European Journal of Pharmacology 775 (2016) 138–148

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Pulmonary, gastrointestinal and urogenital pharmacology

Ambroxol inhalation ameliorates LPS-induced airway inflammation and mucus secretion through the extracellular signal-regulated kinase 1/2 signaling pathway

Shui-juan Zhang [a,1], Juan-xia Jiang [b,1], Qian-qian Ren [a], Yong-liang Jia [b], Jian Shen [b], Hui-juan Shen [b], Xi-xi Lin [b], Hong Lu [a,*], Qiang-min Xie [b,c,**]

[a] Department of Pharmacy, Zhejiang Chinese Medical University, # 548 Binwen Rd., Hangzhou 310053, China
[b] Department of Pharmacology, Zhejiang University School of Medicine, # 866 Yuhangtang Rd., Hangzhou 310058, China
[c] Laboratory Animal Center of Zhejiang University, Hangzhou 310058, China

ARTICLE INFO

Article history:
Received 27 December 2015
Received in revised form
4 February 2016
Accepted 8 February 2016
Available online 10 February 2016

Keywords:
Ambroxol
Drug delivery
Lipopolysaccharide
Mucokinetic activity
Anti-inflammation
Airway inflammatory diseases

ABSTRACT

Ambroxol, a metabolite of bromhexine, is shown to exert several pharmacological activities, including secretolytic, anti-inflammatory and antioxidant actions. Oral and intravenous administration of ambroxol is useful for the airway inflammatory diseases. However, little is known about its potential in inhalation therapy for lipopolysaccharide (LPS)-induced mucous hypersecretion and inflammatory response. In the present study, we compared the pharmacological effects of ambroxol by inhalation with intravenous administration and preliminarily explored its mechanism of action. Our results demonstrated that ambroxol administered by inhalation inhibited MUC5AC expression, reduced glycosaminoglycan levels, enhanced the function of mucociliary clearance and promoted sputum excretion, suggesting that ambroxol increases expectoration of sputum by reducing its viscosity. Moreover, ambroxol significantly alleviated LPS-induced the influx of inflammatory cells and the extracellular signal-regulated kinase 1/2 (Erk 1/2) expression in lung tissues, and inhibited increases in the mRNA expression of the pro-inflammatory cytokines tumor necrosis factor (TNF)-α, CCL-2 (monocyte chemotactic protein-1), KC (keratinocyte cell protein) and interleukin (IL)-1β in lung tissues. The secretolytic and anti-inflammatory effects of inhaled ambroxol at a dose of 7.5 mg/ml was comparable to that of ambroxol at 20 mg/ml i.v. and dexamethasone at 0.5 mg/kg i.p. In addition, we found that ambroxol dose-dependently inhibited LPS-induced increases in the mRNA expression of MUC5AC, TNF-α, and IL-1β in human bronchial epithelial cell (NCI-H292) by inhibiting the Erk signaling pathway. These results demonstrate the beneficial effects of ambroxol in inhalation therapy for the airway inflammatory diseases.

1. Introduction

The increased prevalence of airway inflammatory diseases such as chronic obstructive pulmonary disease (COPD) and asthma place the substantial burden on healthcare systems. Several advances in the treatment of these conditions have been achieved, but current treatment options manage symptoms — curative, preventative and disease-arresting medicines have not yet been introduced. People with airway inflammatory diseases may experience acute, chronic and progressive breathlessness, cough and sputum production, which in turn may lead to restricted activity and a worsening quality of life. Exacerbations occur with increasing frequency as the disease becomes more severe ([Global Initiative for Chronic Obstructive Lung Disease (GOLD], 2015; [Global Initiative for Asthma (GINA], 2015). They are characterized by increased breathlessness, greater volume or purulence of sputum or both. Exacerbations accelerate the decline in lung function and are associated with a worse quality of life and a higher mortality. Thus, treatments that reduce the frequency and duration of acute exacerbations will provide benefits for both individual patients and healthcare systems. Mucolytics are oral medicines that are believed to increase expectoration of sputum by reducing its viscosity, thus making it easier to cough it up (Poole et al., 2015).

Ambroxol {(Amb), 2-amino-3,5-dibromo-N-[*trans*-4-hydroxycyclohexyl] benzylamine} is a metabolite of bromhexine. Both bromhexine (Bisolvon) and ambroxol are semi-synthetic derivatives of vasicine, which is used in the treatment of respiratory disorders with a productive cough. Surfactant stimulation,

* Corresponding author.
** Corresponding author.
E-mail addresses: luhong03@hotmail.com (H. Lu), xieqm@zju.edu.cn (Q.-m. Xie).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.ejphar.2016.02.030
0014-2999/

mucokinetic and secretagogue activity are its major pharmacodynamic actions (Disse, 1987). In addition to mucolytic action, Ambroxol has antioxidant and anti-inflammatory properties *in vitro* (Beeh et al., 2008, Gillissen et al., 1997a, 1997b; Peroni et al., 2013; Nowak et al., 1994a; Gibbs et al., 1999) and *in vivo* (Nowak et al., 1994b, 1995; Su et al., 2004). Ambroxol has been proposed to be a therapeutic agent for the treatment of chronic pulmonary disorders, such as COPD (Malerba et al., 2004; Gupta, 2010; Jahnz-Rózyk et al., 2001; Olivieri et al., 1987), acute lung injury/acute respiratory distress syndrome (Wu et al., 2014; Zhang et al., 2013; Gonzalez Garay et al., 2014), idiopathic pulmonary fibrosis (Gupta, 2014; Tam et al., 2013; Nash et al., 2009) and upper respiratory disease (Nobata et al., 2006). Ambroxol significantly reduced lung hemorrhage, edema, exudation, neutrophil infiltration and total lung injury measured by histological scoring in a murine model of lipopolysaccharide-induced lung injury. Ambroxol treatment significantly reduced the bronchoalveolar lavage fluid (BALF) levels of TNF-α, IL-6, transforming growth factor (TGF)-β1 and total protein compared with control treatment (Su et al., 2004). In addition, ambroxol enhanced LPS-induced secretion of IL-12 and the ratio of IL-12/IL-10 in human alveolar macrophages, suggesting that this compound strengthen the innate immune response and cell-mediated immunity, facilitating Th-1 cell development (Aihara et al., 2000).

Above all of the pharmacodynamic studies of ambroxol are performed by the oral or intravenous administration, and although previous the local pharmacokinetics studies proposed ambroxol dry powder delivered inhalation by tracheal injection (Ren et al., 2008, 2009), little is known about the pharmacodynamic potential and mechanism of inhalation delivery of ambroxol. So far, no molecule signaling pathway of ambroxol effects on airway diseases has also been reported. The extracellular signal-regulated kinase 1/2 (ERK1/2) activation might be important in the development of airway inflammation and sputum viscosity (Shin et al., 2015; Ohnishi et al., 2009; Hou et al., 2015). Moreover, whether ambroxol increases expectoration of sputum is associated with reducing glycosaminoglycans (GAGs) levels. GAGs, these glycomolecules are a major component of the extracellular matrix, distributed in the subepithelial tissue and bronchial walls (Souza-Fernandes et al., 2006). Increased concentrations of GAGs have been found in BALF from patients with COPD (Dentener et al., 2005), asthma (Sahu and Lynn, 1978) and cystic fibrosis (Reeves et al., 2011).

In this study, we hypothesize that ambroxol is a potential expectorant and anti-inflammatory drug with biological and pharmacokinetic properties suitable for delivery by inhalation. Ambroxol administration via inhalation may overcome the low clinical efficacy and side effects of oral or intravenous administration. We aimed to investigate whether ambroxol via inhalation inhibits pathological changes and concentrations of GAGs of lung tissues, and how to regulate molecule signaling pathway in a murine model of LPS-induced acute lung injury *in vivo* and in human bronchial epithelial cells (NCI-H292) *in vitro* in order to demonstrate the beneficial effects of ambroxol administered via inhalation for airway inflammatory diseases.

2. Materials and methods

2.1. Animals

Female Institute of Cancer Research mice (ICR mice, weighing 25 ± 2.5 g) were purchased from Shanghai Slac Laboratory Animal Co. Ltd. (No. SCXK 2012-0002). The animals were housed in isolated ventilated cages (4–5 mice/cage) under a 12-h light/12-h dark cycle and received food and water *ad libitum*. All of the animal experiments performed in this study were approved by the Institutional Animal Care and Use Committee of Zhejiang University School of Medicine. Animals were terminally euthanized by inhalation of CO_2.

2.2. Drug administration

An inhalation solution of ambroxol hydrochloride (Amb, 15 mg/2 ml, Tianjin Institute of Pharmaceutical Research., Tianjin, China) was prepared at concentrations of 1.875, 3.75, and 7.5 mg/ml ambroxol. This solution of ambroxol was aerosolized for 20 min with a jet nebulizer (BARI Co. Ltd., Germany) 20 min before the intraperitoneal injection (i.p.) of phenol red solution (Tracheal phenol red output test) or the exposure of the animals to lipopolysaccharide (LPS, Escherichia coli O127: B8, Sigma Co. *St* Louis, MO, batch: 029k4055). When ambroxol at concentrations of 1.875, 3.75, and 7.5 mg/ml is administered to mice via inhalation for 20 min, the plasma concentrations of ambroxol measured by HPLC are equivalent to that obtained with intravenous injection of 0.7, 1.34, 2.78 mg/kg ambroxol. As a reference drug, 20 mg/kg ambroxol hydrochloride (15 mg/2 ml, Tianjin Institute of Pharmaceutical Research., Tianjin, China) was injected via i.v. 10 min before mice were exposed to LPS. Subsequently, phenol red solution was given i.p. As a positive control, 0.5 mg/kg dexamethasone sodium phosphate (Dex, Tianjin Jin Yao Group Hubei Tianyao Pharmaceutical Co., LTD) was injected intraperitoneally into mice 1 h before their exposure to LPS. As an additional positive control, 200 mg/kg erdosteine (Xian haixin pharmaceutical Co., LTD, China) was orally administered 1 h prior to i.p. administration of phenol red solution. The control mice and model mice received solvent (saline) inhalation.

2.3. Tracheal phenol red output test

A simple method for screening drugs that influence tracheobronchial secretion is a tracheal phenol red output test. After i.p. application of a phenol red solution, part of the dye is secreted into the tracheal lumen. Expectorants are capable of increasing tracheal phenol red output. Therefore, this method is suitable for the study of drugs that may influence tracheobronchial secretion (Engler and Szelenyi, 1984).

Mice were randomly divided into 6 groups of 10 mice each and treated as in Section 2.2. The test was performed as described previously (Engler and Szelenyi, 1984). Briefly, each mouse was treated with a single dose of the test drugs before intraperitoneal injection of phenol red solution (5% in saline solution, w/v, 0.2 ml/20 g body weight). Then, 30 min after application of phenol red solution, the mice were killed by inhalation of CO_2. After being dissected away from adjacent organs, the trachea was removed from the thyroid cartilage to the main stem bronchi and immediately placed into 1 ml normal saline. After ultrasonication for 15 min, 1 ml $NaHCO_3$ solution (5%, w/v) was added to the normal saline, and the optical density of the mixture was measured at 546 nm using a UV spectrophotometer. The standard curve of phenol red was generated as described previously (Engler and Szelenyi, 1984).

2.4. Mucociliary clearance in LPS-exposed mice

The mucociliary clearance in the LPS-exposed mice was performed according to the report of Hosoe and colleagues (Hosoe et al., 1998). Briefly, mice were randomly divided into 7 groups of 10 mice each. Under pentobarbital anesthesia (40 mg/kg, i.p.), carbon solution was instilled to evaluate the mucociliary clearance