International Immunopharmacology 61 (2018) 215–221

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

Montelukast inhibits inflammatory response in rheumatoid arthritis fibroblast-like synoviocytes

Hongyu Dong[a], Feng Liu[b,*], Fengyun Ma[a], Lianna Xu[a], Linna Pang[a], Xuyan Li[a], Bo Liu[a], Liang Wang[c]

[a] Department of Rheumatology and Immunology, Shijingshan teaching hospital of Capital Medical University, Beijing Shijingshan Hospital, Beijing 100043, China
[b] Department of Nephrology, China-Japan Union Hospital of Jilin University, Changchun, Jilin 130033, China
[c] Center of Orthopedics, The 309th Hospital of PLA, Beijing 100091, China

ARTICLE INFO

Keywords:
Fibroblast-like synoviocytes
Montelukast
p65
IL-1β
Rheumatoid arthritis

ABSTRACT

Montelukast, a potent selective antagonist of cysteinyl leukotriene (cysLT) receptors, has displayed its important anti-oxidative and anti-inflammatory effects in various tissues and organs. Rheumatoid arthritis (RA) is an immune disease defined by hyperplastic synovitis and joint destruction. Fibroblast-like synoviocytes in RA (RA-FLSs) are the main cell component of the hyperplastic synovium. Whether montelukast can protect against the inflammatory milieu of RA remains unclear. Here, it is shown that cysLT1R is present in FLSs and unregulated in RA-FLSs. Montelukast has an inhibitory effect on the inflammatory microenvironment of RA by decreasing the secretion of IL-6, IL-8, MMP-3 and MMP-13 in FLSs induced by IL-1β. Notably, treatment with montelukast attenuated IL-1β-induced phosphorylation of IκBα, IκBα degradation, nuclear translocation of p65 and NF-κB activity to express a luciferase reporter gene in FLSs. The findings of the current study provide evidence for a novel therapeutic strategy for RA using montelukast.

1. Introduction

Rheumatoid arthritis (RA) is a destructive systemic autoimmune disease defined by hyperplastic synovitis, infiltration of inflammatory cells and joint destruction [1]. The main contributor to joint destruction is fibroblast-like synoviocytes (FLSs). FLSs are the major component of the synovial membrane and aggressive phenotypes of FLSs are the major component of aberrant pannus tissue. In normal conditions, FLSs form a thin intimal layer that lines the synovium and protect joints by providing a supportive structure and controlling components of synovial fluid and extracellular matrix (ECM) [2]. While under the pathological conditions of RA, FLSs undergo a phenotypic transition to an aggressive phenotype, called RA-FLSs, in the presence of proinflammatory milieu. RA-FLSs are in an abnormal state in terms of their increased capacity for survival, adhesion, and invasion [3]. Aggressive RA-FLSs accumulate to form a hyperplastic lining, and together with infiltrating inflammatory cells (mainly macrophages) form an expansive layer of synovial tissue, a pannus. It has been reported that FLSs exist in a state of duality in RA, and FLSs initially contribute to the inflammatory environment and local production of proinflammatory factors [4]. FLSs respond to inflammation by transforming into an aggressive phenotype that not only has increased proliferation, but also increased production of proteolytic enzymes, which results in excessive degradation of the ECM [5]. However, the specific molecular mechanism underlying the transition of FLSs remains to be fully defined.

Several proinflammatory cytokines, such as IL-6 and IL-8, have been believed to be essential for the activation of FLSs, which cause subsequent cartilage destruction. Therefore, controlling these factors or applying anti-inflammatory reagents are regarded as optimal strategies for the treatment of RA. Anti-IL-6 and anti-IL-8 receptor antibodies are currently available and have significantly advanced the treatment of RA. Meanwhile, it was reported that IL-15 and IL-17 promote the generation of IL-6, IL-8, and TNF-α in RA-FLSs. IL-15 and IL-17 have been considered as novel therapeutic targets in RA [6–8]. The NF-κB pathway is important for the process [9,10], and targeting the NF-κB pathway has been considered as an important therapeutic approach for RA [11,12]. For example, caffeic acid, an anti-oxidative and anti-inflammatory phenolic acid, was found to reduce the secretion of IL-6 and TNF-α in RA-FLSs by inhibiting IκBα to activate NF-κB [13]. Another natural anti-inflammatory product, Gastrodia elata (GE), was demonstrated to attenuate the secretion of IL-6 and IL-8 by RA-FLSs. Blockage of the NF-κB pathway also suppressed activation of MMP-3 and MMP-13 [14].

Montelukast, a potent antagonist of cysteinyl leukotriene (cysLT)

* Corresponding author at: Department of Nephrology, China-Japan Union Hospital of Jilin University, NO.126 Xiantai Street, Changchun, Jilin 130033, China.
E-mail address: liuf0288@sina.com (F. Liu).

https://doi.org/10.1016/j.intimp.2018.04.042
Received 5 February 2018; Received in revised form 22 April 2018; Accepted 24 April 2018

receptors, has displayed an anti-inflammatory effect in bronchial asthma [15–17]. Montelukast also possesses various protective properties against ischemia-reperfusion (I/R) injury in diverse tissues [18–23]. A recent study showed that montelukast could recover motor impairment and inhibited IL-6 production caused by I/R injury [24]. Cysteinyl leukotrienes (cysLTs), metabolites of arachidonic acid, are important modulators of inflammation [22,25]. There are two receptor subtypes for cysLTs, cysLT1R and cysLT2R [26]. These receptors are expressed on mature eosinophils and basophils, while only cysLT1R is expressed on less mature hemopoietic progenitors [27–29]. Thus, montelukast can act as a suppressor to tissue inflammatory responses. Here, we investigated whether montelukast had a therapeutic effect against RA through inhibiting the inflammatory responses of RA-FLSs.

2. Materials and methods

2.1. Isolation of FLSs

FLSs and RA-FLSs were isolated from biopsies of synovial tissues from healthy (n = 10) and RA (n = 10) donors as previously described [27,28]. Briefly, synovial biopsies were incubated with 0.05% trypsin for 10 min at 37 °C. After neutralization with FBS (Sigma-Aldrich, USA), cells were seeded (1–1.5 × 10^6 in a 3 cm dish) and cultured in DMEM (Life Technologies, USA). The adherent cells (which were usually observed after 7–14 d of culture) were cultivated to confluence. The cells were detached from dishes by treatment with 0.05% trypsin and continuously cultured under identical conditions. FLSs were treated with IL-1β (10 ng/ml) with or without montelukast (5, 10 μM) for 24 h.

2.2. Real-time PCR

RNA was extracted from FLSs using a commercial kit (Qiagen). cDNA was produced using an iScript kit (Qiagen). The real-time PCR experiment was run on the StepOne Plus System. Primers for those genes were ordered from Applied Biosystems. Expression levels of each gene were normalized by those of GAPDH in in vitro experiments. Primers used in this study were: CysLTR1 (forward, 5′-ATG TTC ACA AAG GCA AGT GG − 3′; reverse, 5′-TGC ATC CTA AGG ACA GAG TCA − 3′); MMP-3 (forward, 5′-ATG GAC CTT CTT CAG CAA 3′; reverse, 5′-TCA TTA TGT CAG CCT CTC-3′); MMP-13 (forward, 5′-AGG AGC ATG GCG ACT TCT AC-3′; reverse, 5′-TAA AAA CAG CTC CGC ATC AA-3′). Primers for GAPDH (forward, 5′-ACT GGC GTC TTC ACC ACC AT-3′; forward, 5′-AAG GCC ATG CCA GTG AGC TT-3′); β-actin (forward 5′-AAC CCT AAG GCC AACCGT GAA-3′; reverse 5′-TCA TGA GGT AGT CTG TCA GGT C-3′).

2.3. Western blot analysis

Cells cultured in tissue culture plates were lysed in cell lysis buffer. Concentrations in supernatant were measured using a BCA kit (Pierce, USA). Denaturation of protein was achieved by boiling at 95 °C for 5 min in bromophenol blue. Proteins were run on SDS-PAGE (10%) and transferred to PVDF membranes. Then, 5% non-fat milk was used to block non-specific sites for 2 h. Samples were treated with specific primary antibodies, followed by incubation with HRP-conjugated secondary antibodies. Blots were developed by a chemiluminescence assay. Rabbit polyclonal anti-cysLTR1 antibody (1:1000, #ab95492, Abcam, USA) and the following antibodies from Cell Signaling Technology were used in this study:

Mouse monoclonal anti-β-actin antibody (1:5000, #4970); rabbit monoclonal anti-p65 antibody (1:2000, #8242); rabbit monoclonal anti-MMP-3 antibody (1:1000, #14351); rabbit polyclonal anti-MMP-13 antibody (1:1000, #94808); rabbit monoclonal anti-IκBα antibody (1:1000, #4812); rabbit monoclonal anti-phospho-IκBα antibody (1:1000, #2859).

2.4. ELISA analysis of IL-6 and IL-8

Amounts of secreted proteins in supernatants were assessed by ELISA assays using commercial kits from R&D Systems as follows: IL-6 (human IL-6 DuoSet ELISA) and IL-8 (human IL-8/CXCL8 DuoSet ELISA).

2.5. Luciferase assay of NF-κB activity

NF-κB activity was determined by a luciferase reporter assay using a commercially available kit (Thermo Fisher Scientific, USA). Bioluminescence measurements (Caliper Life Sciences) were used for confirmation.

2.6. Small RNA interference (siRNA) transfection assay

cysLT1R siRNA containing 5′-GGAAAAGGCUGUCUACAUU-3′ and negative control siRNA were used for the cysLT1R knockdown experiment (Ambion, USA). Briefly, FLSs were seeded onto plates (5 × 10^6) with 4 μg of negative control or specific siRNA using a RNAiMAX transfection kit (Life Technologies, USA). After culturing for 24 h, cells were used for functional studies.

2.7. Statistical analyses

Data are presented as means ± S.E.M. The Student's *t*-test or ANOVA assays in SPSS 20.0 software were used for statistical evaluation of the differences between independent groups.

3. Results

3.1. Expression of cysLT1R in FLSs

The expression of cysLT1R in FLSs was detected by RT-PCR analysis and western blot analysis. All those methods demonstrated a slightly lower level of cysLT1R expression compared to that of monocytes. Monocytes were used as a positive control (Fig. 1).

3.2. Elevated expression of cysLT1R in RA-FLSs

Real-time PCR of cysLT1R was performed with samples from RA patients (RA-FLSs) and healthy volunteers (FLSs). The results showed an obvious difference in expression levels between RA-FLSs and FLSs (Fig. 2A). Western blot results demonstrated a significantly higher level of cysLT1R in RA-FLSs from RA patients as compared to that of FLSs from healthy volunteers (Fig. 2B).

3.3. Elevated expression of cysLT1R in IL-1β-treated FLSs

After treatment with IL-1β (5, 10, 20 ng/ml) for 24 h, cysLT1R expression in FLSs was measured by RT-PCR and western blot analysis. CysLT1R was dose-dependently increased at both the mRNA (Fig. 3A) and protein (Fig. 3B) levels after IL-1β treatment.

3.4. Montelukast inhibits secretions of IL-6 and IL-8 in FLSs

The secretions of IL-6 and IL-8 in FLSs induced by IL-1β were determined by ELISA assay. Expressions of IL-6 and IL-8 in FLSs induced by IL-1β were blocked by montelukast at both concentrations (5, 10 μM) as compared to cells without montelukast treatment (Fig. 4).

3.5. Montelukast inhibits expressions of MMP-3 and MMP-13

Expressions of MMP-3 and MMP-13 after IL-1β treatment in FLSs with or without montelukast were evaluated using RT-PCR and western blot analysis. Fig. 5 shows that the expressions of MMP-3 and MMP-13