Ursolic acid as a treatment for psoriasis does not affect the immune response of human neutrophils and blood-derived macrophages

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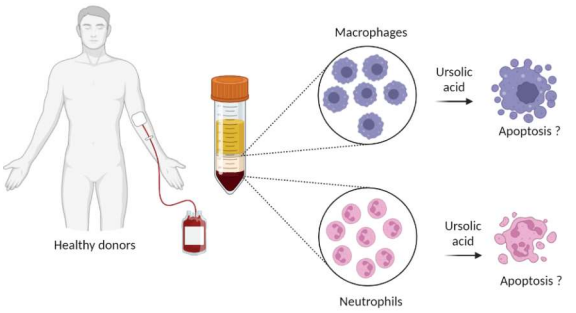
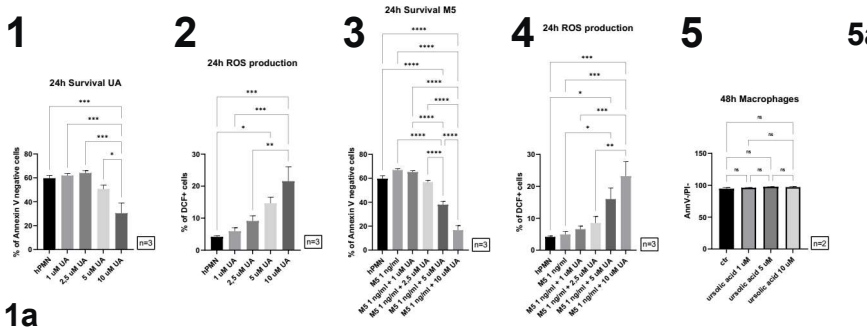
INTRODUCTION

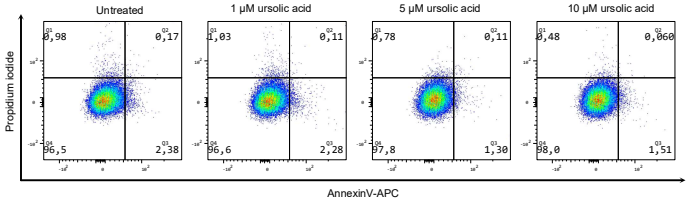
This study aims to examine the impact of ursolic acid (UA) on immune cells in the treatment of psoriasis. UA is a triterpenoid found in many plants (i.e. apples, elder flower, lavender, oregano, peppermint). It is well documented that UA has various beneficial effects, such as being anti-inflammatory, antibacterial, and antiviral. Recently, UA has been proposed as an agent in the psoriasis therapy. Our previous research has shown UA ability to attenuate inflammation in the HaCaT cell line in the psoriasis phenotype model. In this follow-up study, we tested the survival and production of reactive oxygen species (ROS) and secretion of pro-inflammatory cytokines by human neutrophils and macrophages.

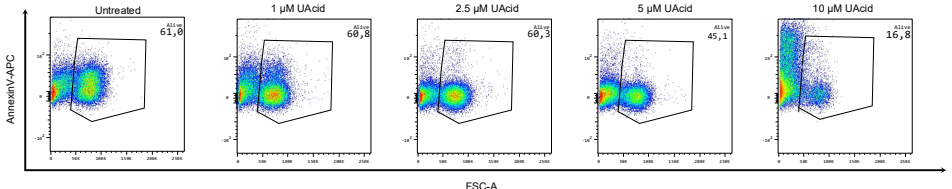
Human blood-derived macrophages have been treated with UA (1, 5 and 10 μM) with or without stimulation with IL-23 (0.1, 1, 10 ng/ml) for 48 h. Cells were stained with Annexin V and propidium iodide. Human neutrophils have been treated with UA (1, 2.5, 5 and 10 μM) with or without stimulation with 1 ng/ml of the M5 cytokine cocktail (IL-17A, IL-22, oncostatin M, IL-1α and TNF-α) for 24 h. Cells were stained with Annexin V and DCFH for ROS production and analyzed by FACS. The secretion of IL-8, IL-17, IL-22, TNF-α, and IFN-β was measured by ELISA.

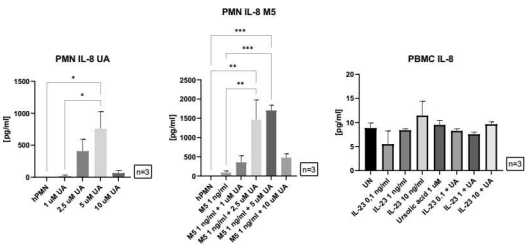


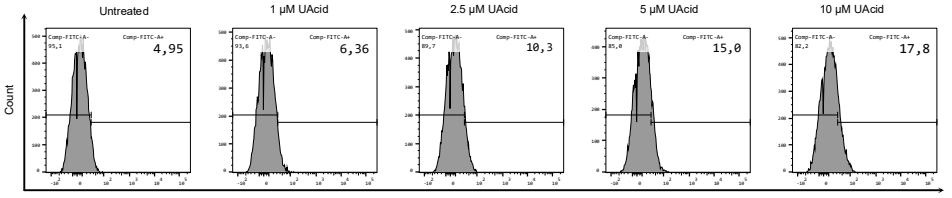
RESULTS

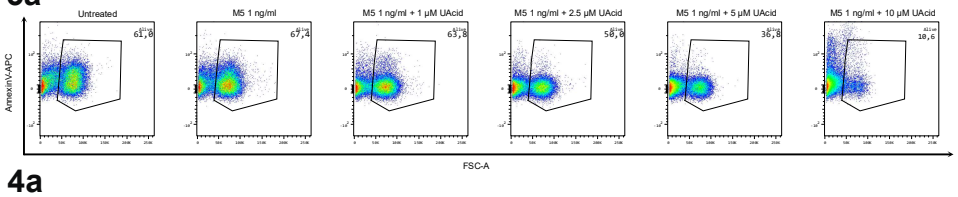


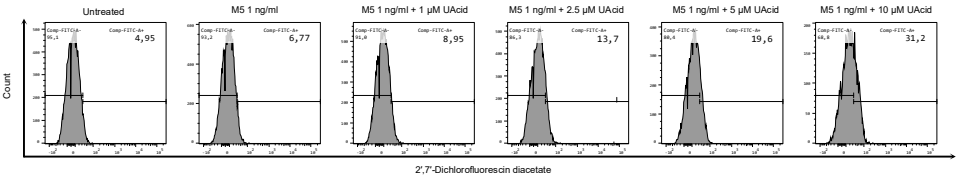












(1, 1a) **Survival of neutrophils**. Human neutrophils were isolated from healthy donors and stimulated for 24 hours with ursolic acid (1, 2.5, 5, and 10 μM). Cells were stained with AnnexinV-APC and analyzed by flow cytometry. The highest concentration of UA decreased neutrophil survival.

(2, 2a) **ROS production of neutrophils**. Human neutrophils were isolated from healthy donors and stimulated for 24 hours with ursolic acid (1, 2.5, 5, and 10 μM). Cells were stained with DCFH for 20 minutes and analyzed by flow cytometry. The highest concentration of UA increased neutrophil ROS production.

(3, 3a) **Survival of neutrophils**. Human neutrophils were isolated from healthy donors and stimulated for 24 hours with an M5 cytokine cocktail (IL-17A, IL-22, oncostatin M, IL-1α, and TNF-α) and ursolic acid (1, 2.5, 5, and 10 μM). Cells were stained with AnnexinV-APC and analyzed by flow cytometry. The highest concentration of UA decreased neutrophil survival.

(4, 4a) **ROS production of neutrophils**. Human neutrophils were isolated from healthy donors and stimulated for 24 hours with an M5 cytokine cocktail (IL-17A, IL-22, oncostatin M, IL-1α, and TNF-α) and ursolic acid (1, 2.5, 5, and 10 μM). Cells were stained with DCFH for 20 minutes and analyzed by flow cytometry. The highest concentration of UA increased neutrophil ROS production.

(5, 5a) **Survival of macrophages**. Human-derived macrophages were isolated from healthy donors and stimulated for 48 hours with ursolic acid (1, 5, and 10 μM). Cells were stained with AnnexinV-APC and propidium iodide and analyzed by flow cytometry. Ursolic acid does not affect macrophage survival.

(6) **Secretion of IL-8 by neutrophils**. Human neutrophils were isolated from healthy donors and stimulated for 24 hours with ursolic acid (1, 2.5, 5, and 10 μM). Cells were stained with AnnexinV-APC and analyzed by flow cytometry. The concentration of 5 μM of ursolic acid increased the secretion of IL-8.

(7) **Secretion of IL-8 by neutrophils**. Human neutrophils were isolated from healthy donors and stimulated for 24 hours with ursolic acid (1, 2.5, 5, and 10 μM). The concentration of 5 μM of ursolic acid increased IL-8 secretion.

(8) **Secretion of IL-8 by macrophages**. Human-derived macrophages were isolated from healthy donors and stimulated for 48 hours with ursolic acid (1 μM) and IL-23 (0.1, 1, 10 ng/ml). Ursolic acid does not affect the secretion of IL-8.

CONCLUSIONS

- Ursolic acid does not negatively affect the immune cells examined in this study in concentrations below 5 μM
- The M5 cytokine cocktail stimulates cells and increases neutrophil survival, an effect limited by ursolic acid
- The results presented indicate that ursolic acid has an inflammation-limiting effect on neutrophils, in the concentration range of 1-5 μM
- Ursolic acid does not induce the secretion of IL-17, IL-22, IFN-β, and TNF-α by neutrophils and macrophages
- Taking into account the previously described inflammatory limiting effect of ursolic acid in HaCaT-based models, ursolic acid could be used as a treatment for psoriasis

ACKNOWLEDGMENTS

The research was funded under the project entitled DEVELOPMENT OF A LINE OF MEDICAL PRODUCTS BASED ON NANO-GEL WITH PROPERTIES RANGING FROM SKIN CARE TO TREATMENT TO ALLEVIATE INFLAMMATORY SKIN CONDITIONS, contract no: RPMP.01.02.01-12-0186/19 implemented by WellNanoPharm Sp. z o.o.
The research has been carried out at the Malopolska Centre of Biotechnology, Jagiellonian University (contract CRUiP UJ nr 1204 z dn. 20.08.2020)

Fundusze Europejskie Program Regionalny | Rzeczpospolita Polska | MAŁOPOLSKA | Unia Europejska Europejski Fundusz Rozwoju Regionalnego