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

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Introduction

Infectious (septic) arthritis occurs when bacteria such as \textit{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\textsuperscript{1}.

Melanocortin peptides display potent anti-inflammatory effects in models of experimental inflammation\textsuperscript{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\textsubscript{1} and MC\textsubscript{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\textsuperscript{2} were plated at $1 \times 10^6$ cells/well in 24-well plates and stimulated with 0.1-3$\mu$g/ml of LPS (\textit{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 $\alpha$-MSH (3$\mu$g/ml), the MC\textsubscript{3} agonist $\text{D}[\text{Trp}^8]\gamma$-MSH\textsuperscript{3} (3$\mu$g/ml) and c-terminal peptide of $\alpha$-MSH KPV (4$\mu$g/ml) (all dissolved in PBS) for 30mins prior or 2h after LPS (0.1$\mu$g/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 (0.1$\mu$g/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 $\alpha$-MSH and $\text{D}[\text{Trp}^8]\gamma$-MSH caused a significant reduction in IL-6 and IL-8 release following LPS stimulation (0.1$\mu$g/ml) with $\alpha$-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\textsubscript{3} agonist $\text{D}[\text{Trp}^8]\gamma$-MSH caused 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. $\alpha$-MSH and $\text{D}[\text{Trp}^8]\gamma$-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[Trp⁸]-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.