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ASP3258, an orally active potent phosphodiesterase 4 inhibitor with low emetic activity

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ABSTRACT

We investigated the pharmacology of a novel phosphodiesterase (PDE) 4 inhibitor, ASP3258 (3-[4-(3-chlorophenyl)-1-ethyl-7-methyl-2-oxo-1,2-dihydro-1,8-naphthyridin-3-yl] propanoic acid), comparing its potency with that of the most advanced PDE4 inhibitors, roflumilast and cilomilast. PDE4 inhibition by ASP3258 (IC_{50} = 0.28 nM) was as potent as that achieved with roflumilast. ASP3258 inhibited lipopolysaccharide-induced tumor necrosis factor (TNF)-α production in rat whole blood cells (IC_{50} = 8.8 nM) and rat alveolar macrophages (IC_{50} = 2.6 nM). Orally administered ASP3258, roflumilast, and cilomilast dose-dependently inhibited production of interleukin-4, TNF-α, and cysteinyl leukotrienes, as well as leukocyte infiltration in bronchoalveolar lavage fluid from the airways of ovalbumin-sensitized Brown Norway rats, and these compounds showed almost complete inhibition at doses of 3, 3, and 30 mg/kg, respectively. PDE4 inhibitors induce emesis by mimicking the pharmacological action of α_2-adrenoceptor antagonist. However, orally administered roflumilast (3 mg/kg) and cilomilast (10 mg/kg), but not ASP3258 (3 mg/kg), inhibited α_2-adrenoceptor agonist-induced anesthesia in rats and induced emesis in ferrets. Although ASP3258 (3 mg/kg) inhibited airway inflammation completely, it had no emetic activity. As such, this compound may be useful in treating airway inflammatory diseases such as asthma and COPD.

1. Introduction

Asthma and chronic obstructive pulmonary disease (COPD) are caused by airway inflammation induced by various initiating factors, such as environmental allergens in asthma or cigarette smoke in COPD [1,2]. In these conditions, the airway is infiltrated by inflammatory cells. Although asthma and COPD share some clinical features, several differences in the characteristics of associated airway inflammation have been noted. For example, eosinophils, mast cells, and $CD4^+$ T lymphocytes are believed to be prominent in asthma patients, whereas neutrophils, macrophages, and $CD8^+$ T lymphocytes are assumed to be prominent in COPD patients. These cells produce inflammatory mediators including cytokines, chemokines, and leukotrienes, which exacerbate and perpetuate the inflammatory state; however, the role of leukotrienes in COPD has not yet been well defined [3,4]. At the very least, inflammation is clearly an important component of the development and progression of both diseases. Although glucocorticoids are the most effective agents in the treatment of inflammatory diseases such as asthma, they have been found to be largely ineffective in attenuating inflammation in COPD patients [5]. For this reason, developing novel anti-inflammatory agents with mechanisms of action differing from those of glucocorticoids is an urgent issue.

Phosphodiesterase (PDE) 4 is the major PDE isozyme in leukocytes. Previous studies have demonstrated that PDE4 inhibitors exhibit a variety of anti-inflammatory effects, including suppression of inflammatory mediators, such as tumor necrosis factor (TNF)-α, interleukins, and leukotrienes (LTs) [6,7]. As such, PDE4 inhibitors have been considered alternative anti-inflammatory agents to glucocorticoids. However, while clinical trials have confirmed the proposed efficacy of PDE4 inhibitors in treating asthma and COPD [8], emesis was reported as a dose-limiting side effect, and therefore research has been focused on developing PDE4 inhibitors with lower emetic activity than presently available compounds [9–14].

ASP3258 (Fig. 1), a novel PDE4 inhibitor, was designed to have oral activity without losing its wide safety margin. In the present study, we measured the anti-inflammatory effects of ASP3258 on production of inflammatory mediators and infiltration of leukocytes into the airway in ovalbumin (OVA)-sensitized Brown Norway (BN) rats. For comparison purposes, the glucocorticoid agent prednisolone was also tested. The emetic activity of ASP3258 was estimated by measuring the duration of α_2-adrenoceptor agonist-induced anesthesia in rats and the incidence of emesis in ferrets. In this manner, we elucidated the pharmacological profile of ASP3258 and estimated the safety margin between the anti-inflammatory dose and the emesis-inducing

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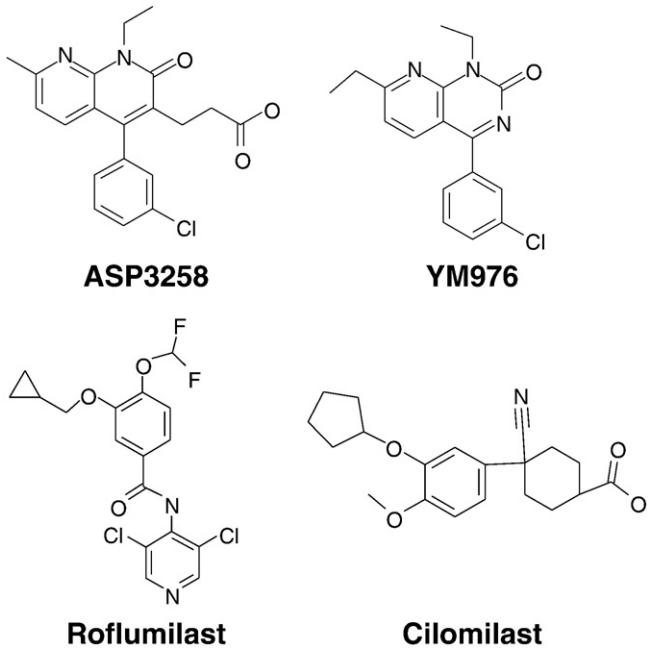

Fig. 1. Chemical structures of ASP3258, YM976, roflumilast, and cilomilast.

dose, comparing our findings with those for structurally different PDE4 inhibitors, such as cilomilast and roflumilast [15,16].

2. Materials and methods

2.1. Animals

Female BN rats and male ferrets were purchased from Charles River Japan (Kanagawa, Japan). Animals were given food and water *ad libitum*, and all animal experimental procedures were approved by the corporate Animal Ethical Committee.

2.2. Chemicals

ASP3258, YM976, roflumilast, and cilomilast were synthesized by Yamanouchi Pharmaceutical Co., Ltd. (Ibaraki, Japan). Prednisolone (Nacalai Tesque, Inc., Kyoto, Japan), 3-isobutyl-1-methylxanthine (IBMX; Sigma-Aldrich, St. Louis, MO, USA), cilostamide (Tocris Cookson Ltd., Bristol, UK), pentobarbital (Nembutal Injection; Dainippon Pharmaceutical Co., Ltd., Osaka, Japan), and heparin (heparin for intravenous injection; Shimizu Pharmaceutical Co., Ltd., Shizuoka, Japan) were purchased from the companies indicated. [5′,8-^{3}H] adenosine 3′,5′-cyclic phosphate, ammonium salt ([^{3}H]cAMP), [8-^{3}H] guanosine 3′,5′-cyclic phosphate, ammonium salt ([^{3}H]cGMP), and polylysine-coated yttrium silicate scintillation proximity assay (SPA) beads were purchased from Amersham Pharmacia Biotech (Piscataway, NJ, USA). Adenosine 3′,5′-cyclic monophosphate sodium salt (cAMP), guanosine 3′,5′-cyclic monophosphate sodium salt (cGMP), phosphodiesterase 3′,5′-cyclic nucleotide activator (calmodulin), prostaglandin E_2 (PGE_2), lipopolysaccharide (LPS; *Escherichia coli*, 0127:B8), and OVA (albumin, chicken egg, grade V) were purchased from Sigma-Aldrich. Aluminum hydroxide gel (Alum) was prepared using aluminum sulfate and sodium hydroxide.

2.3. Isolation of PDE isozymes

PDE1, PDE2, PDE3, and PDE4 were prepared from rat cardiac ventricles, and PDE5 was prepared from rat lungs, as described previously [17,18], with minor modifications. Briefly, all enzymes were partially purified using Q Sepharose Fast Flow ion exchange chromatography (Pharmacia Biotech, Uppsala, Sweden) with 0.02 to 1.0 M sodium acetate gradients from supernatants of tissue homogenates. Each PDE isozyme was identified as follows: PDE1, Ca^{2+}/calmodulin-activated; PDE2, cGMP-activated; PDE3, cGMP-inhibited; PDE4, cAMP-specific; PDE5, cGMP-specific.

2.4. PDE assay

The PDE activity was measured in 100 μL of enzyme reaction mixture using SPA beads in OptiPlates (PerkinElmer Life Sciences, Boston, MA, USA), as previously described [13,19], with some modification. To each well, 1 μL of a dimethyl sulfoxide solution of a test compound, an aliquot of the enzyme solution, and buffer I (40 mM Tris–HCl [pH 8.0], 5 mM $MgCl_2$, 4 mM 2-mercaptoethanol) were added to make a total volume of 75 μL. After incubation of 10 min at 30 °C, $CaCl_2$/calmodulin (final concentration of 30 μM and 10 unit/mL) and cGMP (final concentration of 1 μM) were added prior to assay for PDE1 and PDE2, respectively. Since PDE4 activity might be present in the PDE3 enzyme solution prepared by fractionation of rat ventricular muscle, a PDE4-specific inhibitor (YM976; final concentration of 100 nM) was added prior to assay for PDE3, and the remaining activity was attributed to PDE3. For assay of PDE1, 2, 3, or 4 activity, the enzyme reactions were started by adding tritium-labeled cAMP (final concentration, 1 μM, 1 μCi/mL). For assay of PDE5 activity, the enzyme reaction was started by addition of tritium-labeled cGMP (final concentration, 1 μM, 0.33 μCi/mL). After incubation for 15 min at 30 °C, the enzyme reactions were stopped by addition of 50 μL of 20 mg/mL SPA bead suspension containing 18 mM $ZnSO_4$ and 5 mM IBMX. The enzyme activities were measured by radioactivity detection using a TopCount scintillation plate reader (PerkinElmer Life Sciences).

2.5. TNF-α production in whole blood cells

Blood was collected from male Wistar rats (Japan SLC, Inc., Shizuoka, Japan) via heparinized syringes. RPMI1640 medium was added to whole blood (20 μL) and preincubated with each test compound for 30 min at 37 °C in 5% CO_2 before stimulation by LPS (total volume of 200 μL/well at a final concentration of 3 μg/mL LPS). Twenty hours after addition of LPS, the TNF-α concentration in the culture supernatant was determined using a rat TNF-α ELISA system (Amersham Pharmacia Biotech).

2.6. TNF-α production in alveolar macrophages

The bronchoalveolar lavage (BAL) fluid collected from male Wistar rats (Charles River Japan, Inc., Kanagawa, Japan) was centrifuged, and the sediment (cell fraction) was used as alveolar macrophages. These macrophages were incubated with a mixture of test compound, PGE_2, and cilostamide for 30 min in an incubator kept at 37 °C, with 5% CO_2. LPS was then added, and the mixture was cultured for 17 h (total culture volume, 200 μL/well; final concentrations, 5×10^4 cells/well; 10 nM PGE_2, 1 μM cilostamide, 3 μg/mL LPS). The TNF-α concentration in the culture supernatant was measured using an OPT EIA RAT TNF-α set (Pharmingen, San Diego, CA, USA).

2.7. OVA-induced inflammatory mediator production and leukocyte infiltration in the airways of sensitized BN rats

Four-week-old female BN rats were sensitized by intraperitoneal injections of OVA (1 mg) and Alum (20 mg) in 1 mL of saline solution for 3 consecutive days [20]. The first day of sensitization was on Day 0. On Day 21, the rats were exposed to an aerosol of 1% (w/v) OVA for 60 min with an ultrasonic nebulizer (NE-U12; Omron Corporation, Kyoto, Japan). Test compounds were orally administered 1 h before (Day 21) and 23 h after (Day 22) start of antigen exposure. Animals were fasted