Study of the Effect of *Leonurus Japonicus* Extract on Atopic Dermatitis

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Introduction:

Leonurus Japonicus (LJ) is one of the many plants that constitute Chinese and Kampo traditional medicines and is particularly well known for its antioxidant and anti-inflammatory properties [1], [2]. Millions of people worldwide suffer from inflamed sensitive skin and dryness, which is caused by many external factors such as temperature, or pollution, but also intrinsic factors related to a genetic disposition. For example, atopic dermatitis (AD) is one of the most common chronic skin diseases caused by genetic predisposition [3].

This study aimed to investigate the ability of LJ extract to regulate key genes and protein expression involved in the initiation of AD [4], [5], [6]. After an increase of TSLP in the epidermis induced by the cytokine cocktail, we provide evidence that the LJ extract represses the expression of TSLP at mRNA and protein levels. Moreover, we hypothesized that this active ingredient may also act on this condition by directly controlling key components of the inflammasome, whose dysregulation plays a major role in AD physiopathology. In conclusion, LJ extract may be a promising solution against AD.

Materials & Methods:

For this study, skin biopsies from the abdominal skin of a 59-year-old healthy Caucasian female were cultured under six different conditions and each condition was performed in triplicates and processed separately following the experimental procedures shown in Figure 1: A cytokine cocktail composed of TNF-α, IL-4, IL-5, and IL-13 at a concentration of 200ng/mL was used to induce TSLP production and mimic AD inflammatory response and treatments with an LJ extract at 3 concentrations (0.01%, 0.02%, and 0.03%) were applied. Cyclosporin (1µM), an immunosuppressant drug, was also used as a positive control of inhibition of TSLP production. Their activity was measured using quantitative PCR and immunohistochemistry (IHC). Statistical analysis was performed using a t-test (p value<0.05).

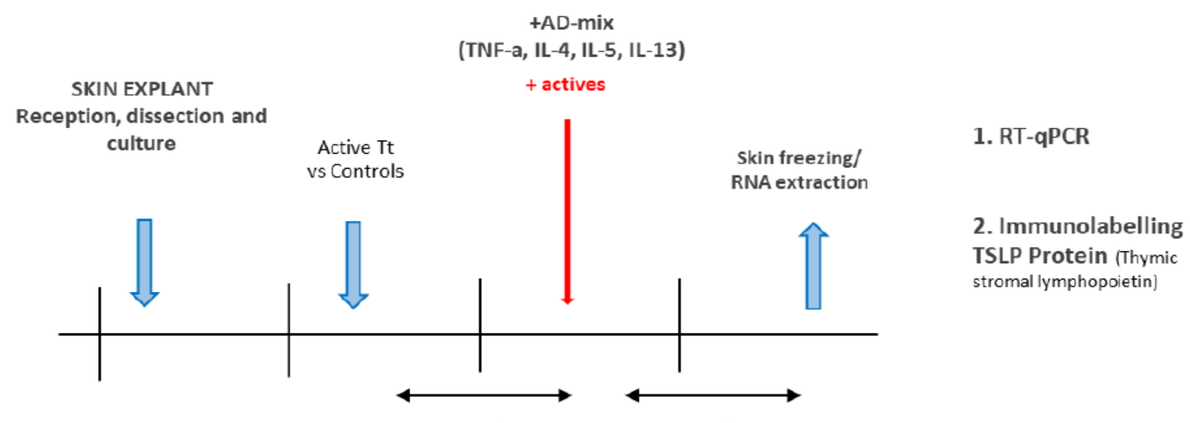

Figure 1: Experimental procedure to study the effect of LJ Extract in skin explants.

Results & Discussion:

Figure 2 shows the effect of LJ on the modulation of IL-1β and PYCARD (coding for ASC protein), two key genes of the inflammasome pathway, and NOD2. Therefore, this active ingredient is a promising molecule against inflammation and specifically AD. We can see the effect of LJ at 3 concentrations (0.01%, 0.02% and 0.03%) on the expression of 2 genes tightly associated with Atopic Dermatitis: TSLP and SPINK5. In this study, we showed that the active ingredient at 3 concentrations decreased the SPINK5 gene expression as well as with the cyclosporin. Moreover, the histogram showed that LJ at 0.03% decreased the expression of the TSLP gene expression at the mRNA level, and the other concentrations did not have any effect on the modulation of TSLP like cyclosporin.

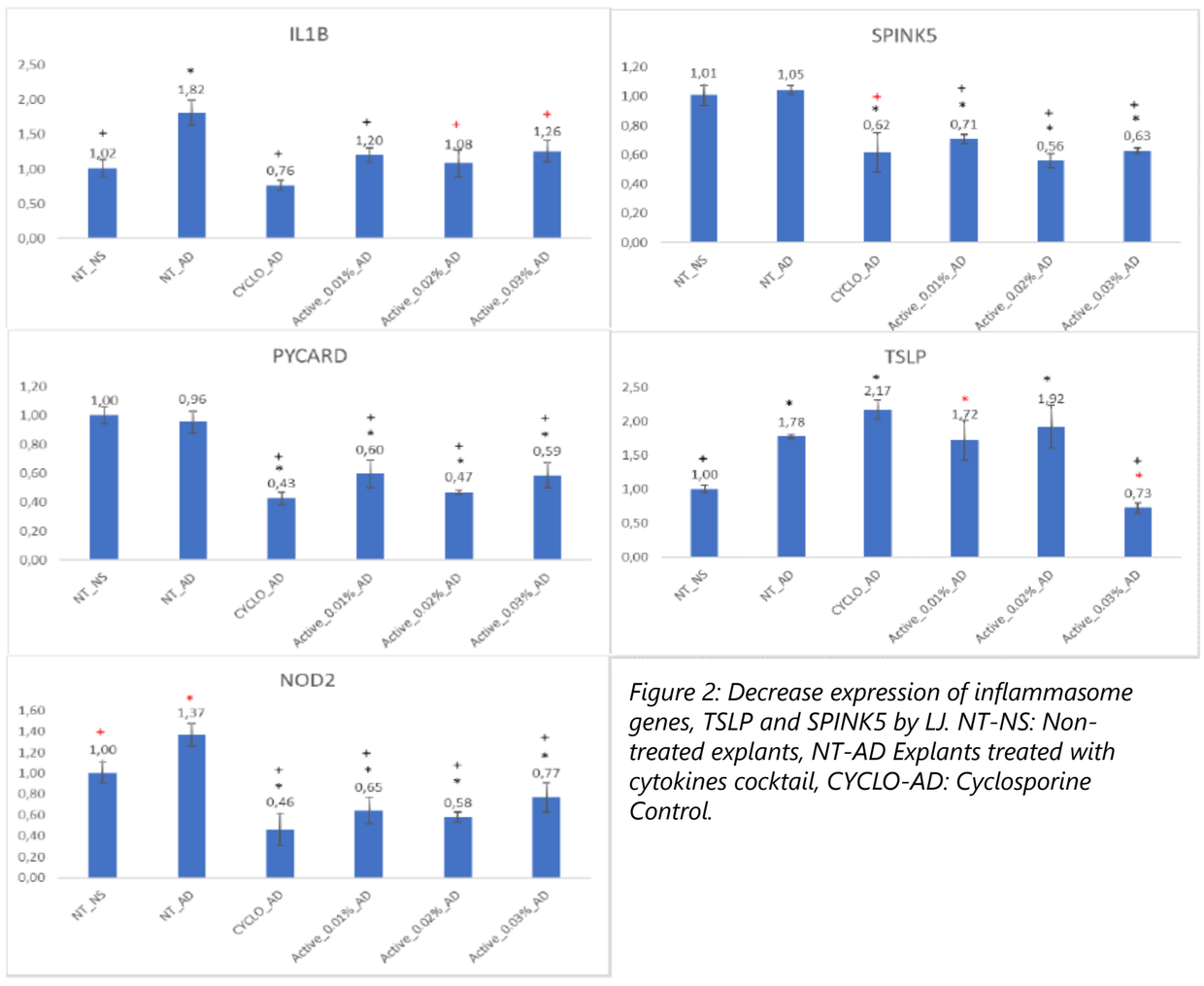

Figure 2: Decrease expression of inflammasome genes, TSLP and SPINK5 by LJ. NT-NS: Non-treated explants, NT-AD Explants treated with cytokines cocktail, CYCLO-AD: Cyclosporine Control.

As LJ showed an effect on TSLP gene expression, we also investigated the effect of the active ingredient on TSLP expression by immunostaining the protein in human skin explants (See Figure 3). The ability of the active ingredient to reduce the production of TSLP after induction by the cytokine cocktail was then assessed by immunohistochemistry and compared to cyclosporin.

As expected, TSLP protein expression increased after AD stress, and the addition of Cyclosporin reduces the TSLP expression. Finally, treating with LJ extract at a concentration of 0.01% or 0.02% also reduces TSLP expression after AD stress at the protein level.

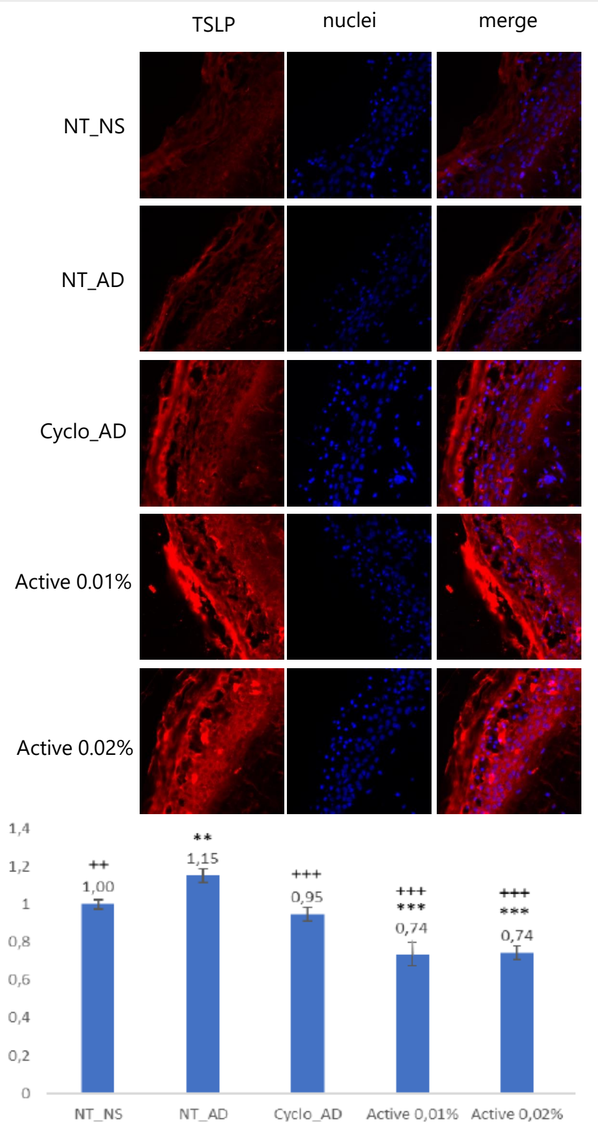

Figure 3: Effect of LJ extract on TSLP at protein expression. NT-NS: Non-treated explants, NT-AD Explants treated with cytokines cocktail, CYCLO-AD: Cyclosporine Control.

Conclusions:

In this study, we provide a new model for mimicking AD disease on explant skin cells, stimulated with a cocktail of cytokines (IL-5, IL-4, IL-13, and TNF-α) triggering a chronic inflammation like the one in AD. We showed that LJ extract represses the SPINK5 and PYCARD gene expressions and additionally it decreases the dependent induction of IL-1β and NOD2 gene expressions. Finally, we found that TSLP mRNA expression was reduced by this active ingredient and such a decrease in TSLP expression was confirmed at the protein level using IHC labelling.

Taken together, LJ extract may be a promising active ingredient against AD. It has also been hypothesized that this extract may also act on AD by directly controlling the key component of the inflammasome and KLKs, as the dysregulation of the latter plays a major role in the pathophysiology of AD.

References:

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