Long Acting Opioid Antagonists Stimulate Mesenchymal Stem Cells Osteoblastic / Chondroblastic Differentiation

INTRODUCTION:
Cellular multiplication and differentiation are still poorly understood and continuously under investigation. Bone repair is a biologic process that is directly dependent upon these cellular changes which affect clinical healing processes as fracture healing and spinal fusion.

Previous research suggests a role of endogenous opioids in bone formation/differentiation and an inverse dose-dependent relationship with stimulation of osteoblastic differentiation when using short acting opioid antagonists (Naloxone) (1, 2) therefore we hypothesize that low dose Naltrexone (long acting opioid antagonist) exposure of human mesenchymal stem cells (hMSC’s) may enhance the multiplication and differentiation towards osteoblastic/chondroblastic lineages.

METHODS:
hMSC’s were obtained from Stem Cell Technologies Inc. Cells were then seeded and expanded in T-75 flasks with hMSC culture media for 7 days. Adherent cells were trypsinized and washed in PBS. After counting, cells were then divided into 4 different groups:

Group 1: Basic media (negative control; DMEM, 10% FBS, 1% Penicillin-Streptomycin).
Group 2: Experimental group: Naloxone 10^-6 M exposure alone.
Group 3: Experimental group: Naltrexone 10^-6 M exposure alone.
Group 4: Positive control: Osteogenic media (hBMP-2; 200 ng/ml). hMSC’s from groups 1-4 were then seeded into 6 ml dishes at a density of 75,000 cells/dish and cultured for 20 days. Media was changed in each group every 4 days. Photographs were taken at days 10 and 20th. Cells were stained by Alizarin Red and Alcian blue. Real time PCR was performed to examine miRNA expression of the osteogenic markers Osteocalcin, ALP and Collagen type II and compared to controls.

RESULTS:
hMSC’s in groups 2 and 3 (Naloxone and Naltrexone) showed obvious morphological changes at Day 10, which is comparable to cells treated with BMP-2 (group 4) (figure 1). Cells treated with opioid antagonists multiplied and differentiated faster than in other groups.

DISCUSSION:
Previous reports showed the presence of an endogenous opioid system and its role in cellular differentiation and bone repair but the understanding of its regulatory mechanisms remains unclear. Our earlier report showed that low doses of opioid antagonists can promote differentiation of murine MSC’s towards the osteoblastic lineage. The present study support our previous findings; exposing hMSC’s to low doses of long acting opioid antagonists (Naltrexone) also promotes their proliferation and differentiation towards osteoblastic and chondroblastic lineages and may allow for a decrease in the need for frequency and amount of medication. Further research is needed to examine the mechanisms of action of opioid antagonist action in osteogenesis. These studies can provide new insights into novel therapeutics to treat clinical problems like back pain surgery and fracture healing.

REFERENCES: