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Gene Expression Characterization of Insulin Mimetic Treated Osteoblasts in vitro

With nearly one million cases per year in the United States, bone fractures are one of the most common injuries in the human population. Immediately following a fracture, the body responses by secreting insulin to stimulate the initial steps of bone fracture repair. Unfortunately, diseases like diabetes that inhibit the body's production of insulin generate multiple downstream, negative effects on the body including impairment in bone fracture healing. In order to help these patients, recover normally from bone fracture, research has shown that supplementation with an insulin mimetic can act like insulin and improve bone healing. Researchers found that vanadium compounds, VSO_4 and VAC mimic insulin's ability to stimulate bone repair. To better understand the mechanism by which these compounds affect bone healing, our study measured the transcriptional response of these vanadium compounds during osteoblast differentiation, an important component of fracture repair. Mc3t3 mouse osteoblast cell cultures were tested with these vanadium compounds (10uM and 100uM), 10uM ascorbic acid as a positive control, and DMEM media as a negative control over time (1, 2, 4, 7, 10, 14, 17, 21, and 28 days). Cell lysates were collected, and RNA was isolated and quantified. Next, RNA was reverse transcribed into cDNA. Polymerase Chain Reaction (PCR) and DNA gel electrophoresis using the housekeeping gene, Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH), was completed to verify the successful production of cDNA for each sample. Osteoblast differentiation markers including Alkaline Phosphatase (ALP) were quantified for each treatment group using quantitative real time PCR (qPCR). On day 2, QPCR analysis showed that 100uM VSO_4 had higher ALP gene expression when compared to controls while 10uM VAC resulted in similar amounts of gene expression to controls. Overall, our data demonstrates that vanadium compounds can modulate osteoblastogenic gene expression. Future experiments will continue to characterize the gene expression patterns that are altered in response to vanadium compound treatment during osteoblast differentiation.

Characterization Gene Expression of Insulin Mimetic Treated Osteoblasts in vitro

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Abstract

With nearly one million cases per year in the United States, bone fractures are one of the most common injuries in the human population which depends on insulin to stimulate the initial steps of bone fracture repair. Unfortunately, diseases like diabetes that inhibit the body's production of insulin generate multiple downstream, negative effects on the body including impairment in bone fracture healing. Researchers found that vanadium compounds, VSO_4, and VAC mimic insulin's ability to stimulate bone repair. To better understand the mechanism by which these compounds affect bone healing, our study measured the transcriptional response of these vanadium compounds during osteoblast differentiation, an important component of fracture repair.

Mc3t3 mouse osteoblast cell cultures were tested with these vanadium compounds (10uM and 100uM), 10uM ascorbic acid as a positive control, and DMEM media as a negative control over time (1, 2, 4, 7, 10, 14, 17, 21, and 28 days). Cell lysates were collected, and RNA was isolated and quantified. Next, RNA was reverse transcribed into cDNA. Polymerase Chain Reaction (PCR) and DNA gel electrophoresis using the housekeeping gene, Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH), was completed to verify the successful production of cDNA for each sample.

Osteoblast differentiation markers including Alkaline Phosphatase (ALP) were quantified for each treatment group using quantitative real time PCR (qPCR). QPCR data showed a higher expression of ALP in VAC than VSO4

Overall, our data demonstrates that vanadium compounds can modulate osteoblastogenic gene expression. Future experiments will continue to characterize the gene expression patterns that are altered in response to vanadium compound treatment during osteoblast differentiation.

Introduction

Osteoblasts function to deposit bone and osteoclasts function remove excess bone. Fully matured osteoblasts are derived from stem cells located within bone marrow that are first turned into mesenchymal stem cells. These turn into osteochondral progenitor cells (OPCs) before finally maturing into osteoblasts. During bone repair, osteoblasts function to create new bone to repair the fracture (1).Since insulin production is impaired in patients with diabetes, they tend to have more difficult and prolonged recovery from bone fractures. A major reason this occurs is because insulin plays a pivotal role in differentiating OPCs to osteoblasts.

Scientific research demonstrates that osteoblast proliferation and maturation increase in the presence of insulin (2). Insulin treatment in this study positively affected osteoblast maturation using both the MAPK and PI3K pathway (2). In certain pathways, insulin acts as a regulatory step that controls the amount of bone growth and remodeling (2,3). ALP, an enzyme responsible for hydrolysis of monophosphate ester bonds functions to support the process of bone mineralization, a key step in bone rebuilding. (4)

Another study also demonstrated that insulin suppresses osteoblast inhibitors like Twist2 (3). Research has also shown that insulin like compounds, known as insulin mimetics (such as vanadium compounds) can have positive effects on endochondral ossification and bone formation during bone healing (5).

The purpose of this study is to test whether insulin mimetics, such as vanadium compounds (VAC, VSO4) affect signaling pathways similar to insulin when promoting endochondral ossification, osteoblast maturation, and bone formation.

Methods and Materials

This project determined the effects that vanadium compounds have on osteoblast gene expression. MC3T3E1 cells, a mouse osteoblast immortalized cell line, were treated and cultured (Figure 1). These cells can differentiate into osteoblasts and make calcified bone tissue.

1. Using this established cell line, cultures were treated with varying amounts of VAC and VSO4, including ascorbic acid as a control. The cells were collected at the following days: 1, 2, 4, 7, 10, 14, 17, 21, 28.
2. The RNA from the cells was then isolated and converted to cDNA using reverse transcriptase (Table 1 & 2).
3. DNA gel electrophoresis was completed to verify successful conversion of RNA to DNA (Figures 2-3)
4. Gene expression was quantified using quantitative real time PCR. Key markers for genes significant to osteoblast function will be quantified. [Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) and Alkaline Phosphatase (ALP)]
5. Gene expression was quantified, statistically analyzed, and appropriate graphs were generated.

The mimetics used were hypothesized to increase osteoblasts proliferation and function similar to insulin. As insulin would normally lead to the stimulation of bone growth through a pathway of proteins, the insulin mimetics, in theory, should stimulate the same proteins.

Sample Isolation Method

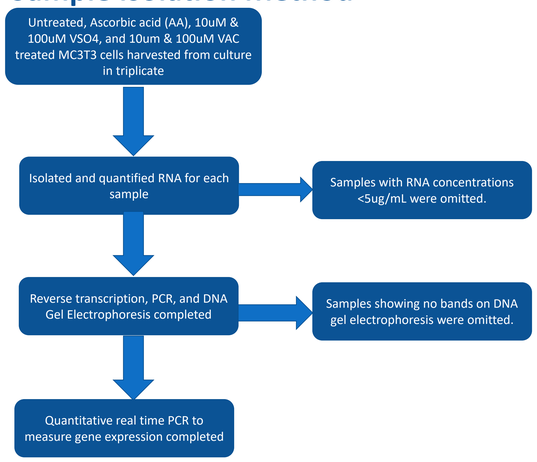

Figure 1. Sample flow throughout experiment. Note: Samples that had low concentrations of RNA or DNA were omitted from experiment.

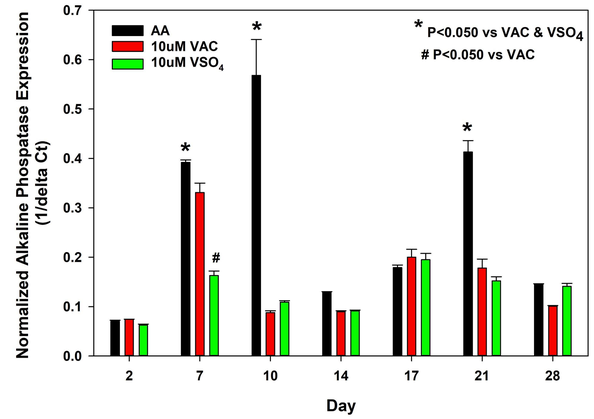

Figure 4. Alkaline Phosphatase Protein Expression in Osteoblasts over Time. On day 10, AA treatment had 460% more ALP expression than either vanadium treatment. This difference was found to be statistically significant.

Results

TABLE 1 Sample	Sample Abbr.	Mean RNA Conc. (ug/mL)
Day 7: AA	AK-AM	6.624 ± 2.870
Day 7: 10uM VSO4	AN-OP	10.068 ± 1.022
Day 7: 10uM VAC	AT-AV	8.747 ± 1.264
Day 7: Media	AW-AY	2.906 ± 1.838
Day 10: AA	BF-BH	7.119 ± 1.172
Day 10: 10uM VSO4	BI-BK	6.710 ± 0.112
Day 10: 10uM VAC	BO-BQ	5.495 ± 0.467
Day 10: Media	BC-BE	5.574 ± 0.408

TABLE 2 Sample	Sample Abbr.	Mean RNA Conc. (ug/mL)
Day 21: 10uM VAC	DJ-DL	5.990 ± 1.439
Day 21: 10uM VSO4	DQ-DS	5.108 ± 2.042
Day 28: 10uM VAC	EC-EE	8.517 ± 1.320
Day 28: 10uM VSO4	EI-EK	7.060 ± 2.579

Tables 1-2. RNA Concentration Summary. Mean RNA concentrations was isolated from Days 7, 10, 21, and 28. Each sample was done in triplicate.

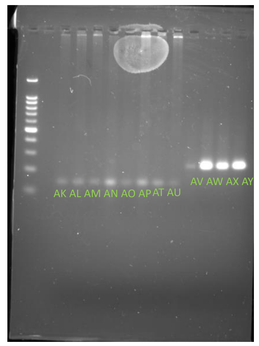

Figure 2. DNA gel electrophoresis for Day 7.

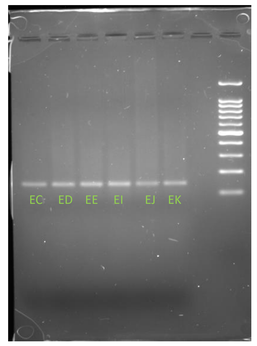

Figure 3. DNA gel electrophoresis for Day 28.

TABLE 3	DAY						
Treatment Group	2	7	10	14	17	21	28
AA	0.68	0.17	0.73	6.12	0.16	0.83	0.27
10uM VAC	1.15	0.12	0.00	0.57	0.23	0.09	0.03
10uM VSO4	0.19	0.01	0.00	0.65	0.21	0.05	0.23

Table 3: Fold Change of Alkaline Phosphatase Expression of Each Treatment Group Compared to Untreated Group (calculated via 2-ΔΔCt).

Conclusions

- Consistent GAPDH expression in cells treated with vanadium compounds showed that the cells proliferated and developed normally. GAPDH is a housekeeping gene that is consistently expressed to maintain normal cellular functions.
- When comparing the untreated samples to AA and vanadium compounds. AA samples positively effected on days 7, 10, 21, and 28 (Table 3)
- Vanadium treatments at the 10uM dose decreased ALP protein expression when compared to controls (Figure 4).
- Generally, VAC treated cells observed higher ALP gene expression when compared to VSO_4 (Figure 4)
- Increasing ALP expression would mean an increase in bone mineralization, a vital part in the bone rebuilding process. Over the 28-day time period, constant increased expression of ALP in all treated cells indicated osteoblast differentiation and function. However, our data indicates that AA treatment stimulates ALP expression better then vanadium compounds.
- Future experiments will measure expression of the gene Twist 2, which functions to inhibit osteoblast function and bone rebuilding. This gene inhibits osteoblast maturation (3) and disrupts bone formation. If Twist 2 gene expression was measured in vanadium treated cells, we hypothesize that it will have higher levels of expression then AA. This would indicate TWIST2 inhibits osteoblast differentiation more then AA treatment.

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