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Summary

Background. Atopic Dermatitis (AD) is a common, chronic skin disorder caused by complex genetic, immunological, and environmental interactions. The current standard treatments for AD are improvable with a low efficiency, particularly in severe cases. Using well characterized molecules the “Ignacio Umbert Dermatology Institute” (IUDI) has developed topical treatments differentiated from the standards (European patent number EP2311454).


Materials and methods. Using a cellular model for human macrophages (THP-1 cell line) the principal components of EP2311454 treatment was evaluated. Expression of proinflammatory cytokines was measured through RNA quantification and ELISA technique. Therapeutically EP2311454 treatment was tested in forty three AD patients and SCORing Atopic Dermatitis (SCORAD) and Dermatology Quality of Life Index (DLQI) was evaluated before and after the treatment.

Results. The effect was significantly anti-inflammatory towards IL6 and COX2. The therapeutic efficacy was demonstrated by obtaining a reduction of initial SCORAD levels in the 97.7% of patients besides EP2311454 treatment reduces DLQI significantly.

Conclusions. Clinically, EP2311454 treatment is effective, especially in severe cases, getting a reduction of 80% initial levels at the end of the evaluation period. Moreover, the effect was detected from the first week.

KEY WORDS: atopic dermatitis; treatment.

Introduction

Atopic Dermatitis (AD) is an inflammatory skin disease with an incidence of 10-20% in children and 1-3% in adults and has a serious impact on the patients’ quality of life (1). AD is associated with defects in the skin barrier that are correlated with the severity of the lesions. According to the clinical tool used to assess the extent and severity of eczema (SCORing Atopic Dermatitis, SCORAD), AD is considered mild when the coefficient does not reach 25, moderate 25-50 and severe over 50. The pathogenesis of AD is poorly understood, and the treatment of recalcitrant AD is still challenging.

The therapeutic efficiency of current standard treatment is higher in mild AD, but it is insufficient to cure moderate to severe patients. Treatment in these cases is limited: phototherapy and systemic immunosuppressive agents, such as systemic corticosteroids, cyclosporine A, methotrexate, and azathioprine (2, 3). For current medication, high doses are usually used on a long-term basis. This regimen of administration is very aggressive and associated with multiple adverse effects, such as tissue inflammation and, more prominently, glucocorticoid resistance (4, 5). This resistance may be caused by a variety of factors, including increased glucocorticoid β receptor expression that acts as a dominant negative receptor so that treatment ceases to have an effect.

Therefore there is much interest in identifying alternative therapies for the treatment. For instance, probiotics have recommended as a therapeutic option. The rationale for their use is that bacterial products may induce an immune response of the Th 1 series instead of Th 2 and could therefore inhibit the development of allergic IgE antibody production. Some report limited benefit in preventive and therapeutic roles (6).

In this scenario antioxidants compounds are suitable candidates to accompany anti-inflammatory molecules like indomethacin (7, 8).

In these regard, IDIU has developed the EP2311454 treatment differentiated from the standards in composition and treatment schedule. A selection of individual components with suitable properties was combined and
the topic treatment resultant (European patent number EP2311454) was tested in patients, so its therapeutic potency evaluated. Besides AD therapeutic drugs, the main ingredients of the mixture are indomethacin, resveratrol, ascorbic acid 6-palmitate, taurine (2-aminoethanesulfonic acid) and cathequin from green tea. Indomethacin is related to anti-inflammatory effect and senescence (9) besides has been shown to affect leukocyte migration to inflammatory sites (10), the other components are antioxidants well described in several publications. Multiple studies correlate treatment with different antioxidants with inhibition of pro-inflammatory gene expression and increased expression of anti-inflammatory genes (11). Concerning resveratrol, a natural non-flavonoid polyphenolic compound, its being related to healthy habits (12). Moreover, emerging evidence on its implication in a number of pathophysiological processes like cancer (13), cardiovascular diseases (14), or inflammatory diseases (15) are arising. Particularly interesting is its effect of mouse model of AD (16). The present study aims to test clinically the efficiency of this treatment and elucidate its way of action through an *in vitro* model.

**Methods**

**Cell culture**  
Human macrophages derived from the THP1 cell line were used as reference model for studies on monocyte/macrophage function. Cells from this cell line (monocytes) were differentiated into macrophages by incubation with Phorbol Miristate Acetate (PMA). The cells from this cell line (monocytes) were differentiated into macrophages by incubation with Phorbol Miristate Acetate (PMA). The cells were then allowed to adhere to the surface of the culture dish for 48 hours.

**Treatment**  
Indomethacin, ascorbyl palmitate, taurine, and resveratrol are components of EP2311454 treatment (EP2311454 patent); they were dissolved according to the manufacturer’s instructions and incubated with THP1 macrophages. Cells were incubated for 2 hours with the compound (different concentrations), then treated with 100 ng/ml lipopolysaccharide (LPS) for 4 hours to stimulate inflammation. At the end of this time, the supernatant and cells were harvested.

**ELISA**  
Protein concentration of detectable cytokines IL-6 was then determined in cell-free supernatants using quantitative commercially available ELISA kits (R&D Systems, Minneapolis, MN, USA) according to the manufacturer’s instructions.

**RNA quantification**  
Cells were then washed with PBS, and total RNA was isolated using TriPure reagent (Roche Applied Science) according to the manufacturer’s instructions. Reverse transcription was performed using the high capacity cDNA reverse transcription kit (Applied Biosystems), and the reaction mix was subjected to quantitative real-time PCR to detect IL-6, COX-2. Human GAPDH from the same provider was used as an endogenous control. Real-time PCR was performed in a PCR-7600HT sequence detection system (ABI PRISM, Applied Biosystems) at 95°C for 10 min for AmpliTaq Gold activation and then run for 40 cycles at 95°C for 15 s and 60°C for 1 min. The relative levels of gene expression were quantized and analyzed using SDS version 2.4 software. The real-time value for each sample was averaged and compared using the *C*_ method, where the amount of target RNA (2−ΔΔ*CT*) was normalized to the endogenous control (Δ*CT*) and related to the amount of target gene in the cells.

**Human study**  
Patients were eligible to participate in the trial if they were at least 18 years of age and had moderate-to-severe atopic dermatitis. Prior to the study, all patients included in the experiment were made to sign confidentiality and informed consent document, as their data could be included in this document. Forty three patients both women and men were selected.

**Monitoring:** For evaluation a total of 5 visits was planned at: 0, 7, 15, 30, 45 and 55 days from the start of treatment, photographs were taken and the signs and symptoms of this disease were evaluated.

**Treatment:** Patients were instructed to use 2 g per application and perform 3 applications per day (every 8 h). The cream was placed first on the fingertips to be later distributed homogeneously in small sub-portions in the affected area. A rotation was performed, until the cream had completely absorbed.

**Evaluation:** To evaluate the effects of the components used for the treatment of AD, the scoring system “SCORing Atopic Dermatitis” (SCORAD) and the Dermatology Life Quality Index (DLQI) were used at the beginning and the end of treatment. SCORAD is a method of clinical standardization of AD that uses a mathematical formula to objectify the extent, degree of eczema involvement as well as pruritus and loss of sleep produced by AD. This way you can measure the severity of the AD as well as measure the improvement of the patient as the treatment progresses (17).

**Statistical analysis**

Results presented are mean ± SEM. Results were analyzed with one ANOVA followed by appropriated post-hoc comparisons. DLQI data were analyzed through 1-Student test. All statistical analyses were completed using the Graphpad Prism program (Prism version 7.0, Graphpad Software Inc).

**Results**

**Treatment in patients**  
As shown in Figures 1 and 2, after treatment SCORAD values decrease drastically the first 7 days, within 15 days, decrease slightly, until they reach constant values. These results indicate that EP2311454 treatment has a...
great efficacy in the reduction of the symptoms of this disease as it can be shown in Figure 1A, B. Interestingly, the total reduction after treatment is present either in moderate and severe AD which is even higher in the last one (Figure 1B).

As seen in Figure 2 A-D patients treated with EP2311454 have a very positive response to treatment. Observing Figure 2C and 2D, the patient was completely bleached after 15 days of treatment. The patient of Figure 2A and 2B took 55 days to be completely bleached. Regarding DLQI index, Figure 3 shows the significant reduction after the treatment.

### In vitro results

The compounds tested reduce the expression of COX-2 and IL-6 over the single treatment with LPS (Figure 4). The levels of IL-6 after combined treatment with indomethacin, resveratrol and taurine are lower than those obtained after incubation of the cells with each of them separately, resveratrol or taurine, in combination with indomethacin. Similarly, ascorbyl palmitate and catechins, in combination with indomethacin, also exert additive actions inhibiting the expression of COX-2. Similar results were obtained for IL-1β (data not shown). These results were confirmed with IL-6 protein levels; ELISA assay was performed to quantify the IL-6 release to the medium after LPS stimuli. Pre-treatment with EP2311454 compounds has significant anti-inflammatory effect, with resveratrol at 25 μM and the combination of indomethacin, resveratrol and taurine having a higher inhibitory effect (Figure 5). Taking all data together seems clear that the mixture of the main components in treatment is more effective, reducing the inflammation than in a separate way.

### Discussion

Current treatments for AD require long management guidelines that encourage the development of resistance and unwanted side effects. Shorter treatments and higher efficiencies are needed but unfortunately these requirements for atopic dermatitis represent an unmet medical need. Therapeutic agents used in the management of AD mostly provide symptomatic relief, in the form of topical emollients and topical anti-inflammatory...
agents, with limited, unspecific options for moderate-to-severe disease. However, emerging data on new anti-inflammatory agents have been published in the recent years (18). New evidences point to an improvement in efficiency not so much in new molecules, but in the adequate combination of existing drugs. In these regards IDIU selected effective anti-inflammatory drugs and promising substances with antioxidant capacity. Antioxidant molecules have demonstrated characteristics as therapeutically treatment against inflammatory diseases (19). Although the clinical application of antioxidants for skin inflammatory disorders is not widespread, we wanted to test it using a combination of natural molecules. Consequently, EP2311454 treatment main components are resveratrol, taurine and ascorbyl-L-palmitate previously defined as antioxidant in humans and indomethacin which is anti-inflammatory in low doses associated to several human diagnosis (20, 21). The aim of this study is evaluate the clinical efficiency of the treatment in moderate and severe AD patients whereas mild AD patients have a better treatment. On the other hand, we wanted to test the anti-inflammatory

Figure 2 - A) Image of a patient with severe AD (hand) before starting treatment. B) Image of the hand after 55 days of treatment. C) Image of a patient with AD (face) before starting treatment. D) Image of the same patient after 15 days of treatment.
Therapeutic efficiency for atopic dermatitis of EP2311454 treatment

As can be seen in the results SCORAD, the patients bleached the skin within a few days of treatment. The treatment achieved a reduction greater than 80% SCORAD reduction in all cases being slightly higher in the severe patients. On the other hand the application time was inferior to the conventional treatments getting this efficiency in less than 60 days in all cases. These results indicate that corticosteroid creams, “NonSteroidal Antinflammatory Drugs” (NSAIDs) and antioxidants are able to regulate inflammation effectively. Another important fact is that the combined use of corticosteroids and antioxidants does not produce resistance to glucocorticoids or other side effects, possibly due to the anti-inflammatory capacity of such antioxidants.

In order to understand cellular mechanisms to these findings individual components of EP2311454 treatments were tested in an inflammatory human

**Figure 3** - Favourable evolution of patients suffering from AD according to the DLQI values obtained. Patients were monitoring after treatment, the reduction was visible in DLQI values. **** P<0.00001.

**Figure 4** - Expression analysis (RNA) of IL-6 and COX-2 following stimulation of THP-1 cells with LPS (100 ng / ml), either alone or in the presence of combinations of indomethacin (5 μM) with different actives: resveratrol (12.5-25 μM), taurine (0.1-0.2 mM), ascorbyl palmitate (5-10 μM) or green tea catechins (0.5-1 μM). Control cells (NO LPS) were not stimulated with any of these compounds. *, P <0.05, versus NO LPS; #, P <0.05, versus LPS (100ng / mL).

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macrophage *in vitro* model. Analysis of the combined action of indomethacin with other antioxidants has shown that the efficacy of some triple combinations is greater than the efficacy shown by combinations of indomethacin with such active compounds alone (double combinations). In conclusion, indomethacin combined with ascorbyl palmitate and catechins of green tea have a synergistic effect on the COX-2 gene. In addition, this combination has an inhibitory additive effect on the IL-1β and IL-6 genes. These results suggest that the combination of these active ingredients together with indomethacin have a superior anti-inflammatory capacity than the use of these antioxidants individually. Regarding way of action PPAR-γ activation could be important as a mediator of its anti-inflammatory capacity (data not shown). These results, together with *in vitro* studies, indicate that the combined use of antioxidants at the same time with corticosteroids or NSAIDs is beneficial in regulating inflammation of the skin, generating fewer adverse effects, since the continuous application of these creams does not generate resistance and the treatment can continue to be applied over time.

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**References**


**Statement of conflicts of interest**

No declared.


