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The suppressive effects of YM-58483/BTP-2, a store-operated Ca^{2+} entry blocker, on inflammatory mediator release in vitro and airway responses in vivo

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Abstract

YM-58483/BTP-2, 4-methyl-4′-[3,5-*bis*(trifluoromethyl)-1H-pyrazol-1-*yl*]-1,2,3-thiadiazole-5-carboxanilide, blocks the store-operated Ca^{2+} entry (SOCE) that mediates the activation of non-excitable cells. This study investigated the pharmacological profile and therapeutic potential of YM-58483 as anti-asthma drug. YM-58483 inhibited DNP antigen-induced histamine release from and leukotrienes (LTs) production in IgE-primed RBL-2H3 cells, a rat basophilic leukemia cell line, with IC_{50} values of 460 and 310 nM, respectively. Prednisolone did not inhibit either of these responses. YM-58483 also inhibited phytohemagglutinin-P (PHA)-stimulated IL-5 and IL-13 production in human peripheral blood cells with IC_{50} values of 125 and 148 nM, respectively, which is approximately 5 times less potent than prednisolone. YM-58483 (30 mg/kg, p.o.) significantly suppressed ovalbumin (OVA)-induced bronchoconstriction in OVA-sensitized guinea pigs, whereas prednisolone did not. YM-58483 (3–30 mg/kg, p.o.) and prednisolone (100 mg/kg, p.o.) both significantly and completely suppressed airway hyperresponsiveness (AHR) caused by OVA exposure. Since YM-58483 inhibits two major characteristic symptoms of bronchial asthma, namely bronchoconstriction and AHR via the suppression of inflammatory mediator and cytokine production, SOCE inhibition is a potential approach for treatment.

Keywords: Store-operated Ca^{2+} entry; Bronchoconstriction; Airway hyperresponsiveness; Mast cell; Leukotrienes; Th2 lymphocytes; YM-58483; BTP-2

1. Introduction

Asthma is characterized by episodic airflow obstruction, airway hyperresponsiveness (AHR) to non-specific stimuli, and inflammation of respiratory tract. It has been suggested that acute inflammation is responsible for episodic airflow obstruction, while chronic inflammation contributes to AHR and fixed airflow obstruction by remodeling the airway [1].

The infiltration of inflammatory cells, such as Th2-type $CD4^+$ T-lymphocytes, eosinophils, and mast cells, into the bronchial mucosa is known to occur with asthma [2–4]. Th2-type $CD4^+$ T-lymphocytes produce Th2-type cytokines such as IL-13, IL-4, IL-5, and granulocyte macrophage-colony stimulation factor. Of these cytokines, IL-4 and IL-13 are thought to be the most closely associated with allergic asthma. IL-4 and IL-13 contribute to B cell class-switching, which leads to IgE production, pulmonary eosinophilia, and AHR [5]. Eosinophils release cytotoxic proteins (major basic protein, eosinophil cationic protein, and eosinophil peroxidase) that damage the airway epithelium and cause structural changes [6]. Mast cells secrete chemical mediators (histamine, leukotrienes (LTs), prostaglandin, and thromboxanes) and cytokines (IL-4 and IL-13) that contribute to bronchoconstriction and AHR [7,8].

In electrically non-excitable cells, such as immune cells, an increase in the cytosolic Ca^{2+} concentration via

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sustained Ca^{2+} influx across the plasma membrane is crucial for their activation. It is mainly mediated by the store-operated Ca^{2+} entry (SOCE) mechanism, in which emptying of intracellular Ca^{2+} stores activates the Ca^{2+} influx [9]. For example, when T-lymphocytes are stimulated by anti-CD3 antibody, Ca^{2+} is released transiently from the endoplasmic reticulum (ER), where intracellular Ca^{2+} is stored, into the cytosol. Depletion of Ca^{2+} in ER triggers a sustained influx of extracellular Ca^{2+}, which results in the activation of the Ca^{2+}-regulated transcription factor (NF-AT) that controls IL-2 gene expression. SOCE is an essential step in T-lymphocyte activation and proliferation [10]. SOCE has been observed in T-lymphocytes, basophils, macrophages, dendritic cells, and mast cells [11,12], as well as in airway smooth muscle cells [13]. At least two types of channels are reported as SOCE-related channels. One is the calcium release-activated calcium (CRAC) channel, which is a highly Ca^{2+}-selective channel, and the others are the non-selective, Ca^{2+}-permeable canonical transient receptor potential channels (TRPC). Recently, Orai1, which is a plasma membrane protein, was identified as a CRAC channel subunit in *Drosophila* cells, human T cells, and human embryonic kidney cells (HEK293) [14–16]. Orai1 and stromal interacting molecule (STIM) 1, which senses the ER Ca^{2+} level, have been reported to mediate the CRAC current as well as SOCE in *Drosophila* cells, Jurkat T cells, and HEK293 [16–18]. However, the molecular mechanisms of SOCE have yet to be determined completely.

The role of SOCE in the pathogenesis of asthma has not been established yet. However, it has been reported that SOCE plays an important role in the activation of inflammatory cells, the degranulation of mast cells [19], and the production of inflammatory cytokines in T-lymphocytes [10]. In addition, sustained contraction of rat trachea requires sustained extracellular Ca^{2+} influx [13]. Therefore, SOCE is expected to be involved in the inflammation and bronchoconstriction seen with asthma. So far, several compounds, such as econazole, SKF-96365, and methyl-β-cyclodextrin, are known to inhibit SOCE [20–22]. However, none of these compounds is very potent or selective.

YM-58483/BTP-2 is a selective SOCE blocker that does not cross-react with voltage-operated Ca^{2+} entry, K^+ channels, or Cl^- channels [22–25]. Although the precise molecular mechanism by which YM-58483 inhibits Ca^{2+} influx is not known, it suppresses CRAC channel, TRPC3 and TRPC5, and also facilitates the transient receptor potential for melastatin (TRPM) 4 channel [23–25]. YM-58483 suppresses cytokine production (IL-2, IL-4, IL-5, IL-13, etc.) in vitro [22,26–28] and T-lymphocyte-mediated immune responses in the in vivo trinitrochlorobenzene-induced delayed-type hypersensitivity model [22]. In this study, the pharmacological profiles of YM-58483 were investigated in vitro and in vivo in order to evaluate its potential as a therapeutic anti-asthma drug.

2. Materials and methods

2.1. Animals

Male Hartley guinea pigs were purchased from SLC Japan Co. (Hamamatsu, Japan). Animals (6–9 weeks old), weighing from 350 to 650 g, were used in spasmogen-induced bronchoconstriction models. Animals (4–5 weeks old), weighing from 250 to 350 g, were used in OVA-induced bronchoconstriction and AHR models. The animals were given food and water ad libitum until the day before the experiments. All experiments were carried out in accordance with regulations of the corporate Animal Ethics Committee.

2.2. Materials

YM-58483, 4-methyl-4′-[3,5-*bis*(trifluoromethyl)-1H-pyrazol-1-*yl*]-1,2,3- thiadiazole-5-carboxanilide was synthesized by Astellas Pharma Inc. (Ibaraki, Japan). Ovalbumin (OVA, Grade V), albumin bovine (BSA), urethane, mepyramine, penicillin–streptomycin and phytohemagglutinin-P (PHA) were purchased from Sigma Chemical Co. (St. Louis, MO, USA), and acetylcholine was purchased from Daiichi Pharmaceutical Co. (Tokyo, Japan), LT D_4 was purchased from Cayman Chemical Co. (Arbor, MI, USA). A 1-*O*-octadecyl-2-*O*-acetyl-*sn*-glycero-3-phosphocholine (PAF) was purchased from Bachem AG (Bubendorf, Switzerland), gallamine triethiodide (Flaxedil®) was purchased from Rhone-Poulenc Rorer (Paris, France), mouse anti-DNP monoclonal IgE was purchased from Seikagaku Co., Ltd. (Tokyo, Japan), DNP-BSA was purchased from Cosmo Bio Co., Ltd. (Tokyo, Japan), rat anti-human/mouse IL-5 antibody, and biotinylated rat anti-human IL-5 antibody were purchased from Pharmingen (San Diego, CA, USA), and recombinant human IL-5 was purchased from PeproTech, Inc. (Rocky Hill, NJ, USA). Prednisolone was purchased from Nacalai Tesque Inc. (Kyoto, Japan). Since prednisolone administered orally exhibited anti-asthmatic effect in rats [29], it was used as a reference compound for in vivo studies. YM-58483 and prednisolone were dissolved in and diluted with 100% DMSO, after which they were added to each assay buffer in volumes of 0.5 µl. The final in vitro concentration of DMSO was less than 0.25%. For oral administration, YM-58483 and prednisolone were suspended in 0.5% methylcellulose solution (Shin-Etsu Chemical, Tokyo, Japan) at a volume of 1 ml/kg and administered 1 and 2 h before spasmogen or OVA challenge.

2.3. DNP-induced histamine release and LTs production in RBL-2H3 cells

RBL-2H3 cells, a rat basophilic leukemia cell line with a phenotype similar to that of mucosal mast cells [30], were purchased from the American Type Culture Collection