

DEXAMETHASONE INHIBITS OSTEOGENESIS AND CHONDROGENESIS AND ENHANCES ADIPOGENESIS IN MURINE ADIPOSE-DERIVED MESENCHYMAL CELLS

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Introduction MSCs are an appealing cell source for tissue engineering applications due to their versatility and multi-lineage potential. Adipose-derived mesenchymal cells (AMCs) possess the additional benefits of accessibility and abundance and are well-suited to osteogenic and chondrogenic applications. In order to successfully drive AMCs to the osteogenic and chondrogenic lineages, it is crucial to determine optimal media formulations for the desired differentiation. While Dexamethasone (Dex) is a common component of chondrogenic, adipogenic and osteogenic media, data regarding its effects upon differentiation is conflicting. The primary objective of this study was to elucidate the role of Dex on differentiation in murine AMCs (mAMCs) cultured in classical osteogenic, chondrogenic and adipogenic conditions.

Methods AMCs were harvested from 25-30d male mice and expanded in growth medium (DMEM, 10% FBS, 1% pen-strep). To investigate osteogenesis, cells were seeded in monolayer at 1,300 cells/cm² and cultured in osteogenic medium (growth medium, 50 µg/mL Ascorbic Acid (AA), 10 mM β-Glycerophosphate, with 0 or 1 µM Retinoic Acid (RA), and 0 or 10 nM Dex). Osteogenic differentiation was assessed by mineralization quantification at 20 d. For chondrogenesis, mAMCs were cultured in pellet conditions at 200,000 cells/well in round-bottom 96-well plates with chondrogenic medium (growth medium, 100 ng/mL BMP-6, 50 µg/mL AA, with 0 or 10 nM Dex). Chondrogenic differentiation was assessed by proteoglycan accumulation via sGAG assay at 12d. For adipogenic differentiation, mAMCs were seeded in monolayer at 26,300 cells/cm² and cultured in adipogenic medium (growth medium, 10 µg/mL insulin, 200 µM Indomethacin, 0.5 µM IBMX, with 0 or 1 µM Dex). Differentiation was assessed by lipid accumulation via Oil Red O quantification at 14d.

Results Addition of Dex to the differentiation medium was detrimental to both osteo- and chondrogenesis but enhanced adipogenesis in mAMCs. Dex inhibited mineralization in mAMCs cultured in osteogenic conditions, despite the presence of RA (Figure 1A) and significantly decreased sGAG accumulation in chondrogenic culture (Figure 1B). In contrast, Dex increased lipid accumulation in mAMCs cultured in adipogenic conditions (Figure 1C).

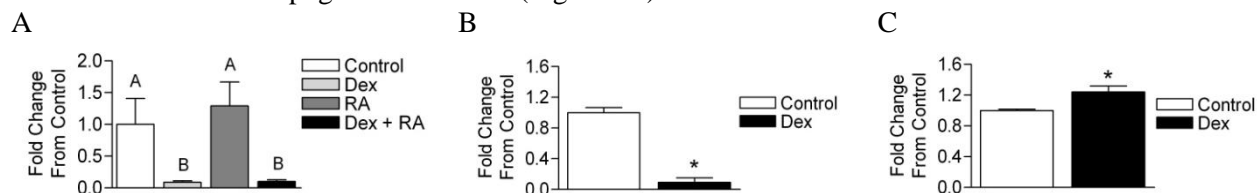


Figure 1. Dex inhibits osteogenesis and chondrogenesis and enhances adipogenesis in mAMCs. Cells were differentiated in monolayer or pellet with or without Dex. A) quantified mineralization of osteogenic monolayer, B) sGAG accumulation in chondrogenic pellet culture, and C) quantified lipid accumulation in adipogenic culture. Differing letters indicates significant difference, ANOVA, Tukey's post test, $p < 0.05$. *indicates difference from control, Student's t-test, $p < 0.05$.

Conclusion Our results indicate that Dexamethasone has a negative effect upon osteo- and chondrogenesis in mAMCs, while it promotes adipogenesis. Further studies will be devoted to investigating species-specific effects of Dex upon osteo-, chondro- and adipogenic differentiation of AMCs by comparing these results to those obtained from human AMCs.