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Evaluating Mannuronic Acid Effect on Gene Expression Profile of Inflammatory Mediators in Rheumatoid Arthritis Patients

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ABSTRACT

Rheumatoid arthritis (RA) is a multisystem disorder. Various studies have shown the important role of inflammatory factors tumor necrosis factor α (TNF-α), interleukin (IL)-6, IL-22, MYD88, and toll-like receptor 2 (TLR2) in this disease. In this study, we investigated the anti-inflammatory effects of B-D-Mannuronic acid (M2000), as a new immunosuppressive drug, on the expression of these inflammatory markers in peripheral blood mononuclear cells (PBMCs) of RA patients.

The blood samples of 24 active RA patients and healthy volunteers were used for PBMCs separation. The cells were cultured with LPS (1 μg/mL), low (5 μg/mL), moderate (25 μg/mL), and high (50 μg/mL) doses of M2000 and a single dose of diclofenac (1 μg/mL) to evaluate *TNF-a*, *IL-6*, *IL-22*, *MYD88*, and *TLR2* genes expression by quantitative real-time (qRT-PCR). Cell surface expression and MFI of TLR2 were assessed; using flow cytometry.

Our findings exhibited a significant reduction of *TNF-a*, *IL-6*, and *MYD88* gene expressions after treatment with three doses of M2000 and an optimum dose of diclofenac. *TLR2* gene expression was significantly diminished by moderate and high doses of M2000 and a single dose of diclofenac. Moreoversurface expression of TLR2 was significantly downregulated by moderate and high doses of M2000, while MFI of this was significantly reduced by three doses of M2000.

The results of this research showed that M2000 was able to significantly reduce the gene expression of inflammatory markers TNF-α, IL-6, MYD88, and TLR2 in patients PBMCs. These data revealed a part of the mechanisms of M2000 in the treatment process.

Keywords: Interleukin-6; Mannuronic acid; MYD88 deficiency; Toll-like receptor 2; Tumor necrosis

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INTRODUCTION

Rheumatoid arthritis (RA) is an inflammatory autoimmune and relatively common disease, characterized by synovial cells hyperplasia and chronic inflammation of the synovium, which leads to bone and cartilage destruction.[1,2] Its spread has been estimated at 0.5-1% worldwide. Both innate and adaptive immune systems have an important role in its immunopathogenesis. In particular, pro-inflammatory cytokines, such as tumor necrosis factor α (TNF-α), interleukin 6 (IL-6), interleukin 22 (IL-22), and innate immune receptors such as toll-like receptor 2 (TLR2) as well as myeloid differentiation primary response 88 (MYD88), as an adaptor protein of this receptor, that have fundamental roles in the pathogenesis of RA. Overall, the levels of these factors are essentially high in serum and synovial fluid (SF) of patients with RA, correlated with the disease activity.[3,4] TNF-α, as one of the most important inflammatory cytokines in RA, is produced mainly by stimulated macrophages and monocytes as well as various subtypes of lymphocytes, dendritic cells (DCs), natural killer (NK), and endothelial cells. It is a key multifunctional factor in the pathogenesis of RA that stimulates inflammatory and tissue-resident cells such as B and T lymphocytes, monocytes, macrophages, and osteoclasts towards the releasing of inflammatory mediators including cytokines, chemokines, proteases, autophagic, angiogenic factors, and osteoclastogenic mediators. TNF-α also increases the differentiation, proliferation, and activation of Th22 and Th17 lymphocytes as well as M1 macrophages.[4-7] Therefore, TNF-α blockade can be an effective target in ameliorating symptoms, inflammatory processes and essentially be considered as the first purpose for therapy in RA. IL-6, as the second cytokine involved in RA, is a multi-practical protein. Macrophages have been ascertained as primary sources of IL-6 production. However, other cells such as B and Th17 lymphocytes, monocytes, fibroblasts, and keratinocytes secret it, as well Given that IL-6level is high in autoimmune disorders such as RA, it has been proposed that its inhibition is an effective therapeutic strategy for the treatment of patients with RA.[8,9] Many investigations clarified that overproduction and hyperactivity of TLRs, particularly TLR2, are crucial in inflammation sustainability and destructive processes in RA.TLR2 diagnoses pathogen-associated molecular patterns (PAMPs) such as exogenous ligands (proteoglycans, peptidoglycan, lipoteichoic acid, etc.) and damage-associated molecular patterns (DAMPs) such as serum amyloid A (SAA) and heat shock proteins (HSPs). These ligands are highly expressed in PB, SF, and synovium tissue of RA patients, and their expression is correlated with the disease activity. In several animal models, bacterial compounds have been used for TLR2 stimulation and induction of experimental arthritis.[10] It has been proved that TLR2 signaling in $CD4^+$ T cells promotes the differentiation of Th17 and Th9 cells. On the other hand, stimulation of TLR2 can lead to M1-polarized macrophages and pro-inflammatory cytokines production.[11,12]

Disease-modifying anti-rheumatic drugs (DMARDs) are selective drugs for RA patients' treatment, whereas,non-steroidal anti-inflammatory drugs (NSAIDs) are considered as these cond line therapy in RA. Despite the efficacy of DMARDs in RA patients, their toxicity has restricted their usage. On the other side, following the NSAIDs consumption, several side effects such as gastrointestinal disorders and cardiovascular diseases (CVD) as well as glomerulonephritis can be seen. Furthermore, TNF-α and IL-6 antagonists increase blood hemoglobin (Hb) levels and infectious diseases risk in these patients.[13] TheM2000, patented (DE-102016113018), as an anti-inflammatory agent with low molecular weight (194.139Da) and biological source belongs to the NSAIDs family, unlike most of the other members of this family has no side effects and toxicity for animals and humans. Moreover, the anti-inflammatory and immunomodulatory effects and safety of this drug have been approved in various animal models like adjuvant-induced arthritis (AIA), experimental autoimmune encephalomyelitis (EAE), nephrotic syndrome, and acute glomerulonephritis as well as several clinical trials. Furthermore, the potent efficacy and safety of β-D-Mannuronic acid, as a new anti-inflammatory drug, have been reported in an international phase III clinical trial of this drug in RA patients.[13-16] Based on the above-mentioned context, we aimed to examine the efficacy of M2000 on TNF-α, IL-6, IL-22, MYD88, TLR2 genes expression, and TLR2 cell surface expression and MFI in PBMCs of RA patients.

MATERIALS AND METHODS

Study Design

Ethical Approval

This study was confirmed by the Ethical Committee of Tehran University of Medical Sciences with permission reference number (IR.TUMS.SPH.REC.1396.2092).

RA Patients and Healthy Volunteers Selection

12 qualified RA patients based on the American College of Rheumatology (ACR) criteria and disease activity score 28-joint (DAS28)>2.6 were enrolled in this study (10 females and 2 males, 30-60-year old) and 12 healthy individuals were considered with the similar sex and age. Moreover, the patients had inadequate responses to conventional drugs and prevalent therapies. These patients did not receive biological and NSAID drugs. The patients were consuming conventional [methotrexate (MTX), sulfasalazine (SSZ), hydroxychloroquine (HCQ), and prednisolone (PRD)] medications. Furthermore, the RA patients with active disease were assigned based on having 3 and/or more than 3 (≥ 3) swollen joints and ≥ 6 tender joints in 28-joint. Inclusion criteria were a minimum of three swollen joints and six tender joints and at least two of the following items: the high levels of erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) (Table 1). The qualified participants were included if they were receiving MTX with a dose of 15–20 mg/week, SSZ 500–1000 mg/day, HCQ 400 mg/day, and PRD 5–15 mg/day for at least 6 months.

Table 1. The clinical and paraclinical characterizations of the rheumatoid arthritis (RA) patients.

Index	patients (n=12)
DAS28	4.46±0.23
ACR	6.58±0.52
Tender joints count	4.00±0.56
Swollen joints count	2.33±0.64
ESR	22.33±3.83
CRP	41.7% (Positive)
RF	58.3% (Positive)
Anti-CCP	207.75±83.43

ACR, American College of Rheumatology; Anti-CCP, Anti-citrullinated peptide antibody; CRP, C-reactive protein; DAS28, Disease activity score 28 joints; ESR, Erythrocyte sedimentation rate; RF, rheumatoid factor.

M2000 Preparation

Production of M2000 (β-D-Mannuronic acid) with the molecular formula $C_6H_{10}O_7$ was carried out at Tehran University of Medical Sciences from Alginic Acid Sodium Salt (Sigma-Aldrich, St. Louis, Mo), based on the modified method of Mirshafiey et al. Its purity was validated via Fourier Transform Infrared Spectroscopy (FT-IR) and Carbon-13 (^{13}C) nuclear magnetic resonance (C-NMR).

Culture and Treatment of PBMCs

Venous blood samples were collected from RA patients and healthy volunteers. After PBMCs isolation by Ficoll-Paque (Amersham Pharmacia Biotech, Sweden), these cells' viability was measured; using trypan blue staining. PBMCs were cultivated in Roswell Park Memorial Institute Medium (RPMI)-1640, enriched by 9% Phosphate-Buffered Saline (FBS) and 1% penicillin/streptomycin (USA). Following the stimulation of the cells with LPS (1 µg/mL) (USA), they were incubated at 37˚C with 5% CO2. After 4 hours, the patients' cells were taken out from the incubator and treated with low, moderate, and high (5, 25, 50 µg/mL, respectively) doses of M2000 and optimum dose (1 µg/mL) of diclofenac. They were then incubated for 18 hours. Afterward, the cells were taken out and wells contents were centrifuged in falcon tubes and cells harvest was done. Oneml Trizole (Korea) was added to half of them and they were assigned to Real-Time PCR tests. Another part of the cells was transmitted to cryotubes for flow cytometry analysis and 1 ml freezing buffer (containing FBS 80%, RPMI10%, and Dimethyl sulfoxide (DMSO 10%) (USA) was added to them. The cryotubes and

microtubes were then stored in a -20˚C freezer. Thereafter, they were transferred to a -70˚C freezer. It should be noted that the cells of healthy volunteers and the untreated cells of the patients were treated with LPS for 18 hours, exclusively. The cells, considered for flow cytometry examination, were put into liquid nitrogen after spending an overnight at -70˚C.

Gene Expression Assay

RNA Extraction and cDNA Synthesis

Total RNA was elicited from the PBMCs of the patients and healthy volunteers; using a total RNA purification kit (Hybrid R Gene All, Seoul, Korea) based on the manufacturer's protocol. RNA concentration and its purity were assessed by Nanodrop 2000 UV spectrophotometer (Isogen Life Science, Netherlands). All of the samples with 260/280 and 260/230 ratios in the range of 2-2.2 were selected for cDNA synthesis. In the end, cDNA was synthesized from the RNA with a concentration of 400 ng based on the protocol of the cDNA synthesis kit (Takara Co., Ltd., Dalian, China).

Quantitative Real-time Polymerase Chain Reaction (qRT-PCR)

The qRT-PCR was accomplished using *SYBER* PREMIX EX TAG II (TAKARA, JAPAN*)* and specific primers for *TNF-α*, *IL-6*, *IL-22*, *MYD88*, and *TLR2* genes (Bioneer, Korea) (Table S1), using ABI STEP ONE PLUS Real-time PCR (*ABI, USA)*. The relative quantification of target genes mRNA compared with internal control, Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) mRNA, was calculated by the $2^{-\Delta\Delta}C_T$ method; using an amplification plot (fluorescence signal *vs.* cycle number).

Flow cytometry

To perform the flowcytometry examinations, the cells were taken out from the liquid nitrogen and thawed at 37˚C. After washing the cells with RPMI-1640 and flow cytometry buffer (FBS 5%, Phosphate-Buffered Saline (PBS 95%), the viability of the cells was measured. Afterward, the sediment of the cells was divided into 2tubes and the cells were then stained with phycoerythrin (PE)-Anti-human TLR2 and mouse IgG2ak as the isotype control, separately. In the next stage, the cells were incubated at 4˚C and darkness for 30 minutes and were washed by flow cytometry buffer, and cell surface expression of TLR2 was then evaluated; using BD Fluorescence-activated cell sorting (FACS) calibur flow cytometer (Partech, United Kingdom). The data were analyzed by FlowJo7.6.1 software.

Statistical Analysis

The statistical analysis was performed; using a statistical package for the social sciences (SPSS) software (24 O: IBM Corporation, Armonk, NY, USA) and the results were reported as Mean±SEM. Independent sample T-test and paired sample T-test were used for intergroup and intragroup comparisons of normally distributed data, while, Mann–Whitney U test and Wilcoxon Signed Rank Test was utilized for the same comparisons of abnormally distributed data. The statistical significance was categorized as *$p\leq0.05$, **$p\leq0.01$, and ***$p\leq0.001$. $p\leq0.05$ was considered statistically significant. The statistical significance was categorized as *$p\leq0.05$, ** $p\leq0.01$, and *** $p\leq0.001$.

RESULTS

Real-time PCR Findings

Effect of M2000 on *TNF-α* Gene Expression

Our findings showed a significant difference in *TNF-α* gene expression in untreated cells of the patients compared to the healthy volunteers *(*2.54-fold, $p<0.01$). In comparison to the untreated cells of the patients, treatment of the patients' PBMCs with a moderate dose of M2000 (25 µg/mL) resulted in a significant reduction in this gene expression (0.03-fold, $p<0.001$). Furthermore, after treatment of these cells with low (5 µg/mL) and high (50 µg/mL) doses of M2000, a significant reduction of this gene expression was observed, statistically (0.02, 0.02-fold, respectively, $p<0.01$). Moreover, treatment of the patients' cells with optimum dose (1 µg/mL) of diclofenac decreased *TNF-α* gene expression in comparison to the untreated cells, significantly (0.18-fold, $p<0.01$). Collectively, treatment of the cells with three doses of M2000 (low, moderate, and high) compared to diclofenac single dose reduced gene expression of *TNF-α*, significantly (0.17, 0.12, 0.12-fold, respectively, $p<0.05$) (Figure 1*)*.

Effect of M2000 on *IL-6* Gene Expression

Our results indicated a significant difference in gene expression of *IL-6* in the untreated cells of the patients compared to the healthy volunteers (1.82-fold, $p<0.05$). In comparison to the untreated cells of the

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patients, treatment of the cells with low (5 µg/mL), moderate (25 µg/mL), and high (50 µg/mL) doses of M2000 diminished this gene expression, significantly (0.15, 0.11,0.05-fold, respectively, $p<0.01$).

The optimum dose (1 µg/mL) of diclofenac declined this gene expression compared to the untreated cells, significantly, as well (0.15-fold, $p<0.01$) (Figure 2).

Effect of M2000 on *MYD88* Gene Expression

Our findings represented a significant difference in this gene expression in untreated cells of the patients compared to the healthy volunteers (2.81-fold, $p<0.01$).Treatment of the patients cells with low (5 µg/mL), moderate (25 µg/mL), and high (50 µg/mL) doses of M2000 declined this gene expression, significantly (0.11, 0.07, 0.06-fold, orderly, $p<0.01$).

Furthermore, in comparison to the untreated cells the optimum dose (1 µg/mL) of diclofenac diminished *MYD88* gene expression, significantly (0.013-fold, $p<0.01$).

The expression of *MYD88* in PBMCs, treated with a high dose of M2000 compared to a low dose of this drug was downregulated, significantly (0.6-fold, $p<0.05$) (Figure 3).

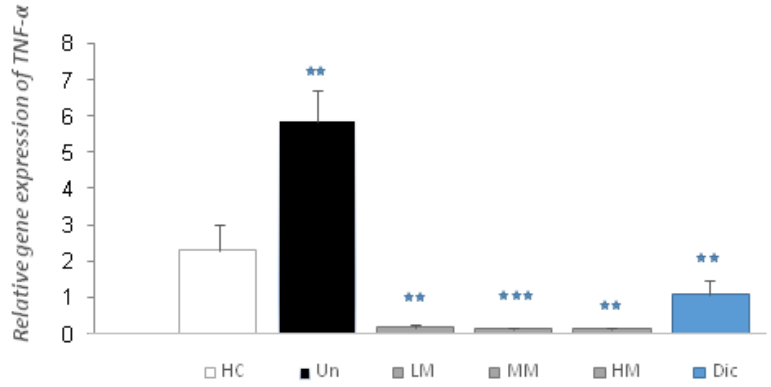

Figure 1. Comparison of *TNF-α* gene expression between the studied groups.
HV: Healthy volunteer; Un: Untreated; LM: Low dose Mannuronic acid; MM: Moderate dose Mannuronic acid; HM: High dose Mannuronic acid; Dic: Diclofenac. The statistical significance was classified as* $p\leq0.05$, ** $p\leq0.01$, or *** $p\leq0.001$. $p\leq0.05$ was considered as statistically significant.

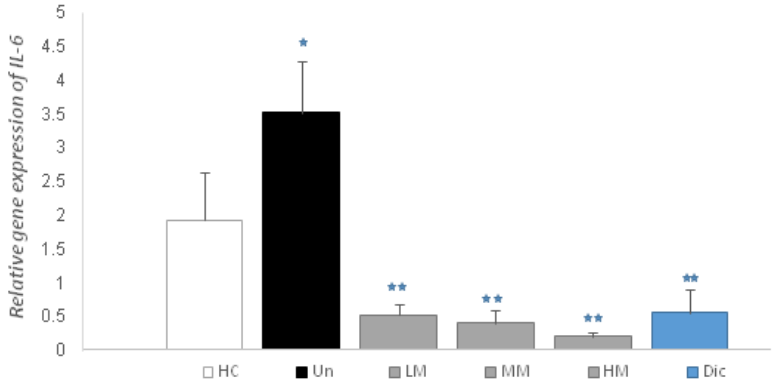

Figure 2. Comparison of *IL-6* gene expression between the studied groups.
HV: Healthy volunteer; Un: Untreated; LM: Low dose Mannuronic acid; MM: Moderate dose Mannuronic acid; HM: High dose Mannuronic acid; Dic: Diclofenac. The statistical significance was classified as* $p\leq0.05$, ** $p\leq0.01$. $p\leq0.05$ was considered as statistically significant.

Effect of M2000 on *TLR2* Gene Expression

Our results represented higher gene expression of *TLR2* (not significant) in untreated cells of the patients compared to the healthy volunteers (2.56-fold, $p>0.05$).

In comparison to untreated cells, treatment of the patients' cells with moderate and high doses of M2000 declined *TLR2* gene expression in these cells, significantly (0.33, 0.16-fold orderly, $p<0.01$). Moreover, treatment of the patients' cells with diclofenac in optimum dose resulted in *TLR2* gene expression reduction, significantly (0.71-fold, $p<0.05$).

Treatment of the cells with moderate and high doses of M2000 in comparison with the low dose formula significantly downregulated the *TLR2* gene expression (0.40, 0.20-fold, respectively, $p<0.01$). Furthermore, moderate and high doses of M2000 caused a significant reduction of *TLR2* gene expression compared to diclofenac (0.46, 0.23-fold, $p=0.010$, $p<0.05$, respectively)(Figure 4).

Flowcytometry Findings

Effect of M2000 on TLR2 Cell Surface Expression

Comparison of TLR2 cell surface expression between the healthy volunteers' cells and untreated cells of the patients demonstrated that TLR2 expression was significantly higher in the patients' cells than the healthy volunteers ($p<0.05$). In comparison with the

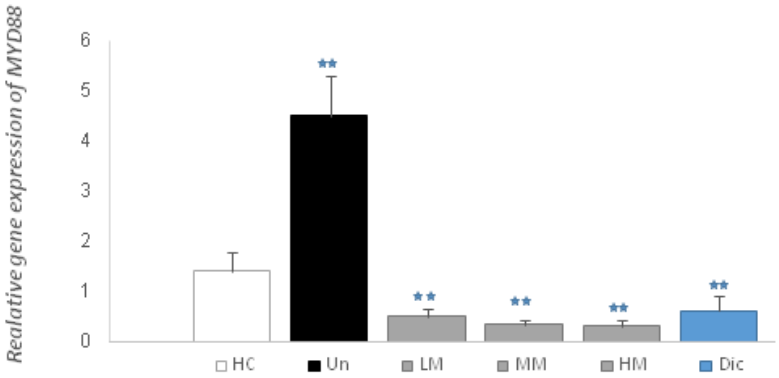

Figure 3. Comparison of *MYD88* gene expression between the studied groups.
HV: Healthy volunteer; Un: Untreated; LM: Low dose Mannuronic acid; MM: Moderate dose Mannuronic acid; HM: High dose Mannuronic acid; Dic: Diclofenac. The statistical significance was classified as *$p\leq0.05$ and **$p\leq0.01$. $p\leq0.05$ was considered as statistically significant.

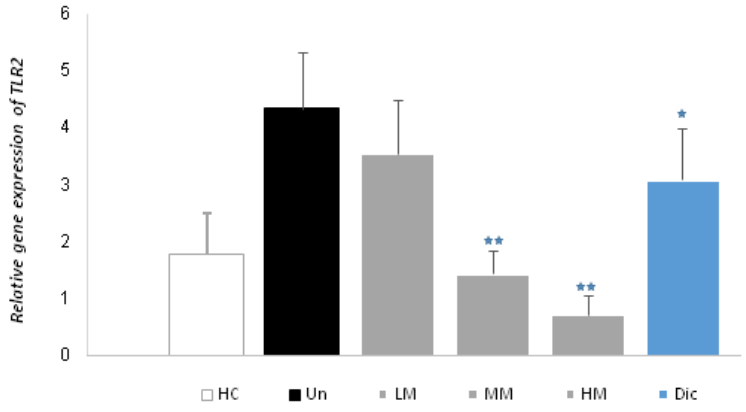

Figure 4. Comparison of *TLR2* gene expression between the studied groups.
HV: Healthy volunteer; Un: Untreated; LM: Low dose Mannuronic acid; MM: Moderate doseMannuronic acid; HM: High dose Mannuronic acid; Dic: Diclofenac. The statistical significance was classified as *$p\leq0.05$ and **$p\leq0.01$. $p\leq0.05$ was considered as statistically significant.

untreated patients' cells, treatment of the cells with moderate (25 µg/mL) and high (50 µg/mL) doses of M2000 decreasedTLR2 cell surface expression, significantly (p<0.001). A moderate dose of Mannuronic acid compared to its low dose reduced TLR2 cell surface expression, significantly (p<0.01).

A high dose of Mannuronic acid in comparison with its low dose reduced cell surface expression of TLR2, significantly (p<0.001) (Figure 5).

Effect of M2000 on Mean Fluorescent Intensity (MFI) of TLR2

Comparison of TLR2 MFI between the healthy volunteers' cells and untreated cells of the patients demonstrated that TLR2 MFI was higher in the patients' cells than the healthy volunteers (p<0.01). In comparison to the untreated patients' cells, treatment of the cells with a low dose of M2000 (5 µg/mL) decreased MFI of TLR2, significantly (p<0.01). In comparison with the untreated patients' cells, treatment of the cells with moderate (25 µg/mL) and high (50 µg/mL) doses of M2000 decreased MFI of TLR2, significantly (p<0.001).

Treatment of the cells with moderate and high doses of Mannuronic acid compared to its low dose resulted in the reduction of TLR2 MFI, significantly (p<0.01). Moderate and high doses of Mannuronic acid compared to a single dose of diclofenac downregulated MFI of TLR2, significantly (p<0.05) (Figure 6).

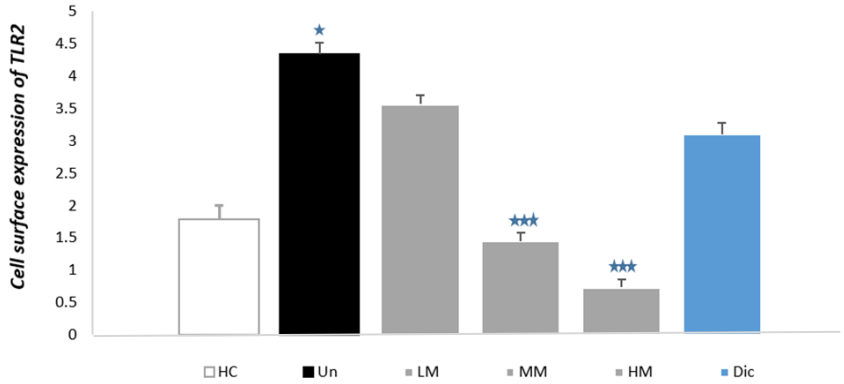

Figure 5. Comparison of TLR2 cell surface expression between the studied groups.
HV: Healthy volunteer; Un: Untreated; LM: Low dose Mannuronic acid; MM: Moderate dose Mannuronic acid; HM: High dose Mannuronic acid; Dic: Diclofenac. The statistical significance was classified as *p≤0.05, **p≤0.01, ***p≤0.001. p≤0.05 was considered as statistically significant.

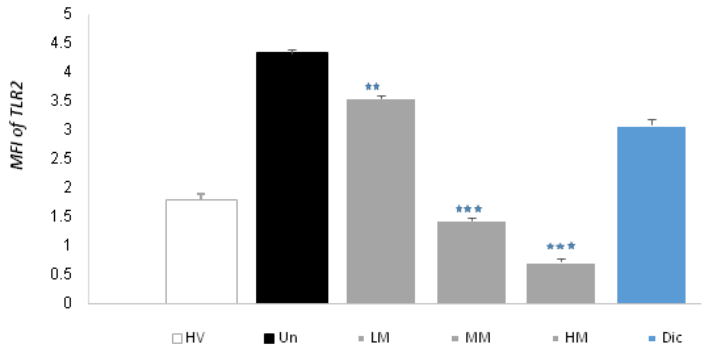

Figure 6. Comparison of TLR2 MFI between the studied groups.
HV: Healthy volunteer; Un: Untreated; LM: Low dose Mannuronic acid; MM: Moderate dose Mannuronic acid; HM: High dose Mannuronic acid; Dic: Diclofenac. The statistical significance was classified as *p≤0.05, **p≤0.01, ***p≤0.001. p≤0.05 was considered as statistically significant.

DISCUSSION

The β-D-Mannuronic acid, extracted from Alginic Acid (a linear polymer formed of β-D-mannuronic acid and α-L-guluronic acid residues) has been marked as a newly designed NSAID with immunosuppressive and antioxidant efficacy. Its anti-inflammatory effects have been identified in various experimental models such as animal models of immune complex glomerulonephritis, nephrotic syndrome, multiple sclerosis, and even in patients with RA. Furthermore, the safety, tolerability along anti-inflammatory, immunosuppressive, anti-aging, and anti-angiogenic properties of M2000 have been proved in numerous studies and clinical trial.[16-30]

As mentioned previously, pro-inflammatory parameters play a crucial role in autoimmune diseases such as RA. Therefore, these biomarkers can be considered as therapeutic targets. There are some reports, which show NSAIDs and DMARDs can reduce the inflammation through the reduction of these parameters. In 2008, Funckoshi-Tago and Colleagues in their investigation indicated that Celecoxib, as an NSAID, could significantly suppress the *TNF-α* gene expression and act as an anti-inflammatory drug in RA.[31] In a study, Huang et al. measured the IL-6 serum level in the parecoxib-treated group with kidney disease and reported a significantly decreased serum level of this cytokine.[32] Grosky and coworkers evaluated the effects of the Lornoxicam on TLR2 mRNA expression as well as serum levels of the TNF-α and IL-6 in patients with acute pancreatitis. The data represented a significant decrease in TLR2 mRNA expression and production of the pro-inflammatory cytokines, followed by the reduction of systemic complications and mortality.[33] In addition, Barcelos et al. studied the efficacy of diclofenac on the TLR4-nuclear factor kappa light chain (NF-κB) signaling pathway for the diminishment of exercise-related inflammation. The results of this study indicated that this drug could significantly reduce the production of the TNF-α, IL-6, and MYD88 adaptor protein.[34] The findings of our research indicated that M2000, as a new anti-inflammatory agent with immunosuppressive properties, could reduce the expression of TNF-α, IL-6, MYD88, as well as TLR2 and downregulate the inflammatory pathways, playing a predominant role in RA pathogenesis, in this way. Given that the TLR2 participates in the immunopathogenesis of RA through the instigation of inflammatory pathways such as NF-κB, (Mitogen-activated protein kinase) MAPK, activator protein*1*(AP-1), P38/extracellular signal-regulated kinase (ERK), Janus kinase signal transducer, and activator of transcription (JAK/STAT), mammalian target of rapamycin TOR/AKT/, phosphatidylinositol-3′-kinase (PI3K), and regarding its involvement in the spontaneous production of cytokines, matrix metal proteinases (MMPs), co-stimulatory factors and induction of major histocompatibility complex (MHC) molecules expression in inflammatory cells.[35-39] reduction of this molecule expression can be beneficial for the improvement of inflammatory diseases such as RA. The inhibitory effects of the drug M2000 on the TLR2-NF-κB signaling pathway have already been observed[40-43] and our obtained results are in agreement with them. It shows that this drug can act as a TLR2 antagonist and reduce the production of a pro-inflammatory cytokine such as TNF-α, IL-6, etc. by blocking this receptor signaling pathway, leading to the disease activity attenuation. It has recently been reported that the MYD88 can induce tumor-initiating cell generation through the NF-κBhypoxia-inducible factor 1-alpha (HIF-1α) activation cascade.[44] As tumor-promoting inflammation is a property of cancers, and chronic inflammatory disease elevates the risk of malignancies, the drug M2000 may be able to act toward cancer risk reduction on the one hand and decrease the inflammation on the other in RA patients via blocking the TLR2-MYD88 signaling pathway. Moreover, both TNF-α and IL-6 are core factors in systemic and local inflammation in RA disease, and generically, both have similar systemic complications like cardiovascular disease (CVD). Furthermore, the IL-6 plays a fundamental role in acute phase reactants synthesis (e.g. C-reactive protein (CRP), SAA, etc.), modifying lipid concentration (hypolipidemia), and Anemia of chronic disease (ACD) by affecting hepcidin expression in hepatic cells.[45,46] Accordingly, declining their expression/production levels can be helpful in disease improvement and lowering complications. As mentioned above, M2000, as a new NSAID with immunosuppressive properties, can target the NF-κB, adaptor proteins, and other components in the signal transmission cascade of TLR2 that leads to the prohibition of pro-inflammatory mediators'

production. Therefore, it may be proposed in a therapeutic approach for RA.[47-49]

High expression levels of inflammatory factors like TNF-α and IL-6 in the PBMCs of RA patients indicate that these markers play a key role in inflammatory signaling pathways and inflammation intensity. Therefore, their decrease can be the principal turning point in inflammation reduction and improvement of RA patients. Based on our investigation results, the drug M2000, as a new NSAID with immunosuppressive properties, could prevent the production of the TNF-α and IL-6 pro-inflammatory cytokines, by targeting the MYD88 adaptor protein, blocking the signal transduction cascade of TLR2, and inhibiting the nuclear translocation of NF-κB. Based on the vital roles of these inflammatory mediators in RA immunopathogenesis and complications development, incorporating the drug M2000 in the therapeutic approach for RA paves the way for better management of the disease and elevates the patients' quality of life.

CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

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