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Therapeutic Effects of β-D-Mannuronic Acid (M2000) on Inflammatory Diseases

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ABSTRACT

The β- D-mannuronic acid (M2000) is a novel nonsteroidal drug with antioxidant, anti-inflammatory and immunosuppressive effects which have been frequently shown in a variety of *in vitro*, *in vivo* and clinical trial studies. In addition, other properties of this drug regarding tolerability, safety, nontoxicity, and biocompatibility were investigated over the recent years. This review will explore the scientific evidence of the therapeutic effects of M2000 on various autoimmune and neurologic disorders and describe the molecular mechanisms involved in regulating anti-inflammatory responses. Collectively, we have summarized all the studies conducted within the last two decades to introduce M2000 as a new drug for treatment of autoimmune diseases.

Abbreviations: NSAIDs: Non-Steroidal Anti-Inflammatory Drugs; EAE: Experimental Autoimmune Encephalomyelitis; AIA: Adjuvant-Induced Arthritis; RA: Rheumatoid Arthritis; AS: Ankylosing Spondylitis; SPMS: Secondary Progressive Multiple Sclerosis; MMP-2: Matrix Metalloproteinase 2; MYD88: Myeloid Differentiation Factor 88; NF-κB: Nuclear Factor-κB; HEK: Human Embryonic Kidney; TNF-α: Tumor Necrosis Factor Alpha; IL-6: Interleukin-6; IRAK1: Interleukin-1 Receptor-Associated Kinase 1; TRAF6: TNF Receptor-Associated Factor 6; miR-146a: microRNA-146a; SLE: Systemic Lupus Erythematosus; SS: Sjögren's Syndrome; SHIP1: Src Homology-2 Domain-Containing Inositol-5'- Phosphatase 1; SOCS1: Suppressors of Cytokine Signaling 1; TIMP1: Tissue Inhibitors Of Matrix Metalloproteinase-1; ECM: Extracellular Matrix; COX: Cyclooxygenase Enzyme; PC3: Prostate Cancer Cell Line; DC: Dendritic Cells; GM-CSF: Granulocyte-Macrophage Colony-Stimulating Factor; PBMCs: Peripheral Blood Mononuclear Cells; iNOS: Inducible Nitric Oxide Synthase; MPO: Myeloperoxidase; GST: Glutathione S-Transferase; RORγt: RAR-Related Orphan Receptor Gamma t; CXCR3: CXC Chemokine Receptor-3; CCR2: C-C Chemokine Receptor-2; MCP-1: Monocyte Chemoattractant Protein-1; STATs: Signal Transducer And Activator Of Transcription; MDS: Myelodysplastic Syndrome; G-CSF: Granulocyte Colony-Stimulating Factor; IBD: Inflammatory Bowel Disease; FOXP3: Forkhead Box P3; IFN-γ: Interferon-γ; BUN: Blood Urea Nitrogen; AIA: Adjuvant-Induced Arthritis; MBP: Myelin Basic Protein; EAE: Experimental Autoimmune Encephalomyelitis; ICG: Immune Complex Glomerulonephritis; PMN: Polymorphonuclear Neutrophil Leukocytes; CAM: Chorioallantoic Membrane; SOD: Superoxide Dismutase; BASDAI: Bath Ankylosing Spondylitis Disease Activity Index; BASFI: Bath Ankylosing Spondylitis Functional Index; ESR: Erythrocyte Sedimentation Rate; DHEAS: Dehydroepiandrosterone Sulfate; SDAI: Simple Disease Activity Index; SPMS: Secondary Progressive Multiple Sclerosis; GGT: Gamma-Glutamyl Transpeptidase

Introduction

β-D-mannuronic acid ($C_6H_{10}O_7$), patented DE/102016113018.4-PCT/ EP2017/067919, is one of the monomers of sodium alginate, reveled to have a variety of anti-inflammatory and immunosuppressive properties. This low weight molecule (194.139 Da), named M2000, has been widely studied to investigate not only its safety, biocompatibility and tolerability, but also the corresponding molecular and immunologic mechanisms as well as therapeutic effects [1-12]. Contrary to other non-steroidal anti-inflammatory drugs (NSAIDs), M2000 showed no side effect and toxicity for both animals and humans [2,4,7,13,14], either in experimental animal models [2,15-17] and a preclinical study [4] or in clinical trials [7,11,14,18,19]. To date, therapeutic effects of M2000 has been shown in a wide range of experimental animal models such as adjuvant-induced arthritis (AIA), experimental autoimmune encephalomyelitis (EAE), nephrotic syndrome, and acute glomerulonephritis [2,3,15-17,20-22] besides clinical trials in human beings including breast cancer and various autoimmune diseases like rheumatoid arthritis (RA), ankylosing spondylitis (AS) and secondary progressive multiple sclerosis (SPMS) [7,9,14,18,19,23-25]. Moreover, there were several *in vitro* studies conducted on cell lines such as WEHI-164, HEK-293, HEK-Blue hTLR2, HEK293 TLR2, 4T1 breast cancer cell line, HT29, THP-1 cells, PC3 cells, and J774 cell line to help a better understanding of the molecular mechanisms of M2000 immunomodulatory effects [1-3,6,10,15,16,20-22,26-32]. In this review, the underlying mechanisms of immune responses induced by M2000 as well as its anti-inflammatory therapeutic effects in management of many autoimmune diseases will be discussed. It would be thoroughly based on the several investigations that have been conducted in the last two decades.

In Vitro Investigations for Anti-Inflammatory Effects of M2000

In Vitro Investigations Using Cell Lines

In order to explore the tolerability, biocompatibility and pharmacotoxicity properties of M2000, a fibrosarcoma cell line, WEHI 164, was used. It was also utilized to evaluate the activity of matrix metalloproteinase 2 (MMP-2). The results demonstrated that M2000 has not only no cytotoxic effect and much higher tolerability compared with certain steroidal and nonsteroidal anti-inflammatory drugs (diclofenac, piroxicam, and dexamethasone), but also a significant greater inhibitory effect on MMP-2 activity compared with the above-mentioned drugs [1-3,15,16,21,22]. Moreover, we found that treatment with M2000 could significantly reduce the mRNA expression of myeloid differentiation factor 88 (MYD88) and nuclear factor-κB (NF-κB) in human embryonic kidney (HEK) 293 cell lines overexpressing Toll-like receptor 2 and 4 (HEK293-Blue-hTLR2/TLR4), and also reduce two inflammatory cytokines; tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) production in both HEK-Blue hTLR2 and HEK293-Blue hTLR4 cells [6]. Furthermore, it was shown that treatment of HEK-Blue hTLR2 cell line with M2000 resulted in the significant reduction in the mRNA expression of interleukin-1 receptor-associated kinase 1 (IRAK1), TNF receptor-associated factor 6 (TRAF6), microRNA-146a (miR-146a) and NF-κB. Regarding the pivotal role of miR-146a in the pathogenesis of many autoimmune and inflammatory diseases such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and Sjögren's syndrome (SS), its down-regulation and target mediators can lead to controlling the inflammation [27].

Employing the same HEK293-TLR2 cell line, Pourgholi et al. indicated that M2000 treatment could lead to significant increase in Src Homology-2 domain-containing inositol-5'- phosphatase 1 (SHIP1) and Suppressors of Cytokine Signaling 1 (SOCS1). Considering the function of SHIP1 as a target in signaling pathways of TLR which is capable of negatively regulate the activation of immune cells and crucial role of SOCS family in regulation of cytokine signaling, these mediators would reduce the inflammation level [28]. Farahani et al. found that M2000 treatment of human leukemia monocytic THP-1 cell line which is differentiated to macrophage by using PMA, would result in significant decrease of the gene expression of MMP-2, MMP-9 and tissue inhibitors of matrix metalloproteinase-1 (TIMP1) as well as the cellular surface expression of CD147 and also inhibition of the gelatinolytic activity of MMP-2 and MMP-9 [31]. In another study on impact of M2000 therapy on activity of MMP-2 and MMP-9, the 4T1 breast cancer cell line was applied. The findings revealed low-cytotoxic effect of M2000 on 4T1 cells together with reduced activity of MMP-2, MMP-9 and decreased adhesion of 4T1 cells to extracellular matrix (ECM) [29]. In order to assess the activity of cyclooxygenase enzyme (COX-1/COX-2), a key enzyme in the initiation of inflammatory pathways, in the presence of M2000, a study was undertaken using the murine macrophage, J774 cell line. The results indicated the significant reduction in the enzymatic activities in the presence of M2000 [32].

In another study on a colonic epithelial cell model (HT29 cell line), M2000 could significantly down-regulate the mRNA expression of both TLR2 and TLR4 [30]. Moreover, M2000 treated prostate cancer cell line (PC3) showed not only the down-regulation in gene expression of MYD-88, IL-8, COX-2 and MMP-9 but reduction in both gene and protein levels of NF-κB [10].

In Vitro Investigations using Human Peripheral Blood Mononuclear Cells (PBMCs)

***In Vitro* Investigations using PBMCs of Healthy Individuals:** In order to investigate how M2000 could influence differentiation, maturation and function of dendritic cells (DC), Fard et al. treated monocytes purified from healthy individuals' PBMCs with M2000 as well as granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-4 to induce DCs. The results showing no significant side effects of M2000 on DCs *in vitro*, recommends M2000 as a safe agent without adverse effects on differentiation, maturation and function of dendritic cells [5]. In another study, also conducted on M2000 treated

DCs, expression of miRNA-155 and miRNA-221 was examined in DCs induced from healthy human PBMCs purified monocytes and once again the results demonstrating no significant side effect of M2000 on expression of regulatory miR-155 and miR-221, could suggest it as a safe drug [33]. Pourgholi et al. have reported the significant down-regulation in mRNA expression of miR-155 in M2000 treated peripheral blood mononuclear cells (PBMCs) of healthy individuals. It is notable that SOCS1 and SHIP1 impacts on inflammatory responses are mainly targeted by miR-155 leading to regulation of the innate immunity and TLR signaling [28]. Investigating the effects of M2000 on the gene expression and activity of COX-1/COX-2 enzymes in PBMCs of healthy donors indicated that M2000 could significantly reduce the gene expression level of COX-2 and the activity of COX-1/COX-2 enzymes [13].

In addition, a study conducted on anti-aging and anti-inflammatory effects of M2000 on gene expression of enzymes involved in oxidative stress in PBMCs of healthy donors indicated that M2000 could significantly reduce the expression levels of the superoxide dismutase (SOD)-2, inducible nitric oxide synthase (iNOS), myeloperoxidase (MPO) and glutathione S-transferase (GST) genes [34]. Moreover, to evaluate the pro-apoptotic and anti-inflammatory effects of M2000 compared to diclofenac, PBMCs of healthy donors were treated with M2000 and the results showed that M2000, despite diclofenac, could significantly decrease the expression level of NF-κB gene together with significant augmentation of apoptosis [35].

***In Vitro* Investigations Using PBMCs of Patients with Rheumatoid Arthritis (RA):** Evaluation of gene expression levels of IL-4, GATA binding protein 3 (GATA3), IL-17 and RAR-related orphan receptor gamma t (RORγt) in PBMCs of RA patients treated with M2000, showed significant decrease in IL-17 and RORγt and increase in IL-4 and GATA3 levels [36]. Furthermore, assessment of the circulating Th17 frequencies among PBMCs of RA patients treated with M2000, showed that the frequency of Th17 cells was significantly decreased [37]. In another study, Mortazavi-Jahromi et al. found the gene expressions of miR-155 and NF-κB were significantly decreased, but expression levels of SOCS1 and SHIP1 gene (as the target molecules of miR-155) were significantly increased among the PBMCs of M2000-treated RA patients [23]. They also indicated the significant decrease in gene expression of miR-146a, IRAK1, and TRAF6 in the PBMCs of M2000-treated RA patients [38]. Regarding that synovium leukocytes infiltration among RA patients is mostly mediated by chemokine ligands and their receptors, Aslani et al. assessing the effects of M2000 on both gene and cell surface expression of these molecules, revealed that M2000 was able to significantly down-regulate not only the mRNA expression of CXC chemokine receptor-3 (CXCR3), CXCR4, C-C chemokine receptor-2 (CCR2), CCR5 and C-C motif chemokine ligand-2/ monocyte chemoattractant protein-1 (CCL2/MCP-1) but also cell surface expressions of CCR2 in the PBMCs of the M2000-treated RA patients [39,40].

Furthermore, Gaafar et al., by assessment of the effect of M2000 treatment on gene expression of the signal transducer and activator of transcription (STATs) proteins among the PBMCs of the M2000-treated RA patients, demonstrated a significant reduction in the gene expression levels of STAT1, STAT3 and STAT4 [41]. They also evaluated the therapeutic efficacy of M2000 on some inflammatory molecules in the progression of RA and found a significant reduction in gene expression levels of MMP2, MMP9 and increase in TIMP2 among the PBMCs of the M2000-treated RA patients [42]. In two other studies on PBMCs from M2000-treated RA patients, it was revealed that M2000 could significantly reduce gene expression levels of inflammatory factors TNF-α, IL-6, IL-22, TLR2 and MYD88 as well as surface expression of TLR2 [12,43].

***In Vitro* Investigations Using PBMCs of Patients with Ankylosing Spondylitis (AS):** Jafarnezhad-Ansariha et al., evaluating the effect of M2000 on gene expression of COX-1/COX-2 in PBMCs of AS patients, showed that M2000 could effectively reduce the expression levels of these cyclooxygenase enzymes in comparison with untreated patients [32]. They also explored the effects of M2000 on the frequencies of helper T cells-17 (Th17) and regulatory T cells (Treg) and expression of leucocyte function-associated antigen-1 (LFA-1) among PBMCs of AS patients and observed significant decrease in Th17 frequency and LFA-1 expression following M2000 treatment of patients [44, 61]. In another study, they investigated the expression of the genes involved in the TLR/NF-κB signaling pathway and showed the down-regulation in gene expression of MYD88, IκB-alpha and NF-κB in PBMCs of AS patients treated with M2000 [45].

***In Vitro* Investigations Using PBMCs of Patients with Multiple Sclerosis (MS):** In order to evaluate effects of M2000 treatment on MS, Najafi et al. investigated the levels of gene expressions in SOCS1, SOCS3, TRAF6, and SHIP1 among the PBMCs of MS patients treated with M2000 and the results showed that the gene expressions of SOCS1, SOCS3, and SHIP1 were increased [24]. In addition, they assessed gene expressions of IL-1β, IL-17A, STAT1 and STAT3 as well as expression of TLR2 and TLR4 molecules in PBMCs of MS patients treated with M2000 and found that M2000 therapy could decrease the gene expressions of IL-1β, IL-17A, STAT1 and STAT3 and also the expressions of TLR2 and TLR4 [25].

***In Vitro* Investigations Using PBMCs of Patients with Other Diseases:** In order to evaluate the effect of M2000 on PBMCs of patients with myelodysplastic syndrome (MDS), the gene expression levels of IL-6, TNF-α, IL-3 and granulocyte colony-stimulating factor (G-CSF) were assessed after treatment of PBMCs with M2000 *in vitro* and the findings indicated a significant rise in gene expression level of G-CSF [46]. Moreover, investigating the potency of M2000 as a better therapeutic candidate in the treatment of inflammatory bowel disease (IBD), showed a significant down-regulation of TNF-α and IL-17 gene expression and a significant up-regulation of forkhead box P3 (FOXP3) gene expression in M2000-treated PBMCs of IBD patients

[47]. Furthermore, studying the PBMCs obtained from patients with breast cancer (BC) treated with M2000, showed reduction in MMP-2, MMP-9, CCL22 and transforming growth factor-beta 1 (TGFβ1) gene expression as well as Tregs frequencies [18]. In another research conducted on PBMCs of patients with coronavirus disease of 2019 (COVID-19), M2000 treatment indicated to cause a decrease in mRNA expression and the PBMC supernatant levels of IL-17, TNF-α, IL-6, and interferon-γ (IFN-γ) [48].

In Vivo Investigations for Anti-Inflammatory Effects of M2000

In Vivo Investigations Using Experimental Animal Models

Experimental animal model was primarily employed by Mirshafiey et al. to examine the therapeutic effect of M2000 in Adriamycin induced nephrotic rats. The treated patient rats showed a significant reduction in proteinuria, blood urea nitrogen (BUN), serum creatinine, cholesterol and IL-6, as well as reduced number of glomerular leukocytes and hypercellularity in capillary network within the renal cortex and decreased tubular casts [20]. In other researches to assess the therapeutic efficacy of M2000, the experimental models of rheumatoid arthritis (Adjuvant-induced arthritis (AIA)), multiple sclerosis (myelin basic protein (MBP)-induced experimental autoimmune encephalomyelitis or experimental autoimmune encephalomyelitis (EAE)), immune complex glomerulonephritis (ICG) (bovine serum albumin nephritis) and nephrotic syndrome (Adriamycin-induced nephropathy) were utilized. Results showed that M2000 could significantly reduce paw edema in AIA model; clinical signs and histological erosions and lymph node cells proliferation in EAE model; the urinary protein excretion, BUN, serum creatinine and cholesterol, as well as glomerular hypercellularity, PMN infiltration and cast formation in kidney in ICG model. Additionally, M2000 had no ulcerogenic stomach effect or influence on the body temperature [21,22]. Moreover, treatment of EAE models with M2000 indicated significant prophylactic and therapeutic effects in suppression of disease development together with marked decrease in numbers of vessels and MBP-specific T-cell reactivity, but no impaired activity of T-cell *in vitro* [2].

EAE models treated with M2000 also showed significant reduction in paw oedema, inflammatory cells infiltrate in joints accompanied with no impact on serum or urine determinants, glomerular histology, body temperature and stomach [15]. Using BSA-induced nephritis (experimental ICG), M2000 therapy revealed to significantly reduce the urinary protein excretion, anti-BSA antibody titer, polymorphonuclear neutrophil leukocytes (PMN) infiltration and glomerular immune complex deposition without any adverse influence on serum determinants, urinary protein excretion and glomerular histology [3,16]. In a preclinical assessment of M2000, a systematic toxicological study on its safety has been conducted to investigate the acute and subchronic toxicity of M2000 in healthy NMRI mice and Wistar rats, respectively. The results of acute toxicity estimated the LD50 of M2000 to 4.6 g/kg combined with no mortality and abnormality in clinical signs, body weight, relative organ weights, or necropsy among animals during subchronic study [4]. Furthermore, the impact of M2000 on a chick chorioallantoic membrane (CAM) was evaluated and the results showed both significant and dose-dependent anti-angiogenesis effects of M2000. It should be noted that the anti-inflammatory effects of M2000 may partly be attributable to its anti-angiogenic activity. Therefore, it could be recommended as a candidate for prevention and treatment of cancer, chronic inflammatory diseases, and other angiogenesis-related disorders [49].

Given that metastasis is the main cause of death in breast cancer patients, M2000 was used in treatment of murine experimental model of 4T1 cell line-induced metastatic breast cancer. The results demonstrated that M2000 not only inhibited tumor growth and increased lifespan, but could decrease tumor mass associated with decreased metastasis, recruitment and frequency of inflammatory cells in tumor tissue [29]. To explore how M2000 treatment influences the Alzheimer's disease (AD), researchers used amyloid β (Aβ)-injected Wistar rats as the experimental animal model of AD and found that M2000 pretreatment of AD would have a potent efficacy on animal's behavior. The therapy, also led to significant inhibition of amyloid plaque production, reduction in the amount of proapoptotic and antiapoptotic proteins like Bcl-2 Associated X-protein/B-cell lymphoma2 (Bax/Bcl2) and P53, malondialdehyde (MDA) and superoxide dismutase (SOD), and normalization in the level of procaspase-3 [50]. Furthermore, investigation of anti-aging property of M2000 in animal model of the Sprague-Dawley rat revealed a slight weight increase, besides the favorable effects of M2000 on gene expression of SOD2, catalase (CAT), glutathione peroxidase (GPX1) and glutathione S-transferase (GST), although the results were not statistically significant [51]. In order to examine the cardioprotective efficacy of M2000, expression profile of oxidized low-density lipoproteins (oxLDL) scavenger receptors was evaluated in the streptozotocin-induced experimental Sprague-Dawley rat model of diabetes treated with M2000.

Results showed the significant decline in gene expression levels of the class A scavenger receptors (SR-A), lectin-like oxLDL receptor-1 (LOX-1), CD36 and CD68 in the diabetic group received M2000 [52]. Moreover, assessment of the anti-diabetic effects of M2000 on blood glucose, insulin production, and inflammatory markers in streptozotocin-induced diabetic rats showed the significant lower levels of fasting serum glucose, high sensitivity C-reactive protein (hs-CRP) and IL-6 combined with significant higher level of serum insulin among M2000-treated animals [53]. Employing animal model of epilepsy, Kamali et al. conducted research on pentylenetetrazol (PTZ)-induced kindling model of Wistar rats treated with M2000 and found that M2000 pretreatment could significantly accelerated epilepsy outcomes. IL-1β, IL-6, TNF-α, interferon-γ (IFN-γ), and cyclooxygenase-2 gene expression levels were higher in the PTZ-induced kindling group and gene expression level of IL-10 increased in the brains of M2000-treated group [54].

Investigations on Human by Randomized Clinical Trials

In a trial conducted on patients with AS to compare the therapeutic effects of M2000, as a novel designed NSAID, with naproxen and placebo, the findings demonstrated that M2000 had beneficial therapeutic effects on pain, stiffness, and inflammation, whereas no adverse effects were observed following the use of M2000 after 12 weeks [32]. In a sub study to trial, Roozbehkia et al. investigated the effect of M2000 treatment on disease activity among AS patients and showed that M2000 could significantly improve the disease activity and physical function as well as decrease in total back pain and duration of morning stiffness [45]. On the other hand, treatment of AS patients with M2000, compared to placebo and naproxen, showed that the incidence of gastrointestinal and other adverse events was higher on naproxen than on M2000 and placebo [14]. Moreover, M2000 therapy in AS could significantly decrease serum levels of IL-17 and IL-6 which were positively correlated with Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) and Bath Ankylosing Spondylitis Functional Index (BASFI) scores [44]. In a trial conducted on patients with RA, the inhibitory effect of M2000 therapy on anti-cyclic citrullinated peptide antibodies (anti-CCP), rheumatoid factor (RF), anti-double strand DNA (anti-dsDNA) and acute phase reactants were evaluated and the results showed a significant decrease in the erythrocyte sedimentation rate (ESR), CRP, serum levels of anti-CCP, RF and anti-dsDNA accompanied with a positive correlation between diminished anti-CCP with disease activity, swollen joint count and CRP [55].

The researchers also observed significant increase in the count of red blood cells, hemoglobin (Hb) concentration and the percent of neutrophils together with significant reduction in serum levels of IL-6, IL-17 and TNF-α in RA patients treated with M2000 [37,56]. Moreover, they found that after 12 weeks of M2000 therapy, the rate of ACR20 response (20% improvement in American College of Rheumatology (ACR) criteria) was significantly higher among M2000-treated patients than conventional-treated controls [7]. In another sub study to RA clinical trial, evaluating sex hormones (estradiol, progesterone, and dehydroepiandrosterone sulfate (DHEAS)) in RA patients treated with M2000, the results indicated reduced serum levels of estradiol and significant increased serum level of progesterone and DHEAS [26]. Moreover, in phase III of the RA clinical trial, M2000 treatment appeared to lead to a significant reduction in ACR20, the swollen and tender joint count and a significant improvement for 28-joint disease activity score (DAS28) beside none or too low adverse events [19]. Scientists also indicated positive correlation of the clinical and paraclinical improvement among M2000-treated RA patients with significant down-regulation of chemokine ligands and receptors mediated leukocytes infiltration into the synovium of such patients [39]. The therapy with M2000 in a RA patient with a long history of migraine, showed a strong clinical improvement in DAS28, simple disease activity index (SDAI) and laboratory parameters.

Moreover, M2000 showed a significant effect on the severity and the duration of migraine pain as well as times of migraine attack [57]. Ghaderi et al, in a randomized clinical trial in myelodysplastic syndromes (MDS), found that M2000 treatment would lead to hematologic improvement in major parameters of erythroid, neutrophil, and platelet responses as well as development of transfusion independence, and/or reduced transfusion requirements among M2000-treated MDS patients [9]. They also showed that a red blood cells (RBCs) transfusion dependent MDS patient treated with M2000, experienced a non-requirement to blood transfusion following increase in blood hemoglobin level accompanied with improvement in patient's life quality. [58]. Furthermore, a randomized controlled clinical trial was conducted on secondary progressive multiple sclerosis (SPMS) patients under treatment with M2000 and the results showed not only a better performance in MRI-related measurements, but also decreased disability progression and expanded disability status scale (EDSS) score among the M2000 treated patients besides observation of no short-term side effects [11]. In addition, a sub study of the MS trial, confirmed the non-toxic effects of drug showing non-significant changes in urea, creatinine, gamma-glutamyl transpeptidase (GGT), and uric acid and anti-phospholipids levels, besides a significant rise in vitamin D3 levels following M2000 therapy of MS patients [59].

Conclusion and Future Perspective

It can be concluded that, M2000 (β-D-mannuronic acid), based on the aforementioned extensive range of studies conducted, can be introduced as a novel immunosuppressive drug with NSAID properties along with antidiabetic, cardioprotective and anti-tumoral efficacy, besides great tolerability and safety profile. In addition, it has no or mild adverse events compared with other NSAIDs or corticosteroid medications. Consequently, this medicine could be considered as a landmark in pharmacology and represent a turning point in the treatment of different diseases based on the described experimental and *in vitro* studies and clinical trials [60-62].

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