International Immunopharmacology 60 (2018) 160–169

Contents lists available at ScienceDirect

International Immunopharmacology

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

MLN3126, an antagonist of the chemokine receptor CCR9, ameliorates inflammation in a T cell mediated mouse colitis model

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ARTICLE INFO

Keywords:
MLN3126
CCR9 antagonist
CCL25
Colitis
Migration

ABSTRACT

C-C chemokine receptor 9 (CCR9) is the homing receptor for C-C motif chemokine ligand 25 (CCL25), and contributes to the maintenance of mucosal immunity and pathogenesis of inflammatory bowel disease (IBD) through the recruitment of T cells into the gut mucosa. Recent reports suggest that the interaction of CCR9 and CCL25 in the large intestine correlate with disease severity of colonic IBD. MLN3126 is an orally available small molecular compound with potent and selective CCR9 antagonist activity. MLN3126 inhibited CCL25-induced calcium mobilization in human CCR9 transfected cells and CCL25-induced chemotaxis of mouse primary thymocytes in a dose-dependent manner. The potential effect of MLN3126 in an activated T cell transfer mouse colitis model was compared with that of an anti-tumor necrosis factor (TNF)-α antibody. CCL25 protein was detected in the colon of mucosal epithelial cells and $CCR9^+$ $CD4^+$ T cells were observed in the lamina propria of the colon of mice with colitis. Dietary administration of MLN3126 to the mice maintained sufficient concentration of the compound in the plasma and dose-dependently inhibited progression of colitis compared to the vehicle control group. Anti-TNF-α antibody, a surrogate for a standard of care for IBD treatment, was also efficacious in the colitis model. These results suggest that MLN3126 would be a promising orally available CCR9 antagonist to treat colonic IBD.

1. Introduction

The migration of leukocytes into site of inflammation is an essential component of the host response in chronic inflammatory disease and thus, chemokines and their receptors are key factors in this process [1,2]. The chemokine C-C motif chemokine ligand 25 (CCL25) and its receptor C-C chemokine receptor 9 (CCR9) play a critical role in the selective homing of lymphocytes to the intestine under inflammatory conditions. It had been reported that $CCR9^+$ T cells isolated from mesenteric lymph nodes draining the small bowel of Crohn's disease (CD) patients had an activated phenotype and $CCR9^+$ T cells isolated from small bowel CD lamina propria (LP) exhibited an enhanced effector Th1 and Th17 cytokine profile in small bowel [3]. Interestingly, a recent report demonstrated a strong correlation of colonic CCL25 gene expression with disease severity in ulcerative colitis patients and it also showed that $CCR9^+$ T-cells were highly enriched in colon tissues of Crohn's disease and ulcerative colitis (UC) patients [4]. Thus, the interaction of CCR9 with CCL25 is considered to contribute to the pathophysiology of inflammatory bowel disease (IBD) [3,5–7]. The association with other intestinal inflammatory diseases such as celiac disease has also been suggested [8]. In addition to intestinal inflammation, associations of CCR9 to primary sclerosing cholangitis [9] and Sjogren's Syndrome [10] are also reported. In addition, targeting CCR9 may be well-tolerated therapeutically because the phenotype of CCR9 knockout mice is similar to normal mice [11]. Antagonizing CCR9 may also be a therapeutic option for several cancers. It has been reported that CCL25 and CCR9 are highly expressed in a wide variety of tumors and this axis has been proposed to be involved in tumor chemoresistance and metastasis [12]. Furthermore, it was demonstrated that a CCR9 monoclonal antibody had antitumor effect on leukemia cell xenografts [13].

Recently, the CCR9 selective antagonist, CCX282-B, showed clinical efficacy in a phase 2b study in CD [14]. CCX282-B is an orally available small molecule compound which potently inhibits chemotaxis of primary CCR9-expressing cells to CCL25 [15]. Anti-inflammatory activity

Abbreviations: CCL25, C-C motif chemokine ligand 25; CCR9, C-C chemokine receptor 9; CD, Crohn's disease; CHO, Chinese hamster ovary; FLIPR, fluorometric imaging plate reader; IBD, inflammatory bowel disease; LP, lamina propria; SCID, C.B-17/Icr-scid mice; TNF, tumor necrosis factor-α; UC, ulcerative colitis

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https://doi.org/10.1016/j.intimp.2018.04.049
Received 8 February 2018; Received in revised form 4 April 2018; Accepted 30 April 2018

of CCX282-B, by subcutaneous (s.c.) administration, was evaluated in tumor necrosis factor (TNF)$^{\Delta ARE}$ mice, which spontaneously develop a syndrome characterized by inflammation of the small intestine [15], and in the mdr1a deficient mice which spontaneously develop colitis [16]. Another CCR9 selective antagonist, CCX8037, administered s.c., has been reported to inhibit lymphocyte trafficking into the intestine in mice, but its anti-inflammatory activity in the intestine has not been investigated [17]. Therefore, there are few studies on the potential ability of oral administration of a CCR9 antagonist to ameliorate disease in colitis models.

MLN3126 is a novel orally available and selective CCR9 antagonist that was discovered by Millennium Pharmaceutical Inc. [18,19]. MLN3126, at 1 and 10 μM, showed potent inhibition against CCL25-induced calcium mobilization in CCR9 expressing cells (> 90% inhibition), but did not affect 12 other chemokines. In addition, MLN3126 dose dependently inhibited CCL25-induced chemotaxis of mouse thymocytes. To evaluate the potential activity of MLN3126 for the treatment of IBD, efficacy of MLN3126 by oral administration was investigated in the activated T cell transfer mouse colitis model. Additionally, efficacy of MLN3126 was directly compared to that of anti-TNF-α antibody, a surrogate for the anti-TNF therapeutics which is standard of care for IBD.

2. Materials and methods

2.1. Animals

BALB/c mice (female) were purchased from Charles River Japan (Japan). C.B-17/Icr-scid mice (SCID, female) were purchased from CLEA Japan, Inc. (Japan). SCID mice were used as recipient mice and bred individually on paper chip. Mice were maintained under specific pathogen-free conditions. All procedures were performed in accordance with the guidelines of Animal Care and treatment of research animal was approved by the Takeda Institutional Animal Ethics Committee.

2.2. Reagents

MLN3126 (Fig. 1A) was synthesized at Albany Molecular Research Institute, Inc. Chemokine ligands, CCL25, biotinylated CCL25 and SDF-1α (CXCL12) were purchased from R&D systems (USA). Monoclonal anti-CCL25 antibody and isotype control antibody were purchased from R&D Systems (USA). Anti-CD4 and CCR9 antibody were purchased from BD Pharmingen. Anti-TNF-α antibody was purchased from Biolegend (USA).

2.3. CCL-25 induced calcium mobilization assay

Chinese hamster ovary (CHO) cell line stably expressing human CCR9 was established and characterized at Millennium Pharmaceuticals, and used for the calcium mobilization assay. Briefly, human CCR9 was cloned from a thymus complementary deoxyribonucleic (cDNA) library by polymerase chain reaction (PCR) using standard procedures. The protein coding sequence of the messenger ribonucleic acid (mRNA) was verified and was identical to the GenBank sequence NM_031200. The insert was placed into vector pEAK10, transfected into CHO cells and placed under puromycin selection. Clone 11e was chosen based on its ability to respond to calcium ion influx in a fluorometric imaging plate reader (FLIPR) based assay and for its ability to bind to anti-CCR9 monoclonal antibody 3C3. Mock vector transfected cells did not exhibit any calcium mobilization response to CCL25 and did not bind CCR9 monoclonal antibodies. To determine the concentration of CCL25 that induced 50% of maximum calcium flux response, CCL25 concentration dose response experiment was performed (Supplement Fig. 1). To determine potential antagonistic activity of MLN3126 against CCR9, a CCL25-induced Ca^{2+} mobilization assay was conducted in the CHO cell line stably expressing human CCR9 [18].

2.4. CCL25 binding to CCR9 assay

A L1.2 cell line stably expressing the sequence-confirmed human CCR9 was generated and characterized at Millennium Pharmaceuticals. Briefly, cDNA clones were inserted into MSPR vector and this construct was used to generate recombinant virus. Recombinant virus was used to infect l1.2 cells, which were then placed under puromycin selection. The L1.2 clone 12 stably expressing CCR9 was chosen on the basis of its ability to respond to CCL25 by Ca^{2+} influx as measured by fluorometric imaging. Expression was also confirmed with CCR9-specific monoclonal antibodies that bound to CCR9-transfected cells but not to the control cells, retrovirally transfected L1.2 cells. Before performing the experiment, the biological ability of the biotinylated CCL25 was confirmed in Ca^{2+} mobilization experiments and shown to be as active as other commercially available CCL25 reagents. The effect of MLN3126 on the binding of the biotinylated CCL25 (100 nM) to CCR9-expressing L1.2 cells were evaluated by the method of Fleming et al. [18].

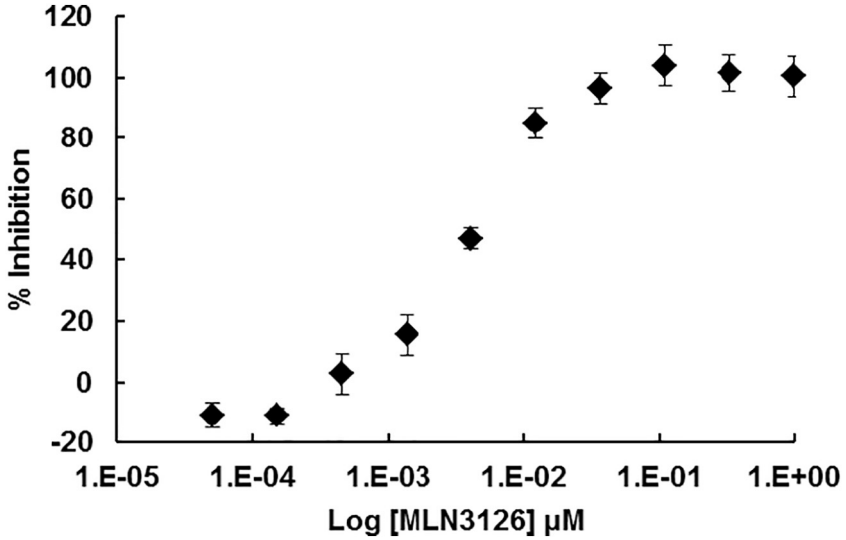

Fig. 1. Representative dose response curves of MLN3126 for the hCCL25-induced Calcium mobilization in human CCR9 expressing CHO/Gα16 cell line. Each concentration of MLN3126 was performed in triplicate well. The same experiment was repeated 5-times and geometric mean IC_{50} was calculated.