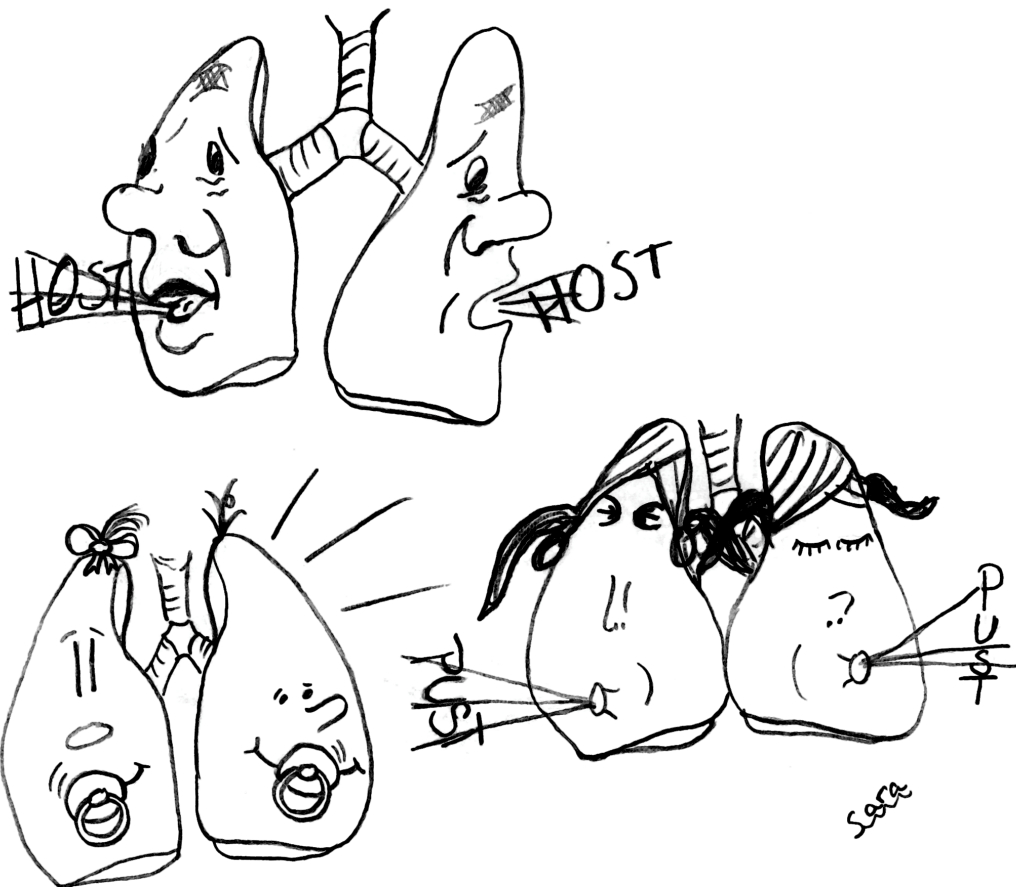


Particles in small airways:
mechanisms for deposition and
clearance
&
Pharmacokinetic assessment
of delivered dose to the lung



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Stockholm 2004

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Cover illustration: “människolungor” drawn by the authors daughter, Sara Lindström, 9 years old.

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To listen is to learn, and to understand is to inspire.

To Pontus and Sara

Summary

BACKGROUND

Knowledge about lung deposition and clearance from airways of inhaled particles/drugs are essential for evaluation of health effects of inhaled pollutants and to achieve optimal drug dose to the lung. The primary defence mechanism in the conducting airways is the mucociliary clearance (MCC). When MCC is defective, as in Cystic Fibrosis (CF) and Primary Ciliary Dyskinesia (PCD), cough can serve as back up in the larger airways. The importance of MCC from the small airways (< 2 mm in diameter) is still unknown. Most studies of lung deposition and clearance are performed with imaging methods using radiation, and are not suitable for routine clinical investigations. A simple pharmacokinetic method to evaluate the pulmonary dose would be beneficial.

OBJECTIVE

The aims of the studies were 1) to investigate the importance of mucociliary clearance to eliminate particles from the small airways, 2) to evaluate if the slow inhalation method is feasible for patients with high airway resistance, and 3) to develop a simple non-radioactive method to assess the deposited dose in the lung.

METHODS

Clearance in small airways was studied in patients with CF and PCD, using the extremely slow inhalation flow method (ESI). The inhalation method deposits particles mainly in the small ciliated airways. Clearance was evaluated by measuring lung retention up to 21 days after exposure, and the results were compared with data from age matched healthy controls.

Inhaled sodium cromoglycate (SCG) was measured both in plasma and urine to estimate the bioavailability and to evaluate what measurement had the best reproducibility. In an other study the SCG method was used in asthmatic children to evaluate the relative humidity effect on droplet size distribution and the effect on lung deposition.

RESULTS

The particle retention (% of deposition) in the lung at 24 h was higher in patients with CF, $67\pm 13\%$, and PCD, $79\pm 11\%$, compared to the healthy subjects, $48\pm 9\%$ ($p < 0.001$), probably due to their defective MCC. There was however a significant clearance after 24 h in all subjects with equivalent velocity during day 7 to day 21. The SCG method with individual plasma analyses showed best correlation between the two exposures and was easy to control. In the study with asthmatic children, the tidal volume corresponded to the deposited amount of drug. No difference in lung deposition measured with the SCG-method however was shown.

CONCLUSIONS

These studies show that despite defective mucociliary clearance, clearance continues in small airways. Apparently there are other clearance mechanisms present in the small airways. The extremely slow inhalation flow technique was shown to be feasible in patients with high airway resistance, and can be used for diagnostic purposes or for delivery of therapeutic drugs. The SCG-method, using plasma analyses, is a simple pharmacokinetic method that can be used in clinical situation, e.g when evaluating individual inhalation techniques. In asthmatic children a larger tidal volume can give greater lung deposition, provided that the droplets are not too small.

Table of contents

Summary	5
Table of contents	6
Abbreviations	7
Original papers	8
Introduction	9
<i>Background</i>	9
<i>Respiratory tract</i>	9
<i>Normal lung development</i>	11
<i>Lung deposition</i>	13
<i>Methods targeting the small airways</i>	15
<i>Assessments of lung deposition</i>	16
<i>Lung clearance</i>	18
<i>Inherited diseases affecting mucociliary clearance</i>	20
Aims of the thesis	22
<i>General aim</i>	22
<i>Specific aims</i>	22
Subjects and methods	23
<i>Pharmacokinetic studies</i>	23
<i>Clearance studies</i>	25
<i>Statistical analyses</i>	28
<i>Ethics</i>	28
Results and comments	29
<i>Pharmacokinetic studies</i>	29
<i>Clearance studies</i>	30
General discussion	32
<i>Pharmacokinetic studies</i>	32
<i>Clearance studies</i>	34
Conclusions	37
Clinical applications and future perspectives	38
Svensk sammanfattning	40
Acknowledgements	42
References	44

Abbreviations

ADS	anatomic dead space
AM	alveolar macrophages
ASL	airway surface liquid
AUC	area under the curve
Bq	bequerel
BPD	bronchopulmonary dysplasia
cAMP	cyclic adenosine monophosphate
CEN	Comité Européen Normalisé
CF	cystic fibrosis
CFTR	cystic fibrosis transmembrane conductance regulator
D_{ae}	aerodynamic diameter
DPI	dry powder inhaler
ESI	extremely slow inhalation flow method
$FEF_{25-75\%}$	forced expiratory flow between 25-75% of exhaled volume
FEV_1	forced exhaled volume in 1 sec
FRC	functional residual capacity
FVC	forced vital capacity
GI	gastrointestinal
GSD	geometric standard deviation
HPLC	high-performance liquid chromatography
ICRP	International Commission on Radiological Protection
IgA	immunoglobulin A
LOD	limit of detection
LOQ	limit of quantitation
MCC	mucociliary clearance
MMAD	mass median aerodynamic diameter
NaF	sodium fluoride
NaI	sodium iodine
PCD	primary ciliary dyskinesia
PCL	the periciliary layer
PFT	pulmonary function test (spirometry)
pMDI	pressurized metered dose inhaler
R_{aw}	airway resistance
Ret_{24}	particle retention at 24 h
RH	relative humidity
SCG	sodium cromoglycate
SD	standard deviation
SV	sievert
VFD	volumetric front depth

Original papers

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals:

- I. **Lindström M.**, Svensson J.O., Meurling L., Svartengren K., Anderson M., Svartengren M. A simple pharmacokinetic method to evaluate the pulmonary dose. – Analyses of inhaled sodium cromoglycate. *Respiratory Medicine* 2004; 98;1: 9-16.
- II. Nerbrink O., **Lindström M.**, Meurling L., Svartengren M. Inhalation and deposition of nebulised sodium cromoglycate in two different particle size distribution in children with asthma. *Pediatric Pulmonol.* 2002; 34:351-360.
- III. **Lindström M.**, Camner P., Falk R., Hjelte L., Philipsson K., Svartengren M. Long term clearance from small airways in patients with Cystic Fibrosis. Submitted revised version 2004-01 *Eur Respir J.*
- IV. **Lindström M.**, Falk R., Hjelte L., Philipsson K., Svartengren M. Clearance from small ciliated airways independent of ciliary function. Submitted.

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Introduction

Background

This thesis is divided in two parts that focus on 1) mechanisms of particle deposition in small airways and clearance from this site in the lung, and 2) non-radioactive assessment to measure the delivered dose to the lung.

The lung is an “external organ” in the sense that it is continuously and directly exposed to the environment. In order to protect the lung from unwanted material, the airways has to be a highly effective filter, and if materials settle in the airways, efficient clearing mechanisms need to come into action.

A region of interest in the airways is the small airways with a diameter less than 2 mm. Many obstructive diseases affect the small airways, but there are a limited number of clinical studies that have tried to assess deposition in, and clearance from this regional site of the lung. Further knowledge about clearance from small airways, both in healthy subjects and in subjects with lung diseases, is needed.

Inhalation therapy is a widely used and well-accepted treatment for many lung diseases, especially asthma. Almost every physician has prescribed pharmaceuticals for inhalation to a patient. Of the nominal, prescribed dose, about 30 % will under good conditions, reach the lower airways, but probably less. One reason for unachieved effect of the inhaled drug could be poor inhalation technique and consequently low dose to the lung. The inhaled pulmonary dose to the lung is difficult to predict, and a simple method to estimate *in vivo* the delivered dose to the lung is desirable.

Respiratory tract

The cardinal functions of the human lung can be divided into two aspects: *ventilation* and *gas exchange*. The human airways consist of the *upper airways*; the nasopharyngeal region, including the nasal cavity down to the epiglottal level in the larynx, and the *lower airways*; the tracheobroncheal region, which includes the ciliated airways from trachea down to the terminal bronchioles, and the alveolar region with non-ciliated airways, which is the site of the gas exchange.

The branching pattern of the lower airways is a complex three-dimensional system of progressively branching with gradual decreasing airway diameter distally, whereas the total cross-sectional area increases. The branching system of the lower airways could be looked upon as an upside-down tree. This branching system provides the maximal surface area for gas exchange within a small volume; the alveolar surface area is larger than the size of a tennis court (100-150 m²), whereas the airway surface area is only about 0.5 m².



The number of branches between the hilum and periphery varies between 8 in some segments of the upper lobe, to 24 in the longest segments of the lower lobes.⁽⁵³⁾ It is therefore difficult to describe the airways in a simple model. One of the most used airway model is the model proposed by Weibel.⁽¹⁴⁰⁾ In the Weibel model the

INTRODUCTION

airways multiply in a regular dichotomy, where each generation corresponds to one branch of the respiratory tree. For each generation the diameter of the airway lumen decreases, but the sum of the total cross-sectional area increases exponentially, Table1.

The large airways consist of the generations 0-8, the small airways consist of the generations 9-15 and the alveolar region consists of the remaining 16-23 generations.

The conducting airways from the nose to the respiratory bronchioles are lined with ciliated epithelium, admixed with numerous mucus-secreting goblet cells and submucosal glands, down to the small bronchi.

The non-ciliated alveolar epithelium is made of type I cells, pneumonocytes, which cover most of the alveolar surface (93%), forming the thin gas-exchange barrier, and the less frequent type II cells (7%), synthesising the surfactant.

Table 1. Dimensions of human airway model “A” by Weibel 1963.⁽¹⁴⁰⁾ Average adult lung with volume of 4.8 L.

Anatomical structure	Generation	Number per generation	Mean diameter (cm)	Mean length (cm)	Cross-sectional area (cm ²)
Trachea	0	1	1.80	12	2.54
Main bronchi	1	2	1.22	4.8	2.33
Lobar bronchi	2	4	0.83	1.9	2.13
Segmental bronchi	3	8	0.56	0.76	2.00
	4	16	0.45	1.27	2.48
Subsegmental bronch	5	32	0.35	1.07	3.11
	6	64	0.28	0.90	3.94
	7	128	0.23	0.76	5.31
	8	256	0.186	0.64	6.95
<hr style="border-top: 1px dashed black;"/>					
Terminal bronchi	9	512	0.154	0.54	9.53
	10	1024	0.130	0.46	13.6
Bronchioles	11	2048	0.109	0.39	19.1
	12	4096	0.095	0.33	29.0
	13	8192	0.082	0.27	43.2
	14	16384	0.074	0.23	70.4
	15	32768	0.066	0.20	112
Terminal bronchioles	16	65536	0.060	0.165	185
Respiratory bronchioles	17	131907	0.054	0.141	300
Alveolar ducts	18	262144	0.050	0.117	534
	19	524288	0.047	0.099	944
	20	1489576	0.045	0.083	1600
Alveolar Sacs	21-23	15000000	0.042	0.060	~140m ²

Small airways

The peripheral or small airways, generations 9-15 according to the Weibel model,⁽¹⁴⁰⁾ and the definition used in this study, are usually defined as ciliated airways that are less than 2 mm in internal diameter in the adult airways, and extend from the non-cartilaginous bronchioles to the alveolar ducts, but not including the acinus.

In the small airways the surface is covered with ciliated epithelium, but unlike the bronchi contains no submucosal glands or goblet cells. Instead of goblet secreting cells, there are non-ciliated granulated Clara cells that secrete the mucus-poor lining protein. In asthma and chronic bronchitis the inflammatory process is considered to be present both in “small and large” airways.⁽⁵⁷⁾ In cystic fibrosis, there is reason to believe that early morphological changes first appear in the small airways,⁽⁶¹⁾ and as a consequence affect mucociliary clearance (MCC).

The small airways are a transitional zone between the conducting airways and the gas exchange site. They are also pathways of low resistance, and contribute only to about 10 % of the total resistance,⁽⁷³⁾ due to the relatively large cross-sectional area with a decrease in velocity of the airway flow rate. If resistance in small airways will double, it would only increase total resistance by 10 %. Therefore, the term “quiet zone” of the small airways seems adequate, since a relatively pronounced inflammation and obstruction will be undetected by usual lung function tests.

Normal lung development

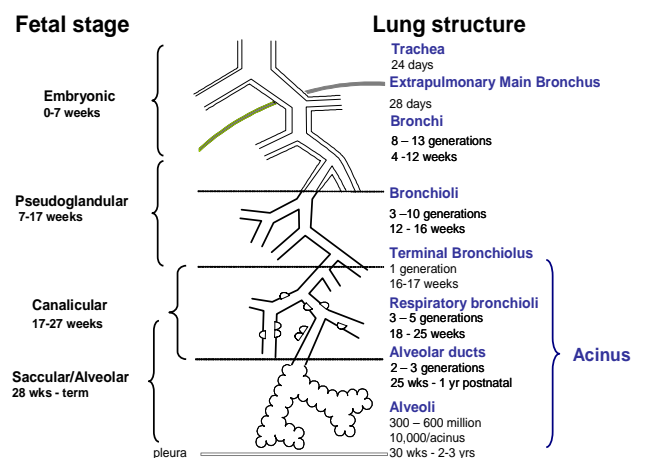
The lung growth in utero begins shortly after the conception during the organogenesis in **the embryonic** period gestational weeks 1-7. Lung buds appear as a ventral outgrowth of the primitive foregut through the primitive hypopharynx. During this period primitive arteries and veins appear.

In **the pseudoglandular stage**, gestational weeks 7-17, dichotomous branching of the bud develops, and all airways to the terminal bronchioles are present by week 16 of gestation.⁽⁶²⁾ Bronchial smooth muscles is present from 6-7 weeks, the smooth muscle are able to respond to nerve stimulation at 8 weeks of gestation. Cartilage appears before the 10th week and reaches the last airway generations by week 25 of gestation.⁽²⁰⁾ Primitive ciliated cells appear at week 10 approximately.

In gestational weeks 17-27, **the canalicular stage**, early acini become visible in the light microscope. Cellular differentiation commences from proximal to distal. The primitive cuboidal cells differentiate into type-1 epithelial cells and type-2 cells. Airway wall structure is mature by 24-26 weeks of gestation, and by this stage type-2 cells are capable of producing and store the surfactant.⁽⁵¹⁾

Later in gestation, surfactant begins to be secreted into airway lumen. Each airway ends in a blind saccule. At this **saccular stage**, the saccules start to divide, and alveolarisation begins. True alveoli appear from about 30 weeks of gestation. The capillary network gets closer together and the walls between the sacs contain a double capillary network.

Figure 1. Lung development in fetus.



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The foetal breathing movements can be observed using ultrasound in the late second trimester. The breathing activity and the circulation of amnioc fluid in the lung is necessary for the lung to develop.⁽¹⁸⁾ In Potter syndrome, oligohydramnios is present due to kidney agenesis, and consequently the lungs are poorly developed and the child usually dies early after birth due to hypoxia.⁽¹⁰⁹⁾

Postnatal lung growth

At birth about one third to half the adult number of the alveoli is formed.⁽⁶⁴⁾ Table 2. The lung continues to grow symmetrically in length and diameter after birth. Alveoli continue to multiply and enlarge and the airways continue to both enlarge and elongate. The complete numbers of alveoli and peripheral airway calibre are reached approximately at 2-3 years of age.^(33, 133)

Nevertheless, the lung continues to grow in volume as alveoli increase in size, complexity and surface area until the end of puberty. The period, during which the lung grows, is longer for boys than for girls, and the trachea of boys becomes relatively larger. There is also some evidence that boys have more alveoli than girls.⁽¹³³⁾

Premature delivery has little effect on the overall alveolar multiplication or airway growth.^(51, 80) However, artificial ventilation leads to abnormalities of alveolar growth, architecture and influences airway wall structure, especially in infants who develop bronchopulmonary dysplasia (BPD) after ventilatory assistance.^(50,52)

Factors influencing airway branching earlier in gestation cannot be corrected once the period of airway multiplication is completed.⁽²¹⁾

Table 2. Approximate measurements of the newborn and the adult lung.

Parameter	Fullterm newborn	Adult
Body weight (Kg)	3.5	70
Lung weight (g)	50	800
Tracheal diameter (mm)	8<	18
Number of airways (x 10 ⁶)	1.5	14.0
Alveolar diameter (µm)	50-100	200-300
Alveolar surface area (m ²)	4	80
Number of alveoli (x 10 ⁶)	124	296
Respiratory rate at rest	40	20
Tidal volume (mL/Kg)	6	7
Functional residual capacity (mL/Kg)	30	34
Vital capacity (ml/Kg)	33	52
Dead space (ml/Kg)	2.2	2.2
Alveolar ventilation (ml/Kg min)	100-150	60
Oxygen consumption at rest (ml/Kg min)	6	3

Lung deposition

Deposition means the event of a particle to adhere to the surface. Inhaled particles are deposited in the airways depending on the interaction of certain physical properties, such as particle size, breathing pattern, airway geometry, and deposition mechanisms.

The most important mechanisms, by which airborne particles can deposit in the respiratory tract, include impaction, sedimentation, Brownian diffusion, and electrostatic attraction.

Deposition mechanisms

IMPACTION:

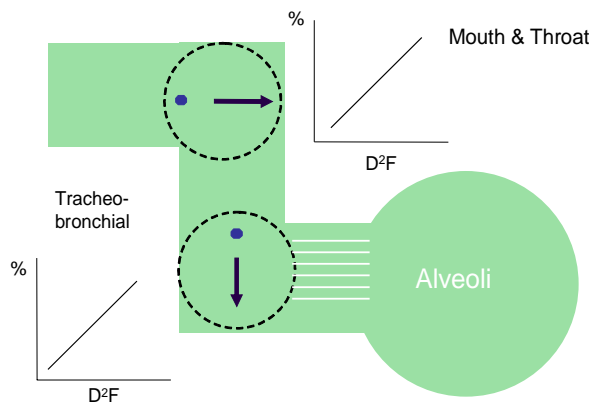


Figure 2

Impaction is most important in the upper airways and in the larger airways. Impaction is a flow dependent mechanism for particles larger than $1 \mu\text{m}$.

The probability of impaction can be described by the parameter D^2F , the square of the aerodynamic diameter (D) multiplied by the inhalation flow (F). With increasing size of the particle and increasing velocity of the airflow the larger probability of impaction.

SEDIMENTATION:

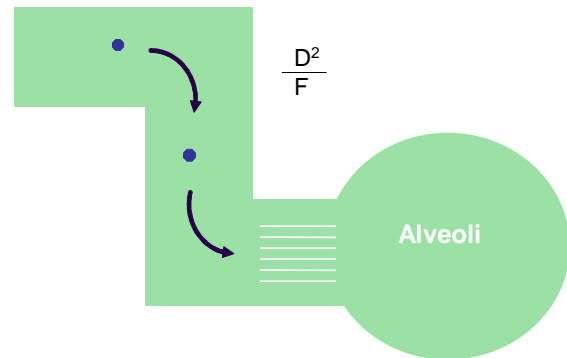


Figure 3

When the velocity of the airflow is low, the deposition is governed by gravity and the particles sediment to the surface.

Sedimentation increases with increasing diameter of the particle (D), inverse to inhalation flow (F), resulting in increasing residence time in the airways.

This mechanism is most important for particles larger than $0.5 \mu\text{m}$ and in the small bronchi, bronchioles, and alveoli where airflow is low.

BROWNIAN DIFFUSION AND ELECTROSTATIC ATTRACTION:

The probability of Brownian diffusion increases with particles of smaller geometric diameter and increasing residence time. The particles random collide and by the motion deposit on the airway surface. Electrical charged particles repel or attract each other, and by the electrostatic force they deposit on the surface. The probability of deposition by electrostatic attraction increases with increasing number of electrical charges and decreasing size of the particles. These mechanisms may be important in the small airways for $0.1\text{-}1 \mu\text{m}$ particles.

Factors determining deposition

PARTICLE SIZE

Aerosols consist of a variation of droplet or particle size distribution. When there is a limited distribution, i.e. all particles have nearly the same size the aerosol is *monodisperse*. Most therapeutic aerosols are *polydisperse*, i.e., they cover a wide range of sizes.

The approximate size of a polydisperse aerosol is referred to as mass median diameter, MMD, where half the aerosol consists of smaller particles and the other half consists of larger particles than MMD. The aerosol geometric standard deviation (GSD) describes how wide the aerosol distribution is. GSD < 1.22 is by definition a monodisperse aerosol.⁽¹³¹⁾

The particle aerodynamic diameter (D_{ae}) is the diameter of a sphere of unit density (1 g/cm^3), which has the same settling velocity in the same gas. D_{ae} can be calculated as $D(P/P_0)^{0.5}$, where D is the geometric diameter and P is the particle density. Particles of different shape, size and density can, with respect to their resistance in moving through still air, be compared.

Deposition due to impaction and sedimentation increases with particle size from $0.5 \text{ }\mu\text{m}$. Ultrafine particles ($<0.1\mu\text{m}$) deposit due to diffusion. Particles $0.5\text{-}1 \text{ }\mu\text{m}$ can follow the breaths in and out.

BREATHING PATTERN

The difference in the inspiratory flow rate has large effect on the regional deposition in human subjects. A fast flow will enhance deposition in the oropharynx and the central airways. A slow and deep inhalation with a breath-holding pause enhances deposition in the airway periphery.⁽⁶⁶⁾

With nose breathing there is no alveolar deposition of particles larger than $8 \text{ }\mu\text{m}$. Hence, mouth breathing will enhance tracheobronchial deposition compared to nose breathing by a factor 3 in children.⁽²⁹⁾ Large volume breaths often increase deposition due to higher flow and/or longer pulmonary residence time.

AIRWAY GEOMETRY

Local deposition depends on the dimension of the airways. The geometry of the larynx may influence the velocity profiles in the trachea and the bronchi. The vocal folds act as an aperture and the sudden increase in downstream diameter will lead to turbulent flow. Turbulent flow increases particle deposition.

A pharyngeal narrowing during inhalation, not related to bronchial obstruction, has been shown to be significantly related to high deposition in the upper airways.⁽¹²²⁾ Increased airway resistance due to bronchoconstriction in diseased airways induces turbulence and increases deposition in larger airways.^(125, 126)

HYGROSCOPIC GROWTH

Inspired air is quickly humidified within the airways. If a particle has hydrophilic surface, the particle absorbs water vapor from the moist air in the airways and grow in size. This is important for aerosols composed of water-soluble particles, e.g. sodium chloride crystals. $0.7 \text{ }\mu\text{m}$ sodium chloride particles were grown to $4 \text{ }\mu\text{m}$ when penetrated to 300 cm^3 lung depth.⁽⁴⁴⁾

In tropic environment hygroscopic growth can occur before inhalation if the relative humidity is high

Site of deposition

The human respiratory tract is an “external” organ in the sense that it is continuously and directly exposed to the environment. During breathing, the airways transport approximately $10\text{-}20 \text{ }000 \text{ L}$ air per day contaminated with a variety of pollutants, particles, viruses, and bacteria. Therefore the airways need to be a highly effective filter to protect the alveolar region.

The respiratory tract can be illustrated as two filters in series. The first filter is the nasopharynx and the second filter is the tracheobronchial region (Figure 2, dotted circles). These two filters have nearly the same characteristics. Hence, any particle that passes through the first filter has also the possibility to pass through the second and deposit in the alveolar region.

It is desirable for therapeutic aerosols that most of the dose is delivered to the lower airways with little losses in the oropharynx. However, a specific region in the lung is hard to target and the precise retained dose in the lung is difficult to predict. Particle size is the most important single factor that determines the site of deposition. Larger particles are deposited mainly by impaction in the first filter. Smaller particles pass through both filters, and deposit in the alveolar region, due to sedimentation or diffusion if the breath-hold is long enough, or else they will be exhaled. Thus, it is difficult to target the small airways; the

particle will either deposit in the oropharyngeal or tracheobronchial region or continue to the alveoli.

The following is a simple rule of thumb; particles larger than 10 μm (pollen) are deposited in the turbulent airflow of the upper airways. Particles 3-10 μm are deposited in the trachea and larger airways due to impaction. Smaller particles about the size of most bacteria 0.5-3 μm are deposited in the terminal airways and in the alveoli. Ultrafine particles, less than 100 nm, are deposited in the alveolar region and a larger fraction is exhaled.⁽¹⁴²⁾

Methods targeting the small airways

Extremely slow inhalation flow (ESI)

For the vast majority of therapeutic aerosols the drug deposits by impaction in the airways. Impaction occurs mainly in the larger airways. Inhalation of particles with an extremely slow inhalation flow, 0.05 L/s, however, will decrease impaction and thereby reduce deposition in the oropharynx as well as in the larger airways, and the particles will continue further down in the airways.

In the small airways, the slow flow allows the particles enough time to settle, and the deposition due to sedimentation will be markedly increased. A large particle ($> 5 \mu\text{m} D_{ac}$) will fall faster than a small particle due to its gravity, and sedimentation increases in the small airways, before reaching the alveolar region. By using this relationship, inhalation with an extremely slow flow (approximately 1 L inspired air will take 20 sec) and rather large particles (6 μm), targeting the small airways is possible.⁽⁴⁾

The method has been shown to be robust and insensitive to airway obstruction.⁽¹²⁷⁾ Calculations of the deposition, using the parameters for ESI, with four different theoretical models, indicate that most of the particles deposit in the small ciliated airways.^(23, 39) Since the particles are inhaled

within a large volume of air and the particles have time for settling in the airways, less than 2 % are exhaled.⁽⁴⁾

Shallow bolus technique

An aerosol bolus is a small volume of air that contains particles, packaged within a larger volume of inhaled air. The depth of which the bolus penetrates into the lung is determined by the volume of the bolus, and the volume of the air inhaled after its insertion into the air stream.

With the “shallow bolus” technique, radiolabelled particles are administered as a small ($< 50 \text{ ml}$) bolus, near the end of the inhalation, so that the bolus should not reach the alveolar region. The inhalation is followed by a breath holding period to maximise deposition in the small ciliated airways.⁽¹⁰⁵⁾

To confine the aerosol to the anatomic dead space (ADS) of the lungs, the boluses are small and delivered to shallow volumetric front depth (VFD), i.e. $< 150 \text{ mL}$. The VFD represents the volume inspired from the point when the first particles enter the mouth to the end of the inhalation. The small boluses within a volume of air could give an uneven distribution, and a left-right asymmetry in particle deposition has been observed.⁽¹³⁾

Assessments of lung deposition

Gamma scintigraphy technique

PLANAR IMAGING

The imaging technique currently used is planar, two-dimensional scintigraphy,⁽⁸⁵⁾ but three-dimensional, single photon emission computed tomography (SPECT)⁽⁹²⁾ and positron emission tomography (PET) could also be used.

The formulation to be deposited in the lung is labelled with an isotope and detected by gamma camera images.⁽³⁵⁾ ^{99m}Tc is the most common isotope, suitable for short term studies and can be bound to insoluble markers such as iron oxide, sulphur colloid, albumin, Teflon and polystyrene latex spheres or to drug formulations.

The standard way of analysing lung images is to use so called "regions of interest", usually dividing the images into central and peripheral zones by equal division of radius vectors by area.⁽¹¹²⁾ SPECT may offer some advantages over two-dimensional imaging to distinguish between deposition and clearance in the small airways and in the alveoli.

Labelling of particles with isotopes, ensuring that the label follows the deposited particles,⁽⁸⁶⁾ is one major limitation for long term studies. Another limitation is the interpretation of the distribution of activity images, i.e that the regions of interest do correspond to the anatomical structures, since there is an overlay of structures of interest (alveoli, small and large airways), which is most marked centrally.⁽¹¹⁴⁾ The methodologies may vary significantly between different laboratories.

PROFILE SCANNING

Another radioactive technique is to label monodisperse insoluble particles and to measure radioactivity in the subjects using a

profile scanner with NaI crystals fitted with collimators. This method can be used for longer studies measuring clearance.⁽³⁷⁾ To determine regional deposition, the radioactivity is often measured at 0 and 24 h. Since the majority of the insoluble particles that deposit in the large ciliated airways is cleared by the mucociliary activity and swallowed, the activity remaining after 24 h represents alveolar deposition. The regional deposition in the ciliated airways is the fraction cleared between 0-24 h.⁽¹²⁵⁾

RISK OF RADIATION

With all radiolabelled methods, the subjects are exposed to radiation. The risk for most gammascintigraphy studies appears however to be very low and often comparable to the radiation received in a 12 h flight or a few weeks back-ground radiation. Recent experimental studies have demonstrated that the distribution of the inhaled radioactive aerosols is non-uniform. Hot spots of deposition in the large airways have been found within the areas of bifurcations; especially at the carinal ridge and at the inner sides of the daughter airways downstream the carini. The mucus clearance in these local areas is decreased. This may have implications for adverse health effects and possible risk of developing lung cancer.⁽⁸⁾ Since children have a longer life expectancy than adults, the risk of a given radioactive dose must be greater for them.⁽³⁶⁾ Minimum numbers of children should be used in studies and the doses of isotopes should produce radiation levels that are only just above background levels to obtain reliable data.

Pharmacokinetic methods

The classic pharmacokinetic methods are non-radioactive approaches to estimate total lung deposition, e.g. indirect methods. The principle is that an inhaled drug (unlabelled) is absorbed from the lung to the systemic circulation. The absorbed drug can then be measured in blood or urine, assuming that the gastrointestinal uptake is negligible or can be blocked and that the drug is not metabolised in the lung. Figure 4.

If the distribution volume is known and constant, the dose or relative dose changes can be estimated. This can be achieved by a reference dose of the drug given intravenously. In order to avoid intra-subject variation between study days, the inhaled and reference dose should be given at the same time, provided that they can be separated in the concentration analyses.

Classical pharmacokinetic studies of inhaled pharmaceuticals have been difficult to perform since the delivered dose in general is very low and the resulting plasma levels correspondingly low, often below the accurate detection limits of standard assay. Recently developed assay systems that are more sensitive have made it possible to determine the pharmacokinetic of the inhaled drug more accurately.⁽⁷¹⁾

The charcoal-block method has been used to assess the total lung deposition for

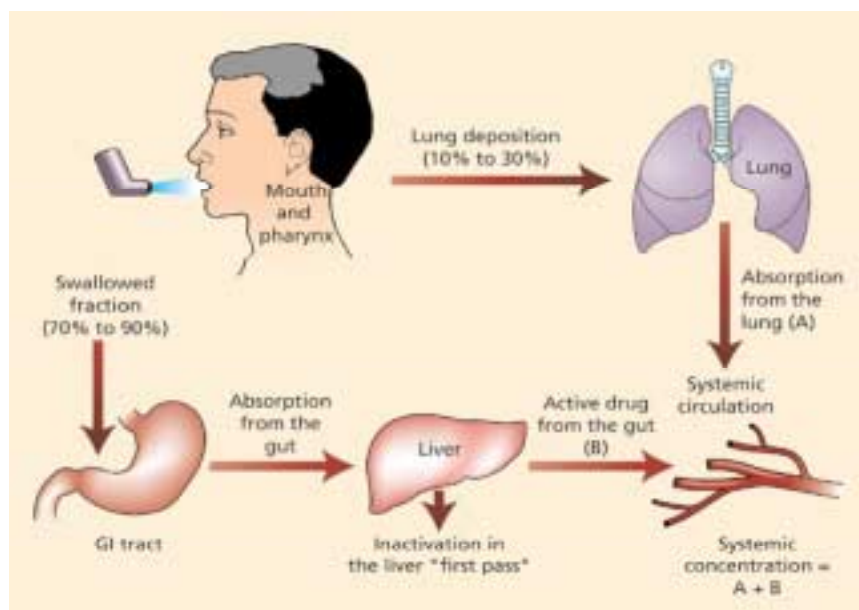
terbutaline sulphate, salbutamol, budesonide, formoterol and ipratropium bromide in 48 h urine recovery, with co-administration of activated charcoal to block the GI absorption.⁽¹⁶⁾ The charcoal-block method correlates well with total lung deposition measured by gamma scintigraphy.⁽⁸⁴⁾

For drugs that are well absorbed through the epithelium in the airways, but do not contribute to systemic uptake by the GI pathway,⁽⁶⁷⁾ for instance sodium cromoglycate (SCG)⁽¹⁰⁸⁾ and fluticasone propionate,⁽⁴⁸⁾ the plasma concentrations or urinary excretion are indicators of the dose absorbed from the airways.^(5, 7)

For drugs whose oral bioavailability is known the concentrations of drug in either plasma⁽⁸⁷⁾ or urine⁽⁴⁹⁾ during the first 30 or 60 min after inhalation can be used as an index of lung deposition, since the contribution of the swallowed drug and the absorption from the GI-tract is slower than from the lung during these first time periods.

The limitations of these methods are that only total lung deposition can be assessed, that expiratory manoeuvres can influence airway absorption, and that the methods are drug specific.

Figure 4. The fate of inhaled drugs. At inhalation the systemic bioavailability is the sum of the pulmonary and the oral components.



Theoretical lung models

Several theoretical models to predict the delivered dose to the lung have been developed within the radiation protection field.^(39,138) These models make use of deposition predictors and clearance kinetics. Data have been obtained almost exclusively from healthy subjects. These models are difficult to apply to aerosols of pharmaceutical drugs.

Data using radiolabelled aerosols in children are, due to ethical reasons, very scarce. During infancy and childhood the lung dynamically changes progressively by growth, and at about 2 years of age the structure is completely developed.⁽¹³³⁾ Hereafter the lung increases in volume. To better mimic the lung of a child, an adjusted child lung model for deposition modelling has been adopted in the report by the Task Group of the ICRP.⁽¹³⁰⁾

In the child model, three different equations are used. The first equation is constructed from the assumption that the dimensions of the trachea and bronchi (generations 0-8) relate to body height.⁽⁹³⁾ In these larger airways, constants are used to calculate scaling of airway diameter and length as a function of body height. The dimensions of the respiratory airways (generations 16-23) are scaled down by one-third power of the functional residual capacity (FRC). The diameter and length of the bronchioles (generations 9-15) are then obtained by interpolating between the reference diameter or length of the last generation of bronchi (generation 8) and the first generation of the respiratory bronchioles (generation 16).

In *paper II*, lung deposition modelling using the KI-model⁽¹²⁴⁾ with adjusted factors for scaled child parameters was used.

Lung clearance

Protection the airways from inhaled particles and keeping the lung sterile require multiple defence mechanisms that cooperate to neutralise and remove inhaled

particles from the lung. The mucociliary clearance (MCC) is the primary defence mechanism to remove insoluble deposited material in the tracheobronchiolar region.

The majority of deposited material in the trachea and bronchi is eliminated within 24 h by the MCC, and it has long been assumed that any particles remaining in the lung at 24 h represent alveolar clearance.⁽²⁶⁾ This is however probably due to the deposition pattern of particles inhaled with normal inhalation flow with limited deposition in small airways.

When insoluble monodisperse particles are deposited in the small ciliated airways by the ESI or the “shallow bolus” methods, a substantial fraction of retained particles was found after 24 h.⁽³⁹⁾ Recently, based primarily on the results of the “shallow bolus” experiments conducted by Stahlhofen et al.,⁽¹¹⁸⁾ this slow phase of bronchial and bronchiolar clearance, has been included in the revised dosimetric model for the human respiratory tract, adopted by the ICRP.⁽¹³⁰⁾

Tracheobronchial clearance

MUCOCILIARY CLEARANCE

MCC consists of the ciliated epithelium and the airway surface liquid. The airway surface liquid (ASL) is a two-fluid model, with a sol phase, the periciliary layer (PCL) of low viscosity, in which the cilia beat, and an overlaying gel phase, the mucus layer of high viscosity, where trapped materials is propelled forward by the ciliary strokes.⁽⁶³⁾

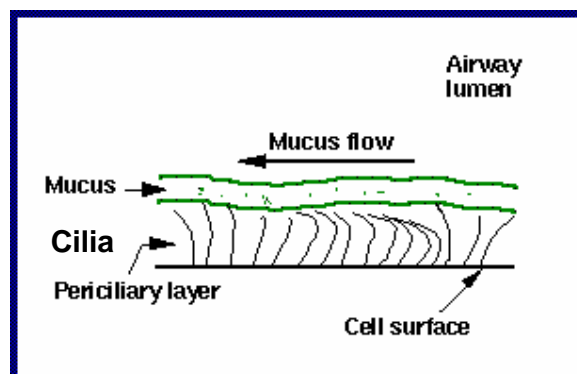


Figure 5. The components of mucociliary clearance

The transport rate of the MMC progressively decreases from the larger airways to the smaller airways.⁽¹³⁴⁾ The rate of MCC is dependent on the rate of ciliary beating,⁽⁷⁶⁾ and can be stimulated for instance by bronchodilators,^(34, 81, 82) and acute exposure to tobacco.⁽⁷⁰⁾ However, MCC is also strongly influenced by the hydration state of the airway surface liquid, and an acute increase in the airway surface liquid increase the rate of MCC.⁽¹¹⁵⁾

CILIA

Ciliated epithelium covers the airways, from the trachea down to the terminal bronchioles, generation 16. Each cilium performs a repetitive beat cycle consisting of a rest, a recovery, and an effective stroke phase. This cyclic activity has a frequency of 5-50 Hz, and a typical ciliary beat occupies about 33 ms. During the effective stroke, the cilium makes contact with the overlying mucus and transport it, together with entrapped particles, forward along the airways for expulsion at the oesophagus.

The respiratory motile cilia (like the sperm flagellum) consist of a basic structure of nine peripheral microtubule doublets circularly arranged around two central microtubules (9+2) axoneme. This is different from the (9+0) arrangement in renal and corneal ciliated epithelium. The microtubules are interconnected by nexin links, radial spokes and dynein arms. The outer and inner dynein arms are periodically attached and distributed along the peripheral microtubules, and generate motion by ATP-dependent reactions.⁽⁵⁵⁾

Nonaka and co-workers have elegantly shown in mouse studies that during embryogenesis, monocilia in the primitive nod are present and generate a clock-wise left rotation of the “nodal flow” which probably determines the normal disposition of the internal organ, situs solitus. When monocilia are immotile or absent, the “nodal flow” does not occur. This leads to randomisation of body situs.⁽⁸⁸⁾ This could be the mechanism behind that situs inversus

randomly occur in 50 % of the patients with primary ciliary dyskinesia (PCD).

AIRWAY SURFACE LIQUID

The components of the ASL, the mucus and the PCL layer are transported at approximately equal rates along airway surfaces via the actions of cilia. The mucus is produced and secreted by the submucosal glands in the airway epithelium. The submucosal glands can rapidly produce copious amounts of mucus in response to neural signals.⁽⁷⁴⁾ Submucosal glands occur at a frequency of about 1 per mm² in the trachea and are scattered down to about the 10th generation.

In normal airways, the thickness of the PCL is about the length of an outstretched cilium, approximately 7 µm, whereas the thickness of the mucus layer varies considerably in height between large and small airways. The mucus layer serve as a reservoir to store and release liquid, i.e swell and shrink.⁽¹²⁹⁾

The ASL is isotonic⁽⁴⁷⁾ and the depth of the PCL is determined by solute and water transport by ciliated epithelia. CFTR and epithelial sodium channel (ENaC) are principal rate-limiting step for Cl⁻ and Na⁺ absorption by the ciliated airway epithelia.⁽¹⁷⁾

The mucus hydration is set by the volume of the liquid present on airway surfaces, which in turn is modified by active ion transport.^(77,116) Mucus osmolarity can increase considerably by rapid evaporative water loss resulting from exposure to dry air.⁽⁵⁶⁾

Cough clearance

Cough is an important defence mechanism of the lungs and can serve as a back up for defective MCC. Cough rarely occurs in healthy subjects except in emergency situations, following the inhalation of a foreign body or bronchial irritants. In diseases with impaired MCC, cough is the major clearance mechanism providing there is an increased mucus production.^(11, 25)

In order to establish an effective cough clearance, sufficient high velocity of airflow is probably needed which can only be obtained in the larger tracheobronchial region approximately down to generation 7.⁽⁶⁹⁾ In the smaller airways, the airflow is much slower due to the large cross-sectional area and consequently cough clearance is less effective. Animal studies indicate that the afferent pathway for cough involves rapidly adapting airway receptors and sensory endings of C-fibres, localised in the larynx down to the smaller bronchi,⁽¹⁴¹⁾ innervated from the vagus nerve. When inhaling an irritant solution with a particle size of 10 μm (more central deposition) coughing is provoked, but when inhaling the same solution with a particle size less than 5 μm (deposition in the alveolar and small airways) coughing is not provoked.⁽¹³⁷⁾

Alveolar clearance

Truly insoluble particles deposited in the alveoli are mainly cleared by phagocytosis of the alveolar macrophages (AM) and subsequent transport to the mucociliary

escalator. There is evidence that this alveolar clearance mechanism is extremely slow, and might take years. In a study of insoluble particles labelled with ^{195}Au , the average half-life was found to be 4-5 years when lung clearance was studied during almost three yrs.⁽⁹⁵⁾

Submicronic (< 0.2 μm) relatively insoluble particles and fibres can be translocated from the alveoli directly to the interstitial region.⁽⁴¹⁾

Macrophages are large complex single cells capable of moving around in the lung and performing a multitude of important functions. In their defensive function, they kill and digest bacteria, degrade antigen, synthesize immunoregulatory substances such as interferon, chemotactic factors, and tumor-inhibiting factors. Macrophages can efficiently dissolve many metal particles which are poorly soluble in water.⁽⁷²⁾ In their non-defence function they synthesize arachidonic acid metabolites, platelet and fibroblast activating factors, enzyme inhibitors and binding proteins.

Inherited diseases affecting mucociliary clearance

Cystic fibrosis

CF is a progressive, and the most common lethal autosomal recessive disease among Caucasians. The incidence varies between populations, lowest in the Japanese population and highest in the Caucasian population. In Sweden the incidence is estimated to be approximately 1/5600, giving about 17 new cases per year.⁽⁶⁵⁾ Predicted survival has steadily increased with a life expectancy today of about 40 years.⁽³¹⁾

The cystic fibrosis transmembrane conductance regulator (CFTR) gene was discovered in 1985⁽¹³⁶⁾ and sequenced in 1989.⁽¹⁰²⁾ The genetic defect is in a single gene located on the long arm of chromosome 7 that encodes the CFTR. Over 1200 different mutations in the CF

gene are known today (www.genet.sickkids.on.ca/cftr/), and they have been classified according to their molecular pathology in five classes.⁽¹⁴⁴⁾

The most common mutation ΔF508 ,⁽⁶⁰⁾ occurring in approximately 70% of all CF alleles,⁽¹⁾ of which approximately 65% of the Swedish CF patients have⁽³²⁾. The mutation cause defective intracellular trafficking of CFTR, resulting in failure of the protein to transport to the apical membrane. Other common mutations are 394delIT, also known as the Nordic mutation⁽¹⁰⁶⁾ and 3569delC.⁽¹⁰⁴⁾ The disease is heterogeneous and there is no typical genotype/phenotype correlation for the development of lung disease.

The gene product, CFTR, is a cAMP regulated chloride channel⁽¹³⁵⁾ expressed in the apical membrane of all respiratory

epithelial cells, and in airway submucosal glands. Defective CFTR function leads to reduced chloride secretion into, and enhanced sodium reabsorption from the airway lumen, resulting in a dehydrated airway lining fluid and consequently defective mucociliary clearance.

Microscopic inflammatory changes develop early in infancy and the subsequent airway inflammation leads to further hypersecretion of mucus, with recurrent bacterial infections, predominantly with *Staphylococcus aureus* and later with *Pseudomonas* species, resulting in a vicious circle of chronic inflammation, bronchiectases and airway damage. This eventually culminates in respiratory failure and premature death.

Defective MCC is one of the central hypotheses for the development of lung disease in CF. However, studies to demonstrate decreased MCC in vivo, using radio-aerosols and planar imaging, have been variously reported as increased, decreased as well as similar MCC to that of healthy subjects.⁽¹⁰⁰⁾ These studies were conducted during a limited time, mostly only up to 24 h, with different methodologies, especially the inhalation procedures, making the results difficult for comparison. A recently published paper with good intrasubject repeatability showed an impaired MCC in whole lung, central intermediate, mid, and apical regions using radiolabelled aerosol with MMAD 5.5µm, inhalation flow of 1 l/s and gamma planar imaging.⁽¹⁰¹⁾ Longer studies of MCC in CF with radiolabelled aerosols than 24 h have to my knowledge not been published.

Primary ciliary dyskinesia

PCD, also known as immotile cilia syndrome, is a rare (about 1/25000) genetic disorder affecting the cilia in the upper and lower respiratory tract, including the sinuses, the middle ear, the ependyma of the brain, the ductuli efferentes of males and the female oviduct. Symptoms characteristic for PCD are chronic rhinosinuitis, otitis, persistent cough and asthma. The disease was first described in 1904 by Siewert⁽¹¹⁰⁾ and then by Kartagener⁽⁵⁸⁾ as the triad of situs inversus, sinuitis and bronchiectasis. Afzelius in 1976 revealed the cause of the disorder, when investigating the sperm tails with electron microscope, from infertile men with situs inversus, finding the structural abnormalities (lack of dynein arms) of the cilium.⁽²⁾ At the same time the mucociliary transport in the tracheobronchial tract in these men were investigated. The mucociliary transport was found to be extremely slow or possibly absent.⁽²⁴⁾

The affected genes have not yet been identified, several chromosomal regions have been suggested; a HLA (human leukocytes antigen) locus on chromosome 6⁽¹⁴⁾ and/or genes located at chromosome 7.⁽¹⁴³⁾ A cilium consists of over 200 different proteins, each encoded by a separate gene, and the number of possible candidates is therefore large. It also explains why this disorder is genetically heterogeneous with a variety of phenotype presentations. Functional studies of the cilia in these patients showed the cilia to have abnormal motility rather than being completely immotile. Immotile cilia syndrome has therefore been renamed to primary ciliary dyskinesia.

Although PCD patients have defective mucociliary clearance and, as in CF, bronchiectasis develops, and sometimes have chronic colonisation with *Pseudomonas aeruginosa*, the prognosis is far better than in CF, with a normal life expectancy.

Aims of the thesis

General aim

The aims of this thesis were to investigate the importance of mucociliary clearance in the role of eliminating particles from the small airways, to evaluate whether the slow inhalation method is feasible in patients with a relatively high airway resistance, and to develop a simple non-radioactive method to assess the dose to the lung.

As background information for the pharmacokinetic studies, information from a pretrial (not yet published) is included.

Specific aims

- I. To compare analysis of sodium cromoglycate in plasma and urine, and to select the measurements that have the best reproducibility, and possibility to be used in clinical practice. To study if the effect of an expiratory manoeuvre could be detected in the plasma or urine analyses.
- II. To investigate the effect on the droplet size distribution in the same nebuliser by altering the relative humidity (RH) of the air carrying the aerosol, and to evaluate the effect of this by *in vitro* and *in vivo* assessments of lung deposition in asthmatic children.
- III. To investigate long term clearance from small airways in patients with cystic fibrosis. The hypotheses were that CF patients have larger retained fraction of inhaled particles at 24 hours and that clearance after 24 h up to 21 days is slower, as a consequence of their defective mucociliary clearance, compared to healthy subjects.
- IV. To investigate long term clearance from small airways in patients with primary ciliary dyskinesia. The findings from study III raised a theory that mucociliary clearance is less important in the small airways. To test this hypothesis we studied clearance from small airways in patients with defected mucociliary clearance of a different origin, abnormal ciliary function.

Subjects and methods

Pharmacokinetic studies

In a pretrial (Lindstrom et al, submitted 2004) we evaluated if high or low oropharyngeal deposition of a polydisperse inhaled dose of terbutaline could be detected using the charcoal-block method⁽¹⁶⁾ as the pharmacokinetic method. In general there is a large inter-subject variability in the oropharyngeal deposition, but a good reproducibility within the subject. Nine patients with obstructive airway disease and known high (>60%) or low (<20%) oropharyngeal deposition (earlier measured with radiolabelled technique)⁽¹²²⁾ inhaled nebulised terbutaline as the test drug. The gastrointestinal uptake was blocked with oral slurry of active charcoal, and urine was collected in three pools, during 24 h. Two subjects who normally used terbutaline in their daily treatment were instructed to use salbutamol instead at least 72 h prior to the beginning of the study.

Paper I

Eleven healthy non-smoking subjects (four males and seven females) aged 24.6 ± 3.3 years, with no history of asthma volunteered for the study. The study was an open randomised cross-over study with two exposures, the base exposure and the exposure with a pulmonary function test (PFT). The routine clinical procedure, a reversibility test, contained PFT (Vitalograph) measuring forced vital capacity (FVC) and forced exhaled volume in one second (FEV_1) before inhalation and 20 min post inhalation. The nebuliser (Pari Inhalierboy LC) was connected to a dosimeter (Spira Electro 2, Spira health care, Finland) that was preset to a nebulisation delay of 20 mL of inspired air prior to the onset of the nebulisation, and a nebulisation period of 1.5 s. The nebuliser was filled with 2 mL sodium cromoglycate solution (10 mg/mL). The subjects inhaled in a sitting position through a mouthpiece,

wearing a nose clip. Each subject made 27 deep inhalations within 3 min, at a preset flow of 0.5 L/s, and the subjects exhaled through a filter. The MMAD of the aerosol was $7.7 \mu\text{m}$ measured with a light-scattering instrument (Malvern Mastersizer).⁽³⁰⁾

The available dose to the subject, calculated from the nebuliser output and nebulisation time was 2.8 mg. In a separate measurement the actual available dose was determined by analysing nebulised SCG on filters with the same set-up as used in the exposures.

Paper II

The study was of an open, randomised, three-way crossover design. A pretest was conducted in which the nebulisers output and the droplet size distribution were characterised at three different levels of relative humidity (RH), low (13%), ambient about (50%), and high (90%), to test the influence of hygroscopic growth. The setup, with a Hudson updraft II nebuliser (Figure 6) was the same in the pretrial as used in the following exposure trial. In the pretest 10 mg/mL sodium fluoride solution (NaF) and a Harvard pump to mimic sinusoidal breathing pattern (500 mL tidal volume, 15 breath/min) were used. NaF was used since it is specific in the CEN methodology,⁽²⁸⁾ and it offers a faster way to assay results than using SCG. However, comparison was made both with high and low RHs to confirm that the SCG and NaF behaved in the same manner.

In the exposure test, nine subjects (two girls) aged 10.4 ± 0.5 years, with a history of mild-to-moderate asthma were recruited from outpatients at the Paediatric Department of Allergy, Karolinska University Hospital, Huddinge. All the children were in a stable clinical condition, with a mean FEV_1 of $93.4 \pm 12.2\%$ of predicted. Each subject was exposed to aerosols having entrained air of low, high or

room RH on three different occasions with at least 48 h apart.

The droplet size distributions in each subject exposure were assessed with an Andersen cascade impactor, and the MMAD and GSD were calculated. Each subject made 50 inhalations through a mouthpiece in a sitting position, wearing a nose-clip. The nebuliser was connected to a dosimeter (Spira Electro 2, Finland), which was preset with a nebulisation delay of 10mL of inspired air prior to the onset of the nebulisation and a nebulisation period of 1 sec. The subjects inhaled with a mean inhalation flow of 0.4-0.5 L/s and a tidal volume of about 0.5-0.7 L, recorded by the attached dosimeter.

Blood sampling and urine collection

Prior to each exposure, a cannula (Venflon; Ohmeda AB, Helsingborg, Sweden) was inserted into a forearm vein for blood sampling. A 5 ml venous blood sample was taken at 15, 30, 60, 120 and 240 min after inhalation of the test drug (SCG). In *paper II* a blood sample was also taken at 5 min post-inhalation. The first 1ml of blood from each sample was discarded and, after collection, the cannula was flushed with 3ml saline (9mg/mL). The blood was drawn into glass tubes containing sodium fluoride heparin. The plasma was separated by

centrifugation, stored in polystyrene tubes, and immediately frozen at -40°C until analysed.

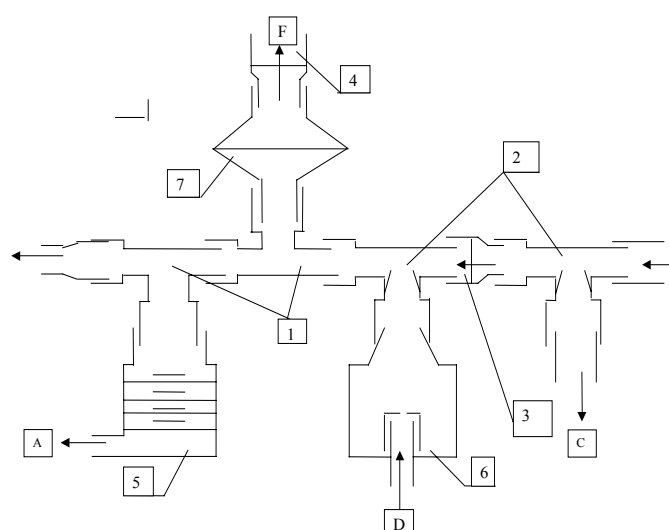
In *paper I* urine was also collected. Prior to the exposures, the subjects were instructed to empty their urine bladder and 20 mL was taken as a baseline sample. Urine was collected in two portions, 0-3 and >3-6 h postinhalation. The volume of each portion was measured, and 20 mL urine was taken from each portion, stored in polystyrene tubes, and immediately frozen at -40°C until analysed.

HPLC method

Sodium cromoglycate concentrations were determined by a high-performance liquid chromatography (HPLC) procedure at the Department of Clinical Pharmacology at the Karolinska University Hospital, Huddinge. The process of the sample analyses are described in detail in *paper I*.

The chromatography procedures to determine SCG on filters were identical to that used for the plasma SCG assay. Before running the HPLC analyses, SCG from filters was dissolved in a 1:1 mixture of ethanol and water (3.0 mg/ml). Aliquots of this solution were put onto blank filters in amounts corresponding to a final quantity of SCG ranging from 0.05 to 3.0 mg per filter. This set constituted seven calibration levels. Control filters were prepared at 1.5

Figure 6. The inhalation set-up



A. Impactor air flow, 2 l/min, B. Inhalation air flow, C. Flow for RH-check, 0,5 l/min, D. Nebuliser air flow, 8 l/min from dosimeter, E. RH controlled excess air with Spira pneumotach and F. Exhalation outlet

1. Tee-piece, Intersurgical
2. Tee-piece, Hudson anti-spill
3. One way valve
4. One way valve
5. Andersen 296 impactor
6. Nebuliser, Hudson Updraft II
7. Pari filterholder with low flow resistance electret filter pad

mg/filter, 0.5 mg/filter and 0.1 mg/filter. The calibrator and control filters were transferred into 100 ml polypropylene tubes to which 50 ml of 1:1 ethanol: water was added and the tubes were shaken for 10 minutes. Aliquots from the tubes were withdrawn and placed in the chromatography auto-injector.

Regression coefficients obtained in calibration curves were better than 0.99. Intraday imprecision and accuracy were found to be over 5.3% and interday imprecision and accuracy over 6.5%. Absolute recovery was 94–96%. The low limit of quantitation (LOQ) for SCG was calculated to 1 ng/ml in serum and 100 ng/ml in urine, based on back-calculation of calibrator and coefficients of variation. However, lower LOQ for urine (10 ng/ml) was possible by introducing lower calibrators, but this sensitivity was not needed in the actual subject urine samples. The limit of detection (LOD) was calculated to 0.3 ng/ml for plasma samples and 3 ng/ml for urine samples.

Clearance studies

Subjects

Patients for the studies were recruited from Stockholm CF-center and the Pulmonary department at Childrens Hospital, at Karolinska University Hospital, Huddinge. Table 3. All patients with CF and PCD were in their stable clinical condition. One CF patient and one PCD patient ended an iv antibiotic treatment at the beginning of the study, initiated because of signs of low grade infection.⁽¹¹⁹⁾

CF was diagnosed in childhood due to

symptoms characteristic for CF and a positive sweat test (>80 Cl⁻ mmol/L).⁽⁴⁶⁾ All PCD patients had clinical and radiological evidence of bronchiectasis; three of them had situs inversus totalis. The PCD patients without situs inversus were examined with nasal or bronchial brush biopsies, and ciliary ultrastructural abnormalities were proven by electron microscopic studies.⁽²⁷⁾

Study design

The CF and the PCD patients inhaled 6 μ m monodisperse Teflon particles labelled with ¹¹¹Indium with an extremely slow inhalation flow (ESI), 0.05 L/s, giving deposition mainly in the small airways. Radioactivity over the mouth, throat, lungs and stomach was measured immediately after the inhalation of the test particles. Lung retention was measured at 24 h, 7, 14 and 21 days. Correction was made for background activity and physical decay of the radionuclide.

For three of the PCD patients, a second exposure was performed. They inhaled the same produced test particles with normal inhalation flow, 0.5 L/s, giving a more centrally deposition. This exposure was performed one month after the first exposure. Lung retention was measured at equal time points as the ESI exposure.

The regional deposition data were estimated using a model developed at the Karolinska Institutet. In the evaluation of the data, the studied period was divided in two phases, a first rapid clearance phase, defined as clearance between 0 and 24 hrs and representing mostly large and medium sized airways, and a second slow clearance phase, defined as clearance between day 1

Table 3. Characterisation of the patients and the healthy subjects in study III and IV. M; male, F; female, BMI; body mass index, FEV₁; forced expiratory volume at one sec, Raw; airway resistance.

Study	Study group	N	Gender M/F	Age	BMI Kg/m ²	FEV ₁ % pred	Raw Kpa*s'L-1
III	CF	11	4 / 7	18.7±2.5	21.6±3.5	72 ± 17.1	0.23±0.09
	Healthy	12	6 / 6	22.3±1.8	23.4±2.6	105 ± 12.8	0.16±0.04
IV	PCD	6	4 / 2	23.5±8.3	22.7±3.6	85 ± 19.7	0.24±0.07
	Healthy	10	6 / 4	22.3±1.9	23.5±2.8	106 ± 16.6	0.14±0.03

and day 21 and representing mostly small airways. Since clearance after 24 hrs up to one week could include cough clearance from larger airways a study period as long as possible is required to estimate small airway clearance.

Lung function test

The pulmonary function was evaluated the same day as the exposure by forced expirograms (Lung Function Laboratory 2100, SensorMedics, USA) giving forced vital capacity (FVC), forced expiratory volume in 1 s (FEV_{1.0}), and forced expiratory flow between 25 and 75% of the exhaled volume (FEF_{25-75%}). The airway resistance (R_{aw}), was measured using a panting technique within a whole-body plethysmograph (Transmural Body Box 2800, SensorMedics, USA). All lung function parameters were determined according to the criteria proposed by Quanjer.⁽⁹⁶⁾

Production of test particles

The Teflon particles were produced and labelled with ¹¹¹Indium (half-life 68 h) by a spinning disc technique.^(22, 94) A suspension of Teflon is added into the disc centre, drops are formed at the edge by centrifugal force and surface tension. The added radionuclide become physically enclosed by heating to 240°C.

The mean geometric particle diameter was 4.2 µm (GSD 1.06), measured in a light

microscope (Visopan projection microscope, Reichert, Austria). The mean aerodynamic diameter was calculated to be 6.2 µm, calculated from the geometric particle diameter and the density of the Teflon particles, 2.13 g/cm³, measured by Philipson.⁽⁹⁴⁾ The calculated aerodynamic diameter of the Teflon particles has been confirmed by direct measurements of the settling velocity in air.⁽¹¹⁷⁾ The leakage of radioactivity in water (37°C) was estimated during the periods of lung clearance measurements by repeated measurements of activities in filter and filtrate. The leakage *in vitro* during the three weeks was less than 2%.

Inhalation of test particles

The Teflon particles were suspended in water with 0.2% tergitol solution. Before use, the particles were allowed to sediment and the supernatant liquid was removed and replaced with distilled water. Distilled water (0.3 ml) together with about 2 mg Teflon particles per ml were aerosolised into a 25 l glass chamber as wet spray.

The subjects wore a nose-clip and inhaled the particles in a sitting position. The participants first made a moderately deep exhalation outside the chamber and then inhaled as deep as they could from the chamber. The flows were monitored using a pneumotachograph placed between the aerosol chamber and the mouthpiece, and were recorded on line, displayed on a recorder. By looking at the recorder needle,

Table 4. Exposure data. Mean ± SD. The PCD patients (n=3) marked with italic font show exposure data when inhaling the test particles with normal inhalation flow.

Subjects	N	Duration of exposure, min	Number of breath	Duration of breath, sec	Flow L/sec	Inhaled volume, L
CF	11	6.3 ± 2	7 ± 2	26 ± 7	0.045 ± 0.003	1.15 ± 0.35
PCD	6	7.5 ± 2	7 ± 2	29 ± 5	0.046 ± 0.002	1.34 ± 0.20
Healthy	10-12	4.9 ± 2	5 ± 1	33 ± 7	0.046 ± 0.002	1.50 ± 0.36
<i>PCD</i>	3	2.9 ± 1	8 ± 3	5 ± 1	<i>0.47 ± 0.013</i>	<i>2.39 ± 0.61</i>

the participants could inhale at a fairly constant rate throughout the inspiration.⁽⁴⁾ All participants were trained to inhale in this manner before they inhaled the test particles. Between each inhalation from the chamber, the participant inhaled and exhaled several times outside the chamber. Exhaled radioactivity has earlier been shown to be 0-2%.⁽⁴⁾ The dose equivalent to the test subject at one single exposure was 50 μ Sv or less.

Measurement of radioactivity

Immediately after inhalation and 24 h later, the radioactivity was measured using two 127 x 51 mm (sodium iodine) NaI crystals fitted with collimators.^(22, 39) Profile scanning over the mouth and throat, lungs and stomach of the supine subject was performed. Factors for self-absorption of radioactivity in the head and throat, lungs and stomach were 2, 2.5 and 4, respectively. These factors were obtained from measurements using an Alderson Rando Phantom. The radioactivity deposited in the lungs was 50-100 kBq (range).

Measurements after the first day were performed at 24 h and 1 week after inhalation using the whole-body scanner in the low activity laboratory at the Swedish Radiation Protection Authority.⁽³⁸⁾ The scanner has three large NaI detectors. The front of each detector facing the subject was equipped with focusing slit collimators of lead. The gamma spectra from each detector were acquired separately giving a total of 210 spectra from one measurement. The spectra were later analysed so that the radioactivity in the lung could be distinguished from the activity in the stomach. The technique has been described in detail by Falk et al.⁽⁴⁰⁾

After one week, when the radioactivity in the gastrointestinal tract was insignificant compared to the lung radioactivity, a more sensitive lung counter with the subject in supine position was used.⁽³⁷⁾ Five 127 x 101 mm (diameter) NaI detectors were placed close to the chest of the subject, two detectors against the back, two under each

armpit and one above the sternum. The relative positions between the five detectors were fixed and the same during all measurements. The relative sensitivity between the scanner and the lung counter was established, for each volunteer, by repeated measurements in the two systems within an hour.

Theoretical calculation of lung deposition

Theoretical deposition data were examined using the model developed at Karolinska Institutet, the KI-model, which is based on the equations for impaction and sedimentation from the model of the Task Group on Lung Dynamics,⁽¹³¹⁾ and on the human airway model A (comprising generations 0 to 23 of which generations 0-16 are ciliated), proposed by Weibel.⁽¹⁴⁰⁾

In the KI-model, inhalation parameters such as airway dimensions, tidal volume, inspiratory flow, post inspiratory pause, and expiratory time can be changed over a wide range. We changed the diameters of generations 0 to 23 by the same factor and calculated deposition for each generation. The factor was chosen so that it gave a calculated airway resistance (using Poiseuille's law) corresponding to the measured ones in the test subjects minus 0.05 $\text{kPa}\cdot\text{sec}\cdot\text{L}^{-1}$, representing the airway resistance in mouth, throat, and larynx based on measurements in healthy subjects by Hyatt and Wilcox.⁽⁵⁴⁾

We used the calculated average airway resistances of 0.234, 0.188 and 0.148 $\text{kPa}\cdot\text{sec}\cdot\text{L}^{-1}$, and a tidal volume of 1.15, 1.34 and 1.50 L, in the CF and PCD patients and the healthy subjects, respectively.

The mean slow inhalation flow was 0.046 L/s. In the three PCD patients exposed a second time using normal inhalation flow, we used the calculated airway resistance of 0.209 $\text{kPa}\cdot\text{sec}\cdot\text{L}^{-1}$, an mean inhalation flow of 0.47 L/s, and a tidal volume of 2.36 L.

Statistical analyses

The data were analysed statistically using SPSS 11.0 for Windows. The descriptive data are presented as mean and SD, and as mean and a 95% confidence interval when appropriate. Data were analysed statistically by using the Pearson correlation coefficient for parametric samples (paper I), and Spearman nonparametric rank correlation (paper II-IV). Differences between the exposures (paper I and II) and the patient groups (paper III and IV) were analysed with Wilcoxon signed test. Three different estimates of assessing the dose to the lung and the following kinetics were analysed (paper I and II): the peak values of SCG (C_{max}), the sum of the three first plasma concentrations after exposure, and the area under the curve AUC. The AUC (0-240 min) were calculated using the trapezoidal rule. The total in vivo lung dose of SCG in percentage of inhaled dose was calculated by the formula: $((Urine_{dose \times 2}) / Inhaled_{dose}) \times 100$, assuming that excretion by the urine pathway is 50%. The numbers of study subjects were chosen to be able to detect an average of 1 SD that could be clinical relevant, i.e about 30% elevation from average.

Ethics

All studies were approved by the Ethic Committee at Karolinska University Hospital, Huddinge, and performed in accordance with the Helsinki declaration of 1975, revised 1983. The Isotope Committee at Karolinska University Hospital, Solna, approved the isotope studies. All subjects have given their written consent to participate in the studies. Parental consent was obtained for the subjects younger than 18 years.

Results and comments

Pharmacokinetic studies

Results from the pretrial with the charcoal-block method showed a mean total deposition of 30 % of the available dose in the both exposures, with a large intra-subject variation ($r=0.49$, $p=0.18$).

A tendency to lower total lung deposition in the three patients with known high oropharyngeal deposition, 24 % (95% CI 19.5;29.0), compared to 32 % (95% CI 22.4;42.3) for the six with low oropharyngeal deposition was seen in the first exposure, but not in the second exposure. Two subjects who normally use terbutaline in their daily asthma treatment showed trace of terbutaline in the baseline urine sample before exposure, despite exclusion of terbutaline 72 h earlier.

Paper I

The mean available dose to the subjects, measured on filter was 2.6 mg. This filter dose was used in the calculation as the delivered dose to the subjects. The small difference between the calculated output dose from the nebuliser (2.8 mg) and the actual delivered dose to the subject was probably due to losses in the mouthpiece.

The exhaled amount of SCG measured on filter corresponded to 2.8 and 2.2 % of the delivered dose in the two exposures, respectively, indicating an almost complete subject deposition. There was a good correlation of the plasma concentrations between the two exposures, especially in the first hour after inhalation, $r=0.80$ ($p<0.001$) (**Figure 7**). The correlation between the two exposures measured in the urine analyses was substantially poorer, $r=0.27$ (**Figure 8**).

Overall increased plasma concentrations were found, except for the last measured point at 240 min, in the exposure with the PFT (**Figure 9**). This difference between the base exposure and the PFT exposure was not detected in the urine analyses.

From the urine analyses it was possible to calculate the total lung deposition in % of the delivered dose, 66 % (50; 82, 95% CI) and 53% (36; 71) in the base exposure and the exposure with the PFT, respectively. One subject was excluded from the results of the urine analyses because of failure to complete the urine collection.

Figure 7.

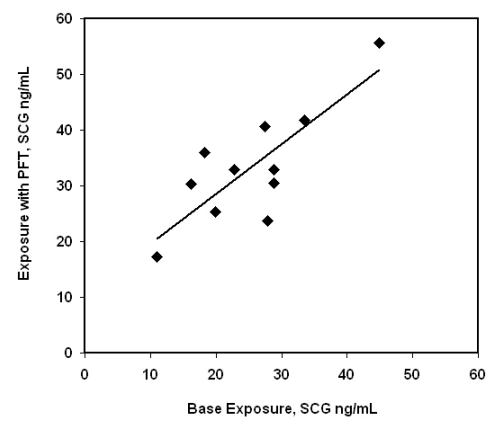


Figure 8.

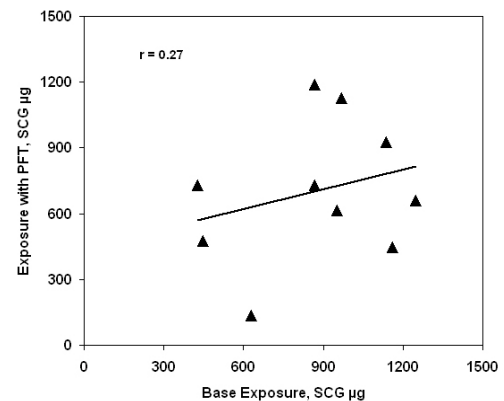
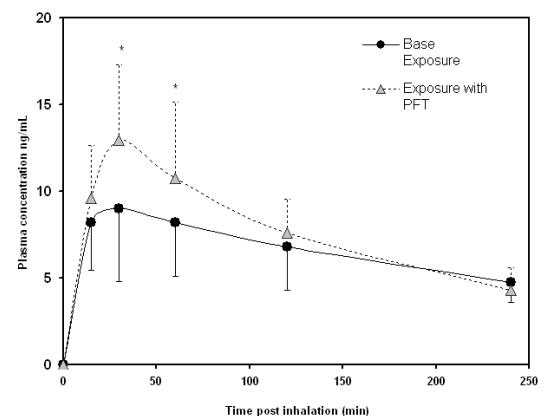


Figure 9.



Paper 11

In this study the droplet size, from the nebulizer characterization in the pretest, using sodium fluoride (NaF) and the Harvard pump, was $1.3\ \mu\text{m}$ (MMAD) for low RH (13%) and $2.3\ \mu\text{m}$ for high RH (90%). The output and the size distribution were almost identical comparing the tracer compound NaF and SCG. In the exposure test the mean droplet size among the subjects, were $1.2\ \mu\text{m}$ for low, $1.6\ \mu\text{m}$ for room, and $2.0\ \mu\text{m}$ for high RH. On average 50% of the available inhaled dose of SCG was found on the exhalation filter. This large amount exhaled probably depends on the small droplet size distribution, where a considerable fraction was smaller than $1\ \mu\text{m}$. The inhaled dose also called deposited dose, calculated by subtracting the amount found in the impactor, and on the exhalation filter from the available delivered dose from the nebuliser, was $832\pm 272\ \mu\text{g}$ for low and $996\pm 162\ \mu\text{g}$ for high RH. This corresponds to a deposited fraction SCG of 33.7 % and 37.7% ($p=0.05$) respectively. From individual plasma samples the AUC was calculated to be $2.3\ \mu\text{g/mL/min}$, average from all exposures. There was no significant difference in the plasma measurements between high or low RH in the exposure test. However, there was a significant correlation between the tidal volume and the amount of SCG deposited, $r=0.72$ ($p<0.01$), and a less correlation ($r=0.46$ $p<0.05$) was also found between tidal volume and AUC. Lung deposition was estimated adjusting to a scaled child lung model,⁽¹³⁰⁾ using length and FRC. In the calculation the droplet sizes and inhalation parameters, such as inhalation flow and tidal volume, obtained from the exposure trial was used. The average calculated lung deposition predicted a droplet size distribution dependency for the total amount deposited SCG, showing that the amount increases with increasing MMAD.

Clearance studies

Estimation of regional deposition

The calculated regional deposition in different airway generations, when inhaling the particles with extremely slow inhalation flow ($0.05\ \text{L/s}$), showed that the main fraction was deposited in the small airways (generations 9-15). There was a similar pattern in the regional deposition calculated for each generation between the CF, the PCD and the healthy subjects (Figure 10). The exposure with normal inhalation flow ($0.5\ \text{L/s}$) in the three PCD patients showed a larger fraction deposited in the larger airways as a consequence of larger impaction due to the particle size and the velocity of the airflow, the few particles that pass the TB region deposited in the alveolar region (Figure 11). This is in agreement with earlier study on healthy subjects.⁽⁴⁰⁾

Figure 10. % Regional deposition in different airway generation.

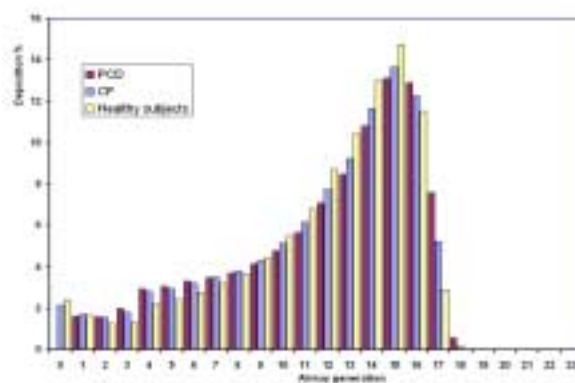
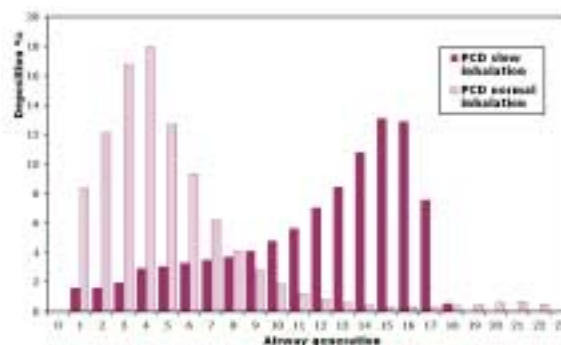


Figure 11. % Regional deposition in different airway generation between slow and normal inhalation flow



Clearance data

Retention of particles measured at 24 h after inhalation in percentage of initial deposition was significantly higher ($p < 0.001$) both in the CF patients, 67 % (95% CI 58;76), and the PCD patients, 79 % (95% CI 67.6;90.6), compared to the healthy subjects, 48 % (95% CI 42;53). The difference in lung retention over time in % of deposition persisted and was still higher at the last measured point at day 21 in the two patients groups compared to the healthy subjects, (Figure 12). In the CF patients there was a larger cleared fraction between day 1 to day 7, 22% (95% CI 15;29) compared to that of the healthy subjects, 14 % (95% CI 12;16) ($p < 0.05$). There was a significant clearance after 24 h in all the subjects. The CF, PCD and the healthy subjects cleared on average 50, 36 and 44 %, respectively, during 20 days of the remaining particle fraction at 24 h to the last measured point at day 21. In an attempt to evaluate if the severity of the lung disease in the CF patients affects MCC, the patients were divided into two groups according to their FEV₁, one group with mild lung disease, FEV₁ > 70 % of predicted, and one group with moderate lung disease, FEV₁ < 70 % of predicted. There was a tendency to difference between the two subgroups in Ret₂₄, but since the rapid clearance phase is more unpredictable due to cough clearance the variability was large in the two CF groups. The slow clearance phase, day 7 to day 21 in percent of Ret₂₄ showed very small differences between the groups (Figure 13).

As a comparison three of the PCD patients were also exposed a second time, inhaling the radiolabelled Teflon particles with normal inhalation flow, 0.5 L/s, giving more centrally deposition. The rapid clearance phase, representing cough clearance in this patient group, continued after 24 h with an average of 39% cleared fraction between 24 h and day 7. After day 7 the clearance turned very slow, the average cleared fraction between day 7 to day 21 was 9 %, representing mainly alveolar clearance, compared to 19 % in the

slow inhalation exposure. The subjects ending an i.v antibiotic treatment had the similar clearance pattern as the average, e.g. was not in the outer range.

Figure 12. Mean retention of particles in % of deposition with ESI.

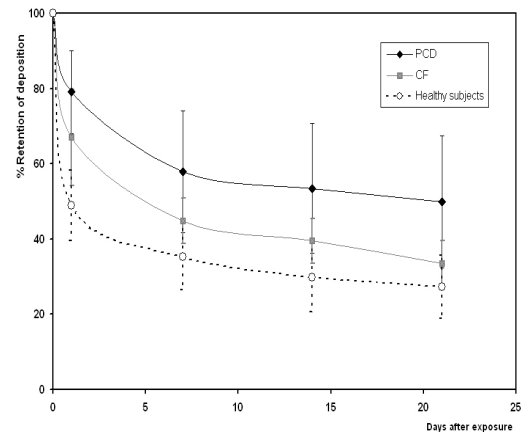
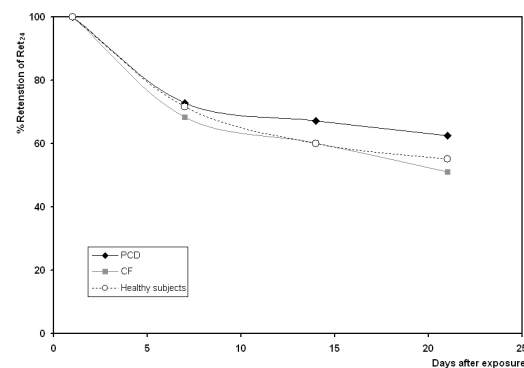


Figure 13. Mean retention of particles in % of Ret₂₄ with ESI.



General discussion

Pharmacokinetic studies

The experience from the pretrial was that the charcoal-block method was not as sensitive as the radiolabelled method, furthermore, bias with urine collection and risk of terbutaline contamination could easily occur. Extra medication outside the study protocol is difficult to control and can distort the data. The method is probably suitable combined with surveillance at an inpatient test department. We concluded that this pharmacokinetic method was not suitable as a simple clinical method. Therefore, another drug formula would be better, one which is not absorbed via the gastrointestinal tract, and which require a shorter urine collection or blood-sampling period. Sodium cromoglycate (SCG) fulfils these criteria.

The HPLC procedure

The purpose of using the present HPLC-method in the study was to evaluate a method with enough sensitivity that can be used in routine laboratories. Several different analytical methods for the determination of SCG in biological fluid have been reported. An ion-change method,⁽⁴³⁾ a HPLC method with manual solid phase extraction⁽⁵⁾ and fluorimetry following post-column photo irradiation to monitor HPLC eluent⁽⁷⁸⁾ in urine has been reported. Since the plasma concentrations of SCG is much lower than the corresponding urine concentrations, methods with a sensitive of 1 ng/ml are needed in order to monitor concentrations after an inhaled therapeutic dose. Hitherto only immuno assay methods have shown this degree of sensitivity.⁽¹⁹⁾ However, these methods are limited due to non-available commercial kits and suitable antibodies. Two other methods have recently been reported, one procedure comprises liquid-liquid extraction followed by back-extraction of

SCG in an aqueous phase,⁽⁵⁹⁾ and the second use LC-MS-MS after solid phase extraction.⁽⁹¹⁾

The present method, using ASPEC solid phase extraction, followed by reverse phase ion-pair chromatography fulfils all the requirements for a rapid, sensitive, convenient and easily accessible method for routine determination of SCG, both in plasma and urine. 50 samples per day can easily be processed.

Paper I

There was a greater individual correlation between the two exposures for the plasma analyses, $r > 0.6$, than for the urine analyses, $r < 0.4$. The highest correlation coefficient was seen for the sum of the first three plasma concentrations. In this study the subjects inhaled a standardized dose of SCG, with a dosimeter that controlled the dose from the nebuliser, together with a constant inhalation flow, which gives better control of the available dose for inhalation and probably contributed to better correlation in plasma analyses. With increasing observation time the intrasubject variation increases, with poorer correlation in the AUC calculation and in the urine analyses. A 1 h post inhalation GI absorption could contribute to this, as reported by Aswania and co-workers. In their studies they use urine samples taken 0.5 h post inhalation to be an index for the amount deposited in the airways.⁽⁶⁾

The reported large variation in plasma concentrations after an inhaled dose of SCG between subjects as well as within subject,^(59, 83) that could depend on not standardized inhalation procedure or the absorptions kinetics from the lung. There are surprisingly few studies that report the reproducibility of the pharmacokinetic methods, probably because it is difficult to achieve. However, good correlation of total lung deposition between the

pharmacokinetic method with charcoal-block and gamma scintigraphy after an inhaled dose of terbutaline has been shown.⁽⁸⁴⁾ The poor correlation for the urine samples could depend on incomplete urine collection, contamination or too short collection time. To diminish this bias the subjects need to be under close observation or use urine catheter.

The influence of the PFT was shown in the plasma concentrations, but could not be detected in the urine analyses. The mechanism for increased absorption through the airway epithelia could be due to mechanical distortion, "stretching" of the epithelium to facilitate passage, or drug displacement to a more distal absorption site.⁽⁷⁵⁾ Since the pulmonary absorption is the rate-limiting step, rather than the elimination rate from systemic circulation, the increased absorption from the airways in the PFT exposure would have been detected also in the urine analyses, especially in the first portion, 0-3 h.^(42, 98) However, the PFT probably accelerate the absorption rather than increase the absorption. Richards et al. showed that plasma concentrations increases within 4 min after an FEV₁ manoeuvre, the plasma concentration then declined to the predicted baseline within 30-60 min.⁽⁹⁹⁾ The time for each examination in a single subject was relatively short, in total 6 h, compared to the charcoal-block method where at least 24 h or preferably 48 h urine collection is necessary.

To evaluate the relative bioavailability from different nebulisers, the pharmacokinetic method with inhaled SCG measured in plasma samples could be a good approach and a tool for inhalation training. Recently this was performed in a study with CF subjects inhaling SCG with different nebuliser bioavailability was evaluated,⁽⁵⁶⁾ except that these measurements of systemic bioavailability were taken from urine collection. The precision would probably increase if plasma samples were used instead.

Paper 11

When the humidity of the air carrying the aerosol decreases, the droplets will start to evaporate and shrink in size, and thus probably affect the deposition pattern in the airways.⁽³⁰⁾ However, small droplets entering the warm and humid airways can increase in size by hygroscopic growth.⁽¹⁰⁷⁾ In this study the monitoring of the droplets size distribution by three different humidity (RH) of the air carrying the aerosol, and the influence of lung deposition in vivo were tested.

The average deposited amount over all exposures was 37.7% of the average amount of SCG available amount for inhalation. This was low and could be explained by the low tidal volumes inhaled. A correlation between tidal volume and amount deposited was shown with a larger fraction exhaled with smaller droplet size distribution. Especially in children this could have a clinical relevance since there is a dose-effect response correlation. Another point is that the fraction exhaled could give undesirable side effects; for instance if the inhaled substance is toxic for the eyes. The conclusion from this is that the droplet size distribution should at least have an MMAD of 2 μm with a small GSD.

There was a positive correlation between RH and tidal volume, and between tidal volume and deposited amount SCG. This was in agreement with the mean deposition of 34% and 41% at high and low RHs, respectively. This effect is probably due to increased residence time for the droplets and they sediment to the surface by gravity. The nebuliser used produced small droplets with small differences between the different RH.

The kinetic measurements with the SCG method as calculated AUC was not sensitive enough to separate this relatively small difference in droplet size distribution. Probably the rate-limiting absorption over the lung and the proceeding time of the sample period influence. The filter estimates maybe more accurate to measure the small differences in droplet size

distribution. There was a small but clear difference in the MMAD between relatively extreme differences in RH, 13 % and 90% respectively. In this study the effect from RH on particle size do not influence the *in vivo* estimates of the lung dose. This might be an effect of higher extrathoracic deposition for the larger particles. Breath size, however, had a small but significant effect.

Clearance studies

The two patients groups examined had both defective mucociliary clearance, but of different origin. In the CF patients the defective MCC is due to abnormal mucus of high viscosity, and in the PCD patients the defective MCC is due to abnormal ciliary activity. By using the method of extremely slow inhalation flow and rather large particles, deposition occurs predominantly in the small airways (generations 9-15).⁽⁴⁾ To our knowledge long term clearance in these patients groups has not been studied before, and the hypothesis was that the long term clearance from small airways would be affected, e.g slower, compared to healthy subjects.

The calculated regional deposition predicted very small differences between the three groups. Even if there was a difference in airway resistance between the patients groups and the healthy subjects our prediction of deposition with slow inhalation flow is rather independent of airway dimension. This has also been investigated in a study where bronchoconstriction was induced 2-3 fold by a cholinergic provocation. The retention at 24 h (Ret_{24}) was similar with normal airway resistance as with induced bronchoconstriction, when inhaling the particles with ESI.⁽¹²⁷⁾

If the particles deposit in the larger airways, due to the increased airway resistance in the patients, the Ret_{24} would have been smaller than what was found in this study and also compared to the healthy subjects.⁽¹²¹⁾ Centrally deposit particles clear from the larger airways even in CF

and PCD patients because of their daily physiotherapy and voluntary coughing. If a larger fraction of the particles were deposited in the alveolar region then the clearance rate after 24 h would have been much slower, especially between 7 and 21 days. Considering this it is reasonable to believe that a main fraction with the slow inhalation flow (0.05 L/s) was deposited in the small airways.

The Ret_{24} , both in the CF and the PCD patients was larger compared to that of the healthy subjects. In the CF patients there was on the other hand a larger cleared fraction between 24 h and day 7. In an earlier study with PCD patients a prolonged rapid clearance phase was also observed. 80% of the deposited particles inhaled with normal inhalation flow, cleared during 0-72 h, compared to 50% for the healthy subjects.⁽¹⁰³⁾ However, after 7 days the clearance rate was similar between the patients and the healthy subjects. The knowledge of airway clearance today of insoluble particles is that clearance occurs from three different compartments. 1) A first rapid clearance phase that clears the larger to middle size airways predominantly by MCC. This phase is considered to be concluded within 24 h in healthy subjects. 2) A very slow clearance phase of particles deposited in the alveolar region that may take years, and 3) a slow clearance phase representing clearance from the small ciliated airways, probably going on for weeks. The larger Ret_{24} found in the CF and PCD patients is probably a result of their defective MCC, and that cough clearance not completely can compensate for this defect the first day. Cough clearance is also more unpredictable. To be effective an increased mucus production is needed.⁽¹¹⁾

Unexpectedly, the slow clearance phase, extended from day 7 to day 21, was similar in CF and in PCD patients compared to healthy subjects. All three studied groups continued to clear their airways with equal clearance rate, on average 50% of the remained fraction at 24 h cleared during this period. Even if CF and PCD still have some MCC activity the curves would have

deviated. In PCD the ciliary activity has been shown not to be completely immotile⁽¹¹¹⁾, there is a good correlation between ultrastructural abnormal findings of the cilium and ciliary activity.⁽⁸⁹⁾ Our patients had the classical ultrastructural abnormalities, lack of outer dynein arms, which is related to very low ciliary activity.

Clearance mechanisms in small airways

Our findings from the clearance studies with similar clearance velocity between the healthy subjects and the patient groups, indicate that MCC is less important in the small airways. Normally, β -adrenergic agonists stimulate MCC,⁽¹⁰⁾ but no increased clearance from small airways was seen in a study with healthy subjects, when inhaling terbutaline together with the radiolabelled Teflon particles.⁽¹²³⁾ This could indicate that MCC is less important clearance mechanism and that a stimulation not is noted in the small airways, or that for stimulation of MCC, a higher dose of β -adrenergic agonists is required^(12, 81) than what was used in the study. Three main possible clearance mechanisms from small airways of insoluble particles are discussed in the literatures. **1)** Phagocytation of particles by airway macrophages. **2)** Penetration of the particles through the mucus layer to the sol layer. **3)** Retransfer of captured particles into the gel layer and then removed by MCC. Since these clearing mechanisms are difficult to assess in human in vivo has in vitro studies been used for modelling.⁽¹²⁰⁾

1) Airway macrophages are rapidly recruited to the sites of the particle deposition and ingest the particles.⁽⁴⁵⁾ Two types of macrophages, based on their location, exist in the airways, the alveolar macrophages (AM) and interstitial macrophages (IM). Other types, like dendritic cells and intravascular macrophages, are also present in the lung. The macrophages could either migrate to the bronchial associated lymphoid tissue and be processed for production of

secretory IgA or leave the airways by the mucociliary escalator. The more loaded the macrophages are the more rapidly they disappear from the conducting airways.⁽⁶⁸⁾

2) The thickness of the mucus layer varies by location in the ciliated airways. Mercer and co-workers suggest that the mucus layer in smaller bronchi and bronchioles consists of discontinuous patches rather than a continuous layer.⁽⁷⁹⁾ Nevertheless, an alternative description has been proposed in which the gel layer in the bronchi consists of a network, and, that glycoproteins influence the formation of this molecular network, thereby altering the rheological properties of the mucus.^(17, 139) In the smallest bronchiolar airways there are no mucus layer, the epithelium is less ciliated and, the secretion of mucus is an active process of other secretory (Clara) cells. Thus, penetration through the mucus layer is more likely to occur with smaller particles, which have a higher chance to penetrate, than the larger particles used in this study.

The third proposed clearing mechanism is less investigated. The alternative clearance mechanisms from the small airways needs further investigation but this was beyond the scope of this thesis.

Two opposing theories have been proposed to explain the pathogenesis of CF. **1)** The isotonic low-volume hypothesis, sodium and water hyperabsorption of airway liquid due to absence of CFTR inhibition of Na^+ absorption leads to decreased volume of ASL and, consequently impaired MCC, because the cilia is unable to beat. Inhaled particles, bacteria and viruses would then be trapped in the viscous ASL and promote inflammation.⁽⁷⁷⁾ **2)** The high salt theory, the defective CFTR leads to high levels of both chloride and sodium in the ASL which could inhibit the activity of antibacterial proteins and peptides.⁽¹¹³⁾ Also a low bicarbonate secretion in CF induces abnormally low pH that decreases antimicrobial functions.⁽⁹⁾ Our findings of functioning clearance from small airways in

CF suggest that the immunology defect is more likely to be responsible for the pathogenesis in CF rather than the defective MCC. This hypothesis also supports the better prognosis for PCD patients than for CF patients. The mechanical clearance mechanism itself has limited effect on the prognosis in PCD and CF, it is probably the

mucus with bacterial deposition that are the pathogenic clue to the more progressive disease and higher mortality in CF. Another hypothesis proposed by Regnis et al⁽⁹⁷⁾ suggested that the cough clearance of secretion is more effective in PCD, whereas in CF, the altered biorheological properties of sputum might make cough less effective.

Conclusions

- ◆ The method using extremely slow inhalation flow (ESI) and particles of 6 μm enhance deposition in small airways.
- ◆ The ESI can be used in patients with obstructive diseases and high airway resistance, and can in the future be used for targeting small airways for therapeutic purposes.
- ◆ The clearance from small airways in subjects with defective mucociliary transport, e.g. abnormal mucus rheology, and abnormal ciliary function, proceeded with equal clearance rate as in healthy subjects with normal mucociliary transport, at least for 21 days.
- ◆ Mucociliary clearance seems not to be the crucial clearance mechanism in the small airways. Other clearance mechanisms co-exist.
- ◆ Analyses of plasma concentrations give a good estimation of the relative bioavailability to the lung after an inhaled dose of sodium cromoglycate.
- ◆ The method can be used as a simple clinical test for biofeedback purposes of inhalation technique and can also be used in children.
- ◆ For individually inhalation evaluation the test should be well standardised including also whether a lung function test should be performed or not since it influences the plasma concentrations.
- ◆ For comparison between individuals the total amount of absorbed dose from urinary excretion might be better. Alternatively an iv dose an internal standard should be used.
- ◆ The small droplet size has a lower total deposition than larger droplet size expressed as exhalation filter deposition.
- ◆ A larger tidal volume can give greater lung deposition.

Clinical applications and future perspectives

Inhalation therapy today in CF and PCD is most often used for nebulised antibiotics and mucus dissolving purposes. The polydisperse aerosol with deposition unselectively in the airways is then accurate. Gene therapy in CF is one of the most promising candidates for curative rather than symptomatic therapy. The aim of gene therapy is to deliver normal copies of the CFTR gene to the airway epithelium. Three classes of gene transfer agents have been used so far: adenovirus, adeno-associated virus, and liposome-plasmid complexes. Since adverse effects such as immunological reactions towards adenovirus can occur, liposomal-plasmid complexes seems more suitable as the vector for the gene product.

Promising *in vitro* studies have been published, but when testing gene therapy *in vivo*, in CF patients, only small and short effects of normal CFTR activity could be measured.⁽³⁾ The low efficiency and short duration is probably due to deposition in larger airways, the vector is cleared by MCC or cough, and the immunological reactions could be expressions of alveolar deposition. A method to diminish these effects could be to use ESI.

Nebulised liposomal sodium cromoglycate and liposomal beclomethasone have been tested. For liposomal cromoglycate a prolonged retention was observed.⁽¹³²⁾ For further studies the pharmacokinetic method with SCG developed from our study could be applicable.

Further therapeutic agents on its way are agents that restore airway surface liquid volume, i.e. blockers of Na⁺ transport, initiators of Cl⁻ transport and osmolytes, which are example of new strategies of treatment for patients with CF. These new coming therapy for inhalation demands specific regional deposition. So far all commercially delivery systems for inhaled drugs delivers polydisperse particles or droplets with a variable size distribution and

consequently gives a large variation of distribution within the lung. For inhaled β -agonist or inhaled steroids this is usually no problem, since β -agonist has a broad therapeutic interval and that the inflammatory process in chronic inflammatory airways disorders probably is present throughout the bronchial tree. New drugs demand higher precision on the deposited location in the lung and the need of monodisperse delivery system. For instance *ribavirin*, to target the bronchioles, to treat bronchiolitis due to respiratory syncytial virus, *pentamidine* used for prophylaxis against *Pneumocystis carinii* pneumonia. Therapeutic peptides, e.g insulin for systemic uptake needs to penetrate to the alveolar region, since insulin absorption to the systemic circulation otherwise can be influenced by different compartment kinetics in the lung.

A desirable new type of device to be develop would be a device delivering monodisperse particles of a size that can be adjusted, simultaneously recording the inhalation flow to control the deposition site within the lung, depending on what drug is to be administrated and what local effect is desirable.

The prevalence of asthma in childhood has increased during the past decades, in Sweden the prevalence from the ISAAC study showed that approximately 10% of children of 6-7 yrs are asthmatics,⁽¹⁵⁾ and a large proportion needs treatment with inhaled pharmaceuticals. Even if inhalation therapy is very common in children there is a lack of relevant data of lung deposition. Dose recommendations, usually derives from estimates of lung deposition data from adults, are scaled down to mimic the child lung. Studies of lung deposition in children are needed, especially in children with obstructive airway disorders. There is some evidence of correlation between total lung deposition and age.^(90, 128) The difference can be as large as 2 % in children (mean age

21 month) compared to 19% in adults. Further investigations needs to be performed to confirm this. To avoid isotope

studies pharmacokinetic techniques are suitable, perhaps the SCG method can be used.

Svensk sammanfattning

Den här avhandlingen handlar dels om 1) mekanismer för deponering och eliminering (clearance) av partiklar i små luftvägar, och dels om 2) framtagning av en icke radioaktiv metod att mäta dosen till lungan av ett inhalerat läkemedel.

Luftvägsträdet i lungan består av stora och små luftvägsgrenar där luften förs till delen där gasutbytet sker (alveolerna). Lungan utsätts hela tiden för omgivningsluften som kan innehålla stora mängder partiklar, föroreningar, bakterier och virus. Lungan behöver därför många skyddsmekanismer.

Den primära skyddsmekanismen i stora och medelstora luftvägar är slemflimmerhårtransport (mucociliär clearance) som för inandad material upp till svalget. Sjukliga förändringar vid många lungsjukdomar anses starta i de små luftvägsgrenarna. Hur stor betydelse slemflimmerhårtransport har i de små luftvägsgrenarna (diameter < 2mm) vet man inte så mycket om. Det beror på att det är svårt att deponera testpartiklar till denna del av luftvägsträdet med vanlig konventionell inandningsmetod och därmed också svårt att mäta elimineringen av partiklarna från denna del.

Inhalationsbehandling är idag den vanligaste formen för behandling av obstruktiva lungsjukdomar, ffa astma. Den vanligaste metoden att mäta lungdeponering är att märka substansen för inhalation med radioaktivitet och sedan detektera deponeringen med gammakamerateknik.

Vid utebliven effekt av ett läkemedel kan orsaken vara dålig inhalationsteknik och följaktligen liten dos till lungan. En enkel farmakokinetisk (icke radioaktiv) metod att mäta lungdeponering i klinisk praxis vore bra att tillgå.

Studiernas syfte:

Att studera om mucociliär transport är den primära försvarsmekanismen i små luftvägar och att utvärdera om långsam inhalationsmetod kan tillämpas på patienter med obstruktiva lungsjukdomar.

Att etablera en icke radioaktiv metod att mäta lungdeponering som kan användas rutinmässigt vid utredning av olika inhalationstekniker samt kunna tillämpas på barn.

Metod:

Clearance av partiklar från små luftvägar har studerats hos patienter med känd medfödd defekt i den mukociliära transporten, dels patienter med cystisk fibros (CF) vars slem i luftvägarna blir mycket segt, dels patienter med primär ciliedyskinesi (PCD) vars flimmerhår (cilier) inte fungerar. Dessa patienter har andats in radioaktivt märkta och relativt stora (6µm) testpartiklar extremt långsamt. Inhalationsmetoden har visats sig deponera partiklarna främst i de små luftvägarna. Därefter har kvarvarande radioaktiviteten över lungorna mätts under tre veckors tid som mått på eliminationen.

Ett vanligt astmaläkemedel, natriumkromoglykat (SCG), absorberas snabbt från lungan till blodbanan, men nedsvält läkemedel tas inte upp i magtarmkanalen. Genom att mäta koncentrationen av SCG i plasma eller utsöndringen i urinen kan man uppskatta dosen till lungan. I studien utvärderades vilken av plasma- eller urinanalyser som hade den bästa reproducerbarheten. Dessutom utvärderades om spirometri (lungfunktionstest) i anslutning till inhalationen påverkade analyserna.

I nästa studie användes SCG som påverkats av luftfuktighet (RH), för att utvärdera droppstorleksfördelningens betydelse vid inhalation hos barn med astma.

Resultat:

Betydligt högre andel kvarvarande partiklar fanns hos CF och PCD patienterna efter ett dygn, jämfört med friska. Detta beror sannolikt på deras försämrade mukociliära transport i de stora och medelstora luftvägarna. Clearance mellan 1-3 veckor som speglar eliminationen från små

luftvägar var likvärdig mellan de båda patientgrupperna och jämförande friska kontroller. Plasma analyser av inhalerat SCG hade bättre reproducerbarhet än urinanalyser.

Exponeringen med spirometri gav signifikant högre plasmakoncentrationer av SCG, detta kunde dock inte detekteras i urinanalyserna. Luftfuktighet påverkar droppstorleksfördelningen i en nebuliserad dos så att låg luftfuktighet ger små droppar och hög luftfuktighet ger större droppar. En korrelation sågs mellan tidalvolym och mängd deponerat SCG ($r=0.72$). Större droppar gav en högre total mängd deponerad substans oberoende av tidalvolym och inhalationsflöde. Någon skillnad mellan hög och låg RH i lungdeponering kunde ej påvisas i plasmaanalyserna.

Betydelse:

Clearance från små luftvägar förekommer trots nedsatt mukociliär transport, med ungefär samma hastighet som för friska. Det finns uppenbart andra clearance-mekanismer i små luftvägar.

Med extremt långsamt inhalation flöde (ESI) deponeras partiklar i små luftvägar. Inhalationsmetoden skulle kunna användas för att selektivt deponera läkemedel i små luftvägar i diagnostiskt eller terapeutiskt syfte.

Plasma-analyser av inhalerat SCG har bättre reproducerbarhet än urinanalyser. Metoden är enkel, kan användas vid barnstudier och kan användas för att mäta individuell biotillgänglighet vid t.ex utvärdering av inhalationsteknik.

Man bör tänka på att en spirometri kan påverka plasmakoncentrationen i omedelbar anslutning.

Större tidalvolum vid inhalation hos barn ger högre lungdeponering förutsatt att dropparna/partiklarna inte för små.

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