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Effects of the selective MC_3 agonists PG990 and PG992 on Interleukin-1 beta induced chondrocyte cell death and pro-inflammatory cytokine release

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Introduction: The catabolic cytokine IL-1β plays a critical role in the development of osteoarthritis, activating chondrocytes to secrete pro-inflammatory cytokines and matrix metalloproteinases (MMPs). These degrade the extracellular matrix promoting chondrocyte apoptosis, leading to progressive and permanent degeneration of cartilage (1). A role for melanocortin peptides exerting anti-inflammatory and chondroprotection effects have been shown, although the receptor subtype involved is unclear. This study aims to assess the chondroprotection and anti-inflammatory effects of the selective human melanocortin-3 (hMC_3) receptor agonists PG990, PG992 and D[Trp^8]-γ-MSH on IL-1β induced cell death, pro-inflammatory cytokine and matrix metalloproteinase release.

Methods: Human C-20/A4 chondrocytic cells were treated with D[Trp^8]-γ-MSH (3.0μg/ml), PG990 (Ac-Nle-c[D-P-P-DNal(2′)-R-W-K]-NH_2) or PG992 (Ac-Nle-c[D-W-P-DNal(2′)-R-W-K]-NH_2) (1.0-30.0μg/ml) for 30mins prior to cAMP analysis, Total Collagen expression, or IL-1β (5000pg/ml) stimulation for 24h. Cell viability was determined by MTT, whilst, IL-8 and MMP-1 release were detected by ELISA in cell free supernatants. Data are expressed as Mean±SD of n=4-8 determinations in triplicate. *p<0.05 vs. control or #p<0.05 vs. stimulus.

Results: PG990 and PG992 caused cAMP accumulation with maximal accumulation at 1.0μg/ml and 3.0μg/ml respectively indicating hMC_3 receptor-functionality, whilst increasing total collagen expression by PG990 (1.0μg/ml) of 113.9±1.1μg/ml (*p<0.05) and PG992 (3.0μg/ml) of 143.5±0.2μg/ml (*p<0.05) compared to control (104.5±0.1μg/ml). IL-1β stimulation caused a maximal cell death of 25% (*p<0.05) which was completely abrogated in the presence of D[Trp^8]-γ-MSH, PG990 (1.0μg/ml) and PG992 (3.0μg/ml). IL-1β caused a significant increase in IL-8 and MMP-1 release, which was not inhibited by D[Trp^8]-γ-MSH. However, PG990 (1.0μg/ml) and PG992 (3.0μg/ml) reduced IL-8 by 80% (#p<0.05) and 47% (#p<0.05) respectively, whilst MMP-1 was reduced only by PG992 (3.0μg/ml) by 16% (#p<0.05).

Conclusion: These data highlight that the selective hMC_3 receptor agonists (PG990 and PG992) exhibited chondroprotection and modulation of IL-8 following IL-1β activation of chondrocytes at 24h, whilst D[Trp^8]-γ-MSH only exerted chondroprotective effects. This data highlights a role for the hMC_3receptor in exerting anti-inflammatory and chondroprotective properties.

References:

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