Ursolic acid as a potential agent in alleviating abnormal keratinocyte behavior in psoriasis

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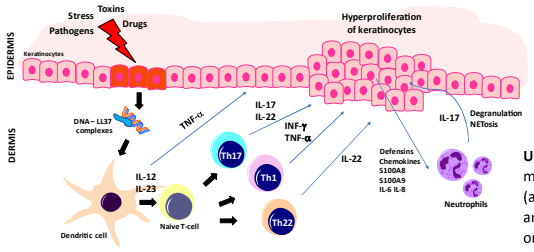
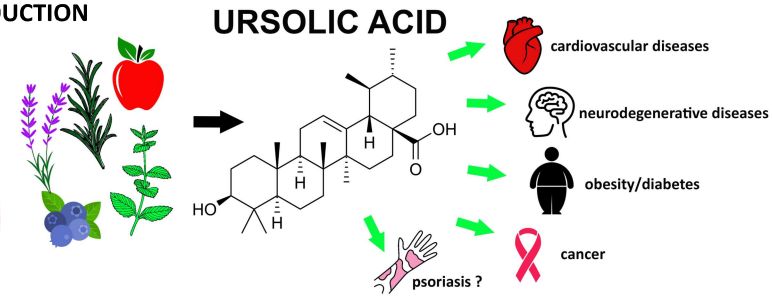
ABSTRACT: Psoriasis is a chronic inflammatory skin disease characterized by excessive proliferation of keratinocytes and infiltration of inflammatory cells into the outer layers of the skin. Observed epidermal hyperplasia and production of pro-inflammatory cytokines by keratinocytes is mainly induced by the action of lymphocytes Th17. Psoriasis treatment focuses on reducing the inflammatory response and limitation of keratinocytes proliferation. Here we report usage of ursolic acid, natural triterpenoid compound present in extracts from herbs and fruits, as an active agent mitigating abnormal keratinocyte behavior typical for psoriasis. Keratinocyte cell line HaCaT treated with cytokine M5 cocktail (IL-1, IL-17A, IL-22, oncostatin M, and TNF-α), characteristically secreted by psoriatic Th17 lymphocytes, showed increased proliferation and proinflammatory cytokine production, mimicking the disease phenotype. Application of ursolic acid limited M5-induced production of IL-6 and -8. This was accompanied by reduction of other psoriatic markers and by a decrease in the hyperproliferation of HaCaT cells. The latest is likely mediated by non-inflammatory apoptotic cell death of keratinocytes. Our data indicate that ursolic acid can serve as a psoriasis mitigating agent and could be considered as an active ingredient in preparations intended for use as a topical cosmetics.

INTRODUCTION

Psoriasis is a relatively common inflammatory skin disease that affects nearly 2-5% of the population. Its clinical symptoms are characterized by an acute inflammatory reaction in the first phase, which turns into a chronic form. Psoriasis is characterized by three typical histological features: (i) epithelial hyperplasia, (ii) dilated, enhanced blood vessels formation in the dermis, (iii) accumulation of inflammatory cells, mainly in the dermis. The detailed mechanism of psoriasis development is not yet clear. Current hypothesis indicate primary activation of the innate immune system, based on the impaired response of keratinocytes, neutrophils and dendritic cells, which leads to the subsequent recruitment of Th17 cells. The disturbance in the functioning of the adaptive immune system including excessive activation of dendritic cells and in turn Th1/Th17 lymphocytes results in the production of cytokines, especially IL-17A, IL-22, TNFα and interferon γ, which generate full clinical outcome of the disease. Current anti-psoriasis treatments focuse on reducing the inflammation state and limitation of keratinocyte hiperproliferation.



URSOLIC ACID



Ursolic acid (3β-hydroxyurs-12-en-28-oic acid), is a pentacyclic triterpenoid and a secondary metabolite identified in many commonly used plants, including herbs (thyme, rosemary, lavender, oregano, mint) and mainly in fruit peels (apples, blueberries, cranberries, hawthorn plums). Ursolic acid can mediate anti-inflammatory, anti-oxidant, antiobesity and anti-carcinogenic effects in different models. Currently studies exploring the usage of ursolic acid focus on prevention/treatment of cancer, diabetes, cardiovascular and neurodegenerative diseases.

RESULTS

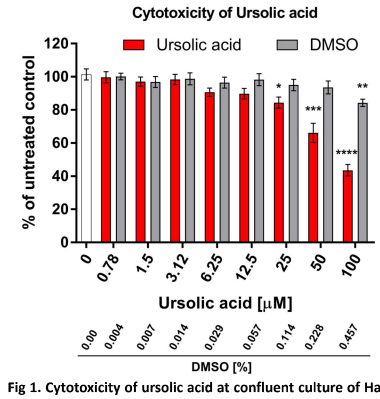


Fig 1. Cytotoxicity of ursolic acid at confluent culture of HaCaT keratinocyte cells. Confluent culture of HaCaT cells were incubated with increasing concentrations of ursolic acid (0-100 μM) or mock (DMSO) for 24h. Viability of the cells was evaluated with MTT assay and shown as a percentage of viability of untreated control. Safe concentration of ursolic acid was assessed at the level of 12,5 μM. Results are shown as an mean with SEM (one-way Anova with Dunnet post test).

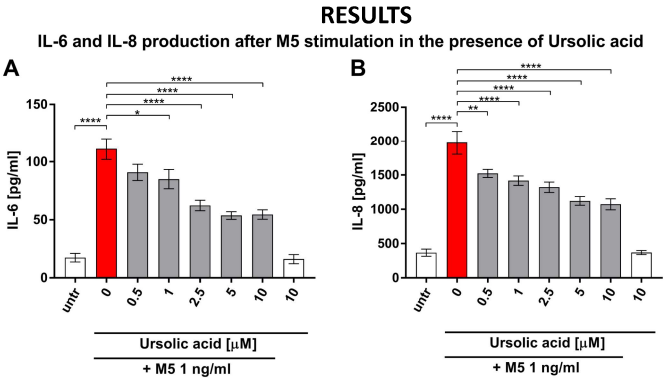


Fig 2. Ursolic acid decreases IL-6 and IL-8 production in HaCaT cells stimulated with M5 cytokin cocktail. Confluent cultures of HaCaT cells were stimulated with 1ng/ml of M5 cytokin cocktail (1ng/ml of each IL-1a, IL-17A, IL-22, TNF-α and Oncostatin M) in the presence of increasing concentrations of ursolic acid (0-10 μM). After 24h cell media were collected and analysed for IL-6 and IL-8 levels with ELISA. M5 stimulation induced significant increase in proinflammatory cytokine production as illustrated by the levels of IL-6 (A) and IL-8 (B). Presence of ursolic acid limited the production of above mentioned cytokines by 50% at the highest used concentration of ursolic acid (10 μM).

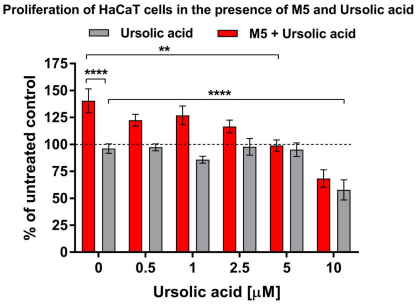


Fig 3. Ursolic acid decreases hiperproliferation of HaCaT cells stimulated with M5 cytokin cocktail. HaCaT cells were seeded on 96 well plate at the density of 2000 cells/well. Cells were then stimulated with M5 cytokin cocktail (2,5 ng/ml) in the presence of increasing concentration of ursolic acid (0-10 μM) in DMEM with 1% FBS. After 72h amount of cells in each sample was estimated using MTT assay. Results are shown as a percentage of untreated control (mean with SEM, one-way Anova with Dunnet posttest). Stimulation of cells with M5 cytokin cocktail increased the proliferation of cells by 40% in comparison to untreated control. Usage of ursolic acid in concentration of 5μM decreased cells hyperproliferation to level of not stimulated control. Concentration of ursolic acid above 10 μM ursolic acid caused decreased proliferation of HaCaT cells.

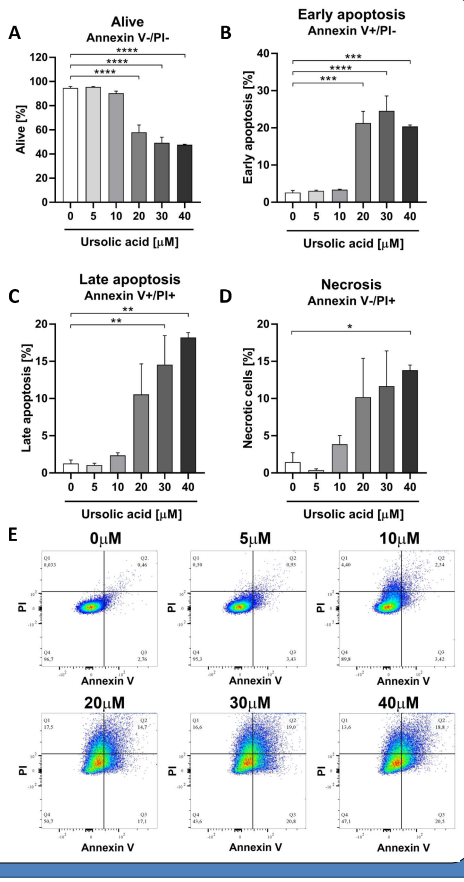


Fig 4. High concentrations of ursolic acid induce apoptotic cell death in HaCaT cells. Cell culture of HaCaT (~80% confluence) were stimulated with increasing concentrations of ursolic acid (0-40 μM). After 24 h cells were harvested and stained with propidium iodine/Annexin V antibodies and analysed with flow cytometer to estimate the amount of alive/apoptotic/necrotic cells. Ursolic acid in concentration above 10 μM induced apoptotic cell death in 30-40% population of HaCaT cells (B-C). Necrosis were observed in ~5-15% of cell population (D). Results are shown as the percentage of counted cells in each population (mean with SEM, one-way Anova with Dunnet posttest). (E) Representative dot plots for each concentration of ursolic acid.

Conclusions

1. M5 cytokine cocktail (IL-1, IL-17A, IL-22, oncostatin M, and TNF-α) is a good stimulant inducing psoriasis-like phenotype in keratinocyte cell lines
2. Ursolic acid mitigate proinflammatory cytokin production (IL-6 and IL-8) by HaCaT cells with psoriatic-like phenotype
3. Ursolic acid limits hyperproliferation of HaCaT cells in psoriatic model, probably by mediation of non-inflammatory apoptotic cell death of keratinocytes

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