Effects of Pyridoxal 5 Phosphate on Mesenchymal Stem Cell Response to Oxidative Stress and Chondrogenic Activity

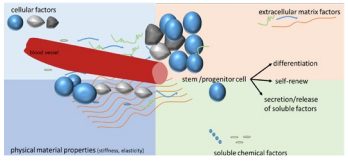
Gengjin Gary Wang[1] , Colleen Gieringer[1], Alexa Lascola[1], Maria Egidio[2], Jiangyinzi Shang[3], Hang Lin[3], Burhan Gharaibeh[2], Bridget M Deasy[4]

[1]Program in Rehabilitation Sciences, University of Pittsburgh, [2]Department of Biological Sciences, University of Pittsburgh [3]Department of Orthopedic Surgery, University of Pittsburgh, [4]Department of Community Health Services and Rehabilitation Sciences

Disclosures: none

INTRODUCTION: BM-MSCs, bone marrow-derived mesenchymal stem cells, can differentiate into osteocytes, chondrocytes, and adipocytes and are a promising approach for regenerative medicine of orthopedic disorders. The potential of MSCs, also called multipotent mesenchymal stromal cells, to treat a range of disorders has led to their clinical approval in several countries, however to date there is no FDA-approved product in the United States. This highlights both the promise of these cells and the challenges of developing a well-characterized therapy. A long-standing focus has been to develop methods to control cell differentiation to various lineages and also control or limit the cells accumulation of a senescence phenotype. The stem cell niche is the microenvironment that helps maintain the undifferentiated and self-renewing state of the stem cells. This niche includes stem cells, progenitor cells, supporting stromal cells, and noncellular soluble signaling factors, and extracellular structural and signaling factors. These signaling molecules are influenced by the organism's nutrition status, disease state or aging. The signaling pathways in the stem cell niche may become disrupted due to poor nutrition or aging and ultimately affect the robustness of the stem cell population; this may result in the inability of stem and progenitor cells to repair and regenerate damaged tissue [4]. Age-related diseases, e.g., have been found to disrupt the stem cells and the niche signaling pathway. Several micronutrients have a role in mitigating cellular damage. For example, vitamin B6, particularly in its active form pyridoxal 5'-phosphate (P5P), plays a multifaceted role in the health and function of MSCs and chondrocyte precursors. First, B6 is essential for amino acid metabolism and collagen formation, which contributes significantly to the structural integrity of cartilage tissue. Second, B6's function as a co-factor in the synthesis of glutathione underscores its importance in mitigating cellular damage, supporting cellular health, and influencing the proliferation and differentiation of MSCs and chondrocyte precursors.
This study aimed to assess the impact of the nutritional coenzyme, vitamin B6, specifically in its active form pyridoxal 5'-phosphate (P5P), on human mesenchymal stem cells (MSCs). The investigation focused on evaluating the capacity of vitamin B6 to support proliferation and chondrogenic differentiation and enhance resistance to oxidative stress in both young and age-induced (expanded) MSCs.

METHODS: hBM-MSCs were isolated as previously described from total joint arthroplasty surgical waste with IRB approval, then cultured and expanded as previously described. Cells were cultured in Dulbecco's Minimal Essential Medium (DMEM containing 10% fetal bovine serum (FBS, Invitrogen), 1% antibiotics-antimycotic, and 1.5 ng/mL FGF-2, at a density of 2E4- 4E4 cells/cm2, and medium was changed every 3 to 4 days. The trilineage mesenchymal differentiation capacity of hBMSCs were previously validated. Cell growth was monitored using time-lapsed microscopy as previously described. To examine, dose-dependent effects, we treated BM-MSCS with 3 does of pyridoxal 5'-phosphate (P5P) – 5uM, 50uM and 500uM P5P; some cells received no P5P. Proliferation characteristics measured include total live cell count, cell death rate, population doubling time, and mitotic fraction. We used hydrogen peroxide (100uM) to induce oxidative stress. Chondrogenic pellet assays were performed to examine the effect of P5P on differentiation. In these assays, cells were treated with 0 or 50uM P5P for 3 weeks, with media changed every 3-4 days. Assays for collagen type II and proteoglycans will be performed. STATISTICS: To compare dose-dependent effects of P5P on unexpanded and expanded (aged), ANOVA or t-tests tests were performed, at alpha=0.05 using Stata.



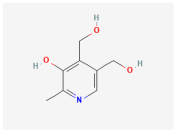


Figure1. A. The stem cell niche includes stem & progenitor cells, supporting stromal cells, soluble signals, & extracellular factors. B. Pyroxidine, or pyridoxal 5 phosphate, the active form of vitamin B6, a small molecule micronutrient, has a key role in cellular response

RESULTS SECTION: MSCS treated with P5P showed an increase in cell growth and a decrease in doubling time with vitamin B6 indicating increased proliferation with vitamin B6 (Figures 2). We also observe that MSC treated with vitamin B6 have increased growth rate in an oxidative stress environment as compared to cells not receiving vitamin B6 (Figures 3). BM-MSCs expanded in culture for > 30 days showed significant increase in rproliferation and resistance to oxidative stress as compared to untreated controls.

.

DISCUSSION: Our results suggest that the micronutrient pyroxidine 5 phosphate may protect cells from oxidative damage due to both metabolic oxidative stress, and stress induced via cell aging. Additional analysis regarding the aging phenotype and are ongoing. In addition, ongoing studies with other human stem cells from old and young donors will be examined.

SIGNIFICANCE/CLINICAL RELEVANCE: The study suggests than an appropriate dose of vitamin B6 (P5P) may protect MSC proliferation and maintain benefits against oxidative stress. Recently, there has been a shift in nutritional guidance to address chronic diseases caused by poor diets. As stem cells serve to maintain tissue of the lifespan, it is essential to understand their proper nutrition as it supports healthspan throughout aging

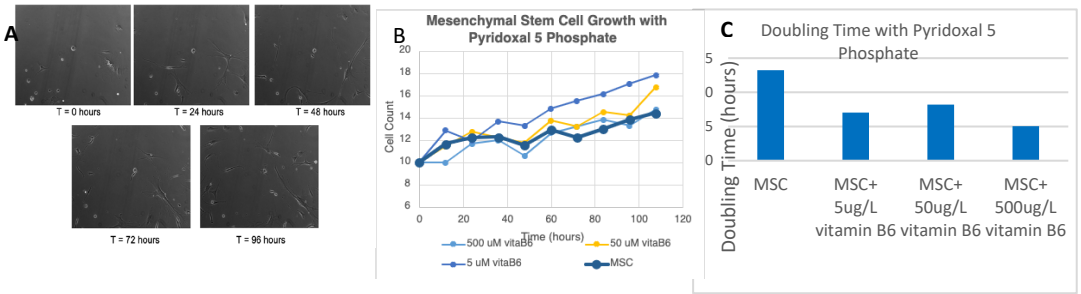


Figure 2. A-C. MSCs respond to soluble P5P at all doses and show an increase in growth after 48 hrs in comparison to untreated cells. Cell doubling time decreased with P5P, indicating faster growth with the micronutrient.