Examining the Paracrine Effects of Adipose-Derived Mesenchymal Stem Cells in a Bovine Model of Osteoarthritis

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1. Abstract

Osteoarthritis is a common joint disease affecting approximately 27 million Americans. Due to stem cells' abilities to differentiate into other specialized cells, they can be utilized in the treatment of many diseases, including osteoarthritis. However, research and many more clinical trials still need to be done before stem cell therapy can have a widespread use. The protective paracrine effects of swine adipose-derived stem cells on bovine cartilage plugs were investigated under the presence of Interleukin-1β and Interleukin-6. IL-1β and IL-6 are inflammatory markers that can induce the degeneration of the extracellular matrix, a major cause for osteoarthritis. In order to test its effects, four groups were created: a regular control which consisted of cartilage not exposed to ADSCs nor IL-1β/IL-6, a negative control which consisted of IL-1β/IL-6 but no ADSCs, a positive control which consisted of ADSCs but no IL-1β/IL-6, and an experimental group which contained both ADSCs and IL-1β/IL-6. Data from three timepoints, day 1, 3, and 7, were collected, and the results were analyzed through histology and immunohistochemistry. The stains revealed that ADSCs prevented the complete degeneration of the extracellular matrix. Furthermore, IHC demonstrated that there was an increased expression of SOX 9, which is present in healthy cartilage. The results promote the idea that paracrine factors of ADSCs can be utilized to treat osteoarthritis, rather than utilizing the ADSCs themselves, to minimize the threat of rejection. It also supports the idea that ADSCs can be used cross-species, making them more easily attainable.

2. Introduction

Osteoarthritis (OA) is a degenerative joint disease characterized by the breakdown of cartilage, which usually acts as a cushion between bones. This degradation causes joint pain and swelling as bones continually rub against one another. Moreover, the body's inflammatory responses result in the production of inflammatory markers and the generation of catabolic enzymes that can further degrade the articular extracellular matrix (ECM) (Mengshol et al., 2000). The production of inflammatory cytokines IL-1β, TNF, IL-6, IL-15, IL-17, and IL-18 are common in OA (Wojdasiewicz et al., 2014). These cytokines stimulate the production of matrix metalloproteinases like MMP 9 and MMP 13, which then degrade collagen and the ECM (Peter, 2006). Due to their ability to differentiate into various cell types, mesenchymal stem cells have provided insight for new treatments to an otherwise debilitating degenerative disease (Mei et al., 2017). Adipose-derived stem cells (ADSCs) have been shown to secrete exosomes, growth

factors, cytokines, and other trophic factors that protect chondrocytes in osteoarthritic conditions and reduce the amount of matrix degrading enzymes (Platas et al., 2016).

The purpose of this study was to examine the protective paracrine effects of ADSCs on cartilage affected by osteoarthritis. By solely utilizing its paracrine effects, which are the factors secreted by the cells, and not the cells themselves, many disadvantages of ADSCs are eliminated, such as the risk of rejection by the host. ADSCs can also be easily isolated and harvested in large quantities, making it very useful in stem cell therapies (Kuroda et al., 2015). It was hypothesized that the use of swine ADSCs would result in an increase in survivability of bovine plug chondrocytes exposed to an in-vitro osteoarthritic condition. To test the hypothesis, ADSCs were co-cultured in a conditioned medium consisting of inflammatory markers Interleukin-1β and Interleukin-6 and allowed to interact with a bovine cartilage plug via a select permeable membrane exposing it to only trophic factors, like exosomes and growth factors. This model replicated osteoarthritic conditions and was used to assess the paracrine effects of ADSCs and determine their chondroprotective potential.

3. Materials & Methods

To establish an in-vitro model of osteoarthritis, the cytokines IL-1β and IL-6 were mixed with a culture medium containing DMEM-F12 with 10% FBS and 1% P/S. Bovine osteochondral plugs were placed at the base of each well in a 24-well plate. Two control groups contained no ADSCs and were exposed to either the cell culture medium with cytokines or without cytokines. In order to investigate the paracrine effects of ADSCs, a transwell co-culture experiment was designed. Isolated swine ADSCs were seeded and expanded in a monolayer on a Falcon Permeable Support for a 24-well Plate with 1 µm Transparent PET. Two experimental

groups comprised of bovine plugs with ADSCs were exposed to either a non-osteoarthritic or osteoarthritic (containing IL-1β & IL-6) medium culture to compare its effects on chondrocytes.

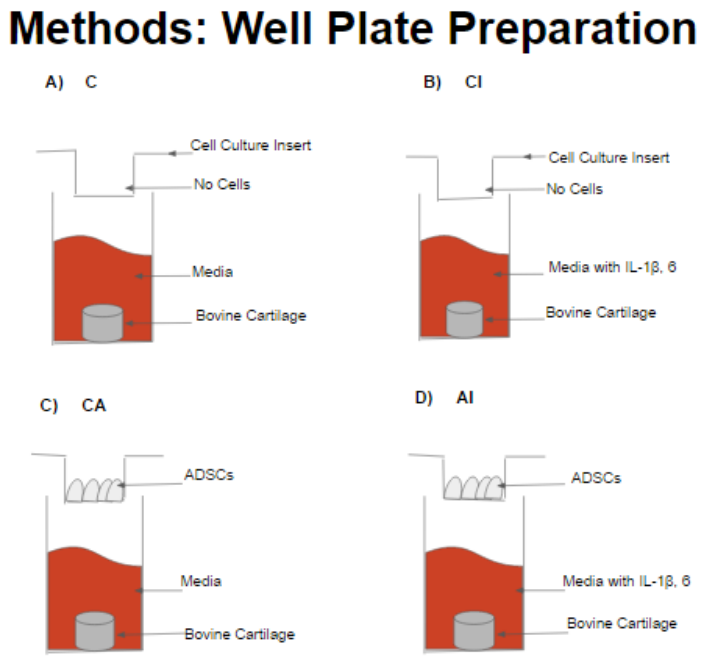

Figure 1: Four different groups were prepared, a regular control (C), negative control (CI), positive control (CA), and experimental group (AI). The media used was DMEM/F12 + 10% FBS. *Figure created by researcher.*

On three separate time points, day 1, 3, and 7, the osteochondral plugs were collected and prepared accordingly for analysis. Histological analysis of the osteochondral plugs using Safranin O and Fast Green staining was used to visualize glycosaminoglycan (GAG) distribution, which are integral parts of cartilage. Immunohistochemistry (IHC) staining was conducted using SOX9 as a chondrogenic marker. ImageJ was used to analyze the Sox9 expression among the groups. The IHC staining was performed by another researcher.

4. Results and Discussion

4.1 Analyzing Results

Histological stains of Safranin-O were compared among the four groups across the three chosen time points: days 1, 3, and 7. The visible degeneration of the CI model over all three timepoints confirmed the conditions of osteoarthritis, whereas the control group demonstrated the conservation of cartilage. The paracrine effects of the ADSCs in IL-1β/IL-6 medium prevented the complete degeneration of cartilage throughout the 7-day observation in comparison to IL-1β/IL-6 with no ADSCs (Figure 1).

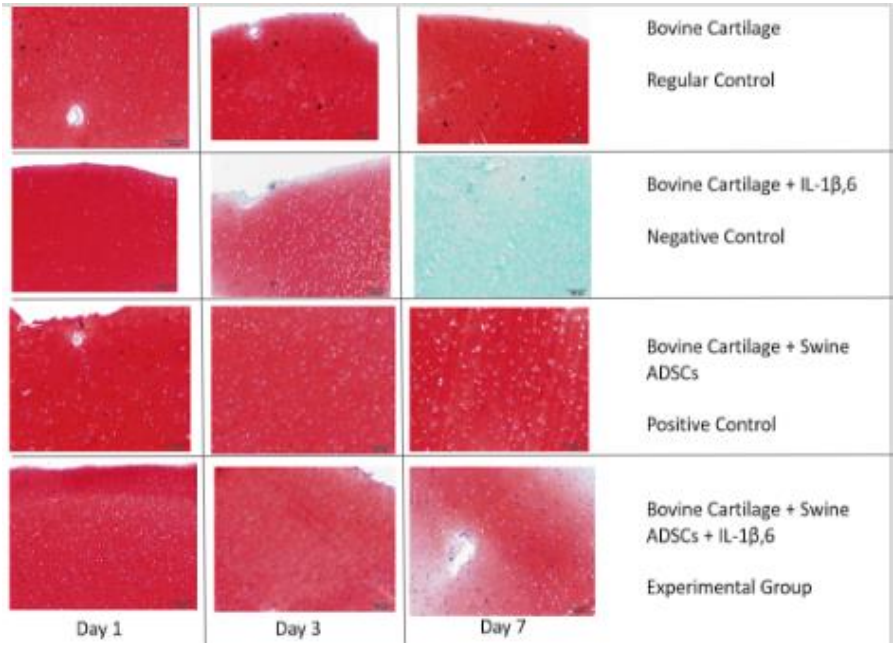

Figure 2. ADSCs show evidence of chondroprotection in an in-vitro OA model: Safranin-O staining of the swine osteochondral plug showed time-dependent cartilage degeneration when exposed to IL-1β/IL-6. ADSCs showed a noticeable decrease in degeneration and preservation of the cartilage matrix. *Image created by researcher.*

IHC results showed a marked decrease in SOX9, an important transcription factor for chondrogenesis, for the bovine cartilage plug in IL-1β/IL-6 medium with no ADSCs. The group with ADSCs, however, had more notable expression of SOX9 visually (Figure 2A). To confirm

this objectively, the integrated density of SOX9 per 10,000 cells was determined using ImageJ. In the given 7 days, the expression of SOX9 between IL-1β/IL-6 with no ADSCs and with ADSCs was significantly different, with the latter showing an increase in expression (Figure 2B). By Day 7, the integrated density of SOX9 for the group with IL-1β/IL-6 was around 100 per 10,000 cells, significantly lower than the group with both ADSCs and IL-1β/IL-6, which was around 240 per 10,000 cells. The positive area of SOX9 expression, a unit of pixel squared, was also used in conjunction and provided further evidence to suggest that ADSCs as an intervention restores chondrogenic expression (Figure 2C). The group with IL-1β/IL-6 had an area of SOX9 expression at 4 units of pixel squared, while the experimental group containing ADSCs nd IL-1β/IL-6 exhibited an area of SOX9 expression at 9 units of pixel squared.

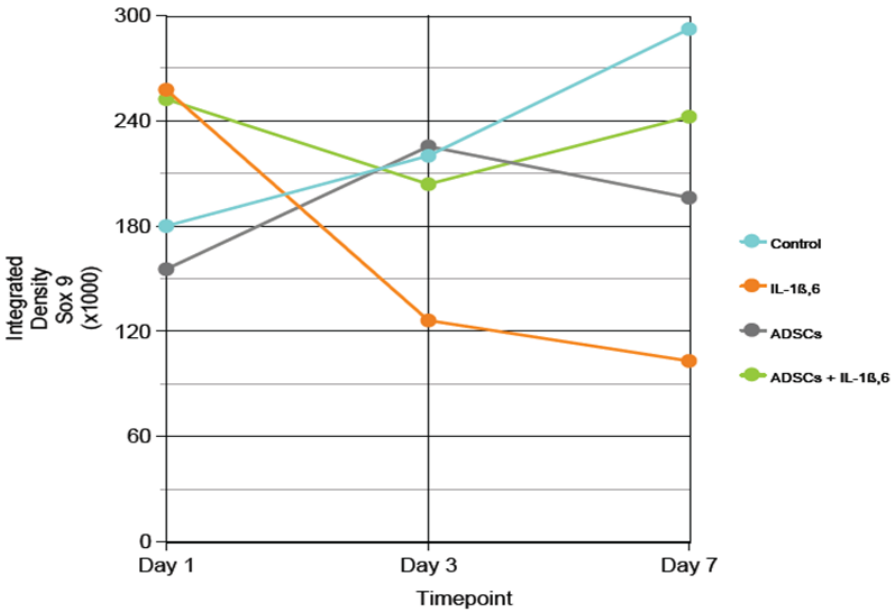

Figure 3. Integrated density of SOX 9: Immunohistochemistry of SOX9 revealed a significant reduction of visible SOX9 expression in chondrocytes exposed to IL-1β/IL-6. The higher integrated density of SOX9, which is expected to be present in cartilage unaffected by osteoarthritis, in the experimental group than in the negative control confirmed the visual restoration of SOX9 by ADSCs. *Graph created by researcher.*

4.2 Hypotheses

The results support the initial hypothesis that the paracrine effects of swine ADSCs would protect the bovine plug chondrocytes when exposed to IL 1-β/6 in osteoarthritic conditions. The histological stains show the preservation of the cartilage by ADSCs during exposure to IL-1β/IL-6. Furthermore, an increased expression of SOX9, a prominent transcription factor active in healthy cartilage, in groups containing ADSCs versus those without it also supports the hypothesis (Hamid et al., 2012).

4.3 Uncontrolled Events, Errors, Repeatability, and Improvements

There were various uncontrolled factors and errors throughout the project that prevented a complete collection of data. During the experimental setup, the contamination of well plates limited time points to only 7 days instead of 2 weeks. The difficulty in preventing contamination in the well-plates is very high because they are often interacted with when collecting other timepoints of data. A way to improve this is to separate the varying time points into different well-plates, so the collection of data from one time point doesn't affect the other wells. Also, the longer the length of the time point, the higher the chance for contamination.

Moreover, qPCR was intended to be included in the research; however, the breaking/cracking of capsules used to grind the tissue prevented the collection of accurate qPCR data. Using an alternative method for obtaining DNA from the samples, such as using spin columns, would allow us to collect the needed DNA for qPCR.

4.4 Significance

The study suggests that condition media is advantageous over whole cell ADSCs because it does not induce a graft rejection and can be allogenic. It also supports the use of ADSCs cross-species, as swine ADSCs were shown to protect bovine cartilage.

4.5 Future Research

Future investigations can be conducted to obtain quantitative qPCR data regarding the chondroprotective effects of ADSCs. Shown by the positive effects of swine ADSCs on bovine cartilage, the research also supports the use of ADSCs across species. More cross-species research can be done, possibly even utilizing human cartilage.

5. Conclusions

The induction of OA with IL-1β/IL-6 was successful in eliciting the complete degradation of the cartilage matrix when ADSCs were not introduced, revealing that an osteoarthritic model was established. The results of the histological findings suggest that ADSCs conditioned medium is able to maintain the matrix of the cartilage in an in-vitro model of OA. Bovine cartilage also demonstrated transcriptional differences among the groups, which was supported by its expression of SOX9.

6. References

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