Anti-inflammatory effect of *Calendula officinalis* L., *Matricaria recutita* L. and *Vitis vinifera* L. aqueous extracts in human keratinocytes

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Keratinocytes, the predominant cell type in the epidermidis, are essential for epidermal renewal and skin's defence. Upon activation keratinocytes actively participate in the cutaneous immune responses expressing different cytokines and chemokines. Dysregulation and abnormal expression of inflammatory mediators, or their receptors in keratinocytes, are relevant to the pathogenesis of chronic inflammatory skin diseases such as psoriasis, and atopic dermatitis. Keratinocytes can act as a primary sensor of cutaneous injury, in particular via Toll-Like Receptors (TLR), activating the NF-κB pathway (Wuallert et al., 2011). Once activated, NF-κB translocates into the nucleus, where it regulates the transcription of several cytokine and growth factor genes, including TNFα, IL-6, IL-8. Several natural compounds possess topical anti-inflammatory activity, including *Calendula officinalis* L. and *Arnica montana* L.. However, the molecular mechanisms underlying the anti-inflammatory activity in the skin is not fully elucidated. The aim of the study was to *in vitro* investigate the anti-inflammatory effect of three aqueous extracts from *Calendula officinalis* L., *Matricaria recutita* L. and *Vitis vinifera* L. on NF-κB pathway in human keratinocytes. Experiments were carried out on spontaneously immortalized keratinocyte cell line HaCaT. The extracts were prepared according to the ESCOP suggestions (ESCOP monographs, 2009): *Calendula* (CE) and *Matricaria* (ME) flowers extracts were obtained by 10 minutes infusion in boiled water (4 g of drugs in 150 and 250 mL of distilled water for CE and ME, respectively), whereas *Vitis vinifera*L. leaves extract (VE) was obtained after 4 hours in cold water (10 g of drug in 100 mL of distilled water). NF-κB driven trascription was evaluated with a plasmid containing the luciferase gene under the control of three NF-κB responsive elements. TNFα, IL-1β (both 10 ng/mL) and LPS (5 μg/mL) were used as pro-inflammatory stimuli. NF-κB nuclear translocation was measured after 1 hour treatment with the previously reported stimuli by ELISA assay. IL-8 release was evaluated after 6 hours treatment by ELISA assay. Characterization of flavonoid occurring in VE was performed with HPLC-DAD. For preliminary evaluation of the anti-inflammatory activity the extracts in keratinocytes, CE, ME and VE, were tested at 50 μg/mL on NF-κB driven transcription. VE showed the highest effect, while CE and ME were inactive. VE displayed a concentration-dependent inhibitory effect, ranging from 2.5 to 100 μg/mL. At maximal concentration (100 μg/mL), VE inhibited NF-κB driven transcription induced by TNFα (-80%), IL-1β (-56%), and LPS (-100%). To further investigate the effect of VE on NF-κB pathway, we focused on NF-κB nuclear translocation. VE (50 μg/mL) was able to decrease NF-κB nuclear translocation induced by TNFα (-55%) and LPS (-100%); the effect was not found when IL-1β was the stimulus. Since it has been demonstrated that the expression of IL-8 is NF-κB dependent, the following experiments were devoted to evaluate the effect of VE on IL-8 secretion induced by TNFα, IL-1β, and LPS in human keratinocytes. VE reduced in a concentration-dependent manner IL-8 release induced by TNFα and LPS (complete inhibition at 100 μg/mL and 25 μg/mL respectively), but showed lower effect when cells were stimulated with IL-1β (-70% at 200 μg/mL). Preliminary characterization of VE showed that quercetin glycosides, hyperoside, and kampferol glucoside, in addition to cyanidin-3-O-glucoside, were the most abudant flavonoids occurring in the extract. These data show for the first time that VE is able to inhibit keratinocytes inflammation by acting on NF-κB pathway. Thus, these results provide new insights on the use of extracts from the leaves of *Vitis vinifera* L. to ameliorate skin inflammatory diseases.

Wuallert et al. (2011). Cell Research 21:146-58
ESCOP monographs (2009), Thieme