Utility of Gamma Scintigraphy in Assessing Mucociliary Clearance in COPD

M Limb[1], R Bacon[1], P Scholes[1], M Longphre[2]

[1]Pharmaceutical profiles Ltd, Mere Way, Nottingham, NG11 6JS; [2]Aerovance Inc, Berkeley, USA

Summary

Gamma scintigraphy was used in this clinical trial to assess the effect of a truncated form of the human serine protease inhibitor AER-002 on mucociliary clearance (MCC) in patients with COPD. In this two period, randomised, placebo-controlled study, patients underwent a baseline assessment of MCC with nebulised colloid followed by a further assessment of MCC with nebulised colloid after five days of dosing with either AER-002 or placebo. Analysis of the scintigraphic data demonstrated that for both central and whole lung over the two study periods MCC improved, but there was no statistical difference between AER-002 and placebo. There was no difference between AER-002 and placebo in the incidence of treatment emergent adverse events.

Introduction

Diagnostic imaging using gamma scintigraphy is a well-established procedure in nuclear medicine (1) and has been used extensively in inhalation drug delivery development for over twenty years (2). It is the only non-invasive method currently capable of providing human data on total and regional lung deposition and mucociliary clearance (MCC) (3).

Chronic Obstructive Pulmonary Disease (COPD) is defined by the Global Initiative for Chronic Obstructive Lung Disease as a disease characterised by airflow limitation that is not fully reversible. This is usually both progressive and associated with an abnormal inflammatory response of the lungs. COPD affects approximately 20-30 million people in the U.S. and is a growing concern worldwide, being designated a Top 5 disease by the World Health Organisation (4).

The test product in this study was AER-002, a truncated form of the naturally occurring human serine protease inhibitor SPINT2. Because it inhibits the serine protease prostasin that is required to activate epithelial sodium channel (ENaC) (5, 6), the major Na^+ channel in the apical airway epithelium, administration of AER-002 to the airways of COPD subjects was expected to improve MCC, lung function and COPD symptoms. Blocking ENaC reduces absorption of Na^+ from the airway surface to the inside of the airway epithelial cells. Higher Na^+ concentrations on the airway surface leads to restoration of the normal depth of the airway surface liquid (ASL), and thus better hydration of the mucus layer leading to enhanced mucociliary transport. In animal models, inhibition of ENaC effects an improvement in tracheal mucus velocity, lung function, and lung clearance (7, 8].

Objectives

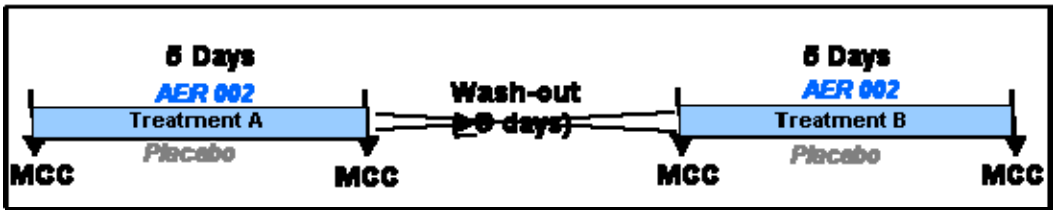
The primary objective of this study was to determine the effect of AER-002 on the MCC rate of a radiolabelled particle from the lungs of patients with COPD after 5 days of treatment with AER-002 versus placebo. Secondary objectives included determination of the initial deposition pattern of radioactivity in the lungs and the effects of AER-002 on symptoms and spirometry.

Methods

The study was a single centre, double-blind, placebo-controlled, randomised, single cross-over, looking at the effects of AER-002 (30mg nebulised twice daily for 5 days) on MCC in male and female patients with COPD. The study was conducted at Pharmaceutical Profiles, Nottingham, UK, with patients referred from three local hospital sites.

Sufficient subjects (maximum of 30) were recruited to ensure 16 completed data sets. Half were randomised to receive 30mg AER-002 twice daily for 5 days then after a 3 day washout period, matched volume placebo for 5 days. The other half received matched volume placebo for 5 days then after a 3 day washout period, 30mg AER-002 twice daily for 5 days. Both AER-002 and placebo were administered by nebulisation using a Pari LC Plus® nebuliser. During the study each subject underwent a transmission scan (^{57}Cobalt [^{57}Co] flood source) of the thorax to provide an outline of the lungs for use in the subsequent analysis. Prior to the first and after the last administration of drug/placebo in each treatment arm, subjects received nebulised particulate colloid radiolabelled with ^{99m}Technetium (^{99m}Tc) for the assessment of MCC. A schematic flow diagram of the study is shown in Figure 1.

Figure 1. **Schematic flow diagram of study**

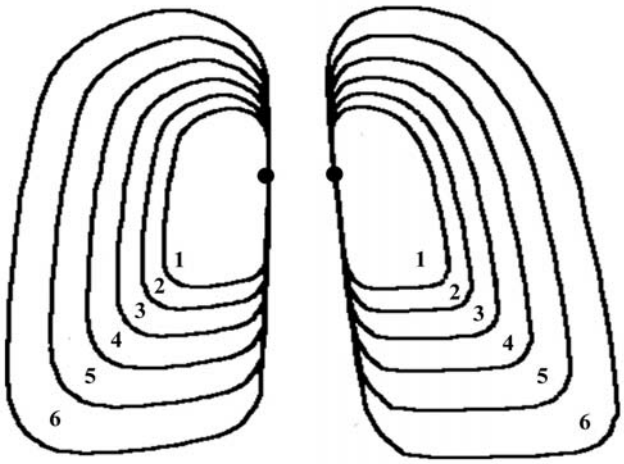


Source: Appendix 16.1.1, Study protocol
MCC = Mucociliary clearance measurements

Scintigraphic imaging and clearance assessment

Gamma scintigraphy was used to quantify the amount of radiolabelled particles deposited in the lungs over a 6 hour period at baseline and the end of each study arm. Anterior and posterior images of the lungs were taken immediately after inhaling colloid (time 0) and then intervals up to 6 hours after inhalation. Initial distribution in the 6 lung zones (see Figure 2.) immediately after inhalation was determined and the amount of ^{99m}Tc in each of six lungs zones was calculated at each time point. The AUCs of the retained amounts of radioactivity were calculated according to the log-linear trapezoidal method. Percentage retention in the central (zones 1-3 in Figure2.) and whole lung was calculated from 0 to 1, 0 to 2 and 0 to 6 hours post-dose.

Figure 2 **Definition of the six lung zones [15]:**



● = Hilum, the point at which the bronchus enters the lungs.

Results

The primary efficacy parameter was the change from baseline of the AUC values for the amount of radioactivity retained in the lungs from 0 to 2 and 0 to 6 hours.

Table 1 **Summary of the mean % change in radioactive counts in the lungs after five days dosing with AER-002 and placebo**

Parameter	Mean % Change in Radioactive Counts from Baseline*		P value
	AER 002	Placebo	
AUC Central lung 0-2 h	-20.9 (93.2)	-24.0 (79.6)	0.968
AUC Central lung 0-6 h	-24.7 (94.6)	-26.1 (77.3)	0.856
AUC Whole lung 0-2 h	-7.5 (741.6)	-19.2 (143.7)	0.456
AUC Whole lung 0-6 h	-18.7 (105.4)	-20.9 (92.7)	0.418

*N = 17

Note: Decrease in radioactive counts equates to an increase in MCC

- For both central and whole lung over the two time periods MCC improved but there was no observable or statistical difference between AER-002 and placebo.
- Many subjects exhibited increases in clearance rates in both study arms, particularly for the 1-hour clearance rate which showed increases from a mean of 4.2%/hr to 19.3%/hr for AER-002, and from 3.4%/hr to 20.3%/hr for placebo.

Figure 3 **Representative scintigraphic images showing lung deposition at t=0 and t=0.5hrs for the baseline assessment and after five days dosing with AER-002**

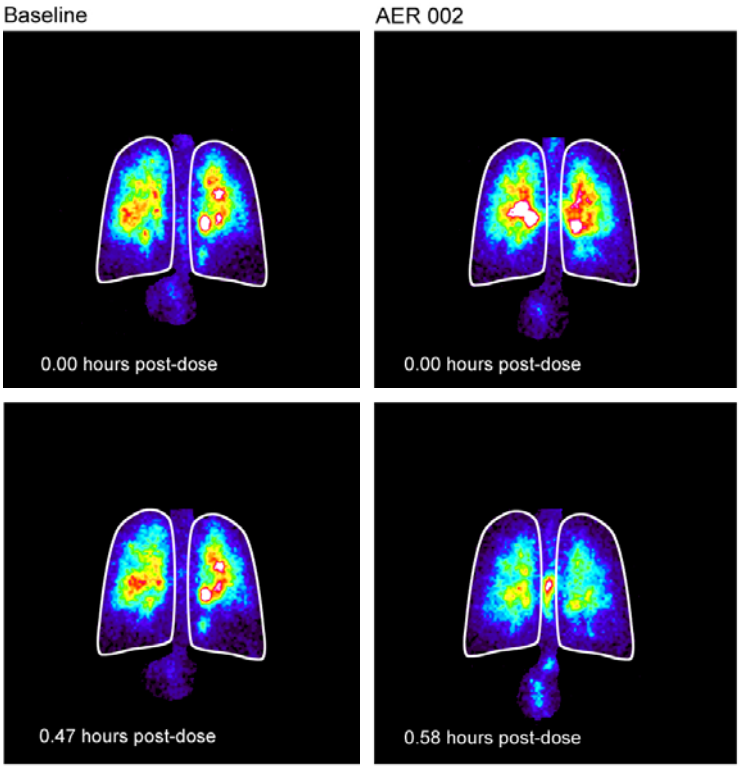
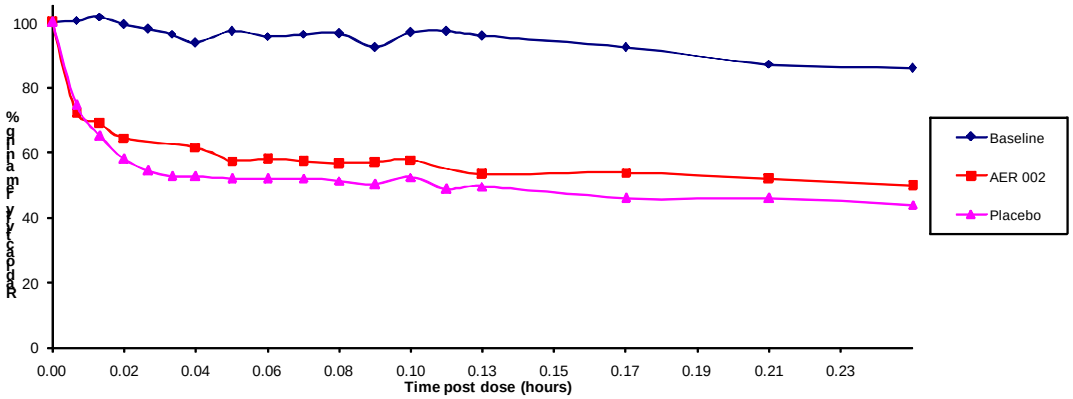


Figure 4 **Graph showing percentage clearance of radioactivity from the whole lung against time for baseline, placebo and test (AER 002) periods for a single subject.**



Safety and tolerability results

- There was no difference between AER-002 and placebo in the incidence of treatment emergent AEs.

Discussion

The data generated during the course of the study demonstrated that although AER-002 was safe and well tolerated in the population studied, there was no significant difference in MCC between AER-002 and placebo. This result was unexpected given the mechanism of action of the product. The majority of enrolled subjects exhibited fairly dramatic increases in clearance rates in both study periods, particularly for the 1-hour clearance rate which showed increases from a mean of 4.2%/hr to 19.3%/hr for AER-002 treatment, and from 3.4%/hr to 20.3%/hr for placebo treatment, but no differences between treatments. Scintigraphy was able to detect changes in MCC after the administration of placebo and test compared to baseline confirming the use of this technique to measure the effect of formulations on MCC in patients with COPD.

Conclusions

In summary, mucociliary clearance improved after 5 days nebulised dosing of AER-002, but no differences were observed between AER-002 and placebo. The treatment was safe and well tolerated.

References

1. Newman, S. (1993), "Scintigraphic assessment of therapeutic aerosols," *Critical reviews in therapeutic carrier systems;* Vol. 10, pp 65-109.
2. Scholes, P. *et al.* (2008), "Future applications of gamma scintigraphy in inhaled drug development," submitted to RDD conference.
3. Newman, S. and Wilding, I. (1996), "The importance of gamma scintigraphy in pharmaceutical development," *J. Pharm. Med.;* Vol. 6, pp 21-33.
4. WHO World Health Report 1999.
5. Tong Z, Illek B, Bhagwandin VJ, Verghese GM, Caughey GH. Prostasin, a membrane-anchored serine peptidase, regulates sodium currents in JME/CF15 cells, a cystic fibrosis airway epithelial cell line. Am J Physiol Lung Cell Mol Physiol 2004; 287:L928-935.
6. Bridges RJ, Newton BB, Pilewski JM, Devor DC, Poll CT, Hall RL. Na+ transport in normal and CF human bronchial epithelial cells is inhibited by BAY 39-9437. Am J Physiol Lung Cell Mol Physiol 2001; 281:L16-23.
7. Verkman AS. Lung disease in cystic fibrosis: is airway surface liquid composition abnormal? Am J Physiol Lung Cell Mol Physiol 2001; 281:L306-308.
8. Widdicombe JH. Volume of airway surface liquid in health and disease. Am J Resp Crit Care Med 2002; 165:1566.