The melanocortin receptor antagonist SHU9119 inhibits the anti-inflammatory/pro-resolving properties of α-MSH and D[Trp\(^8\)]-γ-MSH in lipopolysaccharide stimulated chondrocytes.


**Introduction:** Infectious arthritis occurs when *E.coli or other* microorganisms lead to joint infection and increases in pro-inflammatory cytokines. Investigating how the body naturally produced anti-inflammatory proteins inhibit these pathways, may lead to the development of new therapies for these pathologies (1). The melanocortin agonists α-MSH and D[Trp\(^8\)]-γ-MSH (2) display an important role in inhibiting inflammatory mediators and inducing pro-resolving anti-inflammatory pathways, in models of acute and chronic inflammation (1). The anti-inflammatory effects of these peptides are via a family of G-protein coupled receptors, of which five melanocortin receptors (MC) have been identified, with both MC\(_1\) and MC\(_3\) being promising candidates for modulation of the host inflammatory response in arthritic pathologies (3). This study aims to determine whether the MC\(_{3/4}\) antagonist SHU9119 modulates the anti-inflammatory, pro-resolving effects of α-MSH and D[Trp\(^8\)]-γ-MSH in lipopolysaccharide (LPS) stimulated chondrocytes.

**Methods:** Human C20/A4 cell-line chondrocytes were plated at 1x10\(^6\) cells/well in 24-well plates and were pre-treated with the pan-melanocortin agonist α-MSH (3µg/ml) (Sigma–Aldrich Inc. Poole, UK), and the MC\(_3\) agonist D[Trp\(^8\)]-γ-MSH (3 µg/ml) (Phoenix Pharmaceuticals, Karlsruhe, Germany) (all dissolved in PBS) for 30mins prior to 0.1µg/ml of LPS (*E.coli;111.60*) (Sigma–Aldrich Inc. Poole, UK) stimulation for 6h. In separate experiments the cells were pre-treated with the MC\(_{3/4}\) antagonist SHU9119 (10µg/ml) (Bachem, Bubendorf, Switzerland) 1h prior to LPS stimulation (Time 0h). Cells were harvested to determine the anti-inflammatory protein heme-oxygenase 1 (HO-1) expression by western blot, whilst cell-free supernatants were collected and analysed for IL-6 and IL-8 release by ELISA. Data are expressed as Mean ± SD of n=4 determination in quadruplicate. *P<0.05 vs. control.

**Results:** Cytokine analysis: LPS 0.1µg/ml caused a maximal release of IL-6 and IL-8 with 112.3±6.1 and 314.7±1.9 pg/ml respectively (P<0.05 vs. control). Pre-treatment of cells with α-MSH and D[Trp\(^8\)]-γ-MSH caused a significant reduction in IL-6 (61% and 70% respectively) and IL-8 (71% and 59% respectively) following LPS stimulation. The antagonist SHU9119 completely attenuated the effect of α-MSH on IL-6 and IL-8 release, whilst significantly reducing the effect of D[Trp\(^8\)]-γ-MSH for both cytokines.

HO-1 expression: LPS caused a 30% (0.70 fold) reduction in HO-1 protein expression compared to control, whilst pre-treatment of cells with α-MSH, and D[Trp\(^8\)]-γ-MSH caused a significant increase in HO-1 expression with a 1.25 and 1.57 fold increase respectively. Pre-treatment with the antagonist SHU9119 inhibited both α-MSH and D[Trp\(^8\)]-γ-MSH induced HO-1 expression.
Conclusion: In conclusion α-MSH and D[Trp$^8$]-γ-MSH significantly inhibit pro-inflammatory cytokine release whilst inducing the anti-inflammatory protein HO-1. These effects are attenuated in the presence of the MC$_{3,4}$ antagonist SHU9119, thus suggesting a potential role for the MC$_3$ receptor in mediating their anti-inflammatory effects in this model.

