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Synthesis and biological comparison of enantiomers of mepenzolate bromide, a muscarinic receptor antagonist with bronchodilatory and anti-inflammatory activities

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ABSTRACT

Chronic obstructive pulmonary disease (COPD) is characterized by abnormal inflammatory responses and airflow limitations. We recently proposed that the muscarinic antagonist mepenzolate bromide (mepenzolate) would be therapeutically effective against COPD due to its muscarinic receptor-dependent bronchodilatory activity as well as anti-inflammatory properties. Mepenzolate has an asymmetric carbon atom, thus providing us with the opportunity to synthesize both of its enantiomers ((*R*)- and (*S*)-mepenzolate) and to examine their biochemical and pharmacological activities. (*R*)- or (*S*)-mepenzolate was synthesized by condensation of benzilic acid with (*R*)- or (*S*)-alcohol, respectively, followed by quaternization of the tertiary amine. As predicted by computational simulation, a filter-binding assay in vitro revealed that (*R*)-mepenzolate showed a higher affinity for the muscarinic M3 receptor than (*S*)-mepenzolate. In vivo, the bronchodilatory activity of (*R*)-mepenzolate was superior to that of (*S*)-mepenzolate, whereas anti-inflammatory activity was indistinguishable between the two enantiomers. We confirmed that each mepenzolate maintained its original stereochemistry in the lung when administered intratracheally. These results suggest that (*R*)-mepenzolate may have superior properties to (*S*)-mepenzolate as a drug to treat COPD patients given that the former has more potent bronchodilatory activity than the latter.

1. Introduction

Chronic obstructive pulmonary disease (COPD) is currently the fourth leading cause of death in the world and its prevalence and mortality rates are steadily increasing.[1] The most important etiologic factor for COPD is cigarette smoke, with this disease defined by a progressive and not fully reversible airflow limitation associated with abnormal inflammation and emphysema.[1,2] Reactive oxygen species, such as superoxide anion, are believed to play a major role in this abnormal inflammation. Thus, for the clinical treatment of COPD patients, it is important not only to improve the airflow limitation by inducing bronchodilation, but also to suppress disease progression by controlling inflammation via decreased reactive oxygen species.

Bronchodilators (such as muscarinic antagonists) are currently used for the treatment of COPD owing to their ameliorative effect on airflow limitation.[1,2] On the other hand, steroids are used to suppress inflammation in COPD patients; however recent clinical studies revealed that steroids do not significantly modulate disease progression or mortality,[3,4] because the inflammation associated with COPD tends to be resistant to steroid treatment.[5] This insensitivity can be explained in part by the notion that steroids suppress the expression of pro-inflammatory genes via their action on histone deacetylase (HDAC) 2.[6,7] Importantly, it was reported that cigarette smoke inhibits the activity and expression of this protein.[6] Thus, the development of new types of anti-inflammatory drugs to treat COPD patients is highly desirable.

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The number of drugs reaching the marketplace each year is decreasing, mainly due to the unexpected adverse effects of potential drugs being revealed at advanced clinical trial stages. For this reason, we proposed a new strategy for drug discovery and development (drug re-positioning).[8] In this strategy, compounds with therapeutically beneficial activity are screened from a library of approved medicines with a view of developing them for new indications. The advantage of this approach is that there is a decreased risk for unexpected adverse effects in humans because the safety aspects of these drugs have already been well characterized.[8] From a library of approved medicines, we screened compounds that could prevent elastase-induced pulmonary inflammation and emphysema in mice, and selected mepenzolate bromide (mepenzolate).[9] Mepenzolate is an orally administered muscarinic receptor antagonist used to suppress the gastrointestinal hypermotility associated with irritable bowel syndrome.[10–12] We showed that mepenzolate not only exerts an anti-inflammatory effect via a muscarinic receptor-independent mechanism, but also a bronchodilatory effect via a muscarinic receptor-dependent mechanism.[9] This independence of the anti-inflammatory effect is based on observations that other muscarinic receptor antagonists such as ipratropium bromide (ipratropium) and tiotropium bromide (tiotropium) could not exert ameliorative effects against elastase-induced pulmonary emphysema.[9] Although this animal model (elastase-induced pulmonary inflammation and emphysema) does not reflect some of the pathological features of COPD, it has served as a convenient animal model for studying COPD and we reported that mepenzolate could prevent cigarette smoke-induced pulmonary inflammation and emphysema.[9]

As for the mechanism governing the anti-inflammatory activity of mepenzolate, after confirmation of absence of direct inhibitory effect of mepenzolate on elastase, we found that this drug can restore HDAC activity under inflammatory conditions. We also found that mepenzolate, but not steroids, decreased the pulmonary level of superoxide anions. These results may explain why mepenzolate showed superior anti-inflammatory activity compared with steroids in our animal model of COPD.[9,13] Based on these findings, we proposed that mepenzolate could serve as a candidate drug for the treatment of COPD patients, given that it has both anti-inflammatory and bronchodilatory activities. Anti-inflammatory effect of other muscarinic receptor antagonists was also reported recently.[14,15]

Among the five types of muscarinic receptors ($M_{1-5}R$), the muscarinic M3 receptor (M_3R) expressed in airway and intestinal smooth muscle positively regulates bronchoconstriction and intestinal motility, respectively.[16] Mepenzolate is a subtype-non-specific muscarinic antagonist[12] whose bronchodilatory effect and inhibitory effect on intestinal motility can be explained by its antagonistic action on M_3R. On the other hand, the muscarinic M2 receptor (M_2R) expressed in the sinoatrial node of the heart negatively regulates heart rate,[17] and we recently confirmed that mepenzolate's inhibitory action on this receptor leads to an increased heart rate in mice (Tanaka et al., unpublished results).

Mepenzolate has one asymmetric carbon atom (Fig. 1) enabling it to exist in the form of two enantiomers; a racemic mixture ((±)-mepenzolate) of these two enantiomers has been used in a clinical setting. As the synthesis of one or other of these enantiomers of mepenzolate has not been established, it has thus remained unclear which of them is responsible for the drug's anti-inflammatory and anticholinergic activities. In various types of medicines, including drugs used as muscarinic receptor antagonists, differences in stereochemistry can affect the biochemical and pharmacological activities of these compounds, meaning that the isolation of distinct isomers can lead to the clinical development of more effective or safer medicines,[18–20] however, there are still some advantages of racemates (such as cost). In the present study, we have established a protocol for the synthesis of both (*R*)-mepenzolate and (*S*)-mepenzolate (Fig. 1) and examined their biochemical and pharmacological activities. Results showed that although anti-inflammatory activity was indistinguishable between these enantiomers, the binding activity of (*R*)-mepenzolate to human M_3R (hM_3R) in vitro and its bronchodilatory activity in vivo were superior to that of (*S*)-mepenzolate. These findings suggest that (*R*)-mepenzolate may be preferable to (*S*)-mepenzolate as a candidate drug to treat COPD patients.

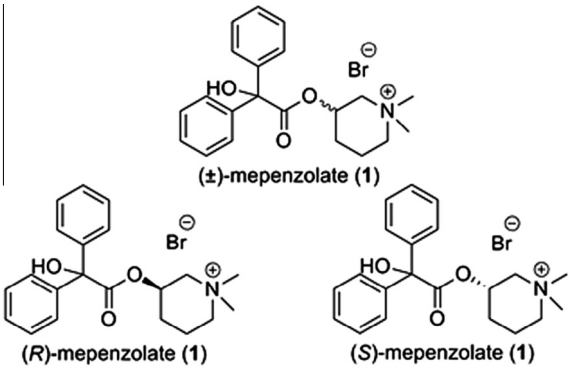

Figure 1. Structures of racemic mepenzolate and its enantiomers.

2. Chemistry

The synthetic route for target compounds is outlined in Scheme 1. The enantiomers of mepenzolate, (*R*)- and (*S*)-mepenzolate ((*R*)-**1** and (*S*)-**1**), were synthesized in two steps from commercially available benzilic acid (**2**) based on a procedure similar to that previously described[21] as outlined in Scheme 1. Condensation of **2** with (*R*)- or (*S*)-3-hydroxy-1-methylpiperidine ((*R*)-**3** or (*S*)-**3**) in the presence of carbonyl diimidazol (CDI) afforded the corresponding enantiomericaly pure tertiary amine ((*R*)- or (*S*)-1-methyl-3-piperidyl benzilate ((*R*)-**4** or (*S*)-**4**)), respectively. Quaternization of intermediate (*R*)-**4** or (*S*)-**4** with methyl bromide in acetonitrile provided desired compound (*R*)-**1** or (*S*)-**1**, respectively.

The final compounds were characterized by nuclear magnetic resonance (NMR), infrared spectroscopy (IR) and high-resolution mass spectra (HR-MS). The enantiomeric purity of each enantiomer of **1** was determined by high performance liquid chromatography (HPLC) with a chiral stationary phase.

3. Results and discussion

3.1. Binding of mepenzolate enantiomers to hM_3R in silico and in vitro

The interaction between hM_3R and (*R*)-mepenzolate (or (*S*)-mepenzolate) was predicted by molecular modelling and docking studies. We constructed the structure of the complex between hM_3R and (*R*)-mepenzolate (or (*S*)-mepenzolate) based on the recent reporting of the crystal structure of the complex between rat M_3R and tiotropium (another muscarinic antagonist[22]) (see Materials and Methods). As for other cases of monoamine receptors,[23] hM_3R has an aspartic acid residue in the third α-helix, identified as $Asp^{3.32}$ (Asp148). This residue of these monoamine receptors strongly interacts with charged nitrogen atoms in the agonists and antagonists,[24] and subsequently we focused on this residue in hM_3R (Asp148).

As shown in Figure 2A, the nitrogen atom (N) in mepenzolate interacts ionically with Asp148; we consider that this interaction