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New Insights in the Skin Protective Activity of a Dexpanthenol Containing Formula (BEPANTHEN®) in a Diaper Rash-Like Model

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Abstract

Background: Dexpanthenol containing formula (BEPANTHEN®), formulated as a water in oil preparation, is currently widely marketed as a diaper care product aiming to protect baby's buttocks and repair diaper dermatitis. Dexpanthenol is a well-known moisturizer with barrier-improving properties and the oily phase of the water in oil preparation forms a lipophilic film on the skin surface that isolates the skin from irritants (feces and urine). Prolonged contact with irritants triggers a local inflammation cascade responsible for the cutaneous erythema. To further investigate the protective properties of skin barrier preparations, we took advantage of an *ex vivo* model of healthy human skin discs especially designed to evaluate protective and/or repairing effects of topical preparations recommended for baby's buttocks through the measurement of interleukin-1 alpha release (a cytokine considered as the *Primum movens* of the skin inflammatory reaction), following the application of different irritants. **Methods:** Healthy human skin discs have been incubated in the absence (control) or in the presence of two irritants, *i.e.* a "urine like + urease" preparation and sodium dodecyl sulfate, and in the presence of three ointments, one containing dexpanthenol, but not the other two. At the end of the incubation period, interleukin-1 alpha (IL-1α) was quantified in the explants culture media. **Results:** "Urine like + urease" preparation (ULU) and sodium dodecyl sulfate (SDS) both increased IL-1α production of skin explants by 181.1% ($p < 0.001$) and 88.3% ($p < 0.001$), respectively. The dexpanthenol containing formula significantly inhibited the ULU- and the SDS-induced IL-1α release by 67.42% ($p < 0.001$) and 46.55% ($p < 0.001$), respectively. Under the same experimental conditions, one of the formulas without dexpanthenol significantly inhibited the ULU-induced IL-1α release by 45.94% ($p < 0.01$) but not the SDS-induced one, and the other tested formulation displayed no significant

effect on the IL-1*α* production regardless of the irritant applied. Moreover, the effect of the dexpanthenol containing formula on the ULU-induced IL-1*α* release was significantly higher than the effect of the other formula; a difference of 19.6 % ($p < 0.05$) was observed. **Conclusion:** Dexpanthenol containing formula (BEPANTHEN®) provides good protection of baby's buttocks against irritants. Its protective effect seems to be superior compared with other products, which did not contain this ingredient. Moreover, the results obtained in the present study suggest that dexpanthenol displays *per se* a real IL-1*α* production inhibitory effect. This work, however, consists of preliminary studies and additional investigations involving more formulas and end-points such as the quantification of other pro- or anti-inflammatory cytokines and/or resolvins for example, are needed to better understand the cutaneous protective effect of dexpanthenol.

Keywords

Dexpanthenol, BEPANTHEN®, Human, Skin Protection, Interleukin-1, Diaper-Rash

1. Introduction

Irritant Diaper Dermatitis (IDD) is the most common dermatosis of the diaper area. Half of all infants experience some form of IDD in the first 12 months of life. The most important factor in the development of IDD is prolonged skin contact with urine and feces that impairs the Stratum Corneum (SC) structure and leads to local inflammation. Indeed, urine can increase the permeability of diapered skin to irritants and can also directly irritate skin when exposure is prolonged [1]. In addition, other irritant agents such as classic soaps and detergents in high concentrations, can worsen IDD by disrupting the skin lipids structure leading to the penetration of substances responsible for subsequent immune reactions and inflammation [2].

Nowadays, the management of IDD mainly comprises frequent diaper changes, gentle cleansing of the area and also frequent application of barrier creams or ointments are able to coat and protect the SC from the IDD triggers. Diaper care products are recommended by dermatologists and pediatricians as a fundamental measure for treatment and prevention of IDD [3].

Topical application of formulas containing dexpanthenol is widely used in clinical practice for the treatment of skin lesions [3]. Dexpanthenol is converted within the skin to pantothenic acid, a constituent of coenzyme A [4]. Coenzyme A then catalyzes the synthesis of fatty acids and sphingolipids which are of crucial importance for SC lipid layers and cellular membrane integrity [3] [5] [6] [7] [8].

It has been shown that dexpanthenol displays skin erythema reduction properties. Proksch *et al.* (2002) for example, showed that dexpanthenol was able to reduce cutaneous redness after Sodium Lauryl Sulfate-induced irritation by using

a "semi-quantitative" scoring of the skin erythema [8]. In 2004, Stozkowska and Piekos showed roughly the same results by using roughly the same methodology (scoring of the skin erythema) in an *in vivo* model of guinea pigs [9]. Finally, the curative effect and the IDD prevention action of a dexpanthenol-containing formula have been assessed in two clinical studies [10] [11] in the pediatric population. The product delivered either faster recovery than controls (standard care and standard care + vehicle) in one study and better prevention than control (standard care) in the second study. However, there are to date no investigations, which explain the cellular mechanisms underlying the skin erythema lowering properties of dexpanthenol.

France-based EPHYSCIENCE (Nantes, France) has developed an *ex vivo* model of healthy human skin explants especially designed to assess protecting and/or repairing effects of cosmetic formulations on baby's buttocks. The methodology measures explants interleukin-1 alpha (IL-1α) release after the application of irritants such as "urine-like + urease" (ULU) to mimic the prolonged effect of urine on skin cells or sodium dodecyl sulfate (SDS) to mimic the irritative action of detergents. This model was notably used to compare topical diaper care preparations and provide scientifically valid efficacy data for formulation selection [12].

Interleukin-1 (IL-1), a cytokine mainly produced in skin by keratinocytes, is considered as the *Primum movens* of the inflammatory reaction and is widely implicated in the occurrence of skin redness [13] in response to external aggressions. In this respect, IL-1 constitutes a very efficient and accurate biomarker to evaluate skin erythema reduction properties of topical products in the developed skin discs' model. Perkins *et al.* [14] and Garcia Bartels *et al.* [15] investigated *in vivo*, using tape stripping the correlation between cytokine IL-1α and IDD. The studies outlined that IL-1α was significantly higher in diapered skin compared to non-diapered skin and significant increases in IL-1α levels were found in skin exhibiting diaper rash, heat rash and erythema compared with normal appearing control skin sites.

Taking advantage of this original and proven model, we choose to evaluate the skin erythema protection properties of a dexpanthenol containing formula (BEPANTHEN®), and to compare it to the skin erythema protection properties of two formulas without dexpanthenol (*SUDOCREM® ANTISEPTIC HEALING CREAM and HIPOGLOS® AMENDOAS*).

2. Materials and Methods

2.1. Reagents and Materials

Dulbecco Modified Eagle Medium (DMEM), Ham's F12 culture medium, fetal bovine serum and antibiotics were purchased from Dominique Dutscher (Illkirch, France). Sodium Dodecyl Sulfate (SDS), urease and all the reagents used to prepare the "urine-like" solution were purchased from Sigma Aldrich (Saint Quentin Fallavier, France). IL-1α assay kit came from R&D systems (Abingdon, United Kingdom).

Tested formula INCI composition:

Dexpanthenol containing formula: *BEPANTHEN® pommade* (water in oil emulsion):

AQUA (WATER), LANOLIN, PARAFFINUM LIQUIDUM, PETROLATUM, PANTHENOL, PRUNUS AMYGDALUS DULCIS OIL, CERA ALBA, CEYYL ALCOHOL, STEARYL ALCOHOL, OZOKERITE, GLYCERYL OLEATE, LANOLIN ALCOHOL

Non-dexpanthenol containing formulas:

SUDOCREM® ANTISEPTIC HEALING CREAM (*lipophilic paste*)

ZINC OXIDE, BENZYL ALCOHOL, BENZYL BENZOATE, BENZYL CINNAMATE, LANOLIN, PURIFIED WATER, SODIUM BENZOATE, PARRAFIN WAX, MICROCRYSTALLINE WAX, LIQUID PARAFFIN, SYNTHETIC BEESWAX, SORBITAN SESQUIOLEATE, PROPYLENE GLYCOL, CITRIC ACID, BUTYLATED HYDROXYANISOLE, LINALYL ACETATE, LAVANDULA ANGUSTIFOLIA LAVENDER

HIPOGLOS® AMENDOAS (*hydrophilic paste*)

AQUA (WATER), ZINC OXIDE, LANOLIN, TALC, PETROLANUM, PARAFFINUM LIQUIDUM, BHA, POLYETHYLENE, DISODIUM EDTA, METHYLPARABEN, PROPYLPARABEN, PRUNUS AMYGDALUS DULCIS OIL, RETINYL PALMITATE, TOCOPHERYL ACETATE, PARFUM, PEG-30DIPOLHYDROXYSTEARATE, ALPHA-ISOMETHYLIONE, LINALOOL, CITRONELLOL, LIMONENE

2.2. Cell Culture and Treatments

Healthy human skin explants caming from chirurgical resections were cultured at 37˚C in an atmosphere containing 5% of CO_2 in 24-well plates in DMEM/Ham's F12 (50:50) containing 10% of fetal bovine serum and antibiotics (penicillin/streptomycin 1% and amphotericin B 0.4%).

For the IL-1α measurements, the three tested formulas were applied or not (control) at the surface of the skin explants (10 µL per skin disc). Skin explants were then incubated for 18 hours in the absence (control) or in the presence of "urine like + urease" (ULU) or of SDS at 0.5% (w/v), applied at the skin surface. At the end of this new incubation period, explants culture media were harvested and frozen at −20˚C until IL-1α quantification.

2.3. ULU Preparation

Artificial urine was prepared according to the formula of Shmaefsky [16] as follows: urea (18.2 g/L), sodium chloride (7.5 g/L), potassium chloride (4.5 g/L), sodium phosphate (4.8 g/L), creatinin (2 g/L), albumin (50 mg/L), pH adjusted at 5.1. According to precedent experiments, "Urine-like + urease" (ULU) was prepared by adding urease (1 U/ml) to the artificial urine.

2.4. IL-1α Measurements

IL-1α was quantified in explants incubation media by using a commercially availa-

ble assay kit purchased from R&D systems.

2.5. Statistics

Results are presented as mean +/− SD of 3 to 6 replicates (n = 3 to 6) coming from 1 to 2 different experiments.

Level of significance between "Control without ointment" and "treated without ointment" conditions has been assessed by using a one factor variance analysis (One way ANOVA) followed by a Holm-Sidak test when necessary.

Level of significance between "treated without ointment" and "treated with ointment" conditions has been assessed by using *Student t-tests*.

Level of significance between "Dexpanthenol containing formula" and "non-dexpanthenol containing formula" has been assessed by using a *Student t-test*.

3. Results and Discussion

As shown in Figure 1, ULU and SDS were both able to significantly enhance the cutaneous production of IL-1α by 181.1% ($p < 0.001$) and 83.3% ($p < 0.001$), respectively. This result was expected and validated the model for the evaluation of the skin erythema protection properties of the three tested products.

As shown in Figure 2, the dexpanthenol containing formula (BEPANTHEN®) significantly limits the ULU- and the SDS-induced IL-1α production of the skin explants by 67.42% ($p < 0.001$) and 46.55% ($p < 0.01$), respectively.

Under the same experimental conditions, one of the two non-dexpanthenol containing formulas (SUDOCREM® *ANTISEPTIC HEALING CREAM*) was able to significantly limit the ULU-induced IL-1α production of skin explants by 45.94% ($p < 0.01$), but not the SDS-induced one (Figure 3).

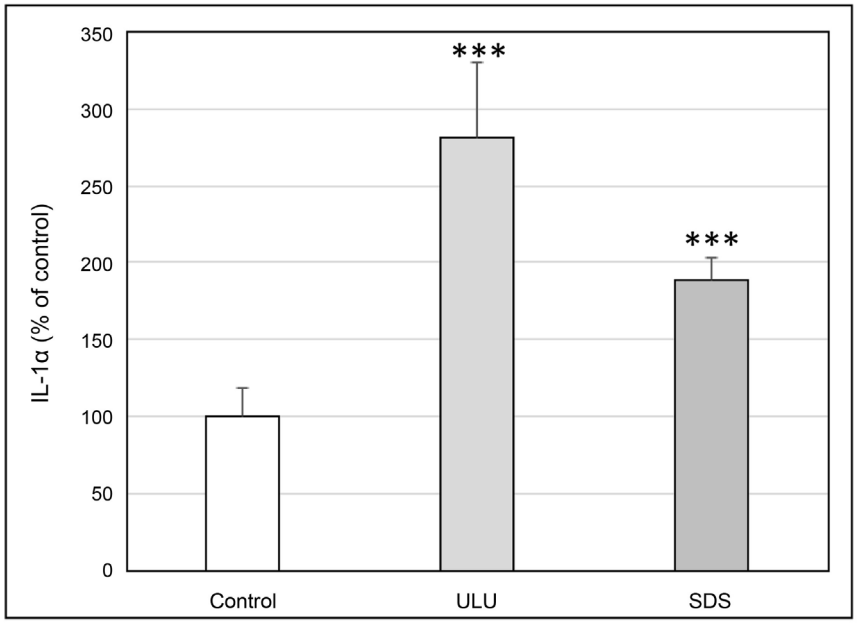

Figure 1. ULU and SDS effect on the IL-1α skin explants production. ***: Significantly different from the "Control" condition ($p < 0.001$).

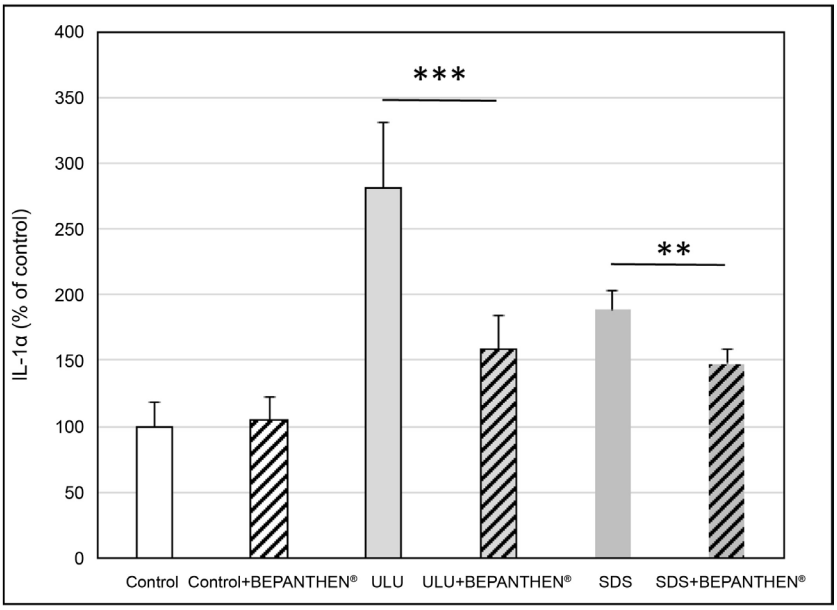

Figure 2. Effect of a dexpanthenol containing formula (BEPANTHEN®) on the ULU- and SDS-induced IL-1α skin explants production. **: Significant difference between the conditions indicated by the bar ($p < 0.01$); ***: Significant difference between the conditions indicated by the bar ($p < 0.001$).

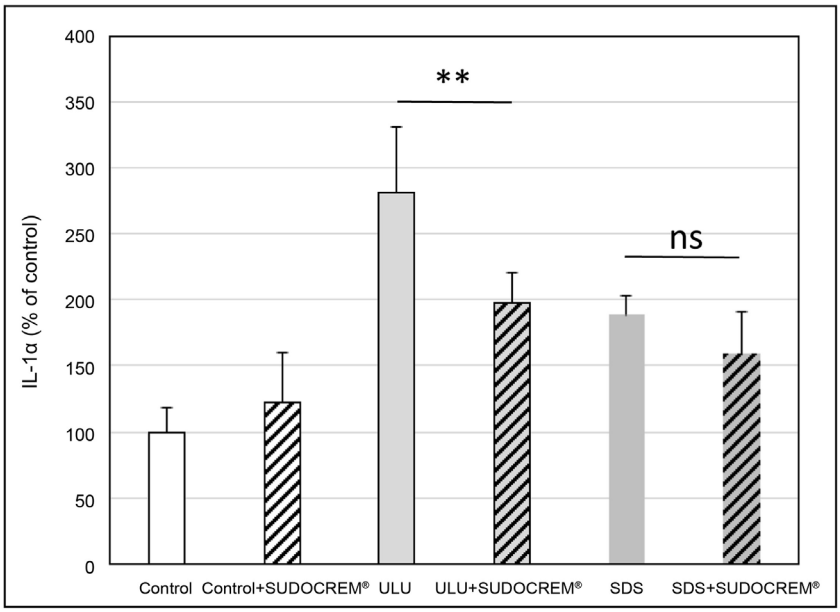

Figure 3. Effect of a formula which doesn't contain dexpanthenol (SUDOCREM®) on the ULU- and SDS-induced IL-1a skin explants production. ns: Non-significant difference between the conditions indicated by the bar ($p > 0.05$); **: Significant difference between the conditions indicated by the bar ($p < 0.01$).

The third tested formula (*HIPOGLOS® AMENDOAS*), which did not contain dexpanthenol, did not show any significant inhibitory effect, neither on the ULU-induced (−32.2%; $p > 0.05$), nor on the SDS-induced (−36.5%; $p > 0.05$) IL-1α production of the skin explants (**Figure 4**).

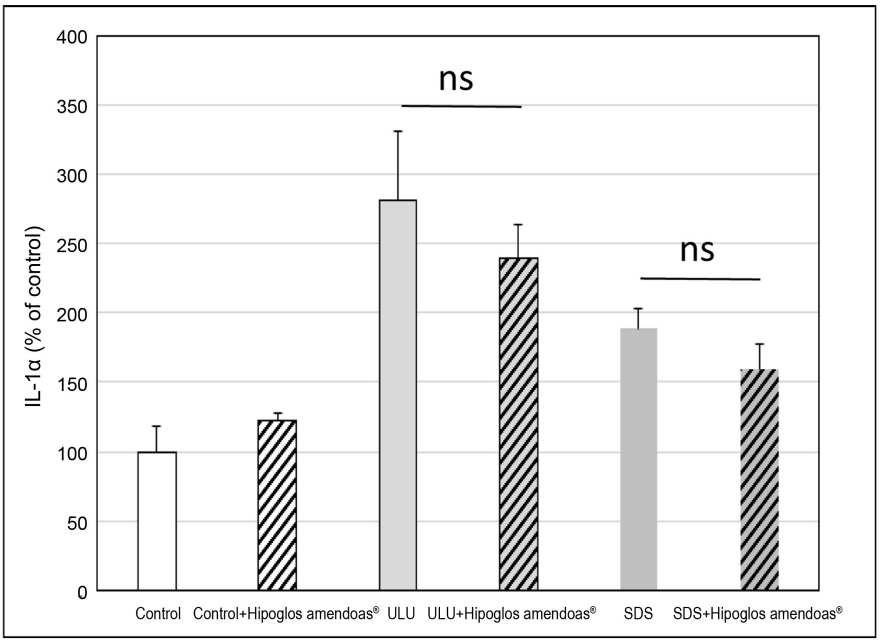

Figure 4. Effect of a formula which doesn't contain dexpanthenol (Hipoglos amendoas®) on the ULU- and SDS-induced IL-1a skin explants production. ns: Non-significant difference between the conditions indicated by the bar ($p > 0.05$).

Interestingly, we can note that the effect of BEPANTHEN® is roughly 20% superior to the effect of SUDOCREM® on the ULU-induced IL-1*α* production ($p < 0.05$).

All obtained results suggest that the Dexpanthenol-containing formula allows to achieve better skin protective effects in the presence of irritations induced by both "natural" (ULU) or "chemical" (SDS) irritants. Dexpanthenol seems to act *per se* on the cellular mechanisms underlying the irritant-induced skin IL-1*α* production and/or skin redness/erythema.

These results are in line with the work of Nitto and Onodera who reported in 2013 that mice lacking the vanin-1 gene (pantotheinase gene) showed less tissue injuries following various irritant treatments (for a review, see [17]). Pantotheinase, an enzyme which hydrolyses pantotheine (a daughter molecule of pantothenic acid), is in fact responsible for the generation of cysteamine which is implicated in the tissue injuries following irritant applications. In these conditions, we can easily reason that pantothenic acid brought into the cell by topical application of dexpanthenol, could counterbalance the effect of this enzyme by restoring "correct" cellular pantotheine levels, thus limiting tissue injuries.

Additionally, we can also imagine that dexpanthenol, due to its implications in the intracellular coenzyme A and lipids synthesis, is able to enhance and/or increase the production of particular fatty acids notably implicated in the resistance to irritant aggressions, the resolvins (for a review, see [18]). However, this explanation of the skin protective effects of dexpanthenol is currently hypothetical and additional work is needed to further investigate and elucidate this aspect.

In many countries, pastes have been a popular product class for IDD prevention and treatment, containing a high proportion of zinc oxide or titanium dioxide, suspended in a water-in-oil (lipophilic) or an oil-in-water (hydrophilic) vehicle. However, hydrophilic paste formulations do not provide a very effective barrier and are generally unsuitable for daily use in a preventive role of IDD. On the other hand, lipophilic formulations will be reasonable barriers but are very difficult to remove and are, in practice, highly occlusive. In general, formulations with a lipid content >50% provide very good protection [12]. Well-known and explained in scientific publications, baby skin has specific characteristics [2] [19] and baby skin care products must be designed in light of these specific needs. Particular attention should be payed to formulation ingredients and product efficacy and safety should be established in scientific studies.

4. Conclusion

This work is the first to unambiguously describe the effect of dexpanthenol on irritant-induced skin IL-1α production, a relevant biomarker of impaired skin barrier and local inflammation, in an established *ex vivo* skin model. The results stipulate first insights to challenge the idea that all diaper care products deliver the same level of protection. Further investigations are required to better understand the cellular mechanisms underlying these newly demonstrated skin protective properties of dexpanthenol containing ointments.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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