Pulmonary Pharmacology & Therapeutics 25 (2012) 357–363

Contents lists available at SciVerse ScienceDirect

Pulmonary Pharmacology & Therapeutics

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

Preclinical efficacy of THRX-200495, a dual pharmacology muscarinic receptor antagonist and β_2-adrenoceptor agonist (MABA)

Alexander McNamara [a,*], Tod Steinfeld [b], Maria Teresa Pulido-Rios [a], Eric Stangeland [c], Sharath S. Hegde [a], Mathai Mammen [d], William J. Martin [a]

[a] Department of Pharmacology, Theravance Inc., 901 Gateway Blvd, South San Francisco, CA 94080, USA
[b] Department of Molecular and Cellular Biology, Theravance Inc., 901 Gateway Blvd, South San Francisco, CA 94080, USA
[c] Department of Medicinal Chemistry, Theravance Inc., 901 Gateway Blvd, South San Francisco, CA 94080, USA
[d] Department of Research and Early Clinical Development, Theravance Inc, 901 Gateway Boulevard, South San Francisco, CA 94080, USA

ARTICLE INFO

Article history:
Received 19 March 2012
Received in revised form
12 June 2012
Accepted 25 June 2012

Keywords:
COPD
Muscarinic
β_2-Adrenoceptor
Ipratropium
Albuterol
MABA

ABSTRACT

Combinations of a muscarinic receptor antagonist (MA) and a β_2-adrenoceptor agonist (BA) improve bronchodilation in COPD patients to a greater extent than drugs with either mechanism alone. Here, using an *in vivo* model of bronchoprotection in guinea pigs, we characterize a single agent with dual-acting MA and BA activity, THRX-200495 (MABA). THRX-200495 was compared to a fixed-dose combination of a short-acting muscarinic receptor antagonist (SAMA) and a β_2-adrenoceptor agonist (SABA). The SAMA/SABA combination consisted of a 1:5.7 ratio of ipratropium and albuterol (the components of Combivent®). Conscious guinea pigs received aqueous nebulized solutions of vehicle or test compound by aerosol exposure. Bronchoprotective potency was estimated in anesthetized, tracheotomized and ventilated guinea pigs at predetermined time points after aerosol exposure by measuring changes in ventilation pressure. The individual (MA, BA) and composite (MABA) pharmacologies were assessed by determining protection against bronchoconstrictor responses induced by methacholine in the presence of propranolol (for MA activity), histamine (for BA activity) or methacholine (MABA activity). Bronchoprotection was calculated as percent inhibition of methacholine or histamine response relative to the vehicle group. THRX-200495 exhibited matched MA (ID_{50} = 11.4 μg/mL) and BA (ID_{50} = 11.2 μg/mL) potency and potent dual pharmacology (MABA ID_{50} = 3.5 μg/mL) that persisted for over 24 h. The combination of ipratropium/albuterol exhibited bronchoprotective activity that was 2.6-fold more potent as a BA (ID_{50} = 5.7 μg/mL) than as an MA (ID_{50} = 14.6 μg/mL) at 0.5 h post-dose and 37-fold more potent as an MA (ID_{50} = 4.3 μg/mL) than a BA (ID_{50} = 159 μg/mL) at 1.5 h post aerosol exposure. Under MABA pharmacological conditions, ipratropium/albuterol produced potent bronchoprotective activity (ID_{50} = 2.0/11.4 μg/mL) and an apparent additive effect of the two pharmacologies. In conclusion, a dual-acting prototypical MABA, THRX-200495, demonstrated potent, balanced and long-lasting bronchodilation in a guinea pig model of bronchoprotection that was greater than either the MA or BA mechanisms alone.

1. Introduction

Muscarinic receptor antagonists (MA) and β_2-adrenoceptor agonists (BA) administered either as monotherapy or in combination improve airway function in patients with chronic obstructive pulmonary disease (COPD) [1–3]. Although MA and BA produce bronchodilation by different cellular mechanisms [4] evidence suggests that these mechanisms are complementary and that biochemical cross-talk exists between M_2/M_3 muscarinic receptors and β_2-adrenoceptors in the lung [4–7]. Indeed, the combination of bronchodilators from these two classes yields greater efficacy in moderate-to-severe COPD patients than either mechanism alone

Abbreviations: MA, Muscarinic receptor antagonist; BA, β_2-adrenoceptor agonist; SAMA, short-acting muscarinic receptor antagonist; SABA, short-acting β_2-adrenoceptor agonist; LAMA, long-acting muscarinic receptor antagonist; LABA, long-acting β_2-adrenoceptor agonist; MABA, muscarinic receptor antagonist plus β_2-adrenoceptor agonist; NMS, N-methyl-scopolamine methyl chloride; DHA 4, 6-propyl-dihydroalprenolol; CHO, Chinese hamster ovary; HEK, Human embryonic kidney; PBS, phosphate-buffered saline; GTPγS, guanosine 5′-O-(3-thio)triphosphate; BSA, bovine serum albumin; BEAS-2B, human bronchial epithelial cells.

* Corresponding author. Tel.: +1 650 808 6091; fax: +1 650 808 6441.
E-mail address: amcnamara@theravance.com (A. McNamara).

1094-5539/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.pupt.2012.06.007

[8–10]. As such, the Global Initiative for Chronic Obstructive Lung Disease (GOLD) Expert Panel recommends combination therapy for the treatment of moderate-to-severe COPD [11]. Despite the potential advantages of combination bronchodilator regimens, mismatched potency and pharmacokinetic profiles, coupled with pharmaceutical incompatibility, limit the potential to develop combination bronchodilator therapies, particularly triple pharmacology therapies that include inhaled corticosteroids (ICS).

Many patients currently are using both inhaled muscarinic receptor antagonists (MA) and inhaled β_2-adrenoceptor agonists (BA) in either two separate inhalers [12] or via the product Combivent® [13], which combines the short-acting muscarinic receptor antagonist (SAMA), ipratropium and the short-acting β_2-adrenoceptor agonist (SABA), albuterol. Products combined in a single delivery device simplify treatment, however, Combivent®, which is the only marketed combination of MA and BA, bronchodilators requires dosing four times per day [14]. The need for frequent dosing lowers patient compliance which, in turn, can increase the rate of hospitalization [15]. Thus, considerable effort has been directed towards identifying long-acting muscarinic receptor antagonists (LAMA) and β_2-adrenoceptor agonists (LABA) that are suitable for concomitant delivery, to reduce dosing frequency and to simplify dosing regimens.

Recent clinical trials have demonstrated that combining the LAMA, tiotropium, with LABAs, salmeterol or formoterol, in COPD patients enhances clinical efficacy without increasing side effects [16–18]. However, the mismatched pharmacokinetics of tiotropium, which is administered once a day, and the twice daily β_2-adrenoceptor agonists suggests that both bronchodilatory mechanisms may not be optimally engaged over 24 h. Several fixed-dose combinations of long-acting bronchodilators (LAMA + LABA), have entered clinical trials and show improved and sustained 24 h bronchodilation [19,20]. The ability to add an inhaled corticosteroid (ICS) to a fixed-dose combination of LAMA + LABA is currently unproven; however, 'triple therapy' including anti-inflammatory activity may offer optimal control of symptoms associated with moderate and severe COPD [21]. The co-formulation of two bronchodilators with an ICS in a single device represents an immense technical challenge [22]. One option to overcome these difficulties is to design a single molecule with dual pharmacology (MA and BA) that could be more readily co-formulated with an ICS. The attractiveness of the MABA concept has led to the development of several MABA candidates, the most advanced being GSK961081 (TD-5959) [23].

In this study we characterized a prototype MABA molecule, THRX-200495, designed with the same multivalent approach [24,25] we used in the discovery of our lead MABA molecule GSK961081 (TD-5959). The dual pharmacological activity of THRX-200495 is attributed to the covalent linkage of a BA moiety, with a carbostyril core [26,27] to a MA moiety, a biphenyl carbamic acid [28]. Using an *in vivo* guinea pig model of pharmacologically-induced bronchoconstriction [29], we characterized the bronchoprotective activity of inhaled THRX-200495 under MA, BA and MABA pharmacological conditions.

2. Material and methods

2.1. Material

THRX-200495 (Fig. 1) was synthesized at Theravance, Inc. Ipratropium and albuterol, methacholine, histamine, atropine and propranolol were purchased from the Sigma–Aldrich Co., St. Louis, MO. All test compounds were dissolved in sterile water (*in vivo*) or DMSO (*in vitro*) and formulated according to the base weight of the compound.

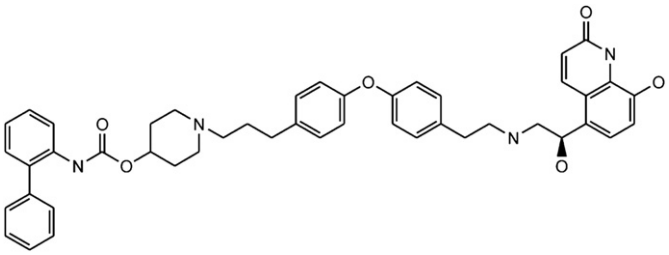

Fig. 1. Chemical structure of THRX-200495, a muscarinic receptor antagonist pharmacophore (biphenyl carbamate piperidine) linked to a β_2- adrenoceptor agonist pharmacophore (carbostyryl group). The linkage is a 4,4-propyl ethyl biphenyl ether. The chemical name is biphenyl-2-ylcarbamic acid 1-{3-[4-(4-{2-[(*R*)-2-hydroxy-2-(8-hydroxy-2-oxo-1,2-dihydroquinolin-5-yl)ethylamino]ethyl}-phenoxy)phenyl]propyl} piperidin-4-yl ester.

[^{3}H]-N methyl scopolamine ([^{3}H]NMS) was purchased from GE Healthcare (Piscataway, NJ). [^{3}H]-dihydroalprenolol ([^{3}H]DHA), [^{125}I]-cyanopindolol ([^{125}I]CYP) and [^{35}S]-Guanosine 5′-(gamma-thio)triphosphate ([^{35}S]GTPγS) were purchased from PerkinElmer (Waltham, MA).

2.2. In vitro pharmacological characterization of THRX-200495 at human muscarinic acetylcholine receptors and beta adrenoceptors: displacement of radioligand binding

Radioligand binding studies were conducted using membranes prepared from CHO-K1 cells stably expressing human recombinant muscarinic M_1, M_2, M_3, M_4 or M_5 acetylcholine receptors or HEK-293 cells stably expressing human recombinant β_1- or β_2-adrenoceptors. Assays were conducted with 1 nM [^{3}H]NMS in a 10 mM HEPES buffer containing 100 mM NaCl, 10 mM $MgCl_2$, and 0.025% BSA, pH 7.4 at 20 °C (muscarinic receptors) or with 1 nM [^{3}H]DHA in a 75 mM Tris/HCl buffer containing 12.5 mM $MgCl_2$, 1 mM EDTA, 0.025% BSA, pH 7.4 at 37 °C (beta adrenoceptors). Nonspecific binding was defined in the presence of 10 μM atropine (muscarinic receptors) or 10 μM propranolol (β-adrenoceptors). Membrane fractions were incubated with radioligand and unlabeled test compounds for up to 6 h at 37 °C to achieve equilibrium. Following separation by vacuum filtration onto GF/B filter plates presoaked with 0.3% polyethyleneimine, the quantity of membrane bound radioligand was measured by scintillation counting using a TopCount scintillation counter (PerkinElmer, Waltham, MA).

2.3. In vitro pharmacological characterization of THRX-200495 at human beta adrenoceptors: agonist Stimulated cAMP accumulation

To determine beta adrenoceptor agonist potencies, cAMP accumulation in HEK-293 cells expressing human recombinant β_1- or β_2-adrenoceptors was measured by a homogeneous [^{125}I]cAMP radioimmunoassay (Flashplate, PerkinElmer NEN). Agonists were incubated with cells for 10 min at 37 °C in vendor supplied stimulation buffer. Assays were terminated with the addition of ice-cold stop solution, provided by the vendor. Scintillation counting was used to quantify antibody-captured radiolabeled cAMP.

To measure intrinsic activity of β_2-adrenoceptor agonists, the cAMP assay mentioned above was performed using human bronchial epithelial cells (BEAS-2B, ATCC, licensed from NIH) expressing low endogenous levels of β_2-adrenoceptors [30]. Cells were grown to 75–90% confluency in complete, serum free medium (LHC 9 MEDIUM containing epinephrine and retinoic acid, Biosource International, Camarillo, CA). The day before the assay, medium was switched to LHC 8 containing no epinephrine or retinoic acid.