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Airways antiallergic effect and pharmacokinetics of alkaloids from *Alstonia scholaris*

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ABSTRACT

Background: *Alstonia scholaris* (L.) R. Br. (Apocynaceae), an important herbal medicine, has been widely used to treat respiratory tract diseases, such as cough, asthma, phlegm, and chronic obstructive pulmonary disease.
Purpose: To evaluate pharmacological effect of alkaloids from *A. scholaris* on ovalbumin induced airways allergic inflammatory model, and explore whether the dosing frequency is related to pharmacokinetics.
Study design: After oral administration of total alkaloids, the pharmacokinetic study of it was investigated. In addition, anti-allergic studies were carried out on ovalbumin-sensitized airways allergic inflammatory model of mice.
Methods: The pharmacokinetics of total alkaloids (TA) was investigated in SD rat plasma by a fully-validated LC-MS/MS method. Then, an ovalbumin (OVA)-sensitized airways allergic inflammatory model was established, in which mice were intra-gastrically administrated by 3 times a day (8.3 and 16.7 mg/kg) based on the pharmacokinetic behavior of TA) and single (25, 50 mg/kg) treatment regimen. Dexamethasone was used as a positive control for corticosteroid drugs. Cellular infiltration was assessed in the broncho-alveolar lavage fluid (BALF). Expressions of interleukin-4 (IL-4) and interleukin-10 (IL-10) in the BALF were determined, levels of immunoglobulin E (IgE) and eotaxin in serum were measured, and superoxide dismutase (SOD) activities as well as malondialdehyde (MDA) content in the serum and BALF were examined. Finally, histopathological examination in the lung was assessed by H. E. staining.
Results: The time course of plasma concentration of 4 bioactive indole alkaloids fitted an open two-compartment model after oral administration of total alkaloids at doses of 10, 25, and 50 mg/kg. The area under the curve and the maximum concentration values of four major alkaloids increased dose-dependently, and half-life suggested a short-lasting pharmacological effect. Then, an ovalbumin-provoked airways allergic inflammatory model indicated that the pharmacological effect of administration of total alkaloids 3 times a day was a little better than that of single dose daily. The percentage of eosinophils in BALF was reduced obviously and the pathological damage of lung was also attenuated. There was also a significant reduction in IL-4 and promotion in IL-10 in the BALF. Serum IgE and eotaxin expression also significantly decreased in treated animals. Furthermore, the activity of SOD elevated remarkably and lipid peroxidation product (MDA) decreased in the administrated mice.
Conclusion: The pharmacological effects administrated for 3 times a day had precedence over single dose daily, which was related to the prolonged retention time and the maintained plasma concentration. Moreover, scholaricine and vallesamine might be responsible for the treatment of allergic asthma, mainly in total alkaloids.

Abbreviations: TA, total alkaloids; Sch, scholaricine; Epi, 19-epischolaricine; Val, vallesamine; Pic, picrinine; DXM, dexamethasone; OVA, ovalbumin; BALF, broncho-alveolar lavage fluid; IgE, immunoglobulin E; MDA, malondialdehyde; SOD, superoxide dismutase; ELISA, enzyme-linked immunosorbent assay; H.E., hematoxylin and eosin; AUC, area under the curve; C_{max}, maximum concentration; $T_{1/2}$, half-life; β_2AR, β_2 adrenergic receptor; T_{max}, time of maximum concentration.

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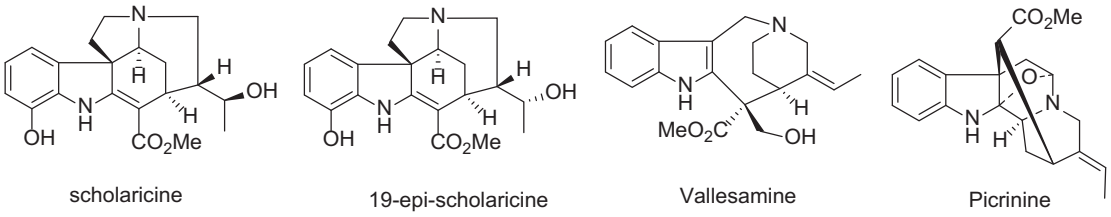

Fig. 1. Four major alkaloids from *A. scholaris*.

Introduction

Asthma is a global health problem, with more than 300 million people worldwide suffering from it (Yin et al., 2009), which results from a complex interplay between genetic and environmental factors. The disease is caused by nonspecific stimuli such as environmental pollutants, allergens, exhaust gases, infections, cold air, and strenuous exercise. Each of these stimuli irritates a variety of effector cells including inflammatory cells, epithelial cells, neurons, and narrowing the airway indirectly through the release of mediators (Factor, 2003). Investigation showed that pro-inflammatory mediators released from mast cells and eosinophils adjusted by T cells were particularly relevant to the progress of asthma (Holgate et al., 1999).

Alstonia scholaris (L.) R. Br. (Apocynaceae) is distributed in deciduous, evergreen forests and even in plains widely over the tropical regions of Africa and Asia (Li et al., 1995). Its leaves have long been used in "dai" ethno-pharmacy for the treatment of respiratory diseases in Yunnan Province, PR China (Compiling Group of Yunnan Traditional Chinese Medicine, 1977). *A. scholaris* extracts and alkaloids have shown antitussive, anti-asthmatic, expectorant (Shang et al., 2010a), analgesic, anti-inflammatory effects (Shang et al., 2010b), and airway anti-inflammation *in vivo* (Zhao et al., 2016). In our previous pharmacological studies, they also triggered β_2 adrenergic receptor (β_2AR) activation (Hou et al., 2012a) and inhibited the nuclear factor-κB (NF-κB) (Hou et al., 2012b) activities *in vitro*. Moreover, the methanolic extract of *A. scholaris* exhibited anti-inflammatory and antioxidant activities against carrageenan induced rat paw oedema (Subraya and Gupta, 2012). These results indicated that alkaloids from the leaves of *A. scholaris* may also possess anti-inflammatory and analgesic effects. In the meantime, chemical profiling and metabolites of alkaloidal extract of *A. scholaris* have been reported (Cao et al., 2016). Based on pre-clinical studies on *A. scholaris*, "total alkaloids" has been registered as investigational new botanical drug (No. 2011L01436) and was approved for phase I/II clinical trials by China Food and Drug Administration (CFDA). A phase-I mono-center, randomized, double-blind, and placebo-controlled trial has been completed, and the results support further phase-II clinical trials.

In our previous phytochemical studies on *A. scholaris*, four indole alkaloids, scholaricine (Sch), 19-epischolaricine (Epi), vallesamine (Val) (Feng et al., 2009), and picrinine (Pic) were isolated as major components in "total alkaloids (TA)" (Fig. 1). Previous pharmacological results indicated the efficiency of "total alkaloids" but adaptions and dosing frequency need to establish before further clinical applications. In the present work, a pharmacokinetic study was designed to develop and validate a highly sensitive liquid chromatography tandem mass spectrometry (LC-MS/MS) method for the quantitative determination of major alkaloids in biological samples collected from rat experiments *in vivo*. This method was applied to elucidate the pharmacokinetic behavior of Sch, Epi, Val, and Pic after oral administration of TA, which is highly important to understand their biological effects. Then, based on the pharmacokinetic profiles of 4 major bioactive alkaloids, evaluation of protective effect on airways allergic inflammation induced by ovalbumin (OVA) in mice was carried out to further provide remedy principle for phase II clinical trials.

Materials and methods

Plant material

The leaves of *A. scholaris* were collected in June 2013 in Pu'er city of Yunnan Province, People's Republic of China, and identified by Dr. Xiao-Dong Luo, Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (Luo20130601) has been deposited in State Key Laboratory of Phytochemistry and Plant Resources in West China, Chinese Academy of Sciences.

Alkaloids preparation

The dried and powdered leaves of *A. scholaris* (1 kg) were extracted with 90% EtOH under reflux conditions (3 h × 4) and the solvent was evaporated *in vacuo* to get the ethanolic extract. The ethanolic extract was dissolved in 0.3% aqueous HCl solution and the residue was recognized as non-alkaloid fraction. Then the acidic solution, adjusted to pH 9–10 with 10% aqueous ammonia, was extracted with EtOAc to give TA fraction (10 g). Sch (6%), Epi (2%), Pic (10%) (Shang et al., 2010a), and Val (6%) were isolated and kept in refrigerator in a previous phytochemical investigation from TA. The established UHPLC/UV method was used to determine four major alkaloids in the TA (See supporting information, S2.4).

Chemicals

Acetonitrile, methanol, and formic acid (Mallinckrodt Baker, Phillipsburg, NJ, USA) were of HPLC grade. Deionized water was obtained from a Milli-Q system (Millipore, MA, USA). Heparin sodium was purchased from Solarbio (Beijing, China). High-purity nitrogen (99.9%) and helium (99.99%) were purchased from Gas Supplies Center of Peking University Health Science Center (Beijing, China). Ovalbumin (OVA) was purchased from Source Biological Technology Co. Ltd. (Shanghai, China). Aluminum hydroxide was purchased from Sigma-Aldrich (St. Louis, MO, USA). ELISA reagents sets for IgE, eotaxin, IL-4, and IL-10 (Lot 10/2014) were purchased from R&D Systems (Minneapolis, MN, USA). Malondialdehyde (MDA), superoxide dismutase (SOD) and nitric oxide (NO) determination kits were purchased from Jiancheng Bioengineering Institute of Nanjing (Jiangsu, China). All the other chemicals and solvents were of highest purity grade.

Preparation of calibration standard, quality control, and internal standard stock solutions

Reference standards (0.5 mg/ml each for Sch, Val, and Pic) were dissolved in methanol to prepare individual stock solutions, and Epi (0.5 mg/ml) was dissolved in 50% aqueous methanol. These stock solutions were mixed and then serially diluted to obtain calibration standard (CS) stock solutions (125 μg/ml for Sch, 62.5 μg/ml for Epi and Val, and 250 μg/ml for Pic). Quality control (QC) stock