TABLE 1 Eosinophil shape change in response to H4R ligand treatment

H_4R agonism			H_4R antagonism[a]	
Compound	pEC_{50}	E_{max} (% change)	Compound	pIC_{50}
Histamine (mean ± SD; n = 3)	7.5 ± 0.3	22 ± 6	ZPL-3893787 (n = 3)	8.4 ± 0.4
Eotaxin[b] (mean ± SD; n = 3)	9.7 ± 0.5	32 ± 5	Toreforant (n = 3)	7.6 ± 0.4

[a]Inhibition of histamine EC_{80} challenge concentration determined for each donor (68–198 nM).
[b]CCR3 agonist used as positive control.

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P096 | A hydrogen sulfide-releasing dexamethasone derivative attenuates atopic dermatitis severity signs and the associated oxidative stress markers in mice

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Background and Purpose: Atopic dermatitis (AD) is a highly prevalent chronic inflammatory skin disease characterized by pruritus and eczematous skin lesions. Considering that hydrogen sulfide (H_2S) is produced in the skin and participates of several processes, such as the regulation of inflammation, pruritus, scarring, and angiogenesis (Coavoy-Sánchez, Costa, Muscará, 2019), we decided to test the effects of a H_2S-releasing dexamethasone (Dexa) derivative in a murine model of AD.

Experimental Approach: The experimental protocol was approved by our Local Ethics Committee for Animal Experimentation (CEUA-ICB/USP; no. 129/2016). Female BALB/c mice (6- to 8-week-old) had the dorsal hair shaved, and 2,4-dinitrochlorobenzene (DNCB) was topically applied on days 1–3. On days 15, 17, 19, and 22, the mice were topically challenged with DNCB on both the dorsal skin and the right ear. On days 19–23 after sensitization, the animals were topically treated with either dexamethasone (Dexa) or the H_2S-releasing thiobenzamide (TBZ)-dexamethasone derivative (Dexa-TBZ; both at 250 nmol per mice), or TBZ (1 μmol per mice). Skin severity score and scratching behaviour were assessed before each challenge and on day 24 before the animal euthanasia. Blood samples were collected for cell counting, and dorsal skin samples were collected for analysis of oxidative stress markers (3-nitrotyrosine [3NT]- and carbonyl group-containing proteins) and antioxidant enzymes. Results are shown as mean ± SEM. Differences among the experimental groups were analysed by one-way ANOVA followed by the Tukey's test for multiple comparisons.

Key Results: Topical DNCB induced AD-like skin lesions, scratching behaviour, ear oedema, and eosinophilia. Similarly to Dexa, topical treatment with Dexa-TBZ significantly reduced the skin severity score (average 27.9%; $P < .001$; $n = 5$), scratching behaviour (88.8%; $P < .001$; $n = 5$), and ear oedema (98.8%; $P < .05$; $n = 5$) and decreased the number of eosinophils to values lower than those observed in the animals without AD. Both 3-NT and carbonyl groups were increased in the AD skins, and treatment with Dexa-TBZ (but not Dexa) resulted in significant reduction of these markers (by average 59% and 100%, respectively; $P < .05$; $n = 5$) in addition to increased glutathione peroxidase activity (by 22.3%; $P < .05$; $n = 5$) in comparison with Dexa.

Conclusion and Implications: The addition of a TBZ moiety to dexamethasone does not interfere with the beneficial effects of this parent corticosteroid and adds the advantage of stimulating antioxidant defences in the animals with AD, thus evidencing the therapeutical potential of this new molecule for the clinical treatment of AD.

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P097 | Synthetic analogues of ferulic acid exert anti-inflammatory and antioxidant effects in human monocytic cells

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Background and Purpose: Flavonoid-rich food and beverage consumption is correlated with a reduced risk of developing cardiovascular disease, potentially due to anti-inflammatory and antioxidative mechanisms in vascular cells. In the gut, flavonoids are extensively metabolised into phenolic acids (PA) via bacterial catabolism. We have previously found that specific flavonoids and metabolites at physiological concentrations modestly inhibit TNF-α secretion and induce haem oxygenase-1 (HO-1) expression in monocytes. In this study, we investigated whether the anti-inflammatory and anti-oxidative effects of three common PA could be enhanced by esterification in THP-1 monocytes.

Experimental Approach: 4-Hydroxy-3-methoxycinnamic acid (FA), 3,4-dihydroxybenzoic acid (PCA), and 4-hydroxy-3-methoxybenzoic acid (VA) and their esters FA methyl (FA-ME), FA propyl (FA-PE), FA hexyl (FA-HxE), PCA methyl (PCA-ME), PCA ethyl (PCA-EE), PCA propyl (PCA-PE), PCA hexyl (PCA-HxE), and VA hexyl (VA-HxE) were utilised. Cell viability was measured by MTS assay (Promega). TNF-α secretion was measured by ELISA (di Gesso et al., 2015), and NQO1 enzyme activity was assessed as previously described (Prochaska & Santamaria, 1988). HO-1 protein expression was measured by a DuoSet® IC ELISA (R&D Systems) and Nrf2 DNA-binding by ELISA (TransAM® Nrf2, Active Motif), all according to the manufacturer's instructions. TNF-α, HO-1, and NADPH quinone oxidoreductase (NQO1) mRNA expressions were measured by quantitative real-time PCR as described earlier (di Gesso et al., 2015).

Key Results: Parent and esterified phenolic acids were screened at 10 μM for their effects on LPS-induced TNF-α secretion in THP-1 cells. None of the parent compounds significantly affected LPS-induced TNF-α secretion. FA-PE and FA-HxE significantly inhibited TNF-α secretion in a concentration-dependent manner (19% and 39% reduction at 20 μM, respectively) but had no effect on TNF-α mRNA expression. FA-PE and FA-HE also significantly induced HO-1 protein expression (2.6 and 3.7 × basal control, respectively) and NQO1 enzymatic activity (1.3 and 1.3 × basal control). FA-HxE significantly increased HO-1 mRNA expression (1.88 × basal control) but neither affected HO-1, NQO1 mRNA, or Nrf2 activation.

Conclusion and Implications: Esterification of FA enhanced its anti-inflammatory and anti-oxidative effects in THP-1 cells, with the longer hexyl ester exerting more inhibitory effects than the shorter propyl ester, suggesting enhanced cellular uptake due to increased lipophilicity. The effect of FA-HxE and FA-PE on TNF-α and NQO1 secretion appears to be post-translational, as mRNA expression was not affected. Furthermore, the effects on HO-1 and NQO1 are independent of Nrf2 activity, similarly to the mechanisms of other common flavonoid metabolites in human monocytic cells.

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P098 | Immunomodulatory effects of alarmins on human monocytes

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Background and Purpose: Alarmins or "damage-associated molecular patterns" (DAMPs) comprise a heterogenous group of endogenous biomolecules released in response to tissue damage. Classically thought to function as "danger" signals to the immune system, recent evidence has associated high plasma levels after cardiac arrest, trauma, and surgery with immunoparalysis. Murine studies suggest that they may induce a "tolerance-like" state in mononuclear cells, including to bacterial motifs; however, whether this occurs in man is unknown.

In this pilot study, the effect of individual alarmins on healthy volunteer monocyte function—either independently or on subsequent LPS challenge—was evaluated.

Experimental Approach: Heparinised whole blood from healthy volunteers (n = 3) was combined with alarmin solutions at a range of physiological and pathophysiological concentrations (1 ml, 48-well plate, triplicate). Alarmins investigated included calprotectin, heparin-binding protein, HMGB1, heat shock protein 70 (HSP70), IL-33, procalcitonin, and S100A12. After 30-min incubation at 37°C, LPS (1 ng·ml^{-1}) was either added or not before incubating for further 4 hr. Monocyte function was quantified using both TNF-α release (R&D DuoSet ELISA) and HLA-DR expression (BD Quantibrite™ system): gold standard assays for identification of clinically relevant immunoparalysis. Ethical approval was granted by the UCL Research Ethics Committee (5060/002). Statistical analysis was conducted in GraphPad Prism using one-way ANOVA (Dunnett's multiple comparisons test) with unstimulated control and LPS-stimulated control as baseline reference.

Key Results: HSP70 alone independently stimulated TNF-α release (Figure 1); mean release was increased by 339 pg·ml^{-1} (95% CI [152, 526], P = .0002) and 2,126 pg·ml^{-1} (95% CI [1,939, 2,313], P < .0001) at 10 and 100 ng·ml^{-1} of HSP70, respectively.