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The anti-inflammatory effects of melanocortin peptides in lipopolysaccharide activated chondrocytes

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Introduction

Infectious (septic) arthritis occurs when bacteria such as *E.coli or other* microorganisms infect the joint leading to inflammation and release of pro-inflammatory cytokines. Harnessing the body's natural anti-inflammatory proteins to target this underlying inflammatory component may provide an effective treatment[1].

Melanocortin peptides display potent anti-inflammatory effects in models of experimental inflammation[1], with effects being mediated via activation of a family of G-protein coupled melanocortin receptors (MC). To date five have been identified, with MC_1 and MC_3 being the most promising candidates for modulation of the host inflammatory response. This study aims to determine whether melanocortin peptides inhibit pro-inflammatory cytokine release and induce anti-inflammatory pro-resolving proteins in a model of lipopolysaccharide (LPS) stimulated chondrocytes.

Methods

Human C20/A4 cell-line chondrocytes[2] were plated at $1x10^6$ cells/well in 24-well plates and stimulated with 0.1-3μg/ml of LPS *(E.coli;*111.60) for 6h, to determine the release of the pro-inflammatory cytokines interleukin (IL)-6 and IL-8. In separate experiments, chondrocytes were pre-treated with the pan-melanocortin agonist α-MSH (3μg/ml), the MC_3 agonist D[Trp^8]-γ-MSH[3] (3 μg/ml) and c-terminal peptide of α-MSH KPV (4μg/ml) (all dissolved in PBS) for 30mins prior or 2h after LPS (0.1μg/ml) stimulation for 6 h. Following stimulation, cells were harvested to determine heme-oxygenase 1 (HO-1) expression by western blot. Cell-free supernatants were analysed for IL-6 and IL-8 release by ELISA. Data are expressed as Mean ± SD of n=4 determination in triplicate. *P<0.05 vs. appropriate control.

Results

LPS (0.1μg/ml) caused a maximal release of IL-6 and IL-8 with 93.6 ± 6.1 and 316.1 ± 2.1pg/ml respectively (P<0.05 vs. control). Higher concentrations of LPS caused a reduction in release of these cytokines at this time-point. Pre-treatment of cells with α-MSH and D[Trp^8]-γ-MSH caused a significant reduction in IL-6 and IL-8 release following LPS stimulation (0.1μg/ml) with α-MSH causing a 30% and 49% reduction in IL-6 and IL-8 with 65.6 ± 6.9 and 160.3 ± 19.2pg/ml respectively (P<0.05). Whilst the selective MC_3 agonist D[Trp^8]-γ-MSH causd a 60% and 29% reduction in IL-6 and IL-8 with 37.8 ± 3.5 and 226.4 ± 8.4pg/ml respectively (P<0.05 vs. control), the peptide KPV failed to inhibit either IL-6 or IL-8.

Pre-treatment of C-20/A4 chondrocytes with melanocortin peptides inhibited LPS induced cytokine release. Next, we investigated the effect of therapeutic peptide treatment on IL-8 release with the melanocortin peptides being administered 2h after LPS stimulation. α-MSH and D[Trp^8]-γ-MSH causing a 23% and 30% reduction in IL-8 release down to 245.0 ± 16.8 and 220.9 ±13.8pg/ml; P≤0.05, respectively.

LPS caused a 21% (0.79 fold) reduction in HO-1 protein expression compared to control, whilst pre-treatment of cells with α-MSH, D[Trp^8]-γ-MSH and KPV caused a significant increase in HO-1 expression with a 1.22,1.59 and 1.2 fold increase respectively.

Conclusion

These results suggest a role for melanocortin peptides at inhibiting pro-inflammatory cytokine release whilst inducing pro-resolving anti-inflammatory proteins following LPS stimulation of human C-20/A4 chondrocytes. Overall suggesting a potential therapeutic application of these peptides in arthritic pathologies.

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