Research Article

Lithium Chloride Induced Osteogenic Differentiation and Immunomodulatory Properties of Adipose Derived Mesenchymal Stem Cells

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Abstract

Transplantation of mesenchymal stem cells (MSCs) is a promising strategy in regenerative medicine. These cells can differentiate into chondrocytes, fibroblasts, or osteoblasts, essential components in bone healing. Dysregulated inflammation, resulting from a decreased or augmented immune response, can suppress bone healing. To overcome this problem, different strategies have been applied to improve the anti-inflammatory and immunomodulatory potencies of MSCs. Several studies have explored the potential of using small molecules to enhance the process of bone formation and regeneration. In addition to the proven safety and efficacy of lithium in managing bipolar disorder over many years, it has been reported in several studies that it could potentially contribute to an increase in bone mass. Some have focused on the role of lithium chloride (LiCl) in activating the WNT/β-Catenin pathway, which is involved in the differentiation of MSCs into osteoblasts. In this study, we evaluated the ability of adipose-derived mesenchymal stem cells (Ad-MSCs) treated with LiCl to differentiate into bone cells. To assess osteogenesis, mineralization was evaluated in cells cultured in an osteogenic induction medium. In addition to checking the expression of genes related to bone formation, we also investigated the expression of several genes related to immunomodulation at the mRNA level. We observed that LiCl enhanced the osteogenesis of Ad-MSCs, as evidenced by an increase in mineralization and the enhanced expression of osteogenic markers. Moreover, the expression of cytokines, which promote the anti-inflammatory behavior of these cells, was augmented. These findings could potentially be clinically relevant to improve conditions associated with bone loss, such as osteopenia and osteoporosis.

***Keywords*:** Mesenchymal stem cells, Lithium chloride, Osteogenesis, Bone healing, Immunomodulation

Introduction

Mesenchymal stem cells (MSCs) have attracted considerable attention in recent years as a promising resource for cell-based therapeutic approaches. Human MSCs can be conveniently isolated from several tissues, including bone marrow, adipose tissue, amniotic fluid, and the umbilical cord, due to their adhesive properties. These cells are capable of extensive expansion and differentiation into various cell types, such as osteoblasts, adipocytes, chondrocytes, and myocytes. Their effectiveness in various therapeutic strategies has been demonstrated, including treatment of osteogenesis imperfecta in children, hematopoietic recovery, and bone tissue regeneration. MSCs have emerged as a promising candidate for cell-based therapies (Chen et al., 2021). They have shown potential for improving cardiac function post-infarction, alleviating symptoms of bone and cartilage defects, and treating neurodegenerative disorders like Alzheimer's disease. Clinical trials are currently being conducted to evaluate their safety and efficacy in the treatment of various diseases (Chen et al., 2021; Götherström et al., 2021).

Small molecules, a variety of substances with a low molecular weight, have been widely utilized in different studies for the discovery and development of new treatments for various diseases. These molecules could potentially reduce the risk of

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secondary infections, chronic pain, and other negative side effects that complicate current treatment methods. Moreover, they have been useful tools for researchers to explore complex biological pathways. Certain small molecules have demonstrated regenerative properties, which hold potential for preserving the function and physical characteristics of natural tissues in areas affected by craniofacial and dentoalveolar trauma or congenital defects (Birjandi et al., 2020; Huang et al., 2021; Mitchell and Lo, 2022; Shang et al., 2021). Using small molecules, recognized for their cost-effectiveness and significant potential in regenerative medicine, along with their simplified synthesis, better storage capacity, higher stability and resistance to physical and environmental changes, and less batch-to-batch variation, proves to be more advantageous compared to alternative strategies (Ahmadi et al., 2022).

Osteoporosis is a medical condition that causes bones to become fragile and prone to fractures. As we age, the rate of bone formation slows down, resulting in a decrease in bone mass and the onset of osteoporosis (Barnsley et al., 2021). Osteoporosis, bone fractures, and other defects have high incidence rates and lead to substantial healthcare expenses, imposing a significant burden on society. As a result, prioritizing bone health is essential, and preventing bone loss throughout adulthood is possible. When it comes to addressing skeletal diseases, there is ongoing research focused on the development of new treatments and medications causing less side effects (Bonjour et al., 2009; Wong et al., 2020).
Lithium chloride (LiCl) has been used for many years in the treatment of bipolar disorder. It is reported to have a neuroprotective effect, which promotes autophagy *in vitro* (Puglisi-Allegra et al., 2021; Wu et al., 2023). Studies have suggested that lithium enhances bone formation and improves bone mass in mice, potentially through the activation of the WNT signaling pathway (Clément-Lacroix et al., 2005). LiCl is known to play a role in regulating the WNT/β-Catenin pathway, which in turn enhances bone formation. LiCl works by inhibiting the function of glycogen synthase kinase 3-β (GSK3β), a component of the complex responsible for β-Catenin degradation. LiCl suppresses GSK3β, causing β-Catenin to accumulate and translocate to the nucleus. This triggers a cellular response through altering the expression of various genes, including those associated with osteoblast differentiation (Posch et al., 2020).

Numerous genes that encode proteins associated with bone structure are indicators of bone formation, and active during the commitment and differentiation of osteoblasts. Generally, alkaline phosphatase (ALP) and bone sialoprotein (BSP) are earlier markers of osteoblast differentiation, while osteocalcin (OCN) is a later marker that appears during the mineralization process. Osteopontin (OPN) exhibits a dual peak pattern in its expression level, with one occurring in the early phase and the other in the late phase of osteoblast differentiation (Posch et al., 2020).

Inflammation plays a pivotal role in the bone healing process. While an initial inflammatory response is essential for this process, an extended period of inflammation can hinder healing. Macrophages play a substantial role in both the innate and adaptive immune systems, and they hold a crucial position in bone formation under normal physiological conditions and during bone repair. MSCs can control macrophage chemotaxis and function, thereby contributing to bone regeneration by adjusting macrophage activity during inflammation. However, chronic inflammation could potentially hinder the efficacy of MSC-based treatments by impairing their capacity to repair. Strategies to enhance bone regeneration through immunomodulation might include preconditioning MSCs with anti-inflammatory cytokines, or using exosomes to boost their immunomodulatory characteristics (Kushioka et al., 2023).

In this research, we explored the effects of LiCl on enhancing the osteogenesis capability as well as the immunomodulatory characteristics of Ad-MSCs. The evaluation was conducted by examining the level of calcium deposition and the expression of osteogenic and immune-related genes at the mRNA level.

Materials and Methods

Isolation and characterization of Ad-MSCs

For the isolation of Ad-MSCs, 100 mL of lipoaspirate was collected and transported to the laboratory after obtaining the patients' approval and informed consent (ethics committee approval ID: IR.ACECR.JDM.REC.1397.020). The separated adipose tissue was washed thrice with equal phosphate-buffered saline (PBS; Gibco, UK). Then it was digested using 0.3 mg/mL collagenase (type I; Gibco, UK) at 37 °C for 45 min, with the container being shaken every 10 min. Then, the digested tissues were neutralized with an equal volume of cell

culture medium supplemented with 10% fetal bovine serum (FBS; Gibco, UK) and centrifuged (600 *g*, 10 min) to obtain the cell pellet. The pellet was resuspended in a culture medium containing FBS and antibiotics, including penicillin and streptomycin (1X; Gibco, UK). After a second centrifugation (400 *g*, 6 min), the cells were seeded into adherent cell culture flasks (Bidkhori et al., 2023).

For characterization of isolated cells, a suspension of 2×10^5 cells were stained with PE-conjugated CD73, FITC-conjugated CD90, CD34, CD14, and HLA-DR, and APC-conjugated CD44 and CD45 antibodies (all from Cytognos, Spain). Then the flow cytometric approach was applied (BD Accuri C6, USA), and the data were analyzed using FlowJo 7.6.1 software. To confirm the differentiation potential of the isolated cells into multiple cell types, lineage-specific differentiation assays were performed. In these experiments, the cells were exposed to adipogenic and osteogenic differentiation-inducing media according to the previous protocols (Bidkhori et al., 2023) for 17-21 days. At specific time points, oil red O staining and both alizarin red S staining and ALP activity assays (Sigma-Aldrich, Germany) were applied to visualize their differentiation into adipocytes and osteocytes, respectively.

Treatment schedule and quantification of osteogenic differentiation via spectrophotometry

Ad-MSCs were grown in 6-well plates for osteogenic induction. Cells were exposed to 20 mM LiCl for 7, 14, and 21 days. Concurrently, osteogenesis was induced using an osteogenic induction medium containing 0.5 mM ascorbate-2-phosphate, 10 mM β-glycerophosphate, and 0.1 µM dexamethasone (Sigma-Aldrich, Germany). Finally, at different time points, they were fixed in 4% paraformaldehyde (PFA) for 30 min and then washed with PBS. The wells were then treated with alizarin red S staining solution (Sigma-Aldrich, Germany) for 5 min, and observed under an inverted phase contrast microscope (Olympus, Japan). Moreover, to quantify the calcium deposition in both treated and untreated cells, the stain was solubilized with acetic acid, and the absorbance was measured at 405 nm.

Assessing the expression levels of osteogenic and immune-related genes after preconditioning Ad-MSCs with LiCl

To investigate the effect of LiCl on the osteogenic potential of Ad-MSCs, cells were constantly exposed to 20 mM LiCl, while they were kept in the osteogenic induction medium. Moreover, two different concentrations of LiCl (2 µM and 20 mM) were also applied to the cells in the presence of an induction medium to evaluate the expression of immune-related genes.

Total RNA was then extracted from the cells at different time points (days 7, 14, and 21) using TRIzol reagent (Ambion, USA). Subsequently and following treatment with DNase I (DNase I Solution; Thermo Fisher Scientific, USA), the single-stranded cDNA was synthesized using the RevertAid First Strand cDNA Synthesis kit (Thermo Fisher Scientific, USA). All reactions were performed according to the instructions provided with the products.

The expression of osteogenic-related genes *ALP*, cyclin D1 (*CCND-1)*, collagen type I *(COL I)*, *COL III*, runt related transcription factor 2 (*RUNX2)*, *OCN*, and *OPN* was measured using specific primers as detailed in Table 1. In addition, the immune-related genes including interleukin (*IL*)-*1β, IL-6*, *IL-4*, cyclooxygenase *(COX)-2*, and tumor necrosis factor- (TNF) stimulated gene (*TSG*)-*6* were also evaluated at mRNA level following the previous steps. Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) was performed using RealQ Plus Master Mix (Ampliqon, Denmark) in the CFX-96 Real-time PCR system (Bio-Rad, USA). The PCR was used to amplify specific sequences under the following conditions: an initial cycle at 94 °C for 10 min, followed by 40 cycles of 94 °C for 15 s, 60 °C (the melting temperature for all primers) for 25 s, and 72 °C for 25 s. The data are presented as $2^{-\Delta\Delta CT}$ and normalized against *RPLP0*.

All experiments were conducted at least three times and the results are presented as the average of triplicate independent experiments. Statistical significance was determined using the *t*-test. A *p*-value less than 0.05 was considered statistically significant. All the analyses were performed using GraphPad Prism version 9.0.0 for Windows (www.graphpad.com).

Results

The identity of Ad-MSCs was confirmed through the expression of specific CD markers and multiple differentiation potential

Flow cytometry was employed to examine the expression of some cell surface markers after Ad-MSCs were isolated from human adipose tissue. Ad-MSCs at passage 3 were applied for all characterization and downstream experiments. The stem cell markers CD105, CD44, CD73, and CD90 were highly expressed in more than 99% of the cells. Desirably, the cells did not exhibit a significant expression of markers associated with hematopoietic stem cells, such as CD14, CD45, HLA-DR, and CD34 (Figure 1A). Moreover, the Ad-MSCs showed the ability to differentiate into adipogenic and osteogenic lineages. This was confirmed using oil red O, and alizarin red S staining, as well as an assessment of ALP activity in comparison to the relevant controls, which were not subjected to the differentiation media (Figure 1B).

LiCl significantly enhanced calcium deposition in the culture media of Ad-MSCs

The mineralization of Ad-MSCs treated with LiCl was verified using alizarin red S staining and compared to untreated cells. As shown in Figure 2A, alizarin red S staining, which indicates the presence of calcium deposits on the 21st day of osteogenesis, denotes successful osteogenic differentiation. The quantification of alizarin red S staining was carried out by measuring optical density at 405 nm on days 7, 14, and 21 following the induction of osteogenesis (Figure 2B).

Table 1. List of primers used in this study to assess the expression of genes related to osteogenesis and immunomodulation properties of cells treated with LiCl.

Target gene	Sequence	Product size (bp)	Accession Number (Reference)
RPLP0	F: TGGTCATCCAGCAGGTGTTCGA R: ACAGACACTGGCAACATTGCGG	119	NM_053275.4 (Designed)
IL-1β	F: CCTCTCTCACCTCTCCTACTCAC R: CTGCTACTTCTTGCCCCCTTTG	186*	NM_000576.2 (Designed)
IL-6	F: ACTCACCTCTTCAGAACGAATTG R: GCAAGTCTCCTCATTGAATCCAG	196*	NM_000600.4 (Designed)
IL-4	F: AGGGCTGCGACTGTGCTC R: GTACTCTGGTTGGCTTCCTTCAC	185*	NM_000589.4 (Designed)
COX-2	F: CCAGAGCAGGCAGATGAAATACC R: ACCAGAAGGGCAGGATACAGC	168*	NM_000963.3 (Designed)
TSG-6	F: GCTGCTGGATGGATGGCTAAG R: CTCCTTTGCGTGTGGGTTGTAG	156*	NM_007115.3 (Designed)
ALP	F: TAAGGACATCGCCTACCAGCTC R: TCTTCCAGGTGTCAACGAGGT	170**	NM_000478.6 (Zhang et al., 2017)
CCND1	F: ATGCCAACCTCCTCAACGAC R: TCTGTTCCTCGCAGACCTCC	159**	NM_053056.3 (Shi et al., 2019)
COL I	F: TGCGATGACGTGATCTGTGA R: TTGGTCGGTGGGTGACTCTG	108**	NM_000088.4 (Designed)
COL III	F: GAGAATCAGGTAGACCCGGAC R: ATCGAAGCCTCTGTGTCCTT	116**	NM_000090.4 (Designed)
RUNX2	F: AGTGGACGAGGCAAGAGTTTC R: CCTTCTGGGTTCCCGAGGT	117**	NM_001015051.4 (Fei et al., 2018)
OCN	F: AGGGCAGCGAGGTAGTGAA R: TCCTGAAAGCCGATGTGGT	151**	NM_199173.5 (Fei et al., 2018)
OPN	F: ATTCTGGGAGGGCTTGGTTG R: GTGAGTTTTCCTTGGTCGGC	150**	NM_000582.3 (Li et al., 2019)

* Immune-related genes, **Osteogenic-related genes

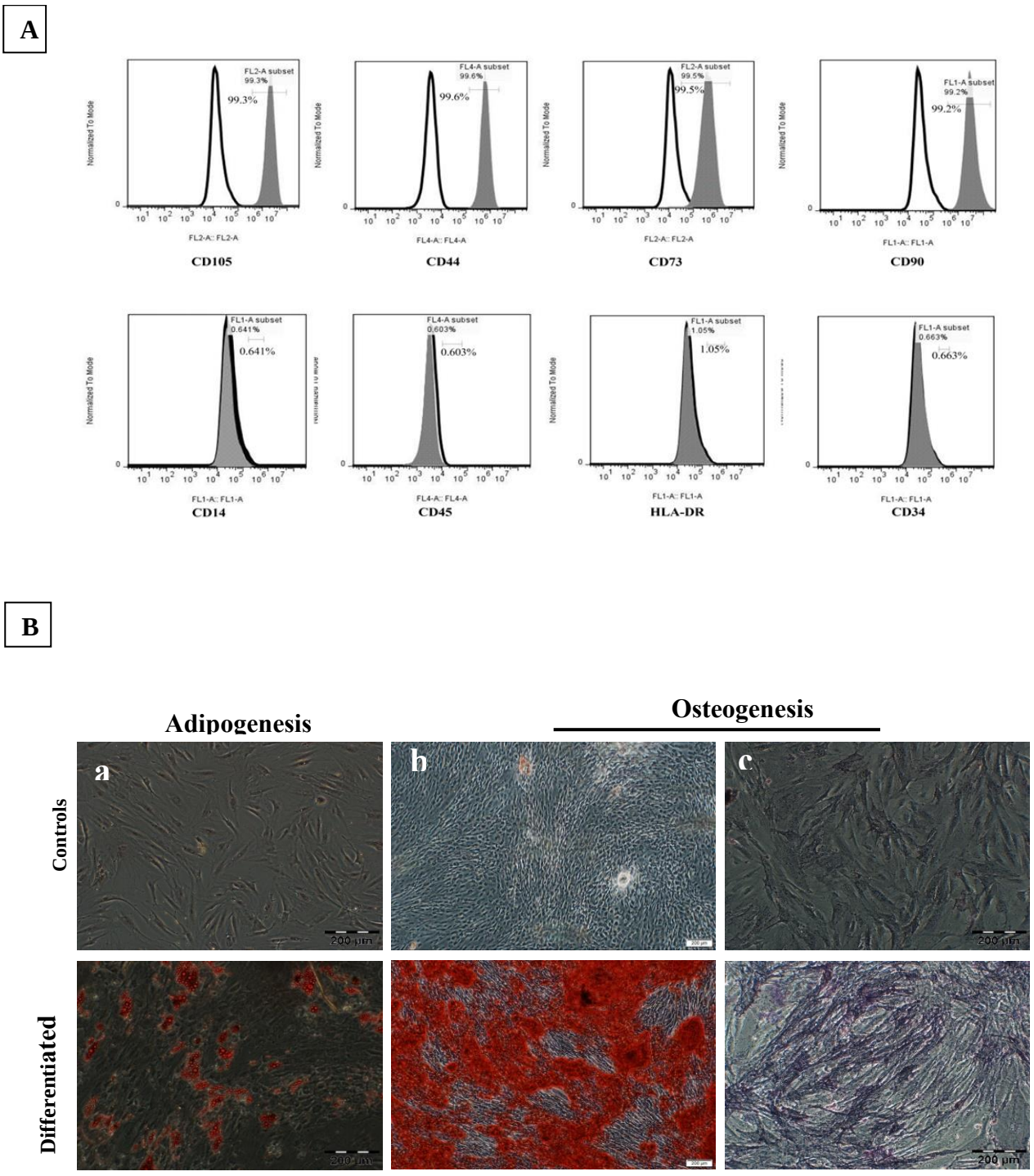

Figure 1. Characterization of isolated Ad-MSCs. (**A**) Ad-MSCs were analyzed using flow cytometry, which indicated the expression of CD105, CD44, CD73, and CD90 in more than 99% of the cells, while they did not significantly express hematopoietic stem cell markers including CD14, CD45, HLA-DR, and CD34. (**B**) Ad-MSCs demonstrated multilineage differentiation into adipogenic and osteogenic lineages, as evidenced by oil red O staining (a), alizarin red S staining (b) and ALP activity (c), respectively. These differentiated cells were evaluated in comparison to Ad-MSCs (controls) that were not subjected to induction media (scale bar = 200 µm).

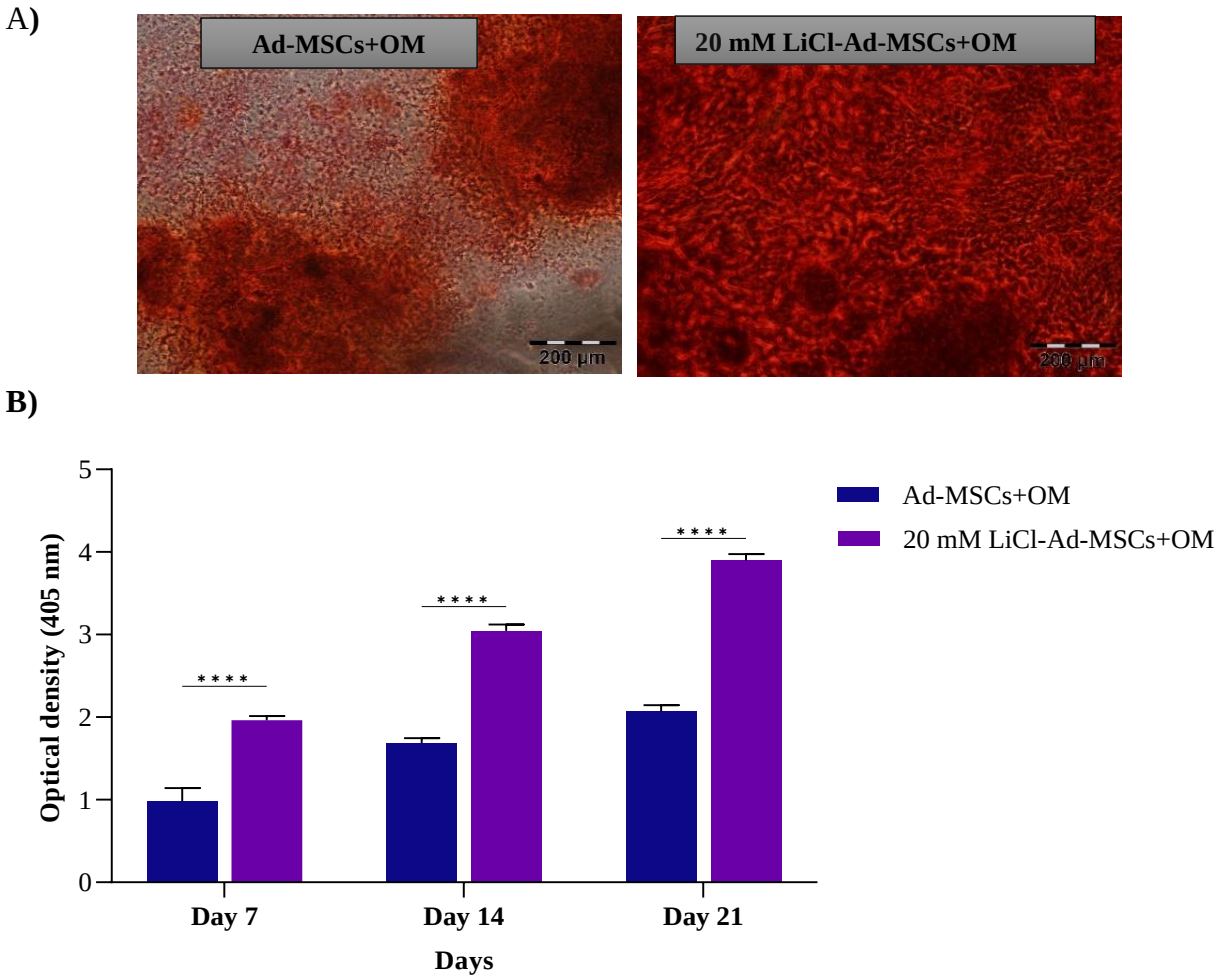

Figure 2. Evaluation of Ad-MSCs mineralization by alizarin red S staining. A) Alizarin red S staining revealed calcium deposition on day 21 of osteogenesis, indicating successful osteogenic differentiation. B) Quantification of alizarin red S was performed through optical density measurements at 405 nm (n = 3) on days 7, 14, and 21 of osteogenesis induction. Results are presented as mean ± SD. (****$p < 0.0001$).
OM refers to the osteogenic-inducing medium.

Expression of osteogenic-related genes in Ad-MSCs increased upon exposure to LiCl

The expression of osteogenesis-specific genes was analyzed using qRT-PCR. The mRNA expression level of osteogenic markers including *ALP*, *CCND-1*, *COL I*, *COL III*, *RUNX2*, *OCN*, and *OPN* was evaluated in Ad-MSCs exposed to an osteogenic-inducing medium in the presence of LiCl (20 mM). These expression levels were compared to the control group at three time points: 7, 14, and 21 days. As shown in Figure 3, the expression of these genes was observed to be significantly elevated in the treated cells compared to the untreated group at all investigated time points.

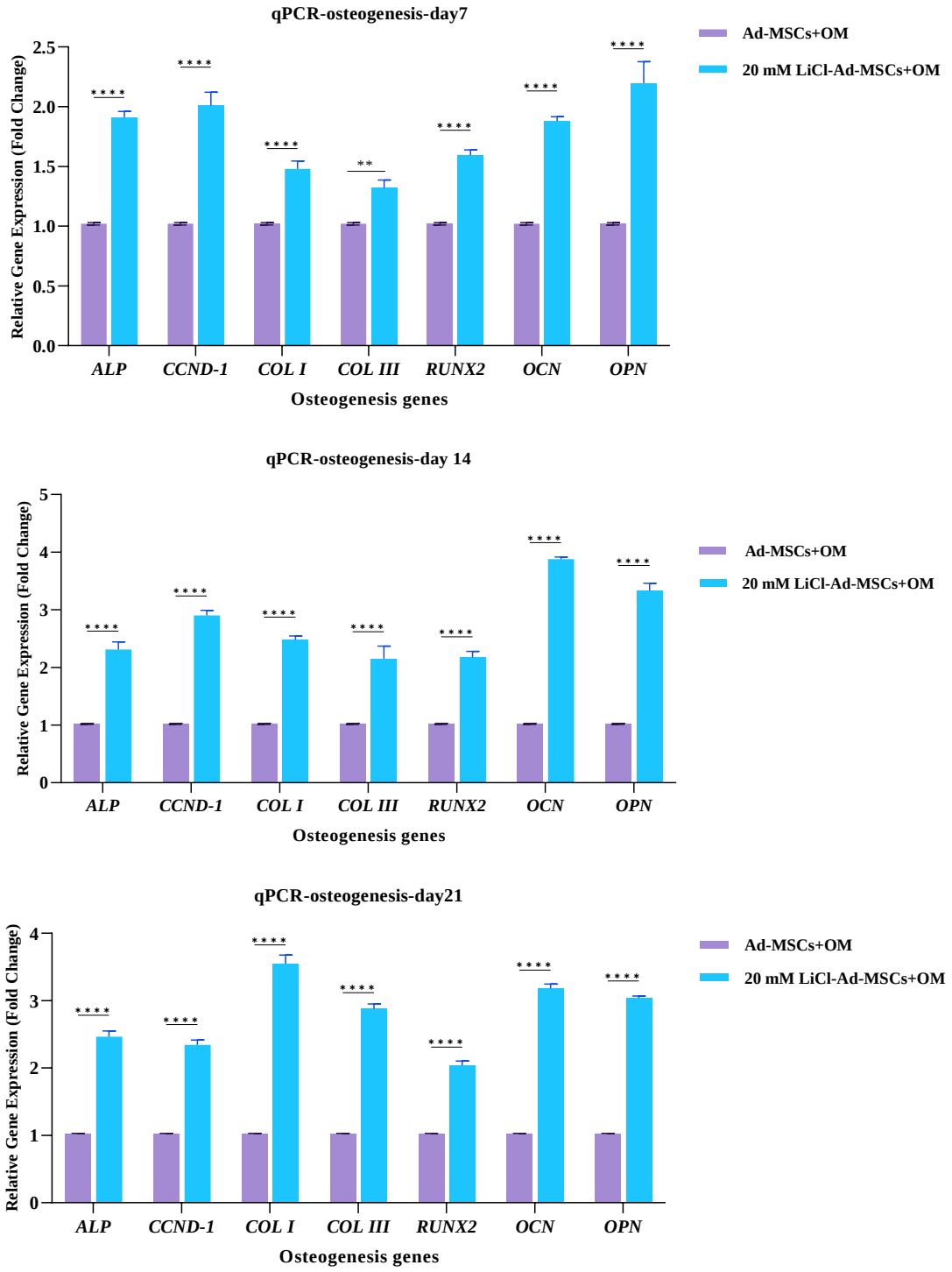

Figure 3. qRT-PCR analysis of osteogenic-specific gene expression in Ad-MSCs treated with LiCl. The relative mRNA expression of osteogenesis markers (*ALP*, *CCND-1*, *COL I*, *COL III*, *RUNX2*, *OCN*, and *OPN*) was measured in Ad-MSCs treated with an osteogenic-inducing medium (OM) supplemented with 20 mM LiCl. These measurements were compared to the control group for 7, 14, and 21 days. Data are represented as the mean ± SEM. $^{**}p < 0.01$, $^{****}p < 0.0001$.

LiCl improved the immunomodulatory properties of Ad-MSCs as assessed by gene expression

The quantitative examination of immune-related gene expression in Ad-MSCs treated with LiCl (both 2 µM and 20 mM) revealed distinct patterns. The expression level of *IL-1β*, *IL-6*, *IL-4*, *COX-2*, and

TSG-6 were analyzed using qRT-PCR and compared with those of the control group. Two notable trends emerged in the expression of these immune-related genes in the LiCl-treated Ad-MSCs compared to untreated cells: a decrease in the expression of pro-inflammatory genes like *IL-1β* and *IL-6* and an increase in the expression of anti-inflammatory genes such as *IL-4*, *COX-2*, and *TSG-6*. This pattern was consistent across Ad-MSCs treated with both 2 µM and 20 mM concentrations of LiCl in a dose-dependent manner (Figure 4). It was observed that higher concentrations of LiCl had a more remarkable effect on the overexpression of the mentioned genes.

Discussion

In this study, we evaluated the potential of LiCl as a safe small molecule to induce osteogenesis and also modulate the expression of some immune-related genes in human Ad-MSCs. We used various assays to monitor the osteogenic markers, gene expression, and calcium deposition in LiCl-treated Ad-MSCs and compared the outcomes with those of untreated cells. We also examined the expression of several immune related genes at the mRNA level.

Given the benefits of small molecules, their implementation could be viewed as a supplementary method to various therapeutic strategies currently used for patients. Studies have shown that LiCl has multiple effects, including mood stabilization, anti-suicide, anti-viral, and anti-tumor properties, as well as immunomodulatory and bone healing effects.

Lithium specifically inhibits GSK3β through two key mechanisms: it directly competes with magnesium ions to block GSK3β and it also indirectly inhibits GSK3β via serine phosphorylation. Notably, GSK3β is involved in the WNT/β-Catenin, phosphatidylinositol 3-kinase (PI3K), and nuclear factor-kappa B (NF-κB) signaling pathways. These pathways play a crucial role in regulating bone metabolism and homeostasis, suggesting the potential of lithium as an osteoprotective agent (Bertacchini et al., 2018; Bonjour et al., 2009; Galli et al., 2013; Wong et al., 2020).

While there have been no previous studies investigating the impact of LiCl on the osteogenic potential and immunomodulatory properties of

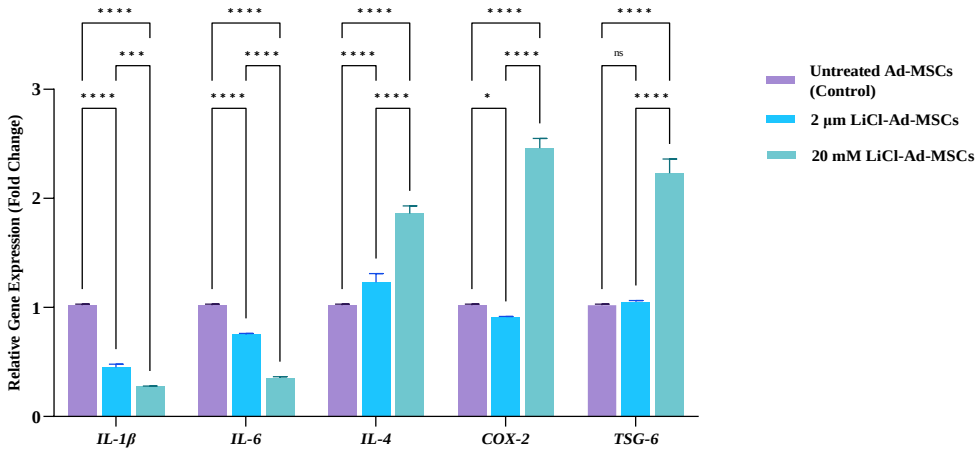

Figure 4. Quantitative analysis of the expression of immune-related genes in Ad-MSCs following LiCl treatment. The expression level of *IL-1β*, *IL-6*, *IL-4*, *COX-2*, and *TSG-6* in Ad-MSCs treated with LiCl (2 µM and 20 mM) was examined using qRT-PCR and compared to the control untreated group. In Ad-MSCs treated with LiCl, the expression of pro-inflammatory genes including *IL-1β* and *IL-6* was decreased, while the expression of anti-inflammatory genes *IL-4*, *COX-2*, and *TSG-6* was increased. A comparable pattern was observed when contrasting Ad-MSCs treated with 2 µM and those exposed to 20 mM LiCl. *RPLP0* was considered as the reference gene. The results from three independent experiments are shown as the mean ± SEM. Statistical significance was determined as $*p < 0.05$, $***p < 0.001$, and $****p < 0.0001$, with 'ns' indicating not significant.

human Ad-MSCs, this research represents the first exploration of this kind.

An examination of potential early inducible biomarkers revealed five distinctive "signature genes". These include *RUNX2*, which is expressed upon differentiation, and four genes that encode extracellular matrix proteins: tissue-nonspecific alkaline phosphatase (*ALP*), *COLIα2*, decorin (*DCN*), and elastin (*ELN*). Together, these genes could provide a one-week osteogenic induction assay that categorizes donor-specific human bone marrow MSCs based on their bone-forming potential (Kulterer et al., 2007; Ofiteru et al., 2020).

Numerous studies have investigated the impact of LiCl on osteoblastogenesis. Exposure of either osteoblast precursor cells or mature osteoblasts to LiCl resulted in overexpression of osteoblast differentiation markers such as *Alp*, *Col I* and *Runx-2* and an enhancement in calcium deposition, as confirmed by alizarin red S staining (Arioka et al., 2014; Bai et al., 2019; Clément-Lacroix et al., 2005; Li et al., 2018; Wong et al., 2020; Yang et al., 2019). The effects of this small molecule on human osteosarcoma cells (MG63) have also been investigated, and the results indicated its potential to enhance the proliferation and differentiation of osteoblasts (Li et al., 2017). The osteogenesis-related genes, including *Runx-2*, *Alp*, *Opn*, and *Ocn* in rat bone marrow MSCs showed increased expression due to the release of lithium ions from mesoporous silica nanospheres doped with lithium (Zhang et al., 2018).

Previous reports have indicated that lithium promotes osteoblastogenesis and bone formation while inhibiting osteoclastogenesis and bone resorption. The regulation of osteogenesis is mainly achieved through the activation of the WNT, PI3K/AKT, and BMP-2 signaling pathways, while the ability of lithium to inhibit osteoclastogenesis is achieved through the signaling axis controlling the inflammatory response via the NF-κB and MAPK signaling pathways (Park et al., 2017; Wong et al., 2020; Yang et al., 2019). Zhang *et al*. investigated the effects of LiCl on glucocorticoid-induced osteonecrosis of femoral heads in rats. The study discovered that less calcium deposition was observed in rats whose MSCs were not able to differentiate into osteoblasts. However, this effect was modulated in rats treated with lithium, resulting in more calcium nodules. The level of mRNAs related to osteogenesis, including *Runx2*, *Alp,* and *Col I,* was notably reduced by glucocorticoids but increased with lithium supplementation (Zhang et al., 2021). The findings of this study are in alignment with the conclusions drawn from previous research. Our results revealed that the expression of genes including *ALP*, *CCND*, *RUNX-2*, *COL I*, *COL III*, *OCN*, and *OPN* increased on days 7, 14, and 21 of bone differentiation induction in the presence of LiCl. Additionally, the amount of calcium deposition, a marker of bone mineralization, significantly increased on days 7, 14, and 21 in the presence of LiCl.

In our previous studies, we observed that overexpression of Nanos2 (Noughabi et al., 2023) and IDO1 (Haghighitalab et al., 2021) through genetic engineering of MSCs, may enhance their immunomodulatory properties and reduce the inflammation in their microenvironment.

Several studies have been conducted on the role of the immune environment in facilitating bone regeneration. It was found that LiCl effectively mitigated the inflammatory response, as evidenced by the reduction in levels of *TNF-α*, *IL-1β*, and *IL-6* and reduced bone loss in a mouse model of calvarial osteolysis (Hu et al., 2017). In addition to altering the expression of osteogenic-related genes, our study revealed that the exposure of Ad-MSCs to LiCl resulted in a decrease in the expression of pro-inflammatory cytokines, namely *IL-1* and *IL-6*. Concurrently, it also led to an increase in the expression of the anti-inflammatory cytokine *IL-4*. An upregulation of *COX-2* and *TSG-6* was also observed. It is evident that the effect of LiCl on immune-related gene expression was dose-dependent, and an increase in the concentration of LiCl amplified its impact on the expression of these genes.

This study investigated the effects of LiCl on the osteogenic differentiation capacity of Ad-MSCs for the first time by evaluating the osteogenic and immunomodulatory-related markers. The results revealed that LiCl can modulate the behavior of Ad-MSCs in the osteogenesis pathway, enhancing their suitability for stem cell therapy. Specifically, it was found that LiCl intensifies alizarin red S staining and amplifies the expression of osteogenic-related genes in differentiated Ad-MSCs. Furthermore, LiCl can affect the expression of immune-related genes, thereby boosting the immunomodulatory properties of Ad-MSCs and leading to an augmentation of their osteogenic potential.

Conclusion

To the best of our knowledge, this research is the first study to illustrate that LiCl can enhance the osteogenic potential of Ad-MSCs. In summary, treatment with LiCl was found to enhance mineralization and the expression of markers related to bone formation. Moreover, it decreased the expression of pro-inflammatory genes and increased the expression of anti-inflammatory markers in these cells, resulting in the amplification of the immunomodulatory characteristics of Ad-MSCs. These observations could potentially have clinical significance in improving conditions related to bone healing.

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Conflict of Interest

The authors declare that there was no conflict of interests.

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