Melanocortin peptides modulate pro-inflammatory mediator relase from TNF-α stimulated C-20/A4 cells.

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Melanocortin peptides exert their anti-inflammatory effects *via* activation of a subgroup of G-protein coupled receptors[1]. To date five melanocortin receptors (MCR) have been identified with the MC1 and 3R bringing about the anti-inflammatory effects of melanocortins[2]. Here we have used an *in vitro* model of chondrocyte stimulation to determine the anti-inflammatory effects of melanocortins.

RT-PCR was used to determine the alteration in mRNA expression of IL-6 and IL-8 in the C-20/A4 chondrocyte cell-line[3] following PBS and TNF-α (20-80 pg/ml) treatment. In separate experiments mRNA for MC1R and 3R was determined in these cells. Cells were plated at 1 x10^6/well in 24 well plates and pre-treated with 1-30 μg/ml of the pan melanocortin agonist α-MSH and the selective MC3R agonist dTrp^8-γ-MSH[1] for 30 mins prior to determination of cAMP accumulation by EIA. In separate experiments, cells were pre-treated with 1-30 μg/ml of α-MSH and dTrp^8-γ-MSH for 30 mins prior to stimulation with either PBS or TNF-α (60pg/ml) for 2-24h. Cell free supernatants were collected and analysed for the cytokines IL-8 and IL-6 by commercially available ELISA. Data is expressed as Mean ± SD of n=4 determination in triplicate. *P<0.05 vs. appropriate control.

RT-PCR showed that C20/A4 cells indicated basal expression of MC1R and MC3R, whilst TNF-α treatment resulted in a significant increase in IL-6 and IL-8 mRNA (*P<0.05) compared to control untreated cells. Functionality of the receptors was demonstrated by stimulating the cells with α-MSH and dTrp^8-γ-MSH, resulting in a concentration dependent increase in cAMP accumulation, with a maximal accumulation of 298.3 ± 5.2 and 175.3 ± 19.6 fmol/well at 10 μg/ml for α-MSH and dTrp^8-γ-MSH respectively (n=4, *P<0.05) increases of 220% and 87% over control (93.3 ± 4.5 pg/ml). The peptides were then evaluated for their ability to modulate TNF-α stimulated IL-6 and IL-8 release. Stimulation of cells with TNF-α resulted in a time and concentration-related release of IL-6 and IL-8 with a maximal release of 154.3 ± 1.3 pg/ml, (*P>0.05 vs. control) and 558.9 ± 11.3 pg/ml, (* P>0.05 vs. control) for IL-6 and IL-8 respectively at 60 pg/ml compared to control (20.5±3.6 pg/ml and 21.7±2.4 pg/ml for IL-6 and IL-8 resepctively). TNF-α pre-treatment of cells with 1-30 μg/ml α-MSH resulted in a significant inhibition of IL-6 (72% *P<0.05) and IL-8 (61% *P<0.05) at 6h but was inactive at 24h post stimulation. The selective MC3R agonist dTrp^8-γ-MSH resulted in a significant reduction in IL-6 (66% *P<0.05, n=4) and IL-8 (74% *P<0.05, n=4) at 6h and a similar degree of inhibition at 24h.

Collectively these data have identified functionally active MCR on the human chondrocyte cell-line C-20/A4. TNF-α caused a time and concentration dependent increase in IL-6 and IL-8 mRNA and protein. IL-6 and IL-8 release was abrogated in the presence of α-MSH and dTrp^8-γ-MSH. Therefore, therapeutic targeting of MCR may be beneficial in the treatment of osteoarthritis.

[1] Getting SJ, *et al.,*FASEB J 20:2234-41, 2006.
[2] Getting SJ, *et al.,* Pharmacol Ther 111: 1-15, 2006.
[3] Goldring M, *et al.,* J. Cell. Physiol. 213(3): 626-634,2007.