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The effect of the selective human MC_3 receptor agonist PG992 on high density human chondrocyte micromass cultures activated by IL-1beta

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Introduction: Osteoarthritis (OA) is a degenerative joint disease partially mediated by the catabolic cytokine IL-1β, which causes progressive and permanent degeneration of cartilage (1). A potential anti-inflammatory and chondroprotective role for melanocortin peptides has been shown via the human melanocortin-3 (hMC_3) receptor subtype. This study aims to assess the chondroprotective and anti-inflammatory effects of the hMC_3 receptor agonist PG992 and the partially selective agonist [$DTRP^8$]-γ-MSH on IL-1β induced cell death, pro-inflammatory cytokine and matrix metalloproteinase (MMP) release in human chondrocyte micromass cultures.

Methods: Micromass cultures of the human chondrocytic cell line C-20/A4 were obtained by seeding cells at a density of 25.0 x 10^6 viable cells/ml into 24-well plates. After 48h micromasses were treated with PG992 (Ac-Nle-c[Asp-Trp-Pro-DNal(2)-Arg-Trp-Lys]-NH_2) (10.0µg/ml) or [$DTRP^8$]-γ-MSH (3.0µg/ml) for 30mins prior to IL-1β (100pg/ml) stimulation for 6h. Micromasses were harvested for RT-PCR gene expression of hMC_1 and hMC_3 receptors, cell viability studies, alcian blue staining for sulphated glycosaminoglycan (GAG) content and western blot detection for hemeoxygenase-1 (HO-1) expression. Cell free supernatants were analysed for IL-6, IL-8 and MMP-1 release by ELISA. Data are expressed as Mean±SD of n=4 determinations in triplicate. #$p \leq 0.05$vs.control or *$p \leq 0.05$vs.stimulus.

Results: RT-PCR showed hMC_1 and hMC_3 receptor expression on micromass C-20/A4 cells. Cell viability (MTT and Neutral Red) showed that IL-1β stimulation caused a maximal cell death of 17% and 19% respectively (#$p \leq 0.05$), with [$DTRP^8$]-γ-MSH inhibiting cell death by 126% and 133% respectively, whilst PG992 inhibited cell death by 135% and 159% respectively (*$p \leq 0.05$). IL-1β stimulation caused a significant increase in IL-6, IL-8 and MMP-1 release. PG992 significantly reduced IL-6 and IL-8 release by 77% and 81% respectively and completely abrogated MMP-1 release. Alcian blue staining showed an increased GAG accumulation treated with PG992 (132.1±1.4µg/ml) compared to IL-1β (81.9±1.2µg/ml), a similar effect was observed for [$DTRP^8$]-γ-MSH (113±1.7µg/ml). IL-1β caused a 33% (0.33 fold) (#$p \leq 0.05$) reduction in the anti-inflammatory protein HO-1 compared to control, whilst pre-treatment with PG992 and [$DTRP^8$]-γ-MSH caused significant increases in HO-1 expression with a 2.5 and 2.4 fold increase respectively when compared to stimulus (*$p \leq 0.05$).

Conclusion: The selective hMC_3 receptor agonist PG992 exhibited both chondroprotection and modulation of inflammatory and tissue destructive pathways following IL-1β chondrocyte activation highlighting a role for the hMC_3 receptor for treatment of OA.

References:

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