Efficacy evaluation of ingredient mixtures using an in vitro model of atopic dermatitis

Mancini F.[1], Mastrocola E.[2], Donelli D.[2], Durando L.[1], Ragni L.[2]
[1]Angelini RR&D (Research, Regulatory & Development) – Angelini S.p.A., Piazzale della stazione snc - 00071 S.Palomba-Pomezia (Rome), Italy
[2]Angelini RR&D (Research, Regulatory & Development) –Angelini S.p.A., Via Vecchia del Pinocchio, 22 – 60131 Ancona, Italy

Introduction

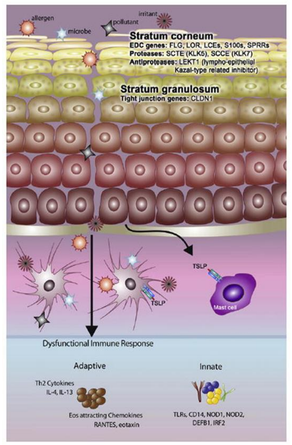


Figure 1. AD pathways [1]

Atopic dermatitis (AD) is an inflammatory skin disease, characterized by erythema and pruritus, affecting 2-20% of the general population and predominantly children [1]. AD is associated with the decrease of barrier integrity and functionality, enhanced allergen penetration and skin colonization by microorganisms such as *Staphylococcus aureus.* Thymic stromal lymphopoietin (TSLP) plays an important role in the initiation and maintenance of the allergic immune response in AD, while chemokines and cytokines such as TNFα and IL-8 play an important role in the pathogenesis of the inflammatory skin diseases [2, 3].

The aim of this study was to verify, using an *in vitro* model of atopic dermatitis, the efficacy of two mixtures of "active ingredients" mixture A and mixture B, specifically created to treat atopic babies' skin.

Methods and Materials

Mixtures of ingredients

Treatments	Ingredients
Mixture A	moisturizer, prebiotics, natural antioxidant mixture
Mixture B	moisturizer, prebiotics, natural antioxidant mixture, lipid molecules, natural anti-inflammatory agent

Endpoints

Hystology

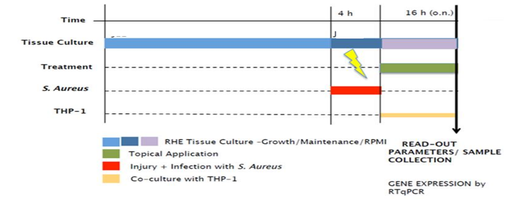
Tissues were fixed in formalin and sections of 5 µm were obtained. Slides were stained with Haematoxilin and Eosin and analyzed under light microscopy (40x). Morphological modification, alterations at the epithelium surface and thickness tissues were analyzed.

Gene expression

Tissues were collected in lysis buffer for RNA extraction, cDNA retrotranscription and gene expression analysis. TSLP, TNFα and IL-8 were assessed. RHE in co-culture with THP1 negative control was used as calibrator control (RHE+THP1=1).

***In vitro* model of atopic dermatitis**

3D Reconstituted Human Epidermis (RHE) model from young donors (age < 6 years) co-cultured with THP-1 human monocytic cells and infected with *S. aureus* after a slight mechanical abrasion on RHE surface [4]. The test items were prepared in PBS and topically applied on the surface of tissues for 16h as described into the following scheme:



Results

Hystology

Results reported in figure 2 show that:

- Mix A treatment was effective in maintaining a conservative morphology. It is possible to notice only a slight swelling on the surface.
- Mix B treatment has preserved tissue morphology in the viable layers: only few signs of toxicity are visible. The structure, thickness and number of stratum corneum layers is fully preserved.

The histology is comparable to a tissue without any treatment (naïve tissue); the thickness of the mix B treated tissue is 125 µm comparable to a naïve tissue [4] as reported in the following table:

Treatments	Thickness tissue (µm)
Naïve tissue	*~ 120/130*
Mixture A	~ 90
Mixture B	~ 125

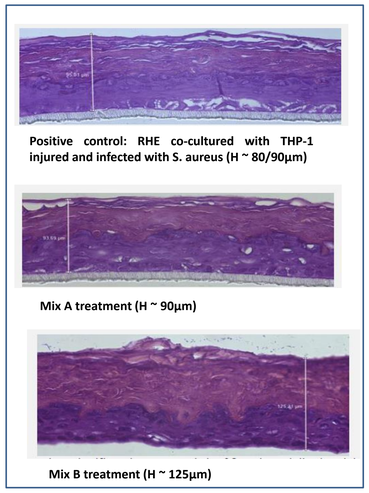


Figure 2. Histomorphological results

Gene espression

Results, expressed as fold with respect to the negative control and reported into figure 3, show that TSLP is highly expressed in the atopic skin model [4] and both mixtures reduced in significantly manner the TSLP overexpression suggesting a role in restoring/enhancing immunity response. Up regulation of IL-8 and TNFα confirms the inflammatory response [4] and mixture B was the most effective in reducing the inflammatory response reducing the IL-8 and TNFα mediated inflammation.

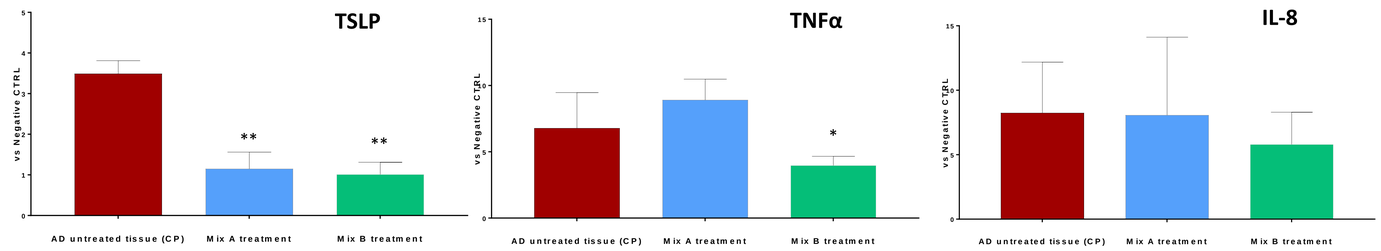


Figure 3. Thymic Stromal Lymphopoietin TSLP, Inflammatory response TNFα and IL-8 genes expression.
Student T-Test has been performed: Mixtures significance vs CP have been calculated (*p<0,05, **p<0,01)
Positive control CP: RHE co-cultured with THP-1 injured and infected with *S. aureus*
Negative control: RHE co-cultured with THP-1

Conclusions

The combined use of prebiotics to boost the growth of the protective friendly skin bacteria, lipid molecules that are effective in improving barrier properties, and a bioactive compound that exhibits potential anti-inflammatory activity, could explain the reduction of inflammatory response and ameliorative effects observed on tissue by mixture B, which can be favourable used in cosmetic formulations for baby care to support atopic dermatitis treatment [5].

Contact Information

Francesca Mancini
Email: f.mancini@angelini.it
Website: https://angelini.it
Phone:+390691045288

References

1. Mark Boguniewicz and Donald YM Leung. Atopic Dermatitis: A Disease of Altered Skin Barrier and Immune Dysregulation. Immunol Rev. 2011 July; 242(1): 233-246.
2. Warner W. Carr. Topical Calcineurin Inhibitors for Atopic Dermatitis: Review and Treatment Recommendations. Pediatr Drugs (2013) 15:303–310.
3. Toshiro Takai. TSLP Expression: Cellular Sources, Triggers, and Regulatory Mechanisms. Allergology International 2012;61:3-17.
4. De Servi B, Semenzato A, Baratto G, Meloni M. Modeling atopic dermatitis in reconstituted epidermis. SFC – Journees Jean Paul Marty, Paris, 8-9th December 2015.
5. Lawrence F. Eichenfield et al. GUIDELINES OF CARE FOR THE MANAGEMENT OF ATOPIC DERMATITIS: Part 2: Management and Treatment of Atopic Dermatitis with Topical Therapies. J Am Acad Dermatol. 2014 July ; 71(1): 116–132.

Acknowledgements

The authors would like to thank Dr M.Meloni and Dr B. De Servi - VitroScreen Srl Via Mosè Bianchi,103 20149 Milano (ITALY) for their kind contribution.