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α-MSH and dTRP^8-γ-MSH inhibit TNF-α induced MMP 1 and 13 expression in human C20/A4 chondrocytes

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Chondrocyte stimulation by tumor necrosis factor-α (TNF-α) leads to interleukin (IL)-6 and IL-8 release with subsequent, upregulation of matrix metalloproteinase (MMP) 1 and 13 leading to degradation of collagen and cartilage. Melanocortin peptides display potent anti-inflammatory effects *via* their ability to activate a family of G-protein coupled receptors termed melanocortin receptors (MC)[1]. To date five receptors have been cloned and MC_1 and MC_3 shown to display anti-inflammatory properties[2]. Here we have evaluated the effects of the melanocortin agonist alpha melanocyte stimulating hormone (α-MSH) and selective MC_3 agonist dTRP^8-γ-MSH on cytokine (IL-6 and IL-8) and MMP 1 and 13 expression following tumor necrosis factor-α (TNF-α) stimulation of human C20/A4 chondrocytes.

Human C20/A4 cell-line chondrocytes[3] were plated at 1 x10^6/well in 24 well plates and stimulated with TNF-α (60 pg/ml) over a 2-48h time-course. In separate experiments, cells were pre-treated with 3 □g/ml of the pan melanocortin agonist α-MSH and the selective MC_3 agonist dTrp^8-γ-MSH[1] for 30 mins prior to stimulation with TNF-α (60 pg/ml) for 6 h. Cells were then harvested and mRNA expression of IL-6, IL-8, MMP1 and 13 analysed by RT-PCR. In separate experiments the effects of α-MSH and dTrp^8-γ-MSH[1] were evaluated in the presence of the MC_3 antagonist SHU9119 (10 α g/ml). Data are expressed as Mean ± SD of n=4 determination in triplicate. *P<0.05 vs. appropriate control.

RT-PCR showed significant (*P<0.05) increases in IL-6, IL-8, MMP1 and MMP13 mRNA following TNF-α stimulation over a time-course compared to non-treated chondrocytes. α-MSH and dTRP^8- α-MSH (3 μg/ml) caused a significant reduction in the cytokines IL-6 (56.4 ± 3.1% and 47.5 ± 2.3% respectively; p≤0.05) and IL-8 (61.6 ± 5.4% and 52.9 ± 4.5% respectively; p≤0.05) as measured by densitometry. The effect of the peptides on MMP expression was then determined with α-MSH inhibiting MMP1 and 13 expression by 35.5 ± 1.4% and 79.0 ± 2.1%, whilst dTRP^8-γ-MSH caused a 40.7 ± 3.3% and 76.7 ± 3.6% reduction in MMP1 and 13 expression respectively (p≤0.05). In the presence of the $MC_{3/4}$ antagonist SHU9119 (10 μ g/ml) the ability of these peptides to inhibit these genes was abrogated.

These data suggest that TNF-α causes a time-dependent increase in IL-6, IL-8, MMP1 and 13 and that pre-treatment with both α-MSH and dTRP^8-γ-MSH inhibit this expression at 6 h, an effect blocked by the $MC_{3/4}$ antagonist SHU9119. Collectively these data highlight a potential role for melanocortin peptide in modulating inflammatory mediator release from stimulated chondrocytes.

[1] Getting SJ, *et al.,* FASEB J 20:2234-41, 2006.

[2] Getting SJ, *et al.,* Scientific World Journal 9: 1394-14141, 2009.

[3] Goldring M, *et al.,* J. Cell. Physiol. 213(3): 626-634, 2007