Anti-inflammatory activity of triterpenoid conjugates on human monocytes/macrophages: evaluation of anti-oxidant activity, PPARgamma expression, and NF-kappaB activation.

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Oleanolic acid (OA) and ursolic acid (UA) are the major triterpenoid acids of dietary relevance, occurring in olive oil and culinary herbs like rosemary and sage (1). OA and UA are pleiotropic agents that target a host of end point involved in inflammation, cancerogenesis, and glucose control (2,3,4,5,6,7,8). The pharmacokinetics of these compounds is poorly known, both in terms of absorption and metabolization. We have previously investigated the effects of an extra-virgin olive oil minor polar extract and we demonstrated that they inhibited p50 and p65 NF-kB translocation in human monocytes and macrophages at concentrations found in human plasma after nutritional ingestion of virgin olive oil (9). Since OA and UA are lipophilic carboxylic acids, the possibility exists that they are conjugated in vivo with biogenic alcohol (e.g. ethanolamine) and amines (e.g. dopamine), in a mechanism somewhat similar to that proposed for the anti-inflammatory activity of paracetamol (10). To this study, a series of conjugates of OA and UA with various biogenic alcohols and amines were synthesized using various coupling strategies. Monocytes were isolated from Buffy coats of healthy volunteers, and differentiated *in vitro* to macrophages. The first screening of the derivatives was done with the viability test MTT of the cell treated for 24h with the compounds in a range of concentration between 20 and 0.01mM. Some of the derivatives resulted significantly cytotoxic and were therefore excluded from further assaying. Selected compounds (used at 1mM) were then evaluated for their anti-oxidant properties through the test of superoxide anion production, and for their activity on NF-kB and on PPARy signalling pathways, by EMSA and Western blot.
The results demonstrated that the Ursolic acid and its synthetic derivatives Ursoil phenylethyl ester (UFE), Ursoil tyramide (UTA), and the Oleanolic acid and its synthetic derivative Oleanoil homovanillyl ester (OOE), resulted not to have pro-oxidant activity by them self but rather presented a significant anti-oxidant activity via inhibition of the superoxide anion production in both monocytes/macrophages induced by PMA (the gold standard for cell activation). Moreover, a consistent modulation of NF-kB and PPARy pathways has been identified in both cellular models: in particular, OA and the UA derivative UFE showed a significant inhibition of PMA-induced NF-kBactivation in a dose-dependent manner (range between 10 and 0.1mM). Vice versa, AU, OA and their derivatives demonstrated a significant effect on inducing PPARy expression. In this case, the comparison were made against the natural receptor agonist 15-deoxy-D(12,14)-prostaglandin J(2) (PGJ2). Compared to AU and AO, the corresponding derivatives UFE, UTA and OOE demonstrated a major efficacy.
In conclusion, out of a library of 20 triterpenoid conjugates, 5 showed superior anti-inflammatory activity on the investigated signalling pathways, with lipophilicy playing a critical role in activity.

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