise comparisons. Background readings at T0 were subtracted from all measurements, and the means and standard deviations of net values are shown in Fig. 1. *p*-Values for differences between antioxidant cream and cream base, and antioxidant cream and barrier cream, respectively, are indicated.

To evaluate the antioxidative capacity of the test products, the radical protection factor (RPF) (9) was determined. The RPF was measured by electron paramagnetic resonance spectroscopy, as previously described (10).

Results

Over the treatment interval of 7 days, the washings with SLS induced strong irritation of the skin. This was

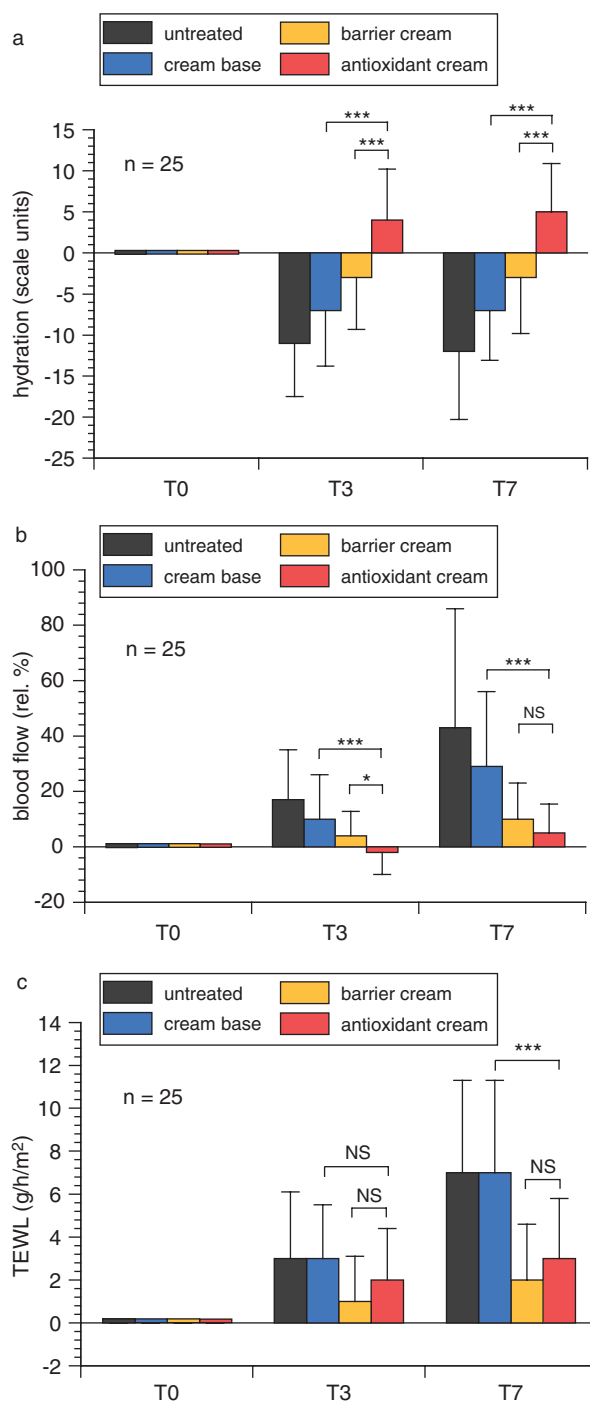


Fig. 1. Results of the repetitive washing test. Instrumental measurements were performed prior to the first treatment (T0), after 3 days (T3), and after 7 days (T7). The mean and standard deviation of each group are shown, and *p*-values for differences between antioxidant cream and barrier cream, and antioxidant cream and cream base, respectively, are indicated. (a) Stratum corneum hydration. (b) Cutaneous blood flow. (c) Transepidermal water loss (TEWL). **p* < 0.05; ****p* < 0.001; NS, not significant.

documented as a loss of stratum corneum hydration, an increase in cutaneous blood flow, and an increase in TEWL (black bars in Fig. 1). Drying of the skin was reduced by both the cream base and the barrier cream. The antioxidant cream, however, was the only test product that even increased stratum corneum hydration over the initial level, despite the repetitive washings (Fig. 1a). Similarly, the cream base and the barrier cream reduced SLS-induced inflammation as indicated by the blood flow measurements (Fig. 1b). Again, the barrier cream was superior to the cream base, but the antioxidant cream was superior to the other products, as it completely suppressed inflammation at T3 and caused the most prominent reduction at T7 (Fig. 1b). The TEWL was significantly reduced at T7 by both the antioxidant cream and the barrier cream, whereas the cream base had no reducing effect on the TEWL at T3 and T7 (Fig. 1c). Analysis of the RPF showed that the antioxidant cream has a very high RPF value as compared with the cream base and the barrier cream (Table 1).

Discussion

There is an increasing body of evidence from *in vitro* and animal studies that ROS play an important role in the development of allergic and irritant contact dermatitis. We have shown recently in mice that contact allergen stimulation generates ROS and causes oxidative degradation of hyaluronic acid, resulting in indirect toll-like receptor activation, eventually amplifying the inflammatory response. The topical application of the antioxidant luteolin prevented sensitization and elicitation in the contact hypersensitivity model (11). Luteolin is one of the most potent natural antioxidants with antioxidative, anti-inflammatory and hyaluronidase-inhibiting properties (12). Systemic and topical treatment of mice with the antioxidant NAC has been shown to reduce irritant and contact hypersensitivity reactions induced by the epicutaneous application of the chemical trinitrochlorobenzene (TNCB) (2). In this study, the mice were treated topically with a 10% cream. In another study, chromium-sensitized albino guinea pigs were treated orally with NAC (1200 mg/kg/day), and this resulted in

a reduction of chromium hypersensitivity by counteracting the generation of ROS (4). Dithranol and SLS, topical inducers of irritant contact dermatitis, strongly reduce the epidermal levels of the antioxidant enzyme Cu,Zn-superoxide dismutase (13). Overexpression of extracellular superoxide dismutase in transgenic mice reduced contact hypersensitivity to TNCB by impairing Langerhans cell migration (14). An ointment with very high vitamin E concentrations (10–20%) reduced chemical-induced irritant and allergic contact dermatitis in rats (3). However, in a small study with 15 volunteers, the topical application of 7.5% quercetin, a plant-derived polyphenolic antioxidant, did not have an effect on SLS-induced skin irritation (5). Given that SLS induces the production of ROS, and that ROS have a very short half-life, the application of quercetin after the chemical irritant might explain the failure of this antioxidant to reduce irritant contact dermatitis in this study.

We have shown previously that different antioxidants protect each other and build an antioxidative network in the skin (10). Therefore, in the present study, we used a combination of antioxidants, namely *R. luteola* extract, tocopherol, and ubiquinone, that show synergistic effects in protecting keratinocytes from solar-simulated generation of ROS (Wölfl U et al., unpublished data 2011). Accordingly, measurement of the RPF of the antioxidant cream in our study showed a very high radical scavenging capacity of the formulation. High RPF values of topical formulations have been shown to provide protection against radical formation in the skin induced by infrared light (15).

The data from the present study show the specific ability of the antioxidant cream to prevent the development of SLS-induced irritant contact dermatitis, as it was superior to the corresponding cream base in all of the parameters evaluated. The flavonoid luteolin that we have used in the antioxidant cream also shows anti-inflammatory effects (12). Therefore, we cannot rule out the possibility that anti-inflammatory properties contribute to the effect of the antioxidant cream. However, its very high RPF probably also plays a role, because there is evidence that SLS induces ROS *in vivo* (13). Future studies must address the role of pure antioxidants in the cumulative SLS irritation model.

The repetitive washing test with SLS induces a disturbance of the epidermal barrier function that is comparable with that caused by working in a humid environment and repeated contact with washing substances (6, 16, 17). The cream base that we used in our study showed less pronounced protective effects than the barrier cream. However, the addition of antioxidants to the cream base significantly improved the protective effect, indicating

Table 1. Radical protection factor (RPF) of the test formulations

Formulation	RPF (10^{14} radicals/mg)
Barrier cream	15 ± 1
Cream base	17 ± 1
Antioxidant cream	320 ± 16

that potent antioxidants may represent a new active principle of a barrier cream.

Conclusion

The topical application of an antioxidant cream with a high RPF may protect the skin from chemical-induced irritation. On the other hand, the superiority of the cream with antioxidants to the vehicle suggests that ROS, at least in part, play a role in the development of irritant contact dermatitis.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. International nomenclature of cosmetic ingredients (INCI) declaration of the test formulations.

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Supplementary Table 1: International nomenclature of cosmetic ingredients (INCI) declaration of the test formulations

Barrier cream:

Aqua, Paraffinum Liquidum, Behenyl Alcohol, Glycerin, Aluminium Chlorohydrate, Ethylhexyl Palmitate, Simmondsia Chinensis Oil, Ceteth-10, Steareth-20, Dimethicone.

Cream base:

Aqua, Caprylic/Capric Triglycerides, Pentylene Glycol, Butyrospermum Parkii Butter, Behenyl Alcohol, Alcohol Denat., Hydrogenated Rice Bran Wax, Squalane, Hydrogenated Lecithin, Raspberry Seed Oil/Palm Oil Aminopropanediol Esters, Sodium Carbomer, Palmitic Acid, C12-16 Alcohols, PVP/Eicosene Copolymer, Acrylates/Vinyl Isodecanoate Crosspolymer, Dehydroxanthan Gum, Benzyl Benzoate, Benzyl Salicylate.

Antioxidant cream:

Aqua, Caprylic/Capric Triglycerides, Pentylene Glycol, Butyrospermum Parkii Butter, Behenyl Alcohol, Alcohol Denat., Urea, Hydrogenated Rice Bran Wax, Squalane, Hydrogenated Lecithin, Reseda Luteola Extract (0.1 % m/v), Luteolin (major constituent of Reseda luteola extract), Tocopherol (0.1 % m/v), Raspberry Seed Oil/Palm Oil Aminopropanediol Esters, Ubiquinone (0.05 % m/v), Sodium Carbomer, Palmitic Acid, C12-16 Alcohols, PVP/Eicosene Copolymer, Acrylates/Vinyl Isodecanoate Crosspolymer, Dehydroxanthan Gum, Benzyl Benzoate, Benzyl Salicylate.

Commento [i1]: Details of the quantitative levels of the antioxidants should be given. The experiment is about them!