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Changes in Polarity and Regeneration-Related Gene Expression in In Vitro Bone Marrow Mesenchymal Stem Cells in a Rheumatoid Arthritis Injury Model and Pharmacological Modulation

Romatoid Artrit Yaralanma Modelinde In Vitro Kemik İliği Mezenkimal Kök Hücrelerinde Polarite ve Rejenerasyonla İlişkili Gen İfadesindeki Değişiklikler ve Farmakolojik Modülasyonu

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ÖZ

Amaç: Bu çalışmanın hedefi patogenez basamakları iyi anlaşılmış kronik, inflamasyon sebebiyle dokusal erozyonun sonucunda oluşan Romatoid Artirit (RA) hastalığının farmakolojik modülasyon ile gen-protein düzeyinde polarite ve rejenerasyondaki değişimleri incelenmiştir.
Araçlar ve Yöntem: Hastalığın patogenez basamaklarının hücre içi polarite ve rejenerasyona verdiği zararı araştırabilmek için Il-1β ve IL-6 ile hasar taklidi yapılmış kemik iliği mezenkimal kök hücrelerinde IL-1β antagonist antikoru canakinumab ve IL-6 antagonist antikoru tocilizumab kombinasyon halinde uygulanmasının hücre canlılığı, sitotoksisite ve hücre içi polarite ve rejenerasyon ile ilgili genlerin ifadesi incelenmiştir.
Bulgular: Analizler sonucunda IL-1β ve IL-6 beklendiği üzere RA hasar taklidi yapılan insan kemik iliği kök hücrelerinde canlılığının azalmasına sitotoksisitenin aynı oranda artmasına sebep olmuştur. Bunun yanında gen ifade analizlerinde polarite yolaklarında görevli genlerin ifadelenmesinde düşük oranda anlamlı değişiklik görülmüş ancak rejenerasyon ile ilgili genlerin ifadelenmelerinde anlamlı değişikliklere rastlanmıştır. Bunun yanında antagonist ajanların uygulanması bu durumu tersine çevirmiş sınırlı seviyede normalleşme hatta hücrelerin canlılık testlerinde canlılığı artıran değişimler gözlemlenmiştir.
Sonuç: Bu durumda hasar taklidi sonrası elde edilen RA benzeri patogenez modelinde hastalığın gelişim basamaklarında gerçekleşen inflamasyonun etkisiyle kök hücrelerin adherasyon, yön bulma, gibi özelliklerini kaybetmeleri, korudukları rejenerasyon özellik verimliliğini etkilemekte olduğu düşünülmüştür.

Anahtar Kelimeler: artrit; ınterlökin; kemik iliği mezenkimal kök hücresi; monoklonal antikor

ABSTRACT

Purpose: The aim of this study was to investigate the changes in polarity and regeneration at the gene-protein level with pharmacological modulation of rheumatoid arthritis (RA) disease, which occurs as a result of chronic, inflammation-induced tissue erosion whose pathogenesis steps are well understood.
Materials and Methods: To investigate the damage caused by the pathogenetic steps of the disease to intracellular polarity and regeneration, bone marrow mesenchymal stem cells subjected to injury mimicry using IL-1β and IL-6 were treated with a combination of the IL-1β antagonist antibody canakinumab and the IL-6 antagonist antibody tocilizumab. The effects on cell viability, cytotoxicity, and the expression of genes related to intracellular polarity and regeneration were examined.
Results: As a result of the analyses, as expected, IL-1β and IL-6 caused a reduction in viability and an acceleration in cytotoxicity in human bone marrow stem cells imitating RA damage. In addition, in gene expression analyses, low significant changes were observed in the expression of genes consisted in polarity pathways, but significant changes were found in the expression of genes take place in regeneration mechanisms. In addition, the application of antagonist agents reversed this situation and limited normalization and even changes that increased the viability of the cells in viability tests were observed.
Conclusion: In this case, in the RA-like pathogenesis model obtained after injury mimicry, it is thought that the inflammation occurring during the disease's developmental stages causes stem cells to lose their properties such as adhesion and navigation, thereby affecting the efficiency of the regenerative properties they maintain.

Keywords: arthritis; bone marrow mesenchymal stem cell; ınterleukin; monoclonal antibody

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INTRODUCTION

Rheumatoid Arthritis (RA) is a persistent, inflammatory disease that occurs in childhood and consists of clinical pictures rather than a single disease.[1,2] Recent scientific studies have shed light on the pathogenesis of RA.[3] Classically, white blood cells such as neutrophils and monocytes in the cell-conjugated phase of inflammation migrate to the damaged tissue area and secrete the early inflammatory factors S100A8, S100A9 and neutrophil-based S100A12 in the extracellular matrix of the area.[4] These alarmins, part of the S100 protein family, play a crucial role in triggering and maintaining the inflammatory response.[5] Their increased levels are frequently associated with disease activity and can act as biomarkers for tracking disease progression.[6] Additionally, these proteins can enhance the local inflammatory response by promoting the synthesis of more chemokines and pro-inflammatory cytokines.[7] These cells, predominantly found in the synovial fluid and synovium membrane of the interacted area in RA pathogenesis, are activated by neutrophils and their metabolites IL-1 and IL-6, leading to the secretion of degradation proteases.[8] IL-1 and IL-6 are crucial pro-inflammatory cytokines that not only assemble an excess of immune cells to the site but also amplify the inflammatory response.[9] These cytokines play a significant role in the feedback loop that sustains the chronic inflammation characteristic of RA.[10] Following these mechanisms, the appearance of blood vessels in the synovial fluid becomes more frequent.[11] This formation of new blood vessels is crucial for maintaining chronic inflammation, as it allows more immune cells to access the joint space.[12] The rise in blood vessel formation is driven by VEGF (vascular endothelial growth factor) is one of the growth factors, which is elevated in the inflamed synovium.[13] This facilitates increased inflammation by enabling the migration of inflammatory cells.[10] CD28 T cells migrating into the synovial fluid produce significant amounts of IL-6 when CD80/86 antigen-presenting cells present antigen in the synovial epithelium. This epithelium acts as a barrier to the synovial liquid in beholds by membrane and defines the boundaries of the joint.[11] Because of their essential role in the adaptive immune response, these T cells contribute to the chronic nature of RA by consistently producing pro-inflammatory cytokines.[12] The persistent activation of these T cells and their interaction with antigen-presenting cells perpetuates the inflammatory cycle.[13] Destructive proteases break down the intercellular desmosomes and tonofilaments in the synovial epithelium and cause the membrane lining the synovial fluid to disappear, initiating an erosion into the synovial fluid.[14] Joint injury and function loss result from the breakdown of these structural proteins, which also undermine the integrity of the synovial membrane.[15] Synovial fibroblasts, stimulated by the inflammatory environment, further contribute to the destruction by releasing MMPs, or matrix metalloproteinases, disintegrate extracellular matrix constituents.[16] The inflammatory state is maintained by these fibroblasts' additional role in the synthesis of pro-inflammatory cytokines.[17] Monoclonal antibody treatment can be used to inactivate and reduce the impact of inflammatory chemicals in the early stages of inflammation.[18] These therapies target specific cytokines such as IL-6 and IL-1β, aiming to reduce inflammation and prevent joint damage.[19] Antagonist antibody agents such as canakinumab, infliximab, and tocilizumab have demonstrated effectiveness in controlling symptoms and enhancing the quality of life for patients with RA.[20] Additionally, newer antagonist antibody targeting IL-6, such as tocilizumab, have been developed and are being used to treat RA.[21] To improve disease control, these therapies are frequently used with traditional disease-modifying antirheumatic medications (DMARDs), such as methotrexate.[22] Recent advances in understanding the genetic and environmental factors such as polarity instability and regeneration capacity decline contributing to RA have also opened up new avenues for potential therapeutic targets in managing this complex disease.[23] For this purpose, we inspected some of the targeted genes that has crucial role in cell polarity and regeneration. Pard3, a gene involved in cell polarity, has been found to play a role in the development and progression of rheumatoid arthritis (RA) through its regulation of synovial fibroblast behavior and inflammatory responses.[24,25] Mapk8 is crucial in RA pathogenesis by mediating pro-inflammatory cytokine production and joint destruction.[26,27] Dmp1, which is related to bone matrix protein, influences bone metabolism and osteoclast differentiation, thereby contributing to the bone erosions seen in RA.[24,25] Yap1, a key regulator of cell proliferation and apoptosis, is implicated in RA by promoting synovial hyperplasia and joint inflammation.[26,27] Junb, a transcription

factor, modulates inflammatory responses in RA by regulating the expression of pro-inflammatory genes and cytokines.[24,25] Mepe impacts bone mineralization and has been linked to altered bone homeostasis in RA.[26,27] Mmp13, a matrix metalloproteinase, contributes to the degradation of cartilage and extracellular matrix in RA, exacerbating joint damage.[24,25] Similarly, Mmp2, another matrix metalloproteinase, is involved in tissue remodeling and synovial inflammation, playing a significant role in RA progression.[26,27] In this study, we aimed to elucidate the cellular mecha-nisms contributing to the pathogenesis of RA and especial-ly the roles of genes associated with cell polarity and tissue regeneration capacity. In this context, the functions of genes such as Pard3, Mapk8, Dmp1, Yap1, Junb, Mepe, Mmp13 and Mmp2 in inflammation and joint destruction were evaluated.

MATERIALS and METHODS

Reagents and Chemicals

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Lactate Dehydrogenase Colorimetric Activity Kit, TRIzol Reagents and dimethyl sulfoxide (DMSO) were purchased from Invitrogen (Waltham, Massachusetts, USA). High-Capacity cDNA Reverse Transcription Kit and TaqMan™ Master Mix for qPCR were purchased from Applied Biosystems (Waltham, Massachusetts, USA). IL-1β and IL-6 obtained from Gibco (Gibco, Waltham, Massachusetts, USA). Canakinumab was obtained as ACZ885-Ilaris from Novartis (Novartis, Basel, Switzerland) and Tocilizumab was obtained as Actemra from Roche (Roche, Basel, Switzerland). Canakinumab and Tocilizumab concentration were adjusted by dissolving in PBS. All other reagents, unless noted otherwise, were obtained from Invitrogen in their highest available purity.

Cell Culture

Human Mesenchymal Bone Marrow Derived Adult Stem Cells are a type of cell line commonly used in cartilage formation research, particularly in the study of Arthritis. Human Mesenchymal Bone Marrow Derived Adult Stem Cells were obtained from Celprogen (SKU: 36094-22, Celprogen; Benelux, Netherlands). The cells were cultured in Human Bone Marrow Derived Mesenchymal Stem Cell Complete Media with Serum (SKU: 36094-21S, Celprogen; Benelux, Netherlands) in a humidified atmosphere (95% humidity) at 37 °C with 5% CO_2. The cells in passages 2–6 were used for testing. Experiments were conducted using cells in the exponential growth phase. Both agents were dissolved in H_2O to create stock solutions, which were subsequently diluted to the required concentrations for analysis. Since this was an in vitro study, ethics committee approval was not required.

Cell Viability Assay

The proliferation of cells treated with different concentrations of IL-1β, IL-6, Canakinumab, and Tocilizumab was assessed using the 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, which measures mitochondrial dehydrogenase activity in living cells.[28] An MTT assay was conducted to determine the viability of Human Mesenchymal Bone Marrow Derived Adult Stem Cells after treatment with cytokine and antagonist antibody agents, and their combination, and the IC_{50} was defined as the concentration of the agent required to block 50% cell viability. For this purpose, an equal number of hBMMSC cells (1 x 10^5 cells/well) were cultured in 96-well plates and allowed to attach overnight. Since the stem cells are adult, too much cell division is not desired. When the cell confluence was almost %80, the cells were then treated as different groups as Group I: 5 ng/mL IL-1β + 50 ng/mL IL-6 (Cyt I), Group II: 10 ng/mL IL-1β + 100 ng/mL IL-6 (Cyt II), Group III: 1 µg/mL Canakinumab + 1 µg/mL Tocilizumab (Mab), Group IV: 5 ng/mL IL-1β + 50 ng/mL IL-6 + 1 µg/mL Canakinumab + 1 µg/mL Tocilizumab (Comb I), Group V: 10 ng/mL IL-1β + 100 ng/mL IL-6 + 1 µg/mL Canakinumab + 1 µg/mL Tocilizumab (Comb II) and incubated at 37°C for 48h and 72h. At the end of the time, 10 µL of 5 mg/mL MTT was added to each well and incubated at 37°C for 4 h. Subsequently, the cell culture medium was removed, and 100 µl DMSO was added to dissolve formazan crystals and the absorbance of each well was measured at 570 nm using a Multiskan SkyHigh Microplate Reader (Thermo Fisher, Waltham, Massachusetts, USA). The percentage of cell growth inhibition was calculated as follows: cell inhibition percentage (%): (mean absorbance in test wells/mean absorbance in control wells) x100. All experiments were repeated six

times for each concentration and independently replicated for a minimum of three times.

Cytotoxicity Assay

Cytotoxicity was evaluated by lactate dehydrogenase (LDH) release assay using Lactate Dehydrogenase Colorimetric Activity Kit (Invitrogen Cat No. EEA013) assay.[28] The Cytotoxicity kit used to determine whether cytokines or antagonist antibodies have any cytotoxic effect on hBMMSC lines. Briefly, hBMMSC were cultured in a 96-well plate at $1x10^5$ cells/well for 24 h. Since the stem cells are adult, too much cell division is not desired. When the cell confluence was almost %80, the cells were then treated as different groups as Group I: 5 ng/mL IL-1β + 50 ng/mL IL-6 (CytI), Group II: 10 ng/mL IL-1β + 100 ng/mL IL-6 (Cyt II), Group III: 1 µg/mL Canakinumab + 1 µg/mL Tocilizumab (Mab), Group IV: 5 ng/mL IL-1β + 50 ng/mL IL-6 + 1 µg/mL Canakinumab + 1 µg/mL Tocilizumab (Comb I), Group V: 10 ng/mL IL-1β + 100 ng/mL IL-6 + 1 µg/mL Canakinumab + 1 µg/mL Tocilizumab (Comb II) for an additional 48h. This assay was performed according to the manufacturer's protocol. The optical density was measured at 490 nm with a Multiskan SkyHigh Microplate Reader (Thermo Fisher, Waltham, Massachusetts, USA). The percentage of LDH release was calculated according to the formula provided in the manufacturer's protocol. All experiments were performed in three replicates in three independent experiments.

RNA Isolation and Quantitative Real-Time PCR Analysis

Cells were treated with agents and antagonist antibodies for polarity and regeneration genes expression study. Treatment groups are Group I: 5 ng/mL IL-1β + 50 ng/mL IL-6 (Cyt I), Group II: 10 ng/mL IL-1β + 100 ng/mL IL-6 (Cyt II), Group III: 1 µg/mL Canakinumab + 1 µg/mL Tocilizumab (Mab), Group IV: 5 ng/mL IL-1β + 50 ng/mL IL-6 + 1 µg/mL Canakinumab + 1 µg/mL Tocilizumab (Comb I), Group V: 10 ng/mL IL-1β + 100 ng/mL IL-6 + 1 µg/mL Canakinumab + 1 µg/mL Tocilizumab (Comb II) for 48h. TRIzol Reagent was used to extract total RNA from excised cell culture with several concentrations of agent combinations (~100 mg) per the manufacturer's instructions. Total RNA concentration was then quantified at 260 nm and 280 nm using Nano-Drop 1000 spectrophotometer (Thermo Fisher Scientific). cDNA synthesis was performed from 1 µg of total RNA with a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, USA), following the manufacturer's protocols. The cDNA samples were stored at -80 °C until use. qPCR reactions were then performed using the Applied Biosystems 7300 Real-Time PCR System with a TaqMan™ Master Mix for qPCR according to the manufacturers' instructions. The qPCR primers for Pard3, Mapk8, Dmp1, Yap1, Junb, Mepe, Mmp13, Mmp2 genes were designed using the Neoformit database program. Table 1 shows the gene-specific primer sequences (in the 5'-3'direction) for each gene used for the qPCR reaction. The mRNA level of Actin β (Beta-actin, housekeeping gene) was used to normalize the levels of the target genes. Thermal cycling conditions were as follows: denaturation at 95 °C for 10 min, 45 cycles of amplification: 95 °C for 10 sec, 60 °C for 20 sec and cooling at 40 °C for 30 sec. Expression levels of target genes were determined employing the $2^{-\Delta\Delta CT}$ method.[29,30] Samples from each experiment were used in triplicate.

Table 1. Gene-specific primer sequences used in qPCR.

Gene	Forward primer (5'- 3')	Reverse primer (5'- 3')
Pard3	TCAGCTCCTACCTATCCCGG	CTCAACTCCCAAGTCAGGCC
Mapk8	AATGGCGTGATCTTGGCTCA	GGGAGGCTGAGGTAGGAGAA
Dmp1	CTTCTCAGAGGAAAGCCCGG	GAGCTGCTGTGAGACTGGAG
Yap1	CCTCTCCAGCTTCTCTGCAG	TGGGCCAGAGACTACTCCAG
Junb	TGGCCTCTCTCTACACGACT	CTTTGAGACTCCGGTAGGGG
Mepe	TGCAACAAGGGTGTGCAGTA	ATGGGGTCTCGCAAATGTGT
Mmp13	CACCATGATGTAGGAGCCCC	GGCTGATCTGCTGATGGACA
Mmp2	TGAAGCACAGCAGGTCTCAG	TCAAACCAGGCACCTCCATC
Actb	AGACCTGTACGCCAACACAG	TTCTGCATCCTGTCGGCAAT

UPL: Universal Probe Library; qPCR: Quantitative Real Time Polymerase Chain Reaction

Statistical Analysis

Statistical analyses were performed with the help of SPSS 16.0 (IBM, USA). GraphPad Prism 6 software (GraphPad Software Inc., San Diego, CA, USA) was utilized to ascertain the concentration at which 50% cytotoxicity (IC50) was achieved.[31] Viability assay and LDH release assay (with Bonferroni post hoc test) results were analyzed using student t-test, qPCR analysis was analyzed using analysis of variance (ANOVA) test. Combination Index curves and Dose-effect curves calculated and visualized using Compusyn (BioSoft, Cambridge UK).[32] The Relative Expression Software Tool (REST® 2009 v2.013) was used to assess the mRNA levels of EMT-related genes, and the GeneGlobe Data Analysis Center (Qiagen) verified the results.[29,30] Every single statistic was displayed as Mean ± Standard Deviation (SD), with *P<0.05* deemed statistically significant. The reporting of this study conforms to STROBE guidelines.[33]

RESULTS

Effects of IL-1, IL-6, Canakinumab and Tocilizumab on Cell Viability

The hBMMSC cell viability was determined by the MTT assay (Figure 1). hBMMSC cells were treated with IL-1β, IL-6, Canakinumab, Tocilizumab for 48 and 72 h. The administration of Mab group for 48, and 72 h did not alter the viability of the cells at the concentrations (P>0.05). Administration of Cyt I group (5 ng/mL IL-1β + 50 ng/mL IL-6) to hBMMSC culture resulted in a slightly diminished cell viability, (P<0.05), but it significantly decreased at higher concentrations when the Cyt II (10 ng/mL IL-1β + 100 ng/mL IL-6) administered (P<0.05). The effects of cytokines and antagonist antibodies combined treatments on cell viability are shown in Comb I and Comb II. After 48 h treatment, the lowest cell viability (37.41%) was observed in the Cyt II group. It was determined that high dosage of cytokine treatments significantly decreased cell viability below 50% in both 48 and 72 h (Figure 1). But the combined treatments led to a normalization in cell viability compared to individual use of the agents. According to the results obtained from the combination index analysis and dose-effect curve, Canakinumab and Tocilizumab showed antagonism against IL-1β and IL-6 in the treatment of RA model of hBMMSC even if there is not immunity cell to effect (Figure 2).

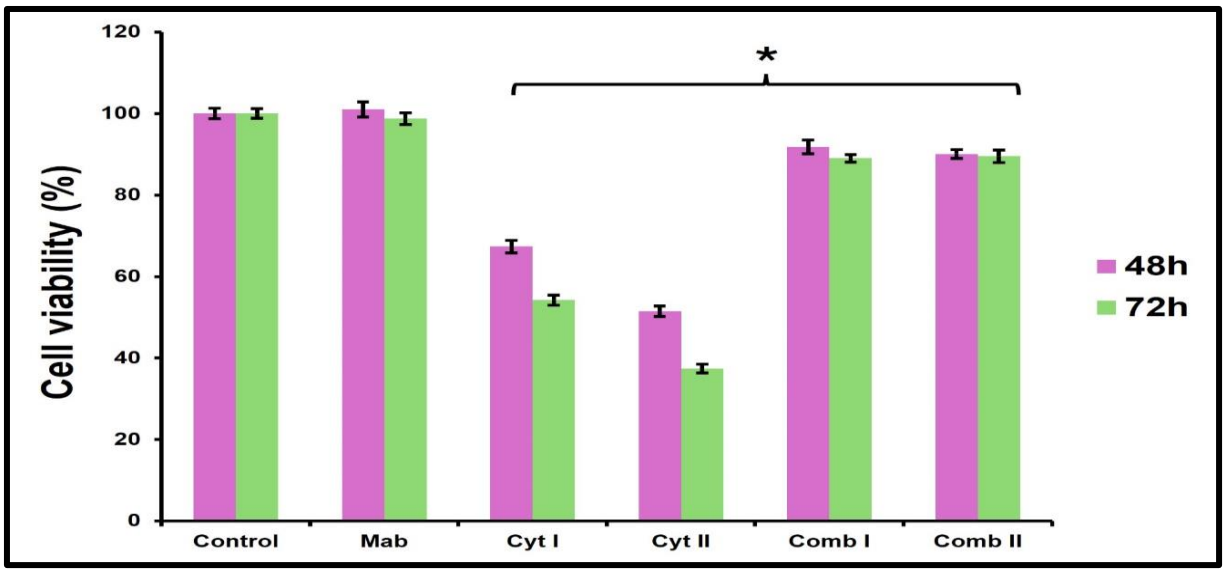

Figure 1. Demonstration of cell viability of groups consist of different combinations of con-centration of IL-1β, IL-6, Canakinumab, Tocilizumab applied to hBMMSC at 48h and 72h. The results shown are representative of the 3 independent experiments. Data were expressed as mean ± standard deviation. *P<0.05 according to the student's t-test.

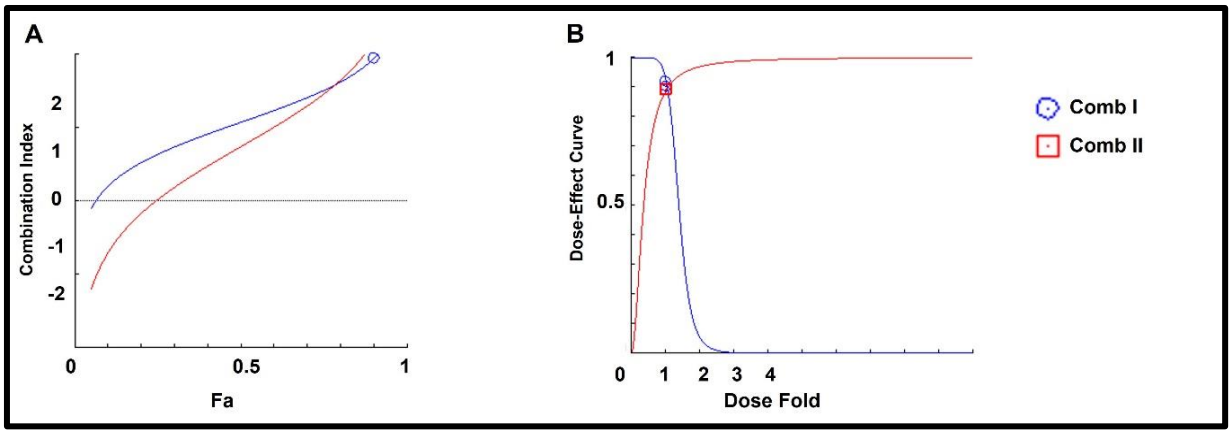

Figure 2. Combination Index Drug Synergy Analysis (CI) of the IL-1β, IL-6, Canakinumab and Tocilizumab synergetic affiliation (A), Dose-Effect Curve demonstration of the combina-tion groups (B). Linear regression was used to render the CI values. Trendlines demonstrate CI values for any expected effect (Fa, fraction affected, 0 to 1; 0-%100 inhibition), and CI values <1, =1, >1 indicate synergy, additivity, or antagonism, respectively.

Effects of IL-1, IL-6, Canakinumab and Tocilizumab on Cytotoxicity

The cytotoxic effects of IL-1β, IL-6, Canakinumab and Tocilizumab on hBMMSC were evaluated by LDH release assay. The individual administration of IL-1β and IL-6 as Cyt I and Cyt II group induced cytotoxicity in the RA model of hBMMSC treated for 48 h. Cytotoxicity was very low and similar in hBMMSC exposed to a selected con-centration of Canakinumab and Tocilizumab as Mab group (1 µg/ml for each) at 48 h. The percentages of LDH release at Cyt I and Cyt II were as follows: 34.05% and 55.47% (P<0.05), respectively. The treatment of hBMMSC with monoclonal antibodies decreased cytokine caused a dose-dependent release of LDH (5 ng/ml IL-1β + 50 ng/ml IL-6) (P<0.05). Results show that combined treatment of cy-tokines and monoclonal antibodies markedly decreased the cytotoxic effects of cytokines alone in hBMMSC (Figure 3).

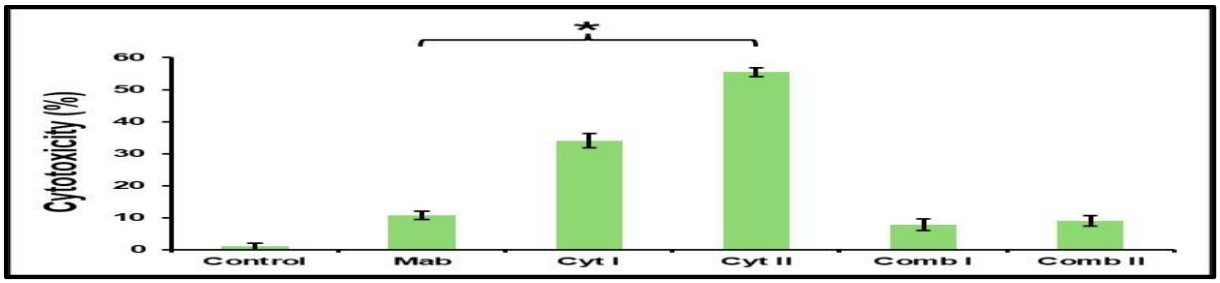

Figure 3. Demonstration of Effect of IL-1β, IL-6, Canakinumab and Tocilizumab on cytotox-icity determined by LDH release in hBMMSC. The results shown are representative of the 3 independent experiments. Data were expressed as mean ± standard deviation. *P<0.05 ac-cording to the student's t-test.

Effects of IL-1, IL-6, Canakinumab, Tocilizumab on mRNA Levels of Polarity and Regeneration-Related Genes In Vitro

The mRNA levels of Pard3, Mapk8, Dmp1, Yap1, Junb, Mepe, Mmp13, Mmp2 genes were evaluated by qPCR. The levels of Pard3 and Dmp1 mRNA significantly de-creased in the Cyt I and Cyt II groups. The mRNA levels of Mapk8, Yap1, Junb, Mepe, Mmp13, Mmp2 genes in Cyt I and Cyt II groups were significantly higher than com-bination groups Comb I and Comb II (P<0.05). Moreover, the mRNA expression levels of Mapk8, Yap1, Junb, Mepe, Mmp13, and Mmp2 were observed to be lower in the com-bination groups, particularly in Comb I, compared to the Cyt I and Cyt II groups, approaching closer to the normal-ization threshold. *(*P<0.05) (Figure 4).

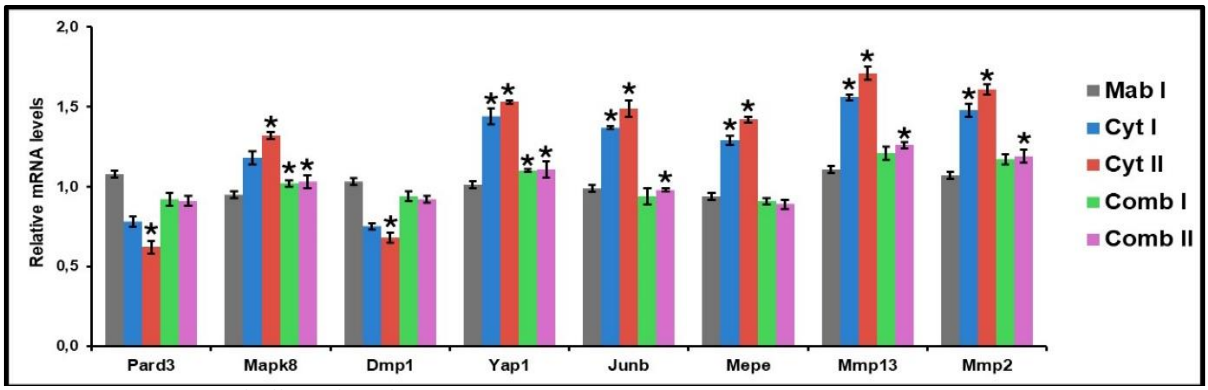

Figure 4. Demonstration of Effects of IL-1β, IL-6, Canakinumab and Tocilizumab on Polarity and Regeneration-related mRNA levels in hBMMSC culture. Data were expressed as mean ± standard deviation. The results shown are representative of the 3 independent experi-ments. *P<0.05 according to the ANOVA test.

DISCUSSION

The effects of IL-1β and IL-6 on human bone marrow mesenchymal stem cells (hBMMSCs) are highlighted in this work, along with the possible therapeutic advantages of combining IL-1β antagonist canakinumab with IL-6 antagonist tocilizumab. The results provide fresh insights into the regulation of inflammatory responses in RA models and are consistent with other studies on the etiology of rheumatoid arthritis (RA).

The observed decrease in cell viability and increase in cytotoxicity in hBMMSCs treated with IL-1β and IL-6 highlight the destructive nature of these cytokines in RA. Even in the recent studies IL-1β and IL-6 are shown to play crucial roles in the inflammatory processes of RA by promoting the recruitment and activation of inflammatory cells, leading to joint damage and erosion.[9,10] It has also been observed that these cytokines stimulate the formation of matrix metalloproteinases (MMPs), which break down components of the extracellular matrix and aid in tissue death.[19]

The combination therapy with canakinumab and tocilizumab demonstrated a significant protective effect on hBMMSCs, reducing cytotoxicity and improving cell viability. This synergistic effect can be attributed to the complementary mechanisms of action of these antagonists. Canakinumab specifically targets IL-1β, a key mediator of acute inflammation, while tocilizumab inhibits IL-6 signaling, which is proven from former studies, involved in chronic inflammation and the promotion of autoimmunity.[24] The combination of these antagonists can effectively reduce the inflammatory burden in RA, offering a potential therapeutic strategy for managing the disease.

In our study, we deliberately selected key genes associated with polarity and regeneration to elucidate the molecular mechanisms influenced by inflammatory events in bone marrow stem cells. Significant alterations in the expression of polarity and regeneration-related genes were seen in hBMMSCs treated with cytokines and antagonists, according to gene expression analysis. The downregulation of polarity-related genes (Pard3, Dmp1) in response to cytokines IL-6 and IL-1β indicates disruption of cellular orientation and structure, which are demonstrated to pivotal for maintaining tissue integrity.[34] As it was already in the literature, the upregulation of regeneration-related genes (Mapk8, Yap1, Junb, Mepe, Mmp13, Mmp2) suggests an attempt by the cells to repair and regenerate damaged tissues in the presence of inflammation.[35]

The application of canakinumab and tocilizumab reversed these gene expression changes, promoting normalization and enhancing the regenerative capacity of hBMMSCs. This finding supports the recent studies about notion that targeting specific inflammatory pathways can restore cellular functions and improve tissue repair processes in RA.[26] The results align with previous studies demonstrating the benefits of cytokine inhibitors in reducing inflammation and promoting tissue regeneration in RA models.[18,22]

The significant reduction in cytotoxicity observed in the combined treatment groups further emphasizes the potential of combination therapy in RA management. By concurrently targeting multiple cytokines, it is possible to

achieve a more comprehensive suppression of inflammatory pathways, leading to better clinical outcomes as previous research claims.[11] This approach is particularly relevant in RA, where multiple cytokines and signaling pathways contribute to disease progression and chronic inflammation.[18]

In addition to the observed cellular and molecular effects, the study also highlights the importance of early interruption in RA. The application of cytokine antagonists in the early stages of inflammation can prevent irreversible tissue damage and preserve joint function. Previous studies have demonstrated that early treatment with such medicines improves outcomes over time in individuals with RA by lowering disease activity and preventing structural damage.[6,19]

The findings of this study have important implications for the development of treatment strategies in RA. Through the identification of distinct cytokine profiles and molecular markers related to the advancement of the disease, guided medicines can be developed, resulting in increased therapeutic effectiveness and reduced side effects.[21] Guided medicine approaches in RA are gaining increasing attention, with ongoing research focused on identifying biomarkers and developing targeted therapies.[11,19]

Future studies should aim to explore and define the detailed molecular mechanisms underlying the synergistic effects of cytokine antagonists in RA models. Researching the connections between various cytokines and the signaling pathways they interact with will shed light on the pathophysiology of RA and help create more potent treatment plans.[25] Furthermore, to confirm the results of this investigation and evaluate the security and effectiveness of combination treatments in RA patients, clinical trials are required.[22,25]

In conclusion, this study demonstrates the potential benefits of combining IL-1β and IL-6 antagonists in an in vitro RA model. The findings support the use of combination therapy to enhance treatment efficacy and improve patient outcomes in RA. The study also underscores the importance of early intervention and guided medicine approaches in managing this complex disease. It is necessary to do additional study to investigate the molecular mechanisms and therapeutic implications of these discoveries.

Conflict of Interest

The authors declare that there is not any conflict of interest regarding the publication of this manuscript.

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Ethics Committee Permission

Since this was an in vitro study, ethics committee approval was not required.

Authors' Contributions

Concept/Design: MO. Data Collection and/or Processing: MO. Data analysis and interpretation: MO. Literature Search: MO. Drafting manuscript: MO. Critical revision of manuscript: MO.

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