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Mini Review

M2000, an Antagonist for Human TLR2 and TLR4

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Abstract

Toll-like receptors (TLRs) especially TLR2 and TLR4 play a crucial role in immunopathogenesis of autoimmune and inflammatory diseases such as rheumatoid arthritis (RA) and ankylosing spondylitis (AS); therefore, selective blockade of these receptors or associated adaptor molecules in their signaling pathways has been developed, as a new therapeutic approach for many inflammatory diseases. The β-D-Mannuronic acid (M2000) is a new anti-inflammatory drug with immunosuppressive properties. Several in vitro and in vivo investigations have shown that this new drug is able to target the TLR2 and TLR4 signaling pathways and act as an antagonist for these receptors.

Keywords: M2000; Mannuronic acid; TLR2; TLR4; NSAIDs

Introduction

TLRs which are single, type I transmembrane glycoproteins are expressed by different types of immune cells such as monocytes/macrophages, dendritic cells and neutrophils. They identify invading organisms including Pathogen Associated Molecular Patterns (PAMPs) and Damage Associated Molecular Patterns (DAMPs) [1-2]. There are 13-15 TLR types in mammals and among them, TLR1-13 have been identified in humans and mice[3]. Structurally, TLRs have several domains, including ectodomains with leucine rich repeat (LRR) motifs which expands into the extracellular region, some transmembrane domains; intracellular Toll–interleukin 1 (IL-1) receptor (TIR) domains and a Toll/interleukin-1 receptor (IL-1R) - interacting (TIR) domain which is vital for intracellular signal transduction. Ligation of TLRs provokes two distinct pathways with different and sometimes overlapping results: the Myeloid Differentiation primary response protein 88 (MyD88)-dependent and the TIR-domain-containing adapter inducing interferon- β (TRIF)-dependent pathways [2,4]. MyD88 interacts with TIR domain of TLRs and then with IL-1R-associated kinase 1 and 4 (IRAK1,4), which leads to activation of NF-kB and AP-1 transcription factors along with the up-regulation of inflammatory cytokines and mediators gene [4]. These receptors regulate a pro-/anti-inflammatory balance. Between the TLRs, TLR2 is able to detect a variety of microbial products, including lipoproteins, lipoteichoic acid (LTA) and peptidoglycan, by formation of heterodimers with other kinds of TLRs [1,3]. Activation of TLR2 results in stimulation of both MyD88-dependent and independent pathways, which can regulate the balance between cell survival and inflammation [3]. TLR4 is also the principal recognition receptor of Lipopolysaccharide (LPS), and can be activated by both MyD88-dependent and independent pathways [4]. TLRs, especially TLR2 and TLR4 have a key role in autoimmune and inflammatory diseases, and single nucleotide polymorphisms in their genes have a close relationship with some disorders [3,4]. They are associated with a variety of diseases, such as RA, AS, inflammatory bowel disease (IBD), multiple sclerosis (MS) and systemic lupus erythematosus (SLE); therefore, they are considered as therapeutic targets and the use of their antagonists as well as their signal transduction inhibitors have been proposed as desirable therapeutic strategies for some autoimmune and inflammatory disorders [2-4].

The β-D-Mannuronic acid (M2000) ($C_6H_{10}O_7$) patented (DE; 102016113018.4), is a new anti-inflammatory drug belongs to the Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) family with a very low molecular weight (194.139 Da), together with immunosuppressive properties, which has been demonstrated in the numerous studies. This monomer is generated directly from Alginic acid, which there is abundantly in the cell walls of brown algae and is used in the cosmetics and food industries. The preliminary researches showed its potential therapeutic effects

in numerous animal models consist of Adjuvant-Induced Arthritis (AIA), experimental autoimmune encephalomyelitis (EAE), glomerulonephritis and nephrotic syndrome. In addition, in some other investigations, its remarkable tolerability and biocompatibility have been proved, which are accompanied by the lack of cytotoxic effects in comparison to the other members of NSAIDs family, such as Diclofenac and Piroxicam and even Corticosteroids, such as dexamethasone [1-34]. A considerable point about this drug, which has been proved in some clinical trial investigations, is that unlike the other kinds of NSAIDs, the oral administration of M2000 not only hasn't any to low side effects, but also it can even decline the side effects of another simultaneous consumed drugs in patients [23]. Recently a phase I/II randomized, controlled, clinical trial for evaluating the effects and safety of M2000 in RA patients, has been done. In this trial, the M2000-treated patients showed a significant improvement, and a great significant reduction in their disease activity score 28 (DAS28) index was observed [23]. Based on the molecular structure of M2000, its cell surface receptors could be probably the members of mannose receptors (MR) family, while, TLR2 and TLR4 can be the second group of receptors of this drug [1].

Effects of M2000 on TLR2 and TLR4 Under In Vitro Investigations

Aletaha S et al. [4], using human embryonic kidney cell line (HEK293-Blue TLR-2/-4), a highly useful cell line for evaluating the TLRs signaling, have shown that in HEK-Blue hTLR2 cells, the mRNA expression of MyD88 and NF-kB were reduced significantly, after treatment of the cells with low and high concentrations of M2000 (5 and 25 µg /well, respectively) alone or in combination with LTA, in comparison to unstimulated control and LTA treated cells; while, in HEK-Blue hTLR4 cells, M2000 (both at 5 and 25 µg /well) in combination with LPS decreased the mRNA expression of MyD88 in comparison to LPS, and the mRNA expression of NF-kB declined in these cells after treatment with both 5 and 25 µg /well of M2000 in combination with LPS, in comparison to unstimulated control cells and LPS, respectively. They have also reported that in both HEK-Blue hTLR2 and HEK-Blue hTLR4 cells, TNF-α and IL-6 production reduced significantly, after treatment of the cells with both low and high concentrations of M2000 in combination with LTA and LPS, respectively [4]. Furthermore, Mortazavi-Jahromi SS, et al [3] using HEK-Blue hTLR2 cell line have shown that after treatment of the cells with both low and high concentrations of M2000 (5 and 25 ug/well, respectively), the mRNA expression of IRAK1, TNF receptor-associated factor 6 (TRAF6), miR-146a and nuclear factor-kB (NF-kB) reduced significantly, in comparison to control group. It should be noted that, miR-146a play a key role in innate immunity and its expression is associated with NF-kB gene activity. It is involved in immunopathogenesis of several autoimmune diseases such as RA, SLE and Sjögren's syndrome (SS); therefore, down-regulation of this molecule and its target mediators can lead to controlling the inflammation [3]. Moreover, Sharifi L et al; 2018, using HT29 cell line (a colonic epithelial cell model) have reported that both low and high doses of M2000 (5 and 25 µg /well) significantly down-regulated the mRNA expression of both TLR2 and TLR4; in addition, treatment of the cells with high dose of M2000 in combination with LPS, reduced the mRNA expression of TLR2 and TLR4, significantly [35]. Pourgholi F et al. [1] by utilizing HEK293-TLR2 cell line have also shown that Src Homology-2 domain-containing inositol-5'-phosphatase 1 (SHIP1) and Suppressors of Cytokine Signaling 1 (SOCS1) increased significantly, after treatment the cells with both 5 and 25 µg/ml of M2000. The SOCS family components play a key role in a negative feedback loop that regulates the intensity and duration of cytokine signaling. SOCS1 controls LPS induced inflammation negatively, thus, inhibits the secretion of pro-inflammatory cytokines such as, TNF-α, IL-6 and INF-γ. SHIP1 is other target in TLR's signaling pathways, which regulates the activation of immune cells negatively, mostly through the phosphoinositide 3-kinase (PI3K) pathway; therefore, down-regulation of these mediators leads to the reduction of inflammation. Furthermore, they have reported that treatment of the Peripheral Blood Mononuclear Cells (PBMCs) of healthy individuals with both low and high doses of M2000 in combination with LPS significantly down-regulated the mRNA expression of miR-155 compared to LPS treated cells. As miR-155 is a substantial regulator of innate immunity and TLR signaling pathway through the targeting SOCS1 and SHIP1 effects in inflammatory responses, therefore, declining this molecule can results in controlling the inflammation[1].

Effects of M2000 on TLR2 and TLR4 Under In Vivo Investigations

Roozbehkia M, et al. [2] using PBMCs of patients suffering from active form of AS, have shown that following the oral administration of 1000 mg/day of M2000 for 12 weeks by the patients, the mRNA expression of IKB-alpha, Myd88 and NF-kB reduced significantly in comparison to before treatment with M2000. Many studies have shown that TLRs have a potential role in the pathogenesis of AS and β-D-mannuronic acid reduced production of inflammatory mediators in these patients, through interfering the TLR/NF-kB Signaling Pathway [2].

Collectively, regarding the key role of the TLRs in the autoimmune and inflammatory diseases, using their antagonists can be considered as an important therapeutic target, and based on the results of the above mentioned investigations, it seems that M2000, as a monomer molecule of sodium alginate, can probably act as an antagonist for TLR2 and TLR4, and consequently, reduce the inflammation.

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Conflict of Interest

No Conflict of Interest.

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