Pulmonary Selectivity and Local Pharmacokinetics of Ambroxol Hydrochloride Dry Powder Inhalation in Rat

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ABSTRACT: The aim of this study was to investigate the local pharmacokinetics and site-specific target efficiency of ambroxol hydrochloride (AH) dry powder inhalation (DPI) by comparing lung epithelial lining fluid (ELF) and plasma AH levels after tracheal administration (TA) with those after intravenous administration. Twelve rats were divided into two groups, one of which was given AH DPI (20 mg/kg) via the trachea and the other was given the same dose AH by intravenous injection (i.v.). Afterwards, each group was subdivided into two groups. The concentration of AH in the ELF was determined by microdialysis in one group while the concentration of AH in plasma was determined in the other group. After AH DPI (20 mg/kg) was given via the trachea, AH achieved a high local concentration in ELF and reached a C_{max} at 1.5 h in plasma. After the same dose AH was given by i.v., AH reached a C_{max} in ELF at 1.25 h. The $(AUC_{0-t})_{ELF}/(AUC_{0-t})_{plasma}$ ratio (1.05–2.25) after TA differed significantly from the ratio (0.029–0.039) observed after intravenous administration ($p < 0.05$). All these results indicate that AH DPI can be delivered to a specific targeted site and achieve high target efficiency in ELF. DPI could be a useful drug delivery system for AH therapy of pulmonary diseases. J Pharm Sci 98:1797–1803, 2009

Keywords: ambroxol hydrochlorides (AH); dry powder inhalation (DPI); microdialysis; pharmacokinetics; lung epithelial lining fluid (ELF)

INTRODUCTION

Ambroxol is an active metabolite of bromhexine and secretory compound[1] which was first used in Germany in 1979 to treat respiratory diseases, and today it is widely used in more than 50 countries. Ambroxol hydrochloride (AH) is often used as a mucolytic agent to increase surfactant secretion in the lung.[2,3] It has also been reported to have a cough-suppressing effect,[4] an anti-inflammatory action,[5] to suppress influenza-virus proliferation[6] and reduce pain symptoms in animal models.[7] Recently, the inhibition of nitric oxide (NO)-dependent activation of the soluble guanylate cyclase was suggested as one of the molecular mechanisms of the therapeutic action of AH.[8] It is frequently used in the treatment of bronchial asthma and chronic obstructive pulmonary disease (COPD),[9] acute upper respiratory disease[4] and infant respiratory distress syndrome.[3] Meanwhile, steam inhalations of AH solution has been applied in the clinical treatment. Clinical experiment showed there were improvements in the expectoration and a reduction in the degree of coughing after AH solution was inhaled with the daily dose (15 mg).[10]

As a specific organ for the treatment of local diseases, the lung is a very attractive target for drug delivery. For *systemic* delivery of drug, it provides an enormous surface area and a relatively low enzymatic activity, and a controlled

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environment for systemic absorption of drugs.[11] The potential advantages of delivering a drug to the lung by inhalation have been well known to scientists, physicians and drug users for many years.[12] Inhalation therapy is gaining importance in the treatment of a number of pulmonary diseases such as asthma and chronic obstructive pulmonary diseases (COPD), by virtue of enhanced local targeting and reduced systemic side effects associated with the administration of low drug dosages.[13] Inhalation therapy is currently being used for the local delivery of drugs to the lung, such as corticosteroids, β-agonists, anticholinergics and mast cell stabilizers, mainly for the treatment of asthma and COPD.[14] Nebulisers, pressurized metered dose inhalers (pMDI) and dry powder inhaler (DPI) systems can all be used to achieve deposition in the target region, but DPI is particularly useful compared with nebulisers and pMDIs, because of its better portability and absence of propellant for drug aerosolization.[15,16]

The aim of this study was to investigate the local pharmacokinetics and site-specific target efficiency of AH DPI by comparing lung epithelial lining fluid (ELF) and plasma AH levels after tracheal administration (TA) with those after intravenous administration.

MATERIALS AND METHODS

Animals

Adult, male Sprague-Dawley rats weighing 250–300 g were provided by the animal laboratory of Shenyang Pharmaceutical University, China. These animals were allowed to acclimatize in environmentally controlled quarters ($24 \pm 1^{\circ}C$ and 12:12 h light–dark cycle) for at least five days before the experiment. These animals were pathogen free. The animals had free access to food and water until 18 h prior to being used in experiments. The experiment complied with the University Animal Ethics Committee Guidelines and was approved by the University Animal Ethics Committee.

Preparation of AH Formulations

Powders were produced using a Spray Dryer SD-1000 (EYELA, Japan) at low relative humidity (30–40%). Aqueous formulations containing AH as the active agent and leucine and mannitol as dispersibility enhancers were prepared, with a total powder mass of 4% (w/v), that is, AH/mannitol/leucine (2.5/1/0.5, w/w/w). When the aqueous solution was sprayed, an inlet air temperature of 110°C, an outlet temperature of $74 \pm 3^{\circ}C$, an atomizing pressure of 17 kPa, a pump rate setting of 1.8 mL min^{-1}, and an airflow rate of 0.70 ± 0.05 $m^3\,min^{-1}$ were used. The powders were collected and stored in a desiccator at 25% relative humidity and 20°C until analysis or *in vivo* assessment.

Parenteral solution (12 mg/mL) was prepared by dissolving AH in physiological saline solution.

Particle Size

The particle sizes of the samples were determined using Beckman Coulter LS 230. Powders were poured carefully into the sample cup. The measurement was then performed for a sample run time of 90 s. Each sample was analyzed in triplicate.

Aerosolization Properties of the Powders *In vitro*

The *in vitro* deposition evaluation of AH DPI was conducted in a twin-stage liquid impinger (TI),[17] using 7 mL purified water as the dilution solvent in the upper impingement chamber D and 30 mL in the lower impingement chamber H, at a continuous air flow rate of 60 L min^{-1} produced by a vacuum pump connected to the outlet F of the impaction apparatus. For each actuation, an approximately half-filled capsule (size 3, loaded with spray-dried powder blends of 24 ± 1 mg) was inserted in the dosage chamber of an Aerolizer® inhaler (Schering Corporation, Kenilworth, NJ) connected to the TI. The pump was switched on and allowed to operate for 10 s.

After 10 actuations for one determination, the apparatus was dismantled. The subsequent steps were:

(1) The upper impingement chamber D, the mouthpiece adapter A, throat B and neck C were washed with the purified water which was transferred to volumetric flasks as stage-1.
(2) The lower impingement chamber H and the coupling E were washed with purified water into volumetric flasks as stage-2.
(3) The capsule and the DPI inhaler were washed separately with purified water into volumetric flasks.

The content of AH in each flask was determined by HPLC assay. Before they were assayed, samples of the solution were passed through a 0.45 μm cellulose acetate filter. These determinations were conducted in triplicate for each preparation.

The deposition in stage-2 represented the respirable fraction (RF), expressed as a percentage indicating the ratio of the mass of drug recovered from stage-2 to the emitted dose.

Animal Experiment

All rats were anesthetized with urethane 0.2 g/mL (1.2 g/kg) by intraperitoneal injection, and placed under an infrared lamp to maintain the body temperature at 37°C. Twelve rats were divided into two groups, one of which was given AH DPI (20 mg/kg) via the trachea and the other was given the same dose of AH by intravenous injection (i.v.). Then each group was subdivided into two, one of which was used to evaluate the concentration of AH in the ELF by microdialysis and the other one for determination of the concentration of AH in plasma.

Six rats in the first group were given AH DPI via the trachea. Each rat was placed on its back with its head towards the investigator, and a 1.5 cm incision was made along the midline of the anterior region of the neck. A 3.5 cm length of PE-240 polyethylene tubing was inserted to a depth of 0.6 cm in the trachea. AH was given by an apparatus used for TA.[18] Six rats in the other group were given the same dose AH by i.v. The rats in which AH in ELF was determined by microdialysis, underwent tracheostomy before drug administration. For the intravenous administration, AH was dissolved in physiological saline and injected into the femoral vein.

Three animals from each group (tracheal and intravenous administration) were used to determine the concentration of AH in the ELF by microdialysis. The microdialysis systems consisted of a microinjection pump (S200, KD Scientific Company, Houston, TX) and a microdialysis probe. The microdialysis probe was positioned within the ELF through the tracheotomy at an angle of about 10° towards the lung as described by Eisenberg and Conzentino et al.[19] (Fig. 1) and then perfused with Ringer's solution[20] (147 mM NaCl, 1.3 mM $CaCl_2$, 4.0 mM KCl (pH 7.2)), at a flow rate of 4 μL/min using an S200 microdialysis syringe pump (KD Scientific Company) for both delivery of the perfusion solution and sample collection. Dialysis samples were collected every 15 min and the dialysate was kept frozen at −20°C for subsequent analysis.

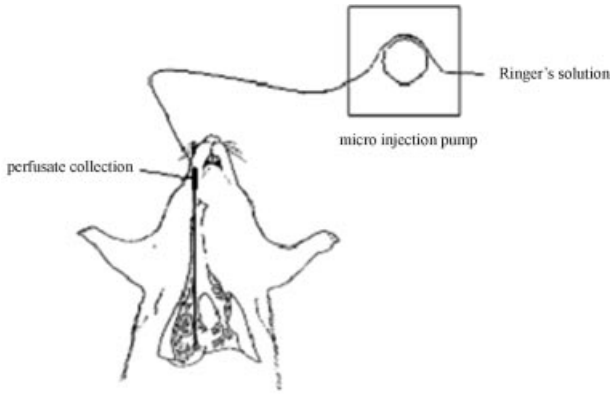

Figure 1. General experimental for ELF pharmacokinetic measurements in the anesthetized rat.

Another three animals from each group (tracheal and intravenous administration) were used to determine the concentration of AH in plasma. Blood samples (0.3 mL) were withdrawn from the fossa orbitalis and transferred heparin stabilized test-tubes at different times, and the sampling time points after TA and after i.v. were 0, 0.083, 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10 h and 0, 0.083, 0.167, 0.25, 0.5, 1, 2, 3, 4, 5, 6, 8, 10 h, respectively. All the blood samples were centrifuged at 4000 rpm for 10 min. The plasma samples were then transferred to new tubes which were then kept frozen at −20°C until analysis.

Analytical Methods

Disposition of Biological Samples

A 0.1 mL plasma sample was transferred to a centrifuge tube, followed by 5 uL internal standard (diltiazem hydrochloride) and 0.1 mL 2.1% citric acid water solution(pH 5.5). The sample was vortexed for 30 s, then two portions of *tert*-Butyl methyl ether was added (2 mL each time), followed by vortexing for 40 s. After the sample was centrifuged at 2000 rpm for 5 min, the organic solvent phase was transferred into another tube, and the two resultant extracts were combined. After adding 30 μL 0.01 mol/L HCl, the tube was vortexed for 40 s and centrifuged at 2000 rpm for 5 min, and then the ether phase was removed. The residue was injected into the HPLC system.

The dialysate was injected directly into HPLC for AH assay without any pretreatment.

Chromatography

The HPLC system consisted of a chromatographic pump (PU-1580, Jasco Corporation, Tokyo, Japan) and an ultraviolet detector (UV-1575). A C18 reverse-phase column (HiQ Sil, 250mm × 4.6 mm, i.d. 5 μm, KYA TECH Corporation, Tokyo, Japan) and its guard column (10 mm × 4.6 mm, i.d.) were used at ambient temperature. The mobile phase consisted of acetonitrile-0.01 mol/L dibasic ammonium phosphate (pH 7.0) (50:50, v/v), operated at a flow rate of 1 mL/min. The mobile phase was passed through a Millipore 0.45 μm filter and degassed prior to use. The UV detector was set at 248 nm and the AUFS of the detector was set at 0.0005.

Data Analysis

To determine the AH concentration in the ELF from the microdialysis data, the concentration of AH in dialysis samples was adjusted with reference to the *in vivo* recovery rate of the probe. The relative recovery (*in vivo* recovery) defined as the ratio of an analytic concentration in the dialysate to its concentration in the surrounding medium, was determined before the experiment. The microdialysis probes were inserted into the ELF of the anesthetized rats. Following the general surgical procedures and insertion of microdialysis probe, more than half-hour equilibration time was allowed for the tissue to recover while the probe was perfused. Then the probes were perfused with Ringer's solution containing AH (1, 5 or 10 μg/mL) at a flow rate of 4 μL/min. The *in vivo* recovery rate of AH was calculated from the following equation: $R_{\text{dial}} = 1 - C_{\text{dial}}/C_{\text{perf}}$,[21] where R_{dial} is the AH *in vivo* recovery rate, C_{perf} is the concentration of AH in the perfusate, and C_{dial} is the concentration of AH in the dialysate.

The area under the plasma concentration–time curve (AUC) value and the ELF concentration–time curve (AUC) value were calculated using the trapezoidal rule. All AUC values and $(\text{AUC}_{0-t})_{\text{ELF}}/(\text{AUC}_{0-t})_{\text{plasma}}$ ratios were calculated for individual animals before applying the paired Student's *t*-test. A value of $p < 0.05$ was considered significant.

The pharmacokinetic model in Figure 2 was used to describe the distribution of AH *in vivo* after TA. The corresponding parameters were

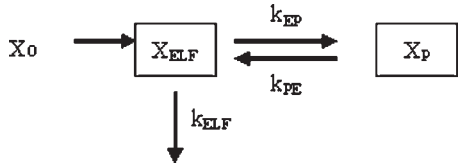

Figure 2. A pharmacokinetic model to describe the distribution of AH *in vivo* after TA.

estimated by Eqs. (1) and (2) using Microsoft Excel software, after Eqs. (1) and (2) were transformed by the Method of Laplace transform. The goodness of fit metrics of the model was attempted to quantify by the coefficient of determination[22]

$$\frac{dX_{\text{ELF}}}{dt} = k_{\text{PE}}X_{\text{P}} - k_{\text{EP}}X_{\text{ELF}} - k_{\text{ELF}}X_{\text{ELF}} \quad (1)$$

$$\frac{dX_{\text{P}}}{dt} = k_{\text{EP}}X_{\text{ELF}} - k_{\text{PE}}X_{\text{P}} \quad (2)$$

where X_{ELF} and X_{P} are the dosage in the ELF and plasma, respectively. The parameters, k_{EP}, k_{PE} and k_{ELF}, are the first order rate constants.

Eq. (3) was obtained, after Eqs. (1) and (2) were transformed by Method of Laplace transform and simplified

$$C_{\text{ELF}} = A\,e^{-\alpha t} + B\,e^{-\beta t} \quad (3)$$

where $A = \text{Co}((\alpha - k_{\text{PE}})/(\alpha - \beta))$, $B = \text{Co}((k_{\text{PE}} - \beta)/(\alpha - \beta))$, $\alpha + \beta = k_{\text{PE}} + k_{\text{EP}} + k_{\text{ELF}}$, $\alpha\beta = k_{\text{PE}}\ k_{\text{ELF}}$. The parameters, k_{EP}, k_{PE}, k_{ELF}, A, B, α and β were calculated using Microsoft Excel software.

RESULTS

Powder Characteristic

Powders from spray drying had a mean particle size of 4.70 μm with a distribution, that is, d [υ, 10] 2.31 μm, d [υ, 50] 4.64 μm and d [υ, 90] 9.27 μm. The percentage of AH in stage-1, stage-2, inhaler and capsule was 38.56%, 30.93%, 13.42%, and 17.08%, respectively. The RF was 44.51%, indicating that the powder was suitable for inhalation administration.

Pharmacokinetic Study

Figure 3 shows the concentration–time profile of AH in rat ELF (corrected by *in vivo* recovery) after AH DPI (20 mg/kg) was given by TA and AH achieved a high local concentration in ELF, then fell rapidly over 2 h, and was finally eliminated slowly until 12 h. Figure 4 shows the concentration–time profile of AH in rat ELF after

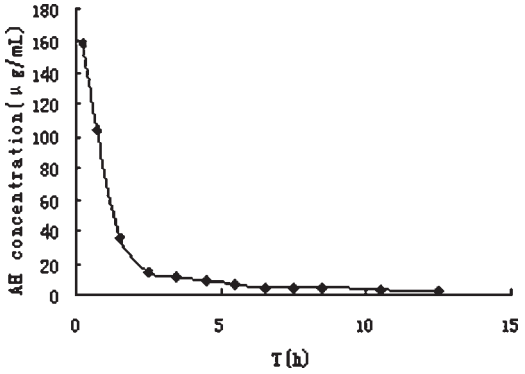

Figure 3. Mean concentration–time profile of AH determined by microdialysis in rat ELF after TA at the dose of 20 mg/kg ($n = 3$).

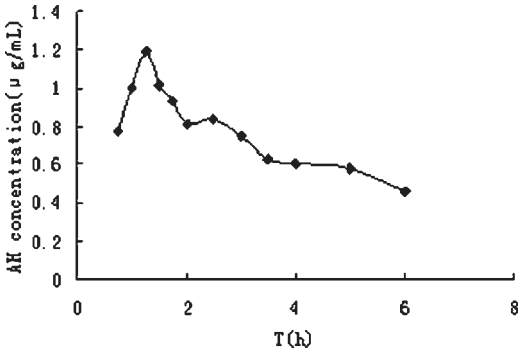

Figure 4. Mean concentration–time profile of AH determined by microdialysis in rat ELF after intravenous administration at the dose of 20 mg/kg ($n = 3$).

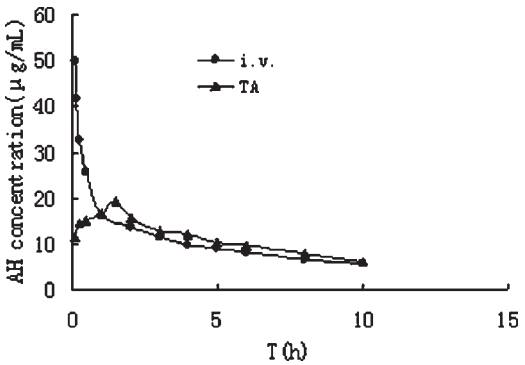

Figure 5. Mean concentration–time profile of AH determined in rat blood after TA and intravenous administration at the dose of 20 mg/kg ($n = 3$).

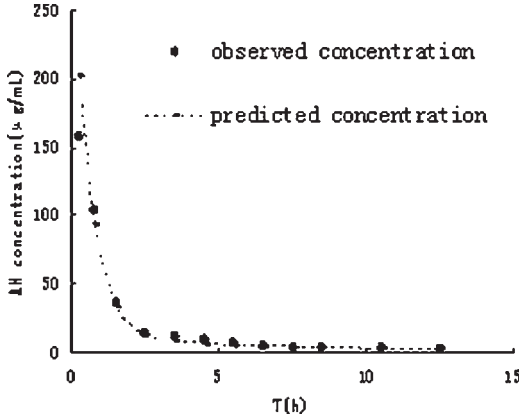

Figure 6. The concentration–time profile of AH in ELF after TA; observed versus model predicted concentrations.

i.v. administration; the C_{max} in ELF was at 1.25 h, while the concentration of AH in ELF remained low and could not be detected after 6 h. The AH in blood after TA reached a C_{max} at 1.5 h (Fig. 5). The concentration of AH in blood after intravenous administration rapidly decreased (Fig. 5). Figure 6 shows concentration–time profile of AH in ELF after TA; observed versus model predicted concentrations. As shown in Table 1, the $(AUC_{0-t})_{ELF}/(AUC_{0-t})_{plasma}$ ratio (1.05–2.25) after TA differed significantly from the ratio (0.029–0.039) observed after i.v. administration ($p < 0.05$). The pharmacokinetic parameters after TA and i.v. administration is listed in Table 2. The coefficient of determination (R^2) were >0.9.

Table 1. AUC_{ELF}/AUC_{plasma} Ratios after TA and i.v. in Rats (Range of Values, n = 3) ($p < 0.05$)

	TA	i.v.
AUC_{ELF} (μg h/mL)	124.80–254.76	3.04–4.18
AUC_{plasma} (μg h/mL)	86.67–113.47	92.97–126.86
AUC_{ELF}/AUC_{plasma}	1.05–2.25	0.029–0.039

Table 2. Pharmacokinetic Parameters of AH after TA in Rats (Range of Values, n = 3)

Pharmacokinetic Parameters	The Range of Values
A (μg/mL)	88.88–391.47
B (μg/mL)	1.21–27.71
α (h^{-1})	0.80–2.32
β (h^{-1})	0.11–0.13
k_{EP} (h^{-1})	0.042–0.90
k_{PE} (h^{-1})	0.13–0.32
k_{ELF} (h^{-1})	0.75–1.55
$t_{1/2(TA)}$ (h)	5.21–9.49

DISCUSSION

The concentration of AH at the site of action is generally regarded as a more important factor than that in plasma in terms of achieving an effective clinical treatment. The data obtained here suggest that AH can achieve a high local concentration in ELF after TA. As shown in Table 1, the $(AUC_{0-t})_{ELF}/(AUC_{0-t})_{plasma}$ ratio (1.05–2.25) after TA differed significantly from the ratio (0.029–0.039) observed after i.v. administration ($p < 0.05$). The results of this study show that AH DPI can be delivered to a specific targeted site and achieve a high local activity with a *higher ratio of concentration of AH in the lung versus blood* and longer-lasting effect compared with that after i.v. administration. Accordingly local treatment with AH DPI is able to achieve its therapeutic goal and drug targeting should reduce the dose required to achieve a desired pharmacological effect. Consequently the systemic load of the drug may be less leading to reduced systemic side effects.

The initial concentration of AH in the ELF was high (Fig. 3), because AH DPI after TA dissolved immediately in the ELF. Because of the huge surface area of the alveoli, there is immediate accessibility of drug and the increased blood flow rapidly distributes the drug throughout the body; the lung has been in fact demonstrated to be an efficient port of entry to the bloodstream[23] and because AH has a high affinity for lung tissue,[2] the concentrations of AH in ELF (Fig. 3) fall rapidly because AH is absorbed rapidly into the blood and lung tissue. However, Figure 3 also shows that the AH concentration in the ELF 2 h after TA; it is eliminated slowly until 12 h. There are several possible explanations for this observation. First of all, it could be that after AH was absorbed rapidly into the blood, it entered the right ventricle through the bronchial veins and then passed into the pulmonary circulation. Secondly, although AH has a high affinity for lung tissue,[2] after AH was transported to lung tissue, some AH could be returned to the ELF.

The alveolar barrier consists of three layers: the capillary lumen, the connective tissue and the alveolar epithelial cells between plasma and ELF.[24] So, even if AH is known to have a high affinity for lung tissue,[2] the lung could be rapidly equilibrating with a compartment that appears to be a large peripheral compartment but, in fact, anatomically is not the lung, it does not represent the situation in ELF. Therefore, very little AH after i.v. administration is transported from the plasma to the ELF via numerous relatively impermeable barriers between the plasma and ELF in the lung. As shown in Figure 4, the concentration of AH in ELF after i.v. administration was low.

As AH is used for the treatment of respiratory diseases, the pharmacokinetic parameters of AH in the lung are more important than those in the blood. Therefore, a simulation model that describes the concentration–time course of AH between plasma and ELF was constructed in rats. Pharmacokinetic parameters of AH in the lung can be obtained from the simulation model. So ELF levels of AH for other doses can be obtained using the simulation. The simulation model could be interesting for preclinical trials and future clinical trials. The tendency of the predicted concentration points of AH by the pharmacokinetic model shown from Figure 6 is identical with the concentration points of AH observed. Therefore, the parameters of the model could be acceptable, which is further confirmed by the goodness of fit metrics of the model. Studies have shown that the pharmacokinetic parameters (half-life) after TA are similar to those in a previous report.[25]

In conclusion, it was found that AH DPI could be delivered to specific targeted sites, that is, possible sites for pulmonary action and achieve high target efficiency in the ELF. DPI could be a useful drug delivery system for AH therapy of pulmonary diseases. However, it is not known whether the lung was damaged or not by AH inhalation powder. Further investigation of this is currently underway.

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