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Characterization of Insulin Mimetic Effects on Gene Expression

Previous data has shown that insulin mimetic such as vanadium compounds can enhance bone healing like insulin without the risk of glycemic changes. Our study used ATDC5 chondrocytes derived from mouse cells to analyze the potential regenerative effects of vanadium compound treatment during chondrogenesis differentiation, a key process in long bone healing. ATDC5 cells were treated with the DMEM/F12 media only (untreated, negative control), 10uM insulin (positive control), and vanadium compounds: vanadyl acetylacetonate (VAC) and vanadium (II) sulfate (VSO4) at concentrations of both 10uM and 100uM. Chondrocyte lysates were harvested at days 1, 2, 4, 7, 10, 14, 17, 21, and 28 for all treatment groups. After RNA isolation, quantification of RNA was completed using a Biodrop, followed by reverse transcription for each sample. Successful conversion of RNA to cDNA was verified using polymerase chain reaction (PCR) and DNA gel electrophoresis for the housekeeping gene, glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Finally, gene expression of key markers of chondrogenesis (i.e. Collagen 2a1, col2a1) was completed using quantitative real time polymerase chain reaction (qPCR) for each treatment group overtime. qPCR analysis demonstrated that col2a1 gene expression was more abundant on Days 4, 7, and 10 when compared to insulin or untreated samples. Our data also demonstrated that col2a1 expression increased over time. Together our data supports the hypothesis that vanadium compounds enhance chondrogenic differentiation which in turn can improve bone healing. Our study also demonstrates that vanadium compounds can serve as an alternative to insulin in modulating chondrogenesis and may be more impactful during the early stages of differentiation. In future experiments, we will continue to characterize the gene expression response to these vanadium compounds in our model and hope to determine if this enhancement occurs through the same molecular pathway as insulin.

Characterization of Insulin Mimetic Effects on Gene Expression during Chondrogenesis in the ATDC5 Cell Line

Caitlin M. Gartley, Ruby M. Pasupuleti, Selam T. Woldegerima, and Jessica Cottrell

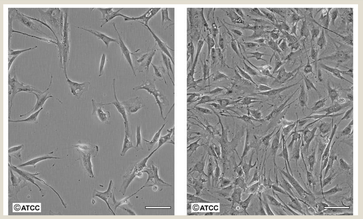
Abstract

Previous data has shown that insulin mimetic such as vanadium compounds can enhance bone healing like insulin without the risk of glycemic changes. Our study used ATDC5 chondrocytes derived from mouse cells to analyze the potential regenerative effects of vanadium compound treatment during chondrogenesis differentiation, a key process in long bone healing. ATDC5 cells were treated with the DMEM/F12 media only (untreated, negative control), 10uM insulin (positive control), and vanadium compounds: vanadyl acetylacetonate (VAC) and vanadium (II) sulfate (VSO4) at concentrations of both 10uM and 100uM. Chondrocyte lysates were harvested at days 1, 2, 4, 7, 10, 14, 17, 21, and 28 for all treatment groups. After RNA isolation, quantification of RNA was completed using a Biodrop, followed by reverse transcription for each sample. Successful conversion of RNA to cDNA was verified using polymerase chain reaction (PCR) and DNA gel electrophoresis for the housekeeping gene, glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Finally, gene expression of key markers of chondrogenesis (i.e. Collagen 2a1, col2a1 and Collage 10a1, col10a1) was completed using quantitative real time polymerase chain reaction (qPCR) for each treatment group overtime. qPCR analysis demonstrated that col2a1 gene expression was increasingly abundant through Days 4, 7, and 10 when treated with vanadium compounds, compared to insulin or untreated samples. Our data also demonstrated that col2a1 expression increased over time, and that col10a increased through days 21 and 28. Together our data supports the hypothesis that vanadium compounds enhance chondrogenic differentiation which in turn can improve bone healing. Our study also demonstrates that vanadium compounds can serve as an alternative to insulin in modulating chondrogenesis and may be more impactful during the early stages of differentiation. In future experiments, we will continue to characterize the gene expression response to these vanadium compounds in our model and hope to determine if this enhancement occurs through the same molecular pathway as insulin.

Introduction

- Chondrogenesis plays a significant part in bone fracture healing.
- ATDC5 cells are useful for investigating chondrogenesis and exploring mechanistic pathways important to this process.
- Differentiating chondrocytes express an abundance of collagen 2a1 (Col2a1), an important component of the cartilage matrix. As chondrocytes mature, they become enlarged and hypertrophic. This stage is associated with collagen 10a1 (Col10a1) expression.
- Past studies have shown that diabetes mellitus can impair fracture healing. Impaired insulin levels disrupt chondrogenesis. (Beam 1213). Insulin therapy can rescue the negative affects associated with diabetic fracture healing.
- Insulin mimetics like vanadyl acetylacetonate (VAC) and Vanadium (II) sulfate (VSO_4) have been shown to improve bone healing like insulin but the mechanism by which this occurs is unclear.

Methods and Materials



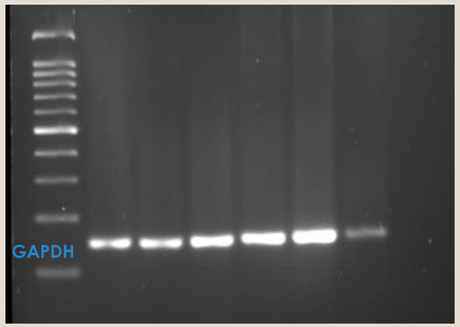
Step 1. ATDC5 cells (shown above) were treated with the DMEM/F 12 media, 10uM insulin, and vanadium compounds

Step 2. RNA Isolation was performed using Qiagen procedure with an RNA Mini Kit.

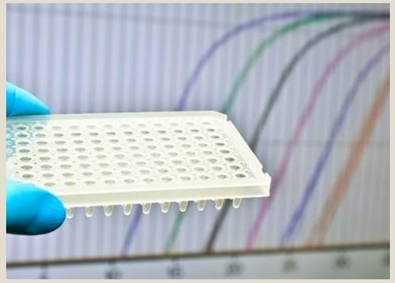
Treatment Group	Mean RNA Concentrations (Mean ±STD)	Common A260/A280
Media	17.85 ± 3.658	2.00
Insulin	6.788 ± 1.638	2.00
10uM VAC	11.235 ± 2.056	2.01
100uM VAC	7.9295 ± 0.852	2.00
10uM VSO4	12.38 3± 2.141	1.88
100uMVSO4	11.081 ± 3.486	1.99

Step 3. RNA was quantified using a Biodrop. Shown above are the Mean ± STD for the samples at Day 10. The purity of each RNA was checked using the A260/A280. A ratio close to 2.00 is considered pure.

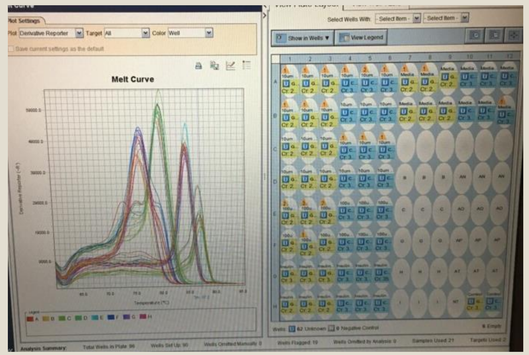
Step 4. The process of Reverse Transcription converted RNA to cDNA and successful reactions were confirmed using polymerase chain reactions (PCR) and BioRad Thermal Cycler.



Step 5. .GAPDH PCR reactions were run using DNA gel electrophoresis and a FluoroChem to visualize the DNA. A band at the correct corresponding nucleotide confirms successful cDNA conversion.



Step 6. Quantitative real time polymerase chain reaction (qPCR) for each treatment group overtime analyzed the presence of glyceraldehyde 3-P dehydrogenase (GAPDH), collagen 2a1 (col2a1), and collagen 10a1 (col10a).



Step 7. qPCR data generated by the machine was outputted. Both melt curves and amplification plots were analyzed during gene expression analysis.

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Results

Figure 1. **Normalized Col2a1 Gene Expression in Different Treatment Groups over Time**.

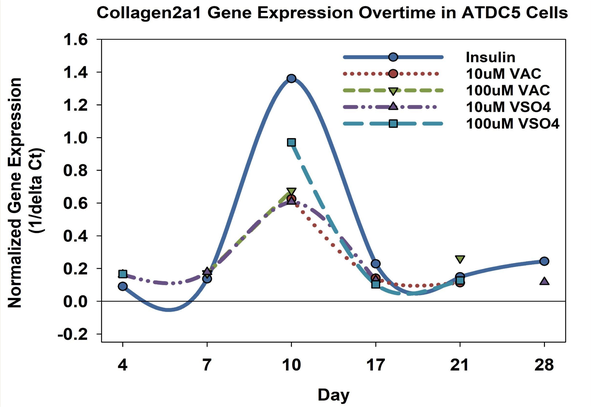

Figure 2. Gene expression of collagen 2a1 relating fold change at Day 10 ATDC5 Cells.

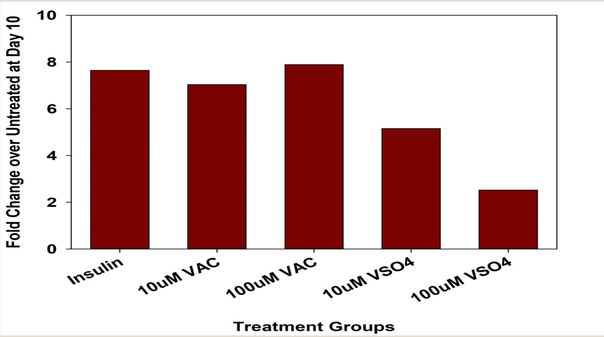

Figure 3. **The progression of Collagen 10a1 expressed in ATDC5 cells when between days 21 and 28.**

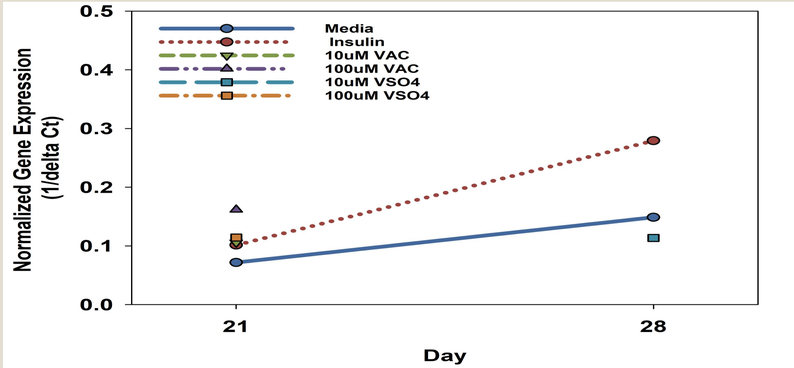

Figure 1 shows Col2a1 gene expression in the ATDC5 cartilage cells over time. Col2a1 increase in concentration between days 4-17, peaking on day 10 and reducing through day 28. Col2a1 gene expression levels shown that insulin is a better stimulator of Col2a1 gene expression in comparison to either vanadium compounds. This is evident by the 3-fold increase in gene expression when comparing insulin treatment to vanadium treatment on day 10. When comparing vanadium compounds (VAC and VSO_4) similar levels of gene expression were found.

Figure 2 shows the fold change in Col2a1 gene expression when comparing the various treatment groups to the untreated control. The data indicates that the VAC compound stimulate col2a1 expression most similarly to insulin on Day 10 (≈7-fold). The VSO_4 compound is also shown to stimulate col2a1 expression 6-fold over the untreated group but at the 100uM dose this fold-change decreases to 2.

Figure 3 shows that when quantifying col10a1 gene expression insulin treatment increases its expression when compared to control.

Conclusions

- Our samples contained a large abundance of Col2a1, one of the largest components in the cartilage matrix, this increased during the first seventeen days of chondrogenesis. However, after this timepoint, the concentration leveled off as the cells matured while col10a1 levels began to increase between day 21 and 28.
- Since vanadium compounds mimic the beneficial effects of insulin and promote bone healing, we had expected that the cell would uptake the vanadium compounds in the same pattern as insulin. The trend in days 4, 17, 21, and 28 shows that the concentration of col2a1 is much greater after insulin treatment than with vanadium compounds. This suggests that vanadium treatment may improve bone healing through a process other than chondrocyte proliferation.
- Previous data in the lab shows that vanadium compounds can increase chondrocyte function. In the future, we will continue to explore genes related to chondrocyte function to better understand the effect vanadium compounds have on their function.

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