



MARISANNA SAARI

Pulmonary Deposition and Clearance of
 ^{99m}Tc -labelled Beclomethasone Liposomes
in Healthy Subjects and in Mild
and Severe Asthma



ACADEMIC DISSERTATION

To be presented, with the permission of
the Faculty of Medicine of the University of Tampere,
for public discussion in the main auditorium of Building K,
Medical School of the University of Tampere,
Teiskontie 35, Tampere, on September 19th, 2003, at 12 o'clock.

Acta Universitatis Tampereensis 952
University of Tampere
Tampere 2003

ACADEMIC DISSERTATION

University of Tampere, Medical School
Tampere University Hospital, Departments of Respiratory Medicine and
Clinical Physiology & Nuclear Medicine
Finland

Supervised by

Professor Markku M. Nieminen
University of Tampere

Reviewed by

Professor Hannu Tukiainen
University of Kuopio
Docent Esko Vanninen
University of Kuopio

Distribution



University of Tampere
Bookshop TAJU
P.O. Box 617
33014 University of Tampere
Finland

Tel. +358 3 215 6055
Fax +358 3 215 7685
taju@uta.fi
<http://granum.uta.fi>

Cover design by
Juha Siro

Printed dissertation
Acta Universitatis Tamperensis 952
ISBN 951-44-5745-5
ISSN 1455-1616

Electronic dissertation
Acta Electronica Universitatis Tamperensis 276
ISBN 951-44-5746-3
ISSN 1456-954X
<http://acta.uta.fi>

Tampereen yliopistopaino Oy Juvenes Print
Tampere 2003

*Work like you don't need the money.
Love like you've never been hurt.
Sing like no one's listening.
Dance like nobody's watching.*

Abstract

The purpose of this study was to evaluate with lung scintigraphic method the distribution and clearance of two ^{99m}Tc -labelled beclomethasone liposome formulations (DLPC and DPPC) in healthy subject (n = 11). In addition, the distribution and clearance of radiolabelled Bec-DLPC liposomes were compared in patients with mild (n = 10) and in severe (n = 10) asthma, after a 1-week treatment period of formoterol in patients with mild asthma (n = 10), and during 4-months treatment period of inhaled corticosteroids in novel steroid-naive asthmatics (n = 9). Healthy subjects were hospital personnel with normal spirometry. Asthmatic patients were recruited from the outpatient clinic of the Department of Respiratory Medicine of Tampere University Hospital. Baseline FEV₁ was ≥ 80 % of the predicted in patients with mild asthma and 60 % or less in those having a severe form of asthma. All study participants were non-smoking.

In healthy subjects both beclomethasone liposomes proved to be suitable for nebulization, although aerosol cloud was more efficiently made from the DLPC liposome suspension. No significant differences were demonstrated in the central/peripheral lung deposition between the DLPC and DPPC formulation. A progressive, slow clearance pattern was seen in both ^{99m}Tc -labelled liposomes. 87 % of the originally deposited radioactivity of DLPC liposomes and 89 % of the DPPC liposomes remained in the lungs 4 hours after inhalation.

In asthmatic patients DLPC aerosol particles were deposited more centrally in lungs of patients with severe asthma than those with a mild form of the disease. The clearance of the DLPC liposomes proved to be slower in the mild asthmatic group, although the clearance rates were strikingly slow in both asthmatic groups. At the 4-hour measurement, 82 % of the initial dose was detected in the lungs of mild asthmatics, while in patients with severe disease it was 69 %.

In asthmatic patients on inhaled steroids, a 1-week medical treatment period of long-acting β_2 -agonist formoterol increased peripheral lung deposition of the beclomethasone liposomes. A systemic positive connection was seen between enhanced lung functions and increased lung deposition measured as $\text{AUC}_{(0-24\text{h})/24}$.

No statistically significant differences in retention curves before and after the formoterol treatment were detected.

A 4-months inhaled corticosteroids treatment period in novel asthmatics did not change the deposition and clearance pattern of Bec-DLPC liposomes observed in lung scintigraphy. However, all lung functions were enhanced, while only improvement of FVC values reached statistical significance.

Lyhennelmä

Tämän tutkimuksen tarkoituksena oli verrata gammakamerakuvaus kahden ^{99m}teknetiumilla leimatun beklometasoniliposomin (DLPC ja DPPC) jakaumaa ja poistumaa keuhkoista terveillä koehenkilöillä (n = 11). Lisäksi seurattiin radioleimatun DLPC liposomin keuhkojakaumaa ja poistumaa lievää (n = 10) ja vaikeaa (n = 10) astmaa sairastavilla potilailla, lievää astmaa sairastavilla (n = 10) 1 viikon formoteroli hoidon jälkeen sekä 4 kuukauden inhaloitavan kortikosteroidihoidon aikana tuoretta astmaa sairastavilla potilailla (n = 9). Kaikilla terveillä koehenkilöillä oli normaali spirometriatulokset. Lievää astmaa sairastavilla FEV₁ oli ≥ 80 % viitearvosta kun taas vaikeassa tautimuodossa FEV₁ oli 60 % tai vähemmän. Kaikki koehenkilöt olivat tupakoimattomia.

Molemmat beklometasoniliposomit osoittautuivat terveillä koehenkilöillä hyvin sumuttimella annosteltaviksi, vaikkakin sumutustehokkuus oli DLPC liposomeilla parempi. Alueellisessa keuhkojakaumassa ei todettu tilastollisesti merkitseviä eroja liposomien välillä. Molemmat liposomit poistuivat keuhkoista progressiivisesti ja hitaasti. DLPC liposomeihin kiinnittyneistä radioaktiivisesta aineesta oli 4 tuntia inhalaation jälkeen havaittavissa gammakuviissa keuhkoissa 87 % ja DPPC liposomien aktiivisuudesta 89 %.

Tutkimuksessamme osoitimme, että inhalaation jälkeen DLPC liposomit jakautuivat vaikeassa astmassa enemmän sentraalisiin hengitysteihin kuin lievässä tautimuodossa, jossa aerosolipartikkelit saavuttivat paremmin perifeeriset hengitystiet. Liposomipartikkelien poistuma keuhkoista kummassakin astmaryhmässä oli hidasta; lievää astmaa sairastavilla tosin vielä hitaampaa. 4 tuntia inhalaation jälkeen lievillä astmaatikoilla oli alkuperäisestä liposomiannoksesta jäljellä keuhkoissa 82 % ja vastaavasti vaikeaa astmaa sairastavilla 69 %.

Astmaatikoilla, joilla oli inhaloitava steroidi anti-inflammatorilääkkeenä, pitkävaikutteisen β₂-agonistin, formoterolin, lisäys yhden viikon ajaksi paransi beklometasoniliposomien perifeeristä depositiota keuhkoissa. Parantuneiden keuhkofunktioiden sekä lisääntyneen keuhkodeposition välillä todettiin systemaattinen positiivinen yhteys mitattuna AUC_{(0-24h)/24} arvolla. Formoterolihoito ei vaikuttanut liposomin poistumaan keuhkoista merkitsevästi.

Astmaatikoilla, jotka aikaisemmin eivät olleet käyttäneet anti-inflammatorilääkitystä, neljän kuukauden inhaloitava kortikosteroidihoito ei muuttanut DLPC liposomien keuhkojakaumaa tai poistumaa. Kuitenkin kaikki keuhkofunktioparametrit paranivat, vain FVC tosin tilastollisesti merkitsevästi.

Contents

ABSTRACT	4
LYHENNELMÄ.....	6
ABBREVIATIONS	12
LIST OF ORIGINAL PUBLICATIONS	13
I INTRODUCTION.....	14
II REVIEW OF THE LITERATURE	15
Asthma	15
Patophysiology	15
Inhaled asthma therapy	15
Inhaled corticosteroids	16
Short acting β_2 -agonists	16
Long-acting β_2 -agonists	17
Other bronchodilators	18
Liposomes.....	18
Structure of the phospholipids.....	18
Liposomes as drug carriers.....	20
Pulmonary delivery of liposomes.....	21
Physical characteristics of the liposomal aerosol.....	21
Degradation of liposomes in alveolar level.....	22
Characteristics of aerosol deposition and clearance in the lower respiratory tract	23
Mechanism of aerosol deposition.....	23
Tracheobronchial mucociliary clearance.....	24
Factors affecting aerosol delivery to the lungs.....	25
Particle size	25
Inhalation flow rate	26
Airway caliber.....	27
Factors affecting mucociliary clearance.....	27

Lung penetration	27
Gender, age, lung functions and smoking.....	28
Drugs.....	28
Obstructive lung diseases and other respiratory tract diseases	29
Lung scintigraphy	30
Gamma camera imaging.....	30
Radiotracers in ventilation scans.....	30
Radiolabelling of liposomes.....	31
Analysis of the lung scans	32
Attenuation and scatter.....	32
Quantitative definition of lung aerosol penetration and clearance.....	33
Radioaerosol images in airway obstruction.....	33
The influence of the liposomal formulation on the clearance from the human lungs.....	34
III AIM OF THE STUDY.....	36
IV SUBJECTS AND METHODS	37
Subjects studied	37
Study population.....	38
Exclusion criteria.....	38
Study designs	38
Liposome preparation and aerosol characteristics	39
Preparation and labelling of Bec-DLPC and Bec-DPPC liposomes with ^{99m} Tc.....	41
SnCl ₂ solution.....	41
Liposome suspension.....	41
Labelling process with ^{99m} Tc.....	41
Liposome suspension delivery	42
Determination of the labelling efficiency	42
Gamma camera measurements	43
Lung scintigraphy with ^{99m} Tc labelled liposomes.....	43
¹³³ Xenon ventilation scan	43
Tissue attenuation.....	43
V DATA PROCESSING	44

Other data acquisition	45
Questionnaires	45
Lung function tests	45
Statistical analyses	45
VI RESULTS	47
Aerosol characteristics of Bec-liposomes (Study I).....	47
Pulmonary distribution and clearance of Bec-DLPC and Bec-DPPC liposomes (Study I).....	47
Deposition and clearance of inhaled ^{99m} Tc-labelled Bec-DLPC liposomes in mild and severe asthmatics (Study II).....	48
Inhaled formoterol therapy and the peripheral lung deposition and clearance in asthmatic patients (Study III).....	48
Inhaled corticosteroid treatment and pulmonary lung deposition and clearance in novel asthma (Study IV).....	49
Summary of the lung deposition and clearance of Bec-DLPC liposomes in Studies I-IV	51
VII DISCUSSION	52
Methodological considerations	52
Attenuation and scatter effect.....	52
Lung ROI delineation.....	52
Overlapping	53
Inhalation and gravitational effect.....	53
Variability of lung scintigraphic method.....	54
Differences in characteristics of nebulized liposomes (Study I)	54
Beclomethasone containing liposomes and pulmonary deposition and retention (Study I)	55
Asthma and inhaled liposomes (Study II).....	56
Centralized deposition	56
Mucociliary clearance	57
Effect of inhaled formoterol on lung functions and aerosol distribution in the lungs (Study III).....	58
Inhaled corticosteroids and novel asthma (Study IV).....	59

VIII CONCLUSIONS.....	62
IX ACKNOWLEDGEMENTS.....	64
X REFERENCES.....	66

Abbreviations

AUC = Area under the curve
Bec = Beclomethasone dipropionate
Bec-DLPC = Beclomethasone dipropionate-dilauroylphosphatidylcholine
C/P ratio = Central/peripheral deposition ratio
DLPC = Dilauroylphosphatidylcholine
DPPC = Dipalmitoylphosphatidylcholine
DSPE = Distearoylphosphatidyl-ethanolamine
DTPA = Diethylenetriaminepentaacetic acid
ECP = Eosinophilic cationic protein
FEV₁ = Forced expiratory volume in one second
FEF₅₀ = Forced expiratory flow when 50 % of the of the forced vital capacity remains in the lung
FVC = Forced vital capacity
GSD = Geometric standard deviation
HMPAO = Hexamethylpropylene-amine-oxime
HYNIC = Hydrazinonicotinamide
ICS = Inhaled corticosteroids
LABA = Long-acting β_2 -agonist
LUV = Large unilamellar vesicle
MCC = Mucociliary clearance
MDI = Metered-dose inhaler
MLV = Multilamellar vesicle
MMAD = Mass median aerodynamic diameter
MMEF = Maximal mid-expiratory flow
PC = Phosphatidylcholine
PI = Penetration index
RES = Reticuloendotelial system
SD = Standard deviation
SUV = Small unilamellar vesicle
V_{max 25} = Maximal expiratory flow when 25 % of the forced vital capacity remains in the lung

List of original publications

1. Saari M, Vidgren MT, Koskinen MO, Turjanmaa VMH, Nieminen MM (1999). Pulmonary distribution and clearance of two beclomethasone formulations in healthy volunteers. *Int J Pharm* 181: 1-9.
2. Saari SM, Vidgren MT, Koskinen MO, Turjanmaa VMH, Waldrep JC, Nieminen MM (1998). Regional lung deposition and clearance of ^{99m}Tc-labelled beclomethasone-DLPC liposomes in mild and severe asthma. *Chest* 113: 1573-1579.
3. Saari SM, Vidgren MT, Herrala J, Turjanmaa VHM, Koskinen MO, Nieminen MM (2002). The possibilities of formoterol to enhance the peripheral lung deposition of the inhaled liposome corticosteroids. *Respir Med* 96: 999-1005.
4. Saari SM, Vidgren MT, Turjanmaa VMH, Koskinen MO, Nieminen MM (2003). No impairment in novel asthmatics treated with an MDI corticosteroids with spacer. *Respir Med* 97: 152-158.

I Introduction

Asthma is a chronic inflammatory airway disease, in which most of therapeutic drugs are administered via an inhalation route. Inhaled corticosteroids are currently accepted as a standard treatment of asthma (Barnes 1995). Successful clinical medication of asthma depends on achieving adequate delivery of inhaled drugs to the lungs. However, pulmonary delivery of drugs is complicated by a rapid absorption of most drugs necessitating frequent dosing which is partly responsible for system side-effects. Patients need to be trained to coordinate breathing and inhaling of aerosols in order to achieve good drug penetration in the lungs (Schreier 1993). Even so, the proportion of the inhaler released dose actually reaching the lower airways, even with the optimal inhalation technique, is relatively small.

Systemic adverse effects of inhaled glucocorticoids depend on the amount of drug present in circulation. This is of particular concern for patients requiring high doses of the anti-inflammatory agent. Reduction in both dosage size and frequency in these patients would have a two-fold benefit: not only by means of avoiding adverse events, but also by improving the compliance of the asthmatic patients in taking medication as prescribed.

Liposomes are phospholipid vesicles composed of lipid bilayers enclosing an aqueous compartment. Hydrophilic molecules can be encapsulated in the aqueous spaces and lipophilic molecules can be incorporated into the lipid bilayers. Liposomes provide an efficient delivery system because they are biocompatible, biodegradable and relatively non-toxic (Shek et al. 1990). As a drug delivery system, liposomes can significantly change the pharmacokinetic and pharmacodynamic fate of a compound by enhancing drug uptake, delaying the loss of rapidly cleared drugs and reducing drug toxicity (Gregoriadis et al. 1993).

This study was designed to evaluate with lung scintigraphic method the distribution and clearance of two beclomethasone liposome formulations in healthy subjects. In addition, the distribution and clearance of Bec-DLPC liposomes were compared in patients with mild and severe asthma, after a treatment period of formoterol in patients with mild asthma, and after treatment period of inhaled corticosteroids in novel steroid-naïve asthmatics.

II Review of the literature

Asthma

Patophysiology

Asthma is a chronic airway disease, the prevalence of which is increasing throughout the world, also in Finland (ISAAC 1998, Pallasaho et al. 2000). Asthma is currently defined as a chronic inflammatory airway disease in which many cells, in particular mast cells, eosinophils, and T lymphocytes, play roles (GINA 2002). Apart from cells conventionally associated with inflammation, structural tissue cells, such as epithelial cells, fibroblast and smooth muscle cells, play significant roles in relation to airway inflammation, throughout release of a variety of mediators (Chung et al. 1999). Airway inflammation in asthma is characterized by vascular leakage, mucus hypersecretion, epithelial shedding, and extensive airway narrowing. Chronic airway inflammation stimulates mechanism of airway healing and repair that can lead to progressive, potentially irreversible, tissue destruction and airway remodelling (Vignola et al. 2000). The structural changes are evident throughout the airways, both in proximal and distal airways sites (Howart et al. 1998, Kraft 1999). In asthmatic subjects, the inflammation causes recurrent episodes of wheezing, breathlessness, chest tightness and cough. The symptoms are usually associated with variable airflow limitation that is at least partially reversible, spontaneously or with treatment (GINA 2002).

Inhaled asthma therapy

The high incidence of side effects associated with oral therapies in asthma led to the development of inhaled therapies in attempt to limit adverse effects. Current asthma therapy is predominantly delivered by inhalation route.

Inhaled corticosteroids

Corticosteroids are by far the most effective therapy currently available for asthma, and a response to steroids is one of the characteristic clinical features of asthma (Barnes 1995, Barnes 1998a). Corticosteroids suppress the eosinophilic inflammation in the airways of patients with asthma by inhibiting many components of the inflammatory response, such as increased transcription and expression of anti-inflammatory proteins and repression of inflammatory genes. In addition, steroids inhibit the activation and recruitment of inflammatory cells, inhibit the survival of mast cells at the airway surface, and also reduce the release of mediators from structural cells in the airways (Schweibert et al. 1996, Barnes 1998b).

Inhaled corticosteroids have revolutionized asthma management and are currently accepted as the standard treatment of asthma (Barnes 1998a). The introduction for guidelines for asthma therapy has led to earlier use of inhaled steroids for both adult and childhood asthma (British Thoracic Society 1997, Dahl et al. 2000), and inhaled steroids have now become first-line therapy for chronic asthma. Inhaled corticosteroids reduce symptoms and use of β_2 -agonist, improve lung functions (Juniper et al. 1990, Haahtela et al. 1991, Djukanovic et al. 1991), reduce and prevent complications, such as exacerbations or irreversible airway damage (Dompeling et al. 1992, Haahtela et al. 1994).

All inhaled corticosteroids exhibit dose-related systemic adverse effects, although these are less than with the comparable dose of oral corticosteroids (Lipworth 1999). In high doses of inhaled corticosteroids above 1.5 mg/d (0.75 mg/d for fluticasone dipropionate) may be associated with marked adrenalin suppression (Agertoft et al 1997, Wilson et al. 1997a, Wilson et al. 1997b, Lipworth 1999) and a significant reduction in bone density (Herrala et al. 1994, Lipworth 1999). Long-term, high-dose inhaled corticosteroids exposure increases the risk for posterior subcapsular cataracts, and in lesser degree, ocular hypertension and glaucoma (Urban et al. 1986). Skin bruising correlates well with the degree of adrenal suppression (Roy et al. 1996). Dysphonia, the most common local side effect of inhaled glucocorticoids, can occur in more than 50 % of patients given high-dose therapy (Willey et al. 1982).

Short acting β_2 -agonists

β_2 -agonists have an important role in the treatment of asthma alongside inhaled corticosteroids. The target for β_2 -agonists are smooth muscle cells in bronchial wall. They are by far the most useful bronchodilators used for treating acute

asthma attack and are most effective when inhaled. Short acting agents, with their fast onset of bronchodilation, provide a rapid relief of asthma symptoms. The major limitation of β_2 -agonists is a short duration of action, typically 4-6 hours (Nelson 1995).

Long-acting β_2 -agonists

The short drug effect of β_2 -agonists gave a challenge to develop bronchodilators with long-acting properties. In the 1980s, salmeterol was developed, and formoterol, originally meant as a conventional β_2 -agonist for oral use, was found to be long-acting when taken by inhalation. Both salmeterol and formoterol share similar properties and provide prolonged bronchodilation (Palmqvist et al. 1997). However, while salmeterol is a partial agonist at the β_2 -adrenoceptor, achieving maximum effect after about 60 min (Broegden et al. 1991), formoterol is almost a full agonist achieving a more rapid onset of action similar to salbutamol (Bartow et al. 1998).

Formoterol is a selective β_2 -adrenoceptor agonist; a dual action mechanism with both fast- and long-acting properties. It is moderately lipophilic, enabling enough inhaled drug to diffuse into the lipid bilayer and produce a long duration of action. On the other hand, formoterol is sufficiently hydrophilic to bind rapidly to the cell surface β_2 -receptors, resulting in a fast onset of action (Johnson 1995a, Linden et al. 1996). In trials with adult asthma patients, 12 μ g and 24 μ g of formoterol dry powder provide a rapid onset of action, 1-3 minutes (Derom et al. 1992, Palmqvist et al. 1997, Seberova et al. 2000). Both salmeterol and formoterol have a duration effect exceeding 12 hours and both are recommended for regular use at 12-h intervals (Kestens et al. 1991, Johnson 1995b, Schreurs et al. 1996).

Formoterol and salmeterol are both effective in the treatment of asthma. The study of formoterol in subjects using inhaled corticosteroids showed improved lung functions with no evidence for worsening asthma (van der Molen et al. 1997). The addition of a long-acting β_2 -agonist to therapy with inhaled steroids produced a greater improvement in symptoms and lung functions than increasing the steroid dose alone (Greening et al. 1994, Woolcock et al. 1996, Pauwels et al. 1997), and a study examining the combined effects of formoterol with inhaled budesonide (the FACET study) demonstrated a significant reduction in asthma exacerbations with the combined therapy (Pauwels et al. 1997). Hence, β_2 -agonists with long-acting properties are now well established as therapeutic options in the management of asthma. They are included in current guidelines as recommended add-on therapy for asthma not controlled with modest dose of

inhaled corticosteroids and occasional use of short-acting bronchodilator (Dahl et al. 2000, GINA 2002).

Other bronchodilators

Methylxanthines such as theophylline, have been used in treatment of asthma since 1930. However, the frequency of side-effects and relative low efficacy of theophylline have recently led to reduced usage since β_2 -agonists are far more effective as bronchodilators and inhaled corticosteroids have a greater anti-inflammatory effect (Barnes et al 1998). Still, theophylline is used as a rescue medication in acute asthma attacks and as add-on therapy in severe asthma (Dahl et al. 2000).

In asthmatic subjects anticholinergic drugs are less effective as bronchodilators than β_2 -agonists. The time course of bronchodilation with anticholinergic drugs is slower than with β_2 -agonists, reaching a peak only 1 h after inhalation, but persists over 6 h. In acute and chronic treatment of asthma anticholinergic drugs may have an additive effect with β_2 -agonist. Nebulized anticholinergic drugs are shown to be effective in acute severe asthma (Barnes et al. 1998).

Liposomes

Structure of the phospholipids

Phospholipids are compound lipids containing, in addition to fatty acids and an alcohol, a phosphoric acid residue. They also have nitrogen-containing bases and other substituents. Phosphatidylcholines (PC), known as lecithins, are phospholipids widely distributed in the cells of the body, having both metabolic and structural functions in membranes (Bangham 1965). Dipalmityl lecithine (DPPC) is a major component of the pulmonary surfactant in respiratory alveoli which principal function is to reduce surface tension in the lungs (Bangham 1987). Surfactant reduces the net contractile force of the surface by forming on it a monomolecular film that keeps the lung from collapsing at resting transpulmonary pressures (Wright et al. 1987).

Phospholipids structurally resemble soap, with molecules characterized having a hydrophilic head attached to a hydrophobic tail. When such compounds are suspended in aqueous media, they spontaneously form a vesicle containing of one or more bilayers in which lipophilic parts of the molecules face inwards and the hydrophilic parts exposed to the aqueous phase surrounding them. This closed membrane systems with water both inside and outside of the bilayer sheets, are called liposomes (Bangham 1965). The lipid bilayer system in liposomes are similar in structure to those found in human cell membranes (Bangham 1968).

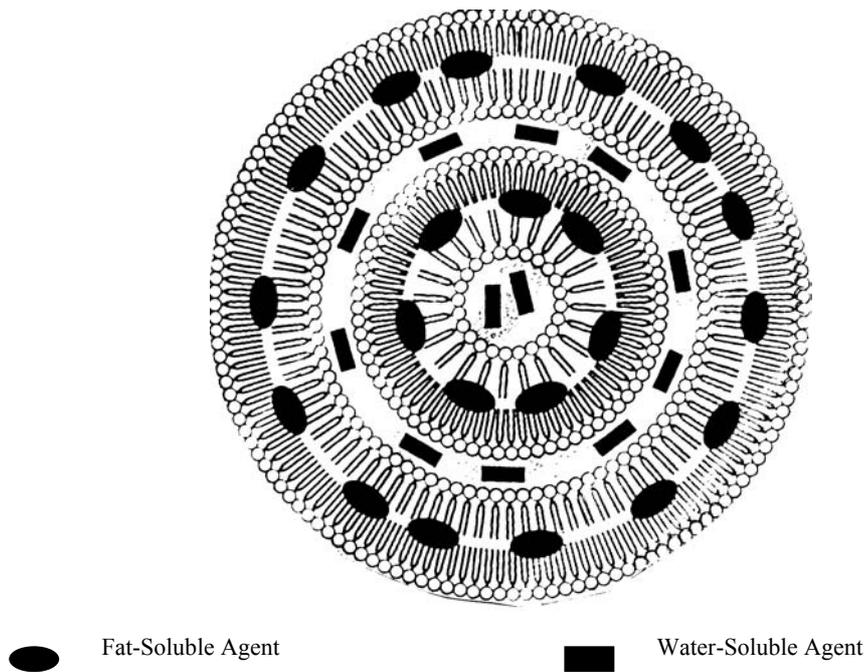


Figure 1. *Liposome (Bangham 1992)*

The liposomes can vary in size considerably, but are usually 0.1-5.0 μm in diameter. When a dried phospholipid mixture is hydrated, liposome particles of heterogenous size are formed. Normally the major components in such a dispersion are large particles consisting of multiple lipid bilayers with aqueous interphases, so called multilamellar vesicles (MLVs). Subsequent ultrasonification or pressure driven filtration causes MLVs (diameter $\geq 4.0 \mu\text{m}$) to form small unilamellar vesicles (SUVs) that are $< 0.1 \mu\text{m}$ in size. Alternatively, large unilamellar vesicles (LUVs) can be formed (1.0-10.0 μm) also consisting of a single phospholipid bilayer but enclosing a large, aqueous phase (Hope et al. 1986, Cullis et al. 1989).

Liposomes as drug carriers

Because of the structural versatility of the liposomes in terms of size, composition, surface charge, bilayer fluidity and their ability to incorporate almost any drug regardless of its solubility, they have been extensively investigated as carriers of drugs (Gregoriadis et al. 1993). Water-soluble substances, such as certain drugs, enzymes, or genes can be encapsulated in the watery regions, whereas fat-soluble entities such as polyene antibiotics and other lipid-soluble drugs can be incorporated within the lipid bilayer (Bangham 1992). Liposomes can be administered orally, transdermally, intravenously, intrabronchially, intramuscularly, intraocularly, subcutaneously, and intraperitoneally.

The rate at which liposome-associated drugs are released for systemic absorption depends on their route of administration. Orally administered liposomes are not optimal drug carriers, since they are easily degraded by bile salts and lipases (Weiner et al. 1988). Thus to date the usage liposomal pharmaceuticals are limited to parenteral and local administration (Fielding 1991).

Liposomes are rapidly taken up after intravenous administration, selectively by the organs of the reticuloendothelial system (RES) such as liver (Kupffer cells, in particular), spleen, lungs, lymph nodes, and to a lesser degree, bone marrow (Senior 1987). This uptake is mediated by the interaction with plasma proteins and lipoproteins (Bonte 1986). In addition liposomes, like other foreign bodies, are ingested by phagocytic cells as macrophages (Schroit 1983, Alving 1983).

The distribution of the liposomal drugs after administration differs from that of the conventional drugs. First, extensive RES uptake of drug-carrier liposomes reduces the systemic exposure of non-RES tissues to the drug. This has significantly improved the safety of agents such as amphotericin B (Mufson et al. 1990, Dupont 2002), doxorubicin (Gabizon et al. 1989) and daunorubicin (Gill et al. 1995, Sparano et al. 2001) by reducing toxicity of drug accumulation in specific organs. Secondly, phagocytic cells play a role of secondary carriers for liposome-encapsulated drugs. Mononuclear phagocytes activated in infection, inflammation or tumour processes tend to act like magnets for liposomes. Disease process enlarges the space between the endothelial cells lining local capillaries in those sites, permitting extravasation of liposomes (Cho et al. 1989). This fact has been successfully used in clinical application of CT- and MRI imaging of liver and spleen diseases (Lokling et al. 2001, Dvorak et al. 2002), for imaging with liposome-labelled gamma emitters in tumours and infections, and as blood pool markers (Boerman et al. 1998, Boerman et al. 2001, Goins et al. 2001). Finally, RES uptake enables the specific targeting of liposomal drug carriers to phagocytic cells. This has been shown to enhance the efficacy of

antibiotics against several intracellular infections such as systemic fungal infections in immunocompromised patients undergoing treatment for cancer (Dupont 2002) or in those with an immunodeficiency disorder, most notably AIDS (Johnson et al. 2002).

Recently, promising results has been achieved in gene therapy using liposomes as gene transfer vector system. Gene therapy is becoming a major contender in treatment of various pathological disorders, including cystic fibrosis, α -1-antitrypsin deficiency, cancer or AIDS (Khaw et al. 2001). Non-viral vector such as cationic liposomes may in future offer an efficient, less toxic and less immunogenic method for intracytoplasmic gene delivery (Schwarz et al. 1996, Kaneda 2001, Bendas 2001).

Pulmonary delivery of liposomes

Liposomes have been considered a good carrier system for the delivery of entrapped drugs to the lungs via a tracheobronchial route. Their use in pulmonary delivery was first investigated as a potential treatment for respiratory distress syndrome (Ivey et al. 1976). Schreier and associates (1993) have listed the characteristics of the liposome aerosols, which may alleviate some of the problems encountered with the conventional aerosol delivery. They provide ability to 1) serve as a solubilization matrix for poorly soluble agents, 2) act as a pulmonary sustained release reservoir, and 3) facilitate intracellular delivery of drugs, especially to alveolar macrophages. In addition, liposomes provide a mean to 4) prevent local irritation of lung tissue and reduce pulmonary toxicity, 5) prolong local therapeutic drug levels, and 6) generate high intracellular drug concentrations, e.g. in infected macrophages.

Physical characteristics of the liposomal aerosol

Size is a critical property determining the deposition site for inhaled particles in the lung. In case of delivering aerosolized liposomes, liposomal particles become part of the aerosol droplets and pulmonary deposition is a function of aerosol droplet size (Farr et al. 1985, Niven et al. 1990, Taylor et al. 1990, Waldrep et al. 1993). May (1973) demonstrated that with nebulization, liposome vesicles were reduced in size by shear forces associated with the continuous recycling through the nebulizer. Aqueous liposome aerosols have been generated with a variety of nebulizers ((Waldrep et al. 1994a). Nebulizers employ compressed gas or ultrasound to generate aerosols from aqueous solutions or suspensions of drugs.

All reported studies of pulmonary liposome delivery to humans have used nebulizers for such administration. With jet nebulizers, the most important factors determining the final aerosol droplet size produced are the design of the device (Waldrep et al. 1994a) and gas stream/ changes in air pressure (Niven et al. 1992). Properties of the liquid being nebulized, such as surface tension, viscosity and drug solubility may also affect final aerosol characteristics (Taylor et al. 1993).

In order to realize the full therapeutic potential of liposome-mediated drug delivery, the liposomal carrier must be sufficiently stable to prevent or minimize premature release of the encapsulated drug. Retention of the drug during aerosolization is greatly dependent on vesicle size and formulation (Niven et al. 1990, Schreier et al. 1993, Abu-Dahab et al. 2001). Temperature rise induced by ultrasonic nebulization can be attenuated by cooling the holding chamber; removal of the thermal effect has shown to reduce substantially the extent of solute leakage (Shek et al. 1994).

Phospholipid aerosols have been employed clinically for many years in the treatment of respiratory distress syndrome in newborns without any reported untoward effects (Jobe et al. 1987). The tolerability and safety of liposome-encapsulated drugs by aerosols has been previously tested in human volunteers, no side-effects have been recognized (Thomas et al. 1991, Waldrep et al. 1997).

Degradation of liposomes in alveolar level

The epochal observation that extracts from the normal lung profoundly reduce the surface tension of water at an air interface while extracts from the lungs of infants dying from hyaline membrane disease lack this property (Clements 1957, Avery et al. 1959) caused intense investigation of the character, metabolism, and replacement therapy of the pulmonary surfactant. Lipids account for nearly 20 percent of the total lung tissue dry weight; of the total lipid pool, phospholipids comprise about 80 percent (Mihalko et al. 1988). In natural lung surfactant, type II pneumocytes (pulmonary epithelial cells) synthesize saturated phosphatidylcholine (mainly DPPC) and phosphatidylglycerole, which are combined with other surfactant components (cholesterol, surfactant protein A) and packed into large secretory vesicles, e.g. lamellar bodies. After secretion, the surfactant is thought to pass through an intermediate state known as tubular myelin before adsorption to monolayer at the air-liquid interface (Kellaway et al. 1990). The mechanism of transformation process from lamellar body through tubular myelin to monolayer is still poorly understood. (Perkins et al. 1996, Quintero et al. 2000)

In the normal lung, surfactant is continuously secreted and degraded. The potential fate of inhaled liposomes is via rapid association with the alveolar surface, and into the intracellular phospholipid pool. Multiple pathways for surfactant clearance exist, including bidirectional flux across the alveolar epithelium, ingestion by macrophages, association with mucus and propulsion up to the airways via mucociliary pathway and lymphatic clearance from the interstitial spaces (Mihalko et al. 1990).

Characteristics of aerosol deposition and clearance in the lower respiratory tract

Mechanism of aerosol deposition

Diffusion, sedimentation and impaction are the most important mechanisms by which particles are deposited in the respiratory tract. Deposition of aerosol particles occurs by inertial impaction in the oropharynx and large “central” airways of the lungs, and also by gravitational sedimentation in smaller “peripheral” airways and alveoli (Brain et al. 1979, Heyder 1981, Brain 1985).

Inertial impaction occurs when aerosol particles are not able to follow the motion of an accelerated gas in which they are suspended at sites of airway branching or changes in the direction of airflow. This major particle transport mechanism in the respiratory tract results the deposition of inspired particles. Impaction increases the larger the particles and the greater the inspired flow rate is (Brain et al. 1979, Heyder 1981, Brain 1985).

Under external mechanical, electrical and thermal force fields, particles can be transported through a gas. Gravitational sedimentation losses in lower airways increase with particles size and the period the aerosol remains in the lungs. Deposition in alveolated regions is enhanced by Brownian diffusion, significantly only for particles smaller than 0.5-0.1 μm in diameter (Brain et al. 1979, Heyder 1981, Brain 1985).

Tracheobronchial mucociliary clearance

Lung mucociliary clearance is one of the lungs' non-specific host defence mechanisms helping to keep lungs clean and sterile. The conducting airways, which in Weibel's mode (Weibel 1963) comprise airway generations 0 to 16 (trachea to terminal bronchioles), are lined with ciliated epithelium. In the trachea each ciliated cell has approximately 200 cilia, i.e. 6 cilia/ μm . The number of cilia, their length, and beat rate decrease in the peripheral airways. A cilia beat consist of a short active propulsive stroke followed by a resting period, and a long backwards recovery stroke. Adjacent cilia beat is functioning in a temporally and spatially co-coordinated manner, thus producing metachronal waves, which drive a layer of mucus from the periphery towards the trachea (Wanner 1977, Pavia et al. 1983, Wanner et al. 1996).

The mucus lining consists of two predominant layers: lower periciliary (sol) layer and upper mucus (gel) layer (Reid et al. 1982). The sol layer consists primarily of transudate, and ions secreted by ion transport and subsequent osmosis from epithelial cells. The gel is a viscous "mucus" layer originating mainly from the submucosal glands, goblet cells, and in most peripheral airways, surfactant. The total volume of mucus-producing structures has been estimated to be 4 ml to 10 ml in human lungs (Reid 1973, Pavia et al. 1983). However, this quantity of mucus secretion can be increased up to 200-300 ml per day during exacerbation in chronic bronchitis (Pavia et al. 1983).

The fate of a deposited aerosol is dependent of the site of deposition and its physico-chemical properties including size, lipid and water solubility, and electrical charge (Clarke et al. 1994). Soluble particles tend to diffuse rather quickly across the epithelium and are mainly removed by the pulmonary or bronchial perfusion. Insoluble particles trapped in the mucus are predominantly removed by mucociliary clearance. Additional clearance mechanism include cough, engulfment by the macrophages, and transepithelial transport (Mortensen 1998). Coughing is an important mucus transport mechanism in the central airways of patients with mucus hypersecretion (Camner et al. 1979). Particles deposited in the alveolar zone may be cleared by surfactant drag to the tracheobronchial tree. Some particles may penetrate the gel layer and reach the periciliary layer and potentially adhere to the ciliated membranes, or they may cross the epithelium by phagocytosis or via paracellular pathways (Mortensen 1998).

Factors affecting aerosol delivery to the lungs

Studies of lung deposition and mucociliary clearance of inhaled particles involve the observation of many interacting mechanisms. Figure 2 summarizes the interrelationships of these processes. The degree of penetration or site of the deposition of aerosol in the airways is dependent on the aerodynamic size of the particle, the pattern of inhalation and airway caliber (Dolovich et al. 1981).

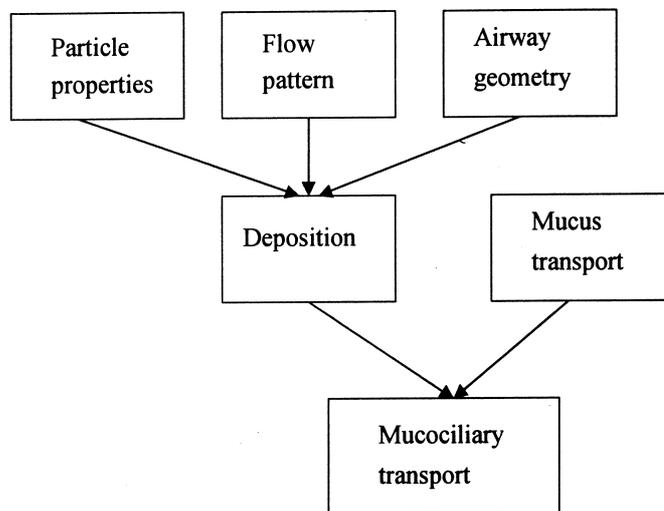


Figure 2. Redrawn and modified from Yeates et al (1981)

Particle size

The goal of the targeted aerosol therapy is to maximize drug delivery and retention while minimizing clearance (Waldrep 1998). For this purpose, aerosol particle size is one of a most important factor in pulmonary delivery. The “ideal” size of the therapeutic aerosol is difficult to specify, partly because it is yet not certain where the aerosol should be deposited within the lung (Howarth 2001), and partly because of the difficulty of predicting the aerodynamic behaviour of therapeutic aerosols (Newman 1985). Aerosol particles $> 5 \mu\text{m}$ are trapped in the

naso/oropharynx, and generally do not enter the respiratory tract (Newman 1985), whereas particles between 1-5 μm are likely reach all peripheral airways parts of the lungs (Rees et al. 1982, Gupta et al. 1991, Waldrep et al. 1994b). Submicron particles tend to have the greatest deposition in the alveoli (Juliano 1984). However, deposition patterns are further complicated by a number of factors including evaporation, hygroscopic growth and particle agglomeration (Newman 1985). The fraction of aerosol containing particles $< 5 \mu\text{m}$ (sometimes defined as $< 6 \mu\text{m}$) in diameter is termed fine particle or respirable fraction (%) of the delivered dose, and has been used to prescribe the quality of the aerosol and its potential for delivery to the lower respiratory tract (Dolovich 2000).

The size distribution of a polydisperse aerosol is best described by the mass median aerodynamic diameter (MMAD) and the geometric standard deviation (GSD), which give information about the average particle size and the scatter sizes, respectively. MMAD is a statistical measure of particle size distribution that characterized the aerosol in terms of its mass (50 % of the mass of aerosol residing in particles less than the MMAD and 50 % in particles greater than the MMAD). A perfectly monodisperse aerosol has a GSD of 1.0, but in practice an aerosol is usually said to be acceptably monodisperse if the GSD is less than 1.22. The therapeutic aerosols are almost invariably polydisperse with the effective aerosol size with an MMAD below 5 μm , and with GSD not exceeding 2.0 (Newman et al. 1985).

Inhalation flow rate

The main intersubject inhalation variables that affect the depth of deposition are the flow rate and the breathing pattern (Bennett et al. 1987). Drug delivery in the lungs may be substantially altered by changes in inhalation mode. With aerosols, slow ($\leq 30 \text{ l/min}$), deep inhalation is likely to deliver an aerosol to peripheral parts of the lungs, and subsequent period of breath-holding enables particles to sediment under gravity in alveolar region. In contrast, rapid inhalation enhances deposition in the oropharynx and in large airways (Agnew et al. 1981, Newman et al. 1982).

Breathing pattern consist of tidal volume and respiratory frequency (Bennett et al. 1987). Tidal volume, if increased, will increase aerosol peripheral penetrance provided the flow rate is constant (Pavia et al. 1977a). A breath holding pause after inhalation of the aerosol favors gravity settlement of a small proportion, which would otherwise remain airborne during expiration (Pavia et al. 1977b).

Airway caliber

There are large intersubject differences in human respiratory tract anatomy in which affect the particle deposition in several ways. First, the diameter and length of the airway determines the displacement required by the particle before it contacts the airway surface. Secondly, the cross section of the airway determines the flow velocity for given volumetric flow rate (Lippmann 1977). Particle velocity varies with both the inspiratory flow rate (IFR) and airflow diameter:

$$V = \text{IFR} / A, \text{ cm/s}$$

where A = airway cross-sectional area, in $\text{cm}^2 = D^2 / 4$ and D = airway diameter, in cm (Dolovich 2000). And finally, the variation in diameter and branching patterns along the bronchial tree affect the mixing characteristics between the tidal volume and the reserve air in the lungs. Airway radius has the greatest effect on impaction and diffusion. Sedimentation and diffusion are dependent on the residence time in the airways, which in turn is directly proportional to length of the airway (Lippmann 1977). The gender differences in regional deposition of inhaled particles are primarily due to differences in airway caliber, which may lead to deposition in the upper airways of women in excess of those in men, despite lower ventilation rates (Prichard et al. 1986).

Factors affecting mucociliary clearance

Lung penetration

Aerosol distribution significantly influences the manner in which mucociliary transport carries out the particle clearance (Yeates et al. 1982, Ilowite et al. 1989). Faster clearance of the central airways is due partly to the fact that particles deposited in the central airways have a shorter pathway to travel when leaving the lung than particles deposited in the peripheral airways. In part, the faster clearance simply reflects that mucus transport is faster in central than in peripheral airways (Sanchis et al. 1972, Yates et al. 1982, Pavia et al. 1983, Mortensen et al. 1994).

Gender, age, lung functions and smoking

The role of gender in mucociliary clearance is still unsettled. In many studies no sex dependency has been observed (Pavia et al. 1970, Yeates et al. 1975, Pavia et al. 1989), while opposite data also exists (Albert et al. 1973, Svartengren et al. 1986, Mortensen 1994). However, there have been speculations, that other confounding factors, such as radioaerosol distribution, could explain the results (Svartengren et al. 1986).

Mucociliary clearance seems to be relatively independent of lung function and age in healthy non-smoking adults (Yates et al. 1975, Yates et al. 1982, Mortensen et al. 1994). However, it is dependent of the tobacco history. Studies have demonstrated slower mucociliary clearance in symptomless smokers than in non-smokers (Lourenco et al. 1971, Goodman et al. 1978, Foster et al. 1985). When the smoking is given up, the retarded mucociliary clearance is gradually improved (Camner et al. 1973), but may be only partial (Camner et al. 1973, Mortensen et al. 1994).

Drugs

Wanner and his associates (1996) in his comprehensive article has collected literature about drug-induced changes in mucociliary clearance and its component functions. Both stimulatory and depressant effects of drugs have a clinical significance: the former in relation to airway therapy, the latter as undesired side-effects of drugs administered for other indications.

Many studies have shown that β_2 -agonist stimulate mucociliary clearance in healthy subjects (Santa Cruz et al. 1974, Camner et al. 1976, Mossberg et al. 1976). The effect seems greater in central than in peripheral airways (Foster et al. 1980, Miyano et al. 1990). In general, β_2 -agonists increase the transport velocity by 50 % in the trachea (Foster et al. 1980). The clearance rate in the main and lobar bronchi may be enhanced even more, e.g. 66 % to 300 %. Reported increases in peripheral zones range from 40 % to 89 % (Foster et al. 1980, Mortensen et al. 1991). The heterogeneity of the effects of β_2 -agonists on different airway generations may be related to the distribution of the drug and/or the β_2 -adrenoceptors within the airways (Mortensen 1998).

The acute effect of β_2 -agonists on mucociliary clearance in patients with various lung diseases is less clear than in healthy individuals (Mossberg et al. 1976a, Mossberg et al. 1976b, Sackner et al. 1979, Bateman et al. 1983a, Pavia et al. 1987, Mortensen et al. 1992). Yet mucociliary clearance appears to be

enhanced with β_2 -agonists in most patients with bronchial asthma (Mortensen et al. 1991) and bronchiectasis (Mortensen et al. 1994). There might be some effect in some patients with cystic fibrosis (Mortensen et al. 1993) and chronic bronchitis (Melloni et al. 1992). From drugs used in obstructive lung diseases, methylxanthines (Sutton et al. 1981) and oral corticosteroids (Agnew et al. 1984) are shown to enhance mucociliary function in patients with airway disease. Cholinergics, e.g. atropine, have a reputation for slowing clearance while synthetic anticholinergic, ipratropium, has no such drawback (Clarke 1989).

Clinically used drug that inhibit clearance in airway diseases are temazepam (Hasani et al. 1992) of the benzodiazepin group, some general anesthetics (Forbes 1976, Forbes et al. 1979) and opiates (Forbes et al. 1977).

Obstructive lung diseases and other respiratory tract diseases

Mucociliary clearance is determined by many factors – the quantity, quality and viscosity of sputum, ciliary function, and epithelial integrity. All of these factors play a roll in the patogenesis of asthma (Djukanovic et al. 1990). Impaired mucociliary clearance in asthma has been detailed in various studies (Foster et al. 1982, Bateman et al. 1983b, Pavia et al. 1985, Messina et al. 1991, O’Riordan et al. 1992). Mucus clearance is slowed during exacerbation of asthma and improved markedly during convalescence (Messina et al. 1991). The noticeable delay in mucus transport in chronic asthma was demonstrated especially in central airways, clearance from the more peripheral airways was reported as being depressed in asthmatic patients (Foster et al. 1982). Tidal flow limitation has reported to correlate with mucociliary dysfunction compared with the patients with milder degrees of airway obstruction (O’Riordan et al. 1992). In addition, mucociliary clearance is demonstrated to be impaired as part of the pathogenesis of other obstructive airway diseases such as chronic bronchitis and COPD (Wanner 1977, Smaldone et al. 1993, Wanner et al. 1996).

Mucociliary clearance is impaired in patients with primary ciliary dyskinesia, as in Young’s syndrome and Kartagener’s syndrome, and in cystic fibrosis. The primary ciliary dyskinesias are a mixed group of inherited autosomal recessive conditions causing impairment of mucociliary transport in the respiratory tract due to the decrease in ciliary beat frequency and uncoordinated ciliary beat. Males are usually infertile because of the sperm immotility. Cardinal features are chronic sinusitis, chronic bronchitis and eventually bronchiectasis, often serous otitis media and, in Kartagener’s syndrome, situs inversus (Van der Baan 1983, de Jongh et al. 1992). Cystic fibrosis leads to hypersecretion of mucus in the lungs and chronic bacterial colonization and infections. Bronchial secretions are

increased in volume and viscosity while mucociliary transport is decreased but not absent; ciliary structure and function appear normal (Clarke 1989).

Lung scintigraphy

Scintigraphy is method currently employed for assessment, diagnosis and monitoring of respiratory diseases. In clinical practice the main use of scintigraphy is for diagnosis or exclusion of pulmonary embolism using both ventilation and perfusion imaging. Ventilation scanning can be used as an independent method for assessment of regional ventilation and small airway function (Miller et al. 1992).

Gamma camera imaging

Gamma cameras have been used in medical diagnosis and research for several decades. Although imaging devices have undergone rapid technical development, the basic design has remained largely unchanged. A modern gamma camera consists of two or three imaging heads in which the detector consists of disc-shaped thallium-activated crystal of sodium iodide. A flat lead collimator, having many thousands of parallel holes is placed between the object being imaged and the crystal. An array of photomultiplier tubes placed behind the crystal records positional (X and Y) signals and enables an image to be built up. A data-processing system records a digital signal image as a matrix consisting of picture elements (pixels), and the data are saved on a computer for subsequent analysis (Newman 1993).

Radiotracers in ventilation scans

Inert gas ^{133}Xe (^{133}Xe) was the first radiotracer used in ventilation scans. Inexpensive, readily available and having a convenient half-life of 5.3 days, it remained for decades the most commonly used agent of ventilation imaging (McCabe et al. 1991). However, ^{133}Xe has several disadvantages including a low principal γ -energy (80 keV) which makes tissue attenuation a greater problem than with high-energy technetium-99m ($^{99\text{m}}\text{Tc}$, 140 keV). β -particle emission increases the radiation dose to the patient and makes the safe discharge of the

exhaled gas essential in minimizing radiation exposure for medical staff. Furthermore, images can only be obtained in one projection (Clarke et al. 1991).

^{99m}Tc-labelled aerosols were first used by Pircher et al. (1965) and Taplin and Poe (1965). Nowadays most ventilation scans with radioaerosols use ^{99m}Tc, which is readily labelled to a variety of compounds, has a suitable half-life of 6 hours and is both easily and cheaply obtainable via a radionuclide generator from the variety of commercial sources (Newman 1993). In deposition studies, it is sometimes possible to incorporate a radiolabelled directly to the drug molecule. Usually this is impracticable and the radiolabelled is incorporated in the formulation associated with the drug but not chemically bound to it (Snell et al. 1998).

Radiolabelling of liposomes

In order to utilize liposomes for diagnostic purposes in nuclear medicine, they are labelled with gamma radiation emitting radionuclides, such as gallium-67 (⁶⁷Ga), indium-111 (¹¹¹In) or, most preferably, technetium-99m.

In general, two different approaches to label preformed liposomes can be distinguished. Firstly, liposomes can be labelled by coupling the radiolabel to the lipid bilayer, either directly to the surface or via chelator. Secondly, the radionuclide can be transported through the lipid bilayer and trapped in the internal aqueous phase of the liposome.

In 1981, Morgan and co-workers were among the first to label liposomes with ^{99m}Tc by reducing pertechnetate with stannous chloride in the presence of the liposomes. This so-called “stannous chloride method” showed in vitro studies relatively high uptake of the radiolabelled in the kidneys and bladder, suggesting in some extent release of the radiolabelled from the liposomes (Love et al. 1989). Alafandy and coworkers (1996) developed the oxido reduction method in which liposomes were prepared with tin(II)dioxinate complexes inserted in the bilayers. However, this method required a series of washing in order to remove the free pertechnetate from the liposomes.

Another approach to label liposomes with radionuclides is to attach chelating agents like diethylenetriaminepentaacetic acid (DTPA) or hydrazinonicotinamide (HYNIC) to the lipid bilayer. The chelator is conjugated to a lipid component of the bilayer, prior to the actual preparation of the liposomes. After hydration of the liposomal lipids the chelator is exposed on the outer surface of the liposomes, thus being easily accessible for the radionuclide. First studies in which DTPA was used to label liposomes with radionuclides showed, however, quite low labelling efficiency (50-60 %) indicating instability of these radiolabelled

formulations (Hnatowich et al. 1981, Goto et al. 1989). Recently, Laverman and coworkers (1999) developed a method to label liposomes with ^{99m}Tc , using HYNIC conjugated to DSPE (distearoylphosphatidyl-ethanolamine). When this conjugate was incorporated in the bilayer, liposomes were labelled with ^{99m}Tc with high efficiency (> 90 %) within 15 min incubation at the room temperature.

Another approach to radiolabelled liposomes, is to encapsulate the radionuclide in the aqueous internal space of preformed liposomes. To achieve the transport of the radionuclide through the lipid bilayer, the radionuclide has to be in a lipophilic form. Hwang et al (1982) showed that ^{67}Ga can be transported through the bilayer in the form of the lipophilic complex ^{67}Ga -oxine and be irreversibly trapped when a hydrophilic chelator is present in the aqueous phase of preformed liposomes. The same procedure can be applied for labelling liposomes with ^{111}In (Gabizon et al. 1988). Phillips and co-workers (1992) developed a method labelling liposomes by using the lipophilic chelator hexamethylpropylene-amine-oxime (HMPAO) for ^{99m}Tc . The ^{99m}Tc -HMPAO complex is able to pass the bilayer of preformed liposomes. When the liposomes contain glutione in the internal phase, the enclosed ^{99m}Tc -HMPAO complex is reduced to its hydrophilic form, and as a result the complex is irreversibly trapped in the interior. The labelling efficiency of this method has proved to be high (approximately 85 %).

Analysis of the lung scans

Attenuation and scatter

Attenuation of gamma photons within the human body prior to reaching the detector and scatter are two major sources of imaging error in planar scintigraphic quantification. Correction to recorded counts are generally applied in order to allow for the effects of gamma-ray attenuations by body tissues, which increases exponentially with the thickness of the media. A variety of solution has been proposed, including calculation of a geometric mean of anterior and posterior counts of lungs (Gonda 1992), use of correction factors derived from tissue attenuation coefficients (Fleming 1979), and a transmission scan using a large flood-field source (Macey et al. 1982).

For ^{99m}Tc -based scintigraphy, scatter can contribute up to 30 % of the photons within the 20 % photopeak window of the acquired image (Buvat et al. 1994). Those scattered photons will decrease the contrast of image, spill the

activity distribution outside the boundary of the target organ, and affect the accuracy of quantitative measurement by either planar scintigraphy or single-photon emission computed tomography (SPET) (Lee et al. 2001). Unfortunately, no universally accepted method for scatter correction has been available for planar scans.

Quantitative definition of lung aerosol penetration and clearance

The planar gamma camera image can readily be sub-divided in zones ('regions of interest') representing relatively central and peripheral parts of the lung. Terms of indices, often a 'penetration index' (PI) or a 'central to peripheral' deposition ratio (C/P) describe the distribution of the radiolabelled aerosol within the lungs (Agnew 1991). Zones taken to represent lung regions have varied in width and in extent to which they have included both apical and basal areas (Sanchis et al. 1972, Short et al. 1979, Greening et al. 1980, Wilkey et al. 1980, Smaldone et al. 1985). The outer lung edges are recommended to define from the image obtained after inhalation of a radioactive gas, such as ^{81m}Kr (krypton-81m) or ^{133}Xe , and used as a reference (Agnew 1991, Newman 1993).

Mucociliary clearance (MCC) has two phases. An initial fast phase, complete in less than 24 hours, represents mucociliary clearance of the tracheobronchial tree (Agnew 1991, Ilowite et al. 1989, Smaldone et al. 1988). The later, slower phase lasts more than 24 hours, and is considered to represent deposition distal to the ciliated airways where MCC does not operate. Although Falk et al. (1997) discovered that a considerable proportion of particles retained at 24 h are actually in the smaller ciliated airways rather than in alveoli, the 24 h retention still gives a good assessment of lung deposition in the bronchioles and distal airspaces.

Radioaerosol images in airway obstruction

Radioaerosol particles of up to 3 or 4 μm aerodynamic diameter inhaled in low to moderate flow rate tend to provide an even distribution of lung radioactivity in normal subjects. In patients with airway obstruction, radioaerosol images are almost always abnormal (Agnew 1984). Aerosol deposition patterns characterized by the excessive amounts of deposition at the sites of airway obstruction are due to turbulence, eddy currents, impingement, and impaction of particles created by the air passing more rapidly in and out of narrowed or irregular bronchial passages (Taplin et al. 1967). Deposition occurs

predominantly in the central airways or may be patchy and irregular with localized zones of hyperdeposition throughout the lung image (Ramanna et al. 1975, Lin et al. 1976, Love et al. 1976, Santolicandro et al. 1979).

One classification scheme for aerosol images in airway obstruction (Taplin et al. 1978) categorizes images as either 'excessive deposition of activity in the major airways' or 'excessive central deposition plus regions of poor aerosol penetrance to the lung periphery'. The first pattern is typically found in subjects with minor abnormalities in lung function tests whereas the latter is associated with moderate or severe impairment of lung functions (Taplin et al. 1978). In patients with airflow obstruction, aerosol deposition patterns depend on the distribution of ventilation and on the sites of obstruction (Agnew 1984). The extra deposition does not necessarily indicate specific sites of obstruction, since central airway aerosol deposition can reflect obstruction in large airways (Chopra et al. 1979) or/and in the peripheral airways (Dolovich et al. 1976).

Several studies have shown that the penetration index correlates well with lung function tests. A significant correlation between FEV₁ and the penetration index have been reported by Greening et al. (1980) and Agnew et al. (1981). A strong correlation between V_{max25} and the penetration index was also seen (Agnew et al 1981). However, data from Garg et al. (1983) suggested that aerosol image scores correlated significantly with 'large airway function' (i.e. FEV₁/FVC) but not with 'small airways function (i.e. MMEF).

The influence of the liposomal formulation on the clearance from the human lungs

In previous studies, the clearance of the aerosolised radiolabelled liposomes suspension proved to be strikingly slow (Farr et al. 1985, Taylor et al. 1989, Barker et al. 1994, Vidgren et al. 1995). Farr and coworkers (1985) conducted a gamma scintigraphy study with nebulized DPPC liposomes. The deposition and clearance of both ^{99m}Tc-labelled large multilamellar DPPC liposomes (average diameter 2.9 µm), and small DPPC liposomes (average diameter 0.07 µm) were similar, being dependent on the site of deposition within the airways, which was determined by the particle size of the aerosol, rather than the size of vesicles. After 6 hour monitoring, mean retention were 88 % and 77 % for MLVs and SUVs, respectively.

In a study of a DPPC/cholesterol formulation of sodium cromoglycate, inhaled from a Hudson jet nebulizer by healthy volunteers, drug was detectable in plasma up to 25 h post-inhalation (Taylor et al. 1989). An equivalent dose of drug inhaled as a solution could not be detected after 8 h, indicating that

liposome entrapment of sodium cromoglycate prolonged drug retention within the lungs and altered its pharmacokinetics.

The pulmonary deposition and clearance of nebulized DPPC/cholesterol liposome formulation containing ^{99m}Tc -DTPA was conducted by Barker with his associates (1994). At 5 h post-inhalation, 59 % of the liposome entrapped radioactivity originally deposited in the lung region still remained compared to 17 % of the free activity at the same time point. Approximately 45 % of originally deposited radioactivity remained in the lungs after 24 hours. Free ^{99m}Tc -DTPA was removed from the airways with the half-life of 75 min (Figure 3).

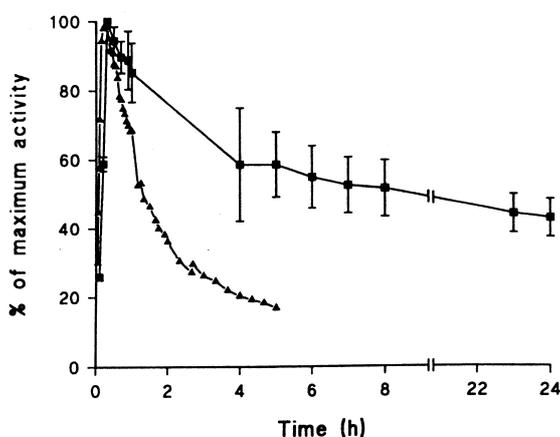


Figure 3. The deposition and clearance of activity following inhalation of ^{99m}Tc -DTPA in solution (▲) and entrapped in DPPC/Chol MLVs (■). (Barker et al. 1994)

Vidgren et al. (1995) conducted trial of Bec-DLPC liposomes in six healthy volunteers using ^{99m}Tc -labelled Bec-DLPC liposome aerosols for visualization of deposition and clearance of the radioactive preparation from the respiratory tract. Inhaled liposomes were cleared slowly; 56-70 % of inhaled radioactivity was still present in the lung after 6 hours, whereas free technetium was cleared from the lungs within minutes.

III Aim of the study

The purpose of the present study was to evaluate the pulmonary deposition and clearance of ^{99m}Tc -labelled beclomethasone liposomes in healthy subjects and in asthmatic patients.

The specific aims of the study were:

1. To compare the influence of liposome composition on the deposition and clearance pattern of inhaled liposomes in healthy volunteers (Study I).
2. To evaluate the pulmonary distribution and clearance pattern of the inhaled Bec-DLPC liposomes in mild and severe asthmatics (Study II).
3. To compare the effect of treatment period of long-acting β_2 -agonist formoterol to the changes in pulmonary distribution and clearance patterns of Bec-DLPC liposomes in asthmatic patients on inhaled steroids (Study III).
4. To monitor the deposition and clearance pattern of the Bec-DLPC liposomes in addition with lung functions during inhaled corticosteroids treatment period in steroid-naïve novel asthmatics (Study IV).

IV Subjects and methods

Subjects studied

Eleven healthy volunteers and thirty-nine subjects with diagnosed asthma participated in these studies (Table 1). Asthma diagnoses were based on clinical evaluation by the pulmonary physician and fulfilled the criteria defined by the American Thoracic Society (1987) with the addition of an increase in FEV₁ > 15 % following a bronchodilation test (after the inhalation of 200 µg of salbutamol). The protocols for the studies were approved by the Ethical Committee of Tampere University Hospital. All the subjects gave their informed consent.

Table 1. *Characteristics of the study population at the baseline*

	Sex (male/ female)	Age (years)	FVC % of predicted	FEV1 % of predicted
Study I				
Healthy subjects (n=11)	3/8	37 (23-50)*	107 (82-121)*	104 (83-117)*
Study II				
Mild asthma (n=10)	5/5	49 (32-60)*	98 (80-121)*	90 (80-101)*
Severe asthma (n=10)	5/5	55 (47-66)*	67 (23-60)*	48 (23-60)*
Study III				
Chronic asthma (n=9)	5/4	48 (25-70)*	90 (56-121)*	73 (56-96)*
Study IV				
Steroid-naive, novel asthma (n=10)	6/4	33 (19-51)*	94 (78-118)*	83 (65-100)*

* Mean (range)

Study population

Study I. Eleven healthy volunteers from the hospital personnel with normal spirometry attended the study.

Study II. Twenty patients with chronic asthma were recruited in the study from the outpatient clinic of the Department of Pulmonary Diseases of Tampere University Hospital. Ten patients had a mild form of asthma and the other ten a severe disease. Baseline FEV₁ measured immediately prior to the study was ≥ 80 % of the predicted in those patients with mild asthma, and 60 % or less in those having a severe form of disease.

Study III. Nine adult subjects with asthma were enrolled in the trial. All patients were asthmatic for at least one year and required regular inhaled corticosteroids treatment. None of the patients were treated with long-acting beta-agonist prior to the study.

Study IV. Ten steroid-naïve patients with novel, mild asthma were included to the trial. All patients had a newly diagnosed asthma with a history of asthma symptoms (cough, wheeze or decreased tolerance to exercise) during the preceding month at least.

Exclusion criteria

None of the subjects had an exacerbation of their asthma or an upper airway infection within 4 weeks prior to the trials. Patients with significant cardiac or metabolic disease were excluded. Other exclusion criteria were: hypersensitivity to sympathomimetics; women pregnant, nursing or of childbearing potential not using contraception; lack of sufficient inspirium; lack of sufficient co-operation; use of tobacco products within the past two months or for more than 10 pack years; remarkable overweight.

Study designs

Study I. The trial was an open, randomised, two-period cross-over study in which each subject inhaled two different beclomethasone-liposome suspensions on separate study days. The time between test days was a minimum of 3 days

and a maximum of two weeks. Gamma scintigraphy was performed immediately after the inhalation.

Study II. The study was designed as an open, parallel group trial in which Bec-DLPC suspension was inhaled by asthmatic patients divided into two study groups based on asthma severity. After inhalation, gamma camera measurements were conducted.

Study III. The study was designed as an open, before-and-after trial. The patients underwent a one-week inhalation treatment of formoterol 12 µg twice daily (Oxis Turbuhaler[®], AstraZeneca) in addition to their own regular anti-inflammatory asthma medication. The gamma camera study and spirometric measurements were conducted before and after formoterol treatment.

Study IV. The study was an open, before-and-after trial in which patients were given 4 months' inhaled steroid treatment of beclomethasone dipropionate (Becotide 250 µg/dose[®], GlaxoSmithKline, U.K) with a daily total dose of 1000 µg. Two doses of corticosteroids aerosol were administered twice daily via a pressurized metered dose inhaler (MDI) with a large-volume spacer device (Volumatic[®], GlaxoSmithKline, U.K). Lung scintigraphy and spirometric measurements were performed and blood tests taken always at the same time of the day at the beginning, after 2 months, and finally after 4 months of inhaled steroid therapy.

The patients were urged to abstain from caffeine-containing beverages for 12 h prior to the gamma camera study. Inhaled long-acting beta-agonists were stopped for at least 24 hours (except Study III), oral controlled-release theophylline preparations 48 hours, and inhaled corticosteroids and short acting bronchodilators for at least 8 hours prior to the study. Oral corticosteroid treatment with one patient was continued throughout the study (Study II, severe asthma group).

Liposome preparation and aerosol characteristics

Multilamellar (MLV) beclomethasone dipropionate (Bec) liposomes were prepared by a freeze-drying technique from phosphatidylcholine (PC) derivatives, dilauroyl phosphatidylcholine (DLPC) and dipalmitoyl phosphatidylcholine (DPPC) as previously described by Waldrep et al. (1994b). Briefly, 1 mg of drug and 25 mg of the phospholipid were dissolved in 10 ml of t-butanol. After mixing, the Bec-phospholipid solution was pipeted into glass vials, rapidly frozen in dry ice-acetone and lyophilised overnight to remove the

organic solvent. In Study II, the Bec-liposomes used were manufactured at Avanti Lipids Corporation, Alabama, USA. In Studies I, III and IV, liposomes were prepared in Finland (Department of Pharmaceutics, University of Kuopio). The acceptance of aerosolized beclomethasone dipropionate liposomes for clinical trials were received from the National Agency for Medicine.

Aerosol characteristics were determined prior to the trials in vitro in the Department of Pharmaceutics, University of Kuopio. In order to analyze the effect of nebulization (Aerotech II CIS-US, Bedford, USA) on liposome size, the liposome aerosol was collected for four minutes in a specially constructed collection chamber (All-glass Impinger, AGI, Ace Glass Co., Vineland, NJ, USA) using an air flow of 12.5 l/min. Liposome size distribution was determined before and after nebulization by using the quasi-elastic light scattering method (Nicomp Submicron Particle Sizer, Model 370, Santa Barbara, USA). Particle size distribution was determined as mean diameter on the basis of vesicle volume. The AGI device was used also to measure the total Bec-aerosol output. The standard sampling period was two minutes after one minute of operation of the nebulizer

Furthermore, the mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD), as well as a fine particle size fraction of the aerosol cloud (< 5.8 microns), were determined by using the Andersen cascade impactor (Andersen Instruments Inc., Atlanta, GA, USA). The Bec-liposome aerosols generated from the jet nebulizer were collected through a metal throat (USP) to the sampler at a sampling time of four minutes. The airflow through the impactor was adjusted to be 28.3 l/min.

The Bec content of the samples was determined by high pressure liquid chromatography (HPLC) analysis using a Promis II autosampler (Spark Holland BV, Emmen, Netherland) and a Supelcosil[®] LC-18-DB column (14 cm x 4.6 mm, 5 µm particle size) (Supelco Inc., Bellefonte, PA, USA) at room temperature. Peak detection for Bec was performed at 254 nm using a variable-wavelength detector (LKB 2151, Bromma, Sweden) with on an integrator (Hitachi D-2500, Hitachi Ltd, Tokyo, Japan). The mobile phase utilized for these studies was methanol/water (80:20) at a flow rate of 1.2 ml per min. Samples for analysis were dissolved into methanol or ethanol.

Preparation and labelling of Bec-DLPC and Bec-DPPC liposomes with ^{99m}Tc

SnCl₂ solution

The preformed Bec liposomes were labelled with ^{99m}Tc in the presence of SnCl_2 as a reducing agent. In the labelling process the same lipid/ SnCl_2 / ^{99m}Tc ratios as described previously by Barratt et al. (1983) were used. In the preparation of the stannous chloride solution, it is important to exclude the possibility of the oxidation of tin to the unreactive stannic form. Therefore, sterile, pyrogen-free water was bubbled for 30 minutes with nitrogen in order to expel most of the oxygen before dissolving stannous chloride (67 mg/100 ml).

Liposome suspension

The MLV-liposomes were reconstructed by adding 1 ml of sterile saline to 13 mg (500 μg beclomethasone dipropionate; 12.4 mg phospholipid) the freeze-dried liposome samples. After mixing, liposome suspension was incubated above the phospholipid phase transition temperature (Bec-DLPC 37°C, Bec-DPPC 50°C). The final drug concentration was 500 $\mu\text{g}/\text{ml}$

Labelling process with ^{99m}Tc

500 μl SnCl_2 was added to liposome suspension and thereafter 1 ml of technetium pertechnetate in sterile saline. The mixture (total volume of 2.5 ml) was shaken vigorously for one minute and left to react at room temperature for 30 minutes. The radioactivity in 1 ml of technetium pertechnetate was approximately 27 mCi.

Liposome suspension delivery

The Tc-labelled Bec-DLPC suspension was delivered from Aerotech II nebulizers connected to an automatic, inhalation-synchronised dosimeter (Spira Elektro 2, Respiratory Care Center, Hämeenlinna, Finland). This dosimeter is triggered by a very low inspiratory flow rate with a threshold of < 2 l/min (Nieminen et al. 1988 and 1987). The volume of each inhalation is displayed digitally, and the inhalation flow rate is controlled by a flow indicator. A breath-actuated, variable-time circuit regulates air through a solenoid valve to a nebulizer, set at a flow rate of 10 l/min. The volume output of the dosimeter with 0.5 sec nebulization periods under these operating conditions is 7 μ l/breath (SD 0.5) (Nieminen et al. 1988 and 1987). In this study, the dosimeter was set to start nebulization in the beginning of the inhalation after the patient had inhaled a volume of 10 ml, with each inhalation lasting approximately 3.0 sec.

A total dose of 500 μ g beclomethasone dipropionate within the labelled liposomes (2.5 ml), having an initial radioactivity of approximately 780 MBq (21 mCi), was placed in the jet nebulizer. Subjects were instructed to place the nebulizer tightly between their lips and inhale deeply. With a noseclip and mouthpiece in place, the subject controlled breathing with a flow indicator (an LED screen) so that the inspiratory flow rate of each breath reached but did not exceed 30 l/min. Inhalation was followed by normal exhalation. Exhaled Bec-liposomes were captured using a Hudson filter. This inspiration procedure was repeated 20 times according to the subject's own inspiratory cycle with no holding of breath between inhalations. Nebulization was practised by each subject with saline before the experiment began.

Determination of the labelling efficiency

The radiochemical purity of liposomes was determined after every labelling by instant thin-layer chromatography (ITCL). In the two-strip mini-ITCL procedure (Robbins 1984), normal saline was used as a solvent and silica gel (ITCL-SG, prod. 61885, Gelman Sciences, Ann Arbor, MI, USA) as an absorbent in order to measure the amount of free ^{99m}Tc . Free pertechnetate migrates to the solvent front, while liposomally entrapped material remains at the application point. The labelling yield was expressed as a percentage of the total amount of the radioactivity in the testing system. ITCL analyses showed a high labelling efficacy throughout the study (96-99 % for DLPC and 97-99 % for the DPPC).

Gamma camera measurements

Lung scintigraphy with ^{99m}Tc labelled liposomes

Immediately after inhalation, anterior and posterior views of the lungs and an anterior view of the oropharynx were measured in a supine position by a large field gamma camera (GE, CamStar XR/T, Wisconsin, USA) equipped with a low-energy high resolution parallel collimator. In order to evaluate the retention of the inhaled liposomes, scans were repeated 1, 2, 4 and 24 h after aerosol delivery. The camera-to-patient distance was standardised by placing the collimator close to the chest for the anterior view and in contact with the imaging bed for the posterior view.

^{133}Xe ventilation scan

A posterior ventilation scan was obtained after the liposome study by inhaling noble gas ^{133}Xe with a radioactive dose of 460 MBq (12.5 mCi). Xenon images were used when defining the outer border of the lungs as well as dividing the lung surface to the central and peripheral lung region in data processing.

Tissue attenuation

An approximate tissue absorption correction was carried out by using the method described originally by Macey and Marshall (1982). Briefly, individual transmission images of each subject's lung region were taken prior to the liposome study using a flat radiation source, keeping the imaging geometry similar both in transmission and ventilation scans. This transmission method was used to correct the individual emission counts recorded with the gamma camera.

V Data processing

All images were stored on a computer (Hermes, Nuclear Diagnostics, Hägersten, Sweden) for subsequent data analysis. ^{133}Xe posterior images were used when regions of interest (ROI) were manually drawn around central and peripheral lung zones. ROIs were subsequently superimposed upon each liposome aerosol view, enabling the quantity of aerosol dose in each of the zones to be determined. Each image was manually aligned i.e. each lung view was shifted to adapt to the superimposed ROIs. The lungs were divided into inner and outer regions, with the central zone encompassing 33 % (± 2 %) and outer the remaining of the total lung area (Smaldone 1989, 1988). Lung distribution of the liposome aerosol was described as the ratio between central and peripheral lung areas (C/P ratio). The total lung retention curve was described as a plot of the percentage of initial lung burden versus time after inhalation.

The number of counts and pixels in each region of interest were measured and saved to a file in the Hermes computer. Subsequently, the data was transferred via a local area network to a personal computer and analysed with a program specially made for this study. Counts from the anterior and posterior views of the lungs were combined by taking geometric mean values. Geometric mean counts were corrected for the room's background - measured separately from each image - and for radioactive decay.

Study II: In order to determine fractional distribution patterns of $^{99\text{m}}\text{Tc}$ Bec-DLPC liposomes after inhalation, radioactivity in the nebulizer reservoir was measured before and after inhalation with a dose calibrator (Capintec; Ramsey, NJ, USA). In addition, the filter collecting exhaled liposomes was measured. Mean activity was calculated to be 19 MBq in the lungs and 25 MBq in the whole body.

Other data acquisition

Questionnaires

Details of study participants' medical history were collected by a clinical questionnaire dealing with respiratory symptoms and use of current asthma medication. Patients' smoking history was examined and total past smoking history was calculated as pack years (one pack year is defined as smoking of 20 cigarettes daily for one year). Questions concerning concurrent diseases and concomitant medication were included.

Lung function tests

Spirometric measurements (Vitalograf, Buckingham, UK) were performed before each inhalation study. The subjects were instructed to inspire to total lung capacity and then exhale with maximal effort through the mouthpiece as rapidly and as far as possible. The nose was closed with a nose-clip. At least three technically correct maneuvers for forced maximal expiratory flow-volume curves were performed, and the curve with the greatest sum of FEV₁ and FVC was utilized. Finnish reference (predicted) values for adults were used in interpretation of the results (Viljanen et al. 1982).

Statistical analyses

In all studies, the analyses of variance for repeated measurements were used to test the differences between distribution and clearance of different Bec liposomes (Study I), groups of asthmatic patients (Study II) and during medical treatment (Study III and Study IV). In Study I, the cross-over design was used and the treatment, period and carryover effect of the treatment were tested. The within-patient changes for lung functions, retention, C/P ratio and for AUC

variables were analysed using the unpaired and paired t-test and were described as mean with 95 % confidence intervals. In Studies III and IV, the corresponding results for C/P ratio and retention were summarized using the area under the curve (AUC) statistics. Spearman's rank correlations were calculated to test the associations between the within-patient changes in the lung functions vs. C/P ratio and retention. Data were expressed as mean \pm the standard deviation (SD) unless stated otherwise.

VI Results

Aerosol characteristics of Bec-liposomes (Study I)

The mean liposome size, was determined using quasi-elastic light scattering method, was 3.49 (1.16) and 5.07 (2.06) μm before nebulization and 0.83 (0.08) and 0.91 (0.26) μm after nebulization for the DLPC and DPPC liposomes, respectively. The cascade impactor analysis showed fine particle fraction for the DLPC liposome to be 93 %, and for the DPPC 96 %. The MMAD (GSD) were 1.3 (2.7) μm for the DLPC and 1.3 (2.2) μm for the DPPC liposomes. There was a considerable difference in drug output rate between the liposome formulations. The output of Bec-DLPC and Bec-DPPC liposomes nebulized via the Aerotech II nebulizer was 68.6 and 18.3 $\mu\text{g}/\text{min}$, respectively. The total rate of output of the DLPC aerosol was 11.4 μg and that of the DPPC liposome 3.1 μg .

Pulmonary distribution and clearance of Bec-DLPC and Bec-DPPC liposomes (Study I)

Immediately after inhalation, no significant differences existed in central/peripheral deposition between DLPC and DPPC formulations. The C/P ratio at 0 h was 0.66 (0.05) and 0.64 (0.07), respectively ($p = 0.21$). The similar homogenous distribution pattern was evident during the entire follow-up period. The central/peripheral ratio of the DLPC liposomes at 24 h was 0.60 (0.06) and of the DPPC formulation 0.59 (0.05).

The progressive clearance pattern after inhalation of radiolabelled Bec liposomes was clearly seen in both formulations. 4 hours after inhalation, a mean of 87 % (4.8) of the total pulmonary dose was detected in the lungs after Bec-DLPC inhalation, whereas after Bec-DPPC inhalation the dose was 89 % (4.8). After 24 h, on average 79 % (6.8) of the DLPC liposomes was detected in the whole lung area, whereas of the DPPC liposomes retention on average was 83 % (8.1). The difference in the retention of these two formulations after 24 h was 4 % (95 % CI: 0.9-7.2 %, $p = 0.001$)

Deposition and clearance of inhaled ^{99m}Tc -labelled Bec-DLPC liposomes in mild and severe asthmatics (Study II)

The asthma patient groups consisted of ten patients with a mild form of asthma and ten patients having a severe form of the disease. The mean values of FEV₁ as percentages of the predicted values were 47.6 (16.0) for patients with severe asthma and 90.1 (6.7) for patients having mild disease.

The percentage of inhaled dose deposited in the lungs in patients with severe asthma (68 %) was similar to that of the mild asthmatics (66 %). The combined values of oropharyngeal and GI-tract deposition were 20 % and 23 %, respectively.

Immediately after liposome inhalation, gamma scintigraphy demonstrated an asymmetric distribution radioactivity with increased number of counts in the central airways of patients with severe asthma. In patients with mild asthma, however, there was a more uniform distribution of counts within the central and peripheral lung fields. C/P ratio in patients with severe asthma was significantly higher than in those having a mild form of the disease: 1.07 (0.29) and 0.76 (0.07) ($p = 0.008$), respectively. Thereafter, only a slight decline in the difference in lung distribution was found during follow-up period which showed a significant inequality between the two groups as 24 h; 0.76 (0.17) vs. 0.67 (0.05) ($p < 0.001$).

A progressive clearance of radiolabelled DLPC liposomes was demonstrated in both asthmatic groups during 24-hour follow-up. However, clearance was more rapid among severe asthmatics ($p < 0.0001$). 4 hours after inhalation, a mean of 82 % (5.9) of the total pulmonary dose was detected in the lungs of the patients with mild asthma, while in severe asthmatics the dose was 69 % (10.9).

Inhaled formoterol therapy and the peripheral lung deposition and clearance in asthmatic patients (Study III)

Study III consisted of the group of asthmatic patients on inhaled steroids. The mean daily dose of inhaled steroid in use was 680 μg beclomethasone or 500 μg

fluticasone. Lung function variables were measured before and after the formoterol treatment. Prior to the study, FVC and FEV₁ values were 90.1 % (SEM 7.5) and 72.7 % (SEM 5.1) of the predicted, respectively. The baseline FEV₁/FVC ratio was 66.7 % (SEM 3.2). All measured lung function values except FEV₁/FVC improved following the medication period, although statistically significant levels were not reached. The mean improvement in FEV₁ was 0.15 l and FVC 0.18 l. FVC, FEV₁ and FEF₅₀ values of the predicted increased approximately 4.0-5.7 %.

One-week usage of inhaled formoterol enhanced peripheral lung deposition of the beclomethasone liposome and thereby diminished the C/P ratio. Measured immediately after inhalation, the C/P ratio was 0.87 (SEM 0.06) before formoterol treatment and 0.77 (SEM 0.04) afterwards. The positive effects of the medication treatment maintained throughout the 24 h follow-up period. The difference in AUC_{(0-24h)/24} was statistically significant (p = 0.011).

In this as in our previous studies, the progressive clearance of inhaled radiolabelled Bec-liposome at 24 h follow-up was shown. However, no statistically significant differences in retention curves before and after formoterol treatment were detected, AUC_{(0-24 h)/24} being 72.0 (SEM 2.1) and 73.6 (SEM 3.6), respectively. At the 4-h measurement, 76 % (SEM 2.5) of the radioactivity still remained in the lungs before and 75 % (SEM 4.2) after the 1-week medication period. After 24 h, a minor difference in retention rates of the corticosteroids liposomes was detected: the mean dose being 63 % before and 68 % after treatment. The difference between clearance rates was 4.8 % (95 % CI: -0.6 to 10.2 %, p = 0.075).

A systematic positive connection between changes in lung functions and changes in retention and C/P ratios measured as AUC_{(0-24h)/24} was seen due to the formoterol treatment. Statistically significant correlations were not, however, reached.

Inhaled corticosteroid treatment and pulmonary lung deposition and clearance in novel asthma (Study IV)

In all ten patients who completed the study, asthma symptoms (wheezing, dyspnea, excess mucus production) were reduced with no need for additional asthma medication. Baseline FVC and FEV₁ values were 94 % (11.4) of predicted and 83 % (11.5) of predicted, respectively. Lung function increased gradually during 4-month treatment period. The mean FVC improved by 0.20 l (p < 0.04, 95 % CI 0.02-0.38). A trend of increased FEV₁ values (0.24 l),

although statistically insignificant ($p = 0.15$), was seen. Baseline FEV₁/FVC ratio (75.4, SD 9.1) was slightly reduced, and remained so in spite of corticosteroids therapy.

Serum ECP levels showed a tendency towards reduction during ICS therapy but the change was insignificant. However, the correlation between changes in reduced ECP levels and improved FVC values proved to be statistically significant ($r = -0.934$, $p = 0.001$)

Immediately after inhalation, the ratio of distribution of the radiolabelled liposomes between central and peripheral parts of the lungs (C/P ratio) was 0.77 (0.10) prior to the anti-inflammatory therapy and 0.78 (0.13) after the 4-month medication period. Pulmonary distribution pattern remained similar during the follow-up period, being 0.69 (0.06) and 0.67 (0.06) at 24 h, respectively. No statistically significant changes in pulmonary deposition pattern were observed in the follow-up period. The AUC_(0-4h) values for C/P ratios were 2.96 (0.38), 2.96 (0.29) and 2.92 (0.37) at baseline, and after 2 and 4 months (time effect $p = 0.93$, ANOVA for repeated measurements).

The progressive clearance pattern ^{99m}Tc-labelled Bec-DLPC was seen at baseline, and also during the 4-month inhaled corticosteroid treatment period. However, no statistically significant changes were detected during 4-month follow-up period. Prior to the corticosteroid treatment, a mean of 84 % (4.9) of the total pulmonary dose was detected in the lungs after inhalation at the 4-h measurement point, whereas after 4-months' treatment the dose was 80 % (9.6). The AUC_(0-4h) values for retention at the baseline, and after 2 and 4 months were -41.4 (14.6), -48.2 (25.6) and -49.4 (25.7) (time effect $p = 0.39$, ANOVA for repeated measurements).

Summary of the lung deposition and clearance of Bec-DLPC liposomes in Studies I-IV

Table 2 and Table 3 summarize the data of mean C/P ratios and clearance curves of healthy subjects and asthmatic patients in Studies I-IV.

Table 2. C/P ratio in healthy subjects and in mild and severe asthmatics

Subjects	C/P ratio	
Healthy	0,66	
Mild, steroid-naive asthma	0,77	Before ICS
	0,78	After ICS
Asthmatics on ICS	0,87	Before formoterol therapy
	0,77	After 1-week formoterol therapy
Severe asthma on ICS	1,07	

Table 3. Clearance of ^{99m}Tc from the lungs of healthy subjects and patients with mild and severe asthma

	Time hours	Healthy	Severity of asthma		Formoterol treatment		ICS	
			Mild	Severe	Before	After	Before	After
% of total cpm	0	100	100	100	100	100	100	100
	1	94	92	85	87	88	92	92
	2	91	88	77	82	83	89	84
	4	87	82	69	76	75	84	80

VII Discussion

Methodological considerations

Attenuation and scatter effect

In planar imaging, attenuation and scatter effects are difficult to correct. In planar gamma scintigraphy, the original 3-D activity distribution is recorded as a 2-D image which results in the loss of distribution information along the depth of the projection. Assumptions about the depth distribution must therefore be made. Two assumptions are commonly used: 1) uniform distribution of activity within the region, and 2) homogeneous attenuation within each region (Lee et al. 2001). Unfortunately, these assumptions are not valid for aerosol inhalation studies with uneven distribution of radioaerosol in the lungs, which could lead to variation among the results of different studies.

In our studies no scatter correction were made since, while to the best of our knowledge, no dedicated scatter correction has been implemented for inhalation gamma scintigraphy. To perform quantification, proper compensation for scatter should be applied (Buvat et al. 1994).

Lung ROI delineation

There are presently no reliable automatic edge detection methods for delineating the contour. Therefore, we delineated the lungs' ROIs manually. They were drawn on ¹³³Xenon images due to the more easily defined lung borders and were very useful where the contour was not easily detected (severe asthma). The same person delineated all the lung scans as well as fixed display conditions i.e.

intensity, contrast and color were used in each study in order to minimize operator-dependent errors. In our data, clear differences between study groups and logical results imply that this manual delineation method can produce reliable results, even in small sample sizes.

The stomach sometimes produces a “hot-spot” in pulmonary planar images and was therefore excluded from the ROI of the left lung contour in PA projection.

Overlapping

The use of two-dimensional (2-D) planar imaging means that there is an overlay of structures of interest (alveoli, small and large airways), which is most marked centrally. Three-dimensional (3-D) imaging techniques (e.g. single photon emission computed tomography, SPET and position emission tomography, PET) provide more detailed and precise information about regional lung distribution (Dolovich 2001), but are more expensive, employ higher radiation doses, and are less validated than 2-D imaging (Snell 1999).

Inhalation and gravitational effect

Different inhalation techniques may influence the deposition of the radiotracer (Lloyd 1994). For that reason the inhalation technique was practiced prior to the experiment. The inhalation volume and flow rate was controlled as well as a slow inspiratory flow rate used to minimize the impaction of the aerosols in the upper parts of the respiratory tract.

The subject’s position has also effect on ventilation images owing to the gravitational influence on alveolar size. In the upright position, the lung bases are compressed by the weight of the lung itself and alveoli are less extended than those at the lung apices, where the more negative intrapleural pressure makes the alveoli larger and less compliant and ventilated. In the supine position, regional ventilation changes so that lowermost alveoli ventilate more efficiently than the upper (Clarke et. al. 1991).

Variability of lung scintigraphic method

Several authors have reported substantial variability between healthy subjects in scintigraphic measurements of lung aerosol deposition (Ilowite et al. 1987, Bennett et al. 1987, Thomas et al. 1991a and b). In series of experiments with ^{99m}Tc -labelled colloidal human serum albumin using jet nebulizer and air compressor Thomas et al. (1991b) showed a great between-subject variability in total pulmonary deposition. Between-subject coefficients of variation was 47 % and 54 %, depending of the tissue attenuation method. Within-subject variability (coefficients of variation) was 37 % between occasions and 23 % within occasion.

Inter-subject variability in mucociliary clearance has reported to be high. The coefficients of variation for particle retention at 120 min varies between 18 % and 45 % (Wilkey et al. 1980, Yeates et al. 1982, Ilowite et al. 1989, Mortensen 1998). Even when standardizing the deposition, the 95 % confidence interval in normal subjects averaged 19.4 % (Ilowite et al. 1989). Because intra-subject variability is measured to be smaller (Wilkey et al 1980, Mortensen 1998), it probably represents true variability among subjects rather than technical problems related to measurement (Ilowite et al. 1989).

Differences in characteristics of nebulized liposomes (Study I)

The pulmonary deposition of the MLV liposome vesicles delivered from the jet nebulizer is dependent on the droplet size of the aerosol product rather than the liposome vesicle diameter before nebulization (Farr et al.1985, Taylor et al. 1989, and Waldrep et al. 1993). In our studies, Aerotech II was chosen for liposome nebulization for having a beneficial aerosol droplet MMAD (1.3 μm) for efficient peripheral lung penetration.

May (1973) demonstrated that with nebulization, liposome vesicles were reduced in size by shear forces associated with the continuous recycling through the nebulizer. Our results confirmed these earlier published data, since a significant decrease in mean vesicle size was found after nebulization of the liposome suspensions which suggesting that liposomes were broken down on passage through the nebulizer. No significant differences in vesicle size between these corticosteroids liposomes was found. The high respirable fraction for both aerosols showed the ability of the nebulizer to filter out large primary aerosol droplets.

The output of the nebulized DPPC liposomes was constantly lower than that of the Bec-DLPC liposomes. This smaller output led in scintigraphic imaging to lower levels of radioactivity measured in the respiratory tract after inhalation. The higher gel-to-liquid phase transition temperature (T_c) above the operating temperature of an air jet nebulizer thus produced more solid and rigid liposomes, which were inefficiently nebulized and hence, needed higher initial radioactivity doses in gamma imaging. Therefore, Bec-DLPC was chosen for the following studies.

In lung imaging, it is sometimes possible to incorporate a radiolabelled directly into the drug molecule; usually this is impractical or even impossible. In most cases the radiolabelled is incorporated in the formulation associated with the drug but not chemically bound to it. In our studies, ^{99m}Tc is attached to the outer surface of the phospholipid. A previous study by Vidgren et al. (1995) showed that the ^{99m}Tc remained associated with Bec-DLPC liposomes during nebulization and in the aerosol particles, with homogenous distribution of the radioactive tag throughout the lipid phase.

In contrast to soluble drugs entrapped within aqueous liposome vesicles, which are subsequently released upon nebulization (Taylor et al. 1990), the beclomethasone-DLPC (GC-PC) bond has demonstrated to remain associated (Waldrep et al. 1994). Earlier Taniguchi et al. (1987) discovered that highly lipophilic GC esters were 99-100 % incorporated into PC liposomes and remained intact after prolonged sonication in vitro. However, the fate of the chemical bond between radiolabelled and the liposome surface as well as between beclomethasone and the phospholipid during long-term follow-up is still somewhat obscure, and a better understanding of the pathways and kinetics of the drug will be needed.

Beclomethasone containing liposomes and pulmonary deposition and retention (Study I)

Although there have been a number of studies investigating the fate of the liposomal drugs in the lungs, most have been conducted in animal models by instilling liquid formulation. The pharmacokinetics of intratracheally instilled formulations in animals may differ considerably from those of inhaled by humans. In addition, since the success of aerosol delivery to the lungs is determined by many interdependent factors that can result in a high variability of nebulized drug delivery, great care must be taken when comparing the lung deposition findings. However, several studies have shown prolonged fate on

phosphatidylcholine entrapped glucocorticoids (Vidgren et al. 1995, Gonzalez-Rothi et al. 1996, O’Riordan et al. 1997, Suarez et al. 1998) which are congruent with our data with two different beclomethasone liposomes. The retention of the both radiolabelled phospholipids in the lungs were strikingly high and the clearance patterns were similar. The slightly accelerated clearance of DLPC liposome might be due to the differences in pharmacokinetical characteristics and phase transition temperatures of the liposomes.

Asthma and inhaled liposomes (Study II)

Centralized deposition

Our findings on the intrapulmonary pattern of distribution of inhaled liposome aerosols in asthmatic patients demonstrate that peripheral deposition of the particles is dependent of the severity of asthma; the more severe asthma, the more centralized deposition. We also found differences in particle distribution between mild asthmatics and healthy subjects; central/peripheral ratios being 0.76 and 0.66, respectively. Even in mild disease, inhaled particles were not able to reach the peripheral parts of the lungs as effectively than in healthy, open airways. Our data conforms and extend those of other studies (Dolovich et al. 1976, Pavia et al 1977a, Pavia et al. 1977b, Chopra et al 1979, Svartengren et al. 1984, Svartengren et al. 1986, Chung et al. 1988). Pavia et al (1977a and 1977b) demonstrated that alveolar particle deposition is a function of airway caliber as measured in FEV₁. Dolovich et al. (1976) found a good relationship between lung aerosol distribution and maximal mid-expiratory flow rate. Chopra et al. (1979) were able to show that aerosol penetrance was a good positive function of both FEV₁ and airway conductance. Similarly, Chung et al (1988) showed that intrapulmonary penetrance is a positive linear function of baseline airway conductance, and conductance changes produced by methacholine, salbutamol and saline. Thus normal subjects, when bronchoconstricted, demonstrated a pattern of aerosol deposition similar to that seen in asthmatic subjects.

This centralized distribution pattern in asthmatics is probably largely due to narrowing of these central airways. Narrowing causes turbulent airflow, particularly at bifurcations, and thus promotes particle deposition. The problem with the 2-D lung scintigraphic method is that central airways can only be visualized by scanning through the overlying peripheral lung. C/P or PI indexes, therefore, underestimate actual penetration where peripheral deposition is normal

or high as in subjects with good airway functions. Thus the method may underestimate the differences seen between normal and asthmatic subjects regarding the peripheral aerosol deposition.

Mucociliary clearance

Many previous studies have shown that mucociliary clearance is impaired in asthmatics compared to normal subjects. Lack of a standardized method in measuring mucociliary clearance of the respiratory tract and consequently variety of methods used in evaluation make an objective comparison of the results of the studies complicated. Earlier studies (Santa Cruz et al. 1974, Mossberg et al. 1976) considered small numbers of patients and mucociliary clearance has been assessed only in central airways due to the observation technique or the large particle size. Foster et al. (1982) found that mucus velocities in central airways were markedly reduced compared with those in normal volunteers. Their asthmatic patients had a mean FEV₁ of 49 % of predicted, consistent with the results of O’Riordan et al. (1992) who demonstrated prolonged retention of particles in central airways patients with FEV₁ of 68 %. Even in mild, stable asthma with normal lung functions, the mucociliary function has shown to be impaired compared to the healthy control subjects (Bateman et al. 1983b, Pavia et al. 1985).

Our study demonstrated that clearance of the ^{99m}-technetium attached to the beclomethasone liposomes were accelerated in both in mild and severe asthmatic groups compared to the healthy subjects. In patients with severe asthma, the clearance rate proved to be somewhat quicker than among those with the mild disease. The fact that the mucociliary clearance was enhanced as a function of asthma severity is probably largely dependent on the method we used in assessing MCC. In studies done by Bateman et al. (1983b) and Pavia et al. (1985) mucociliary clearance were measured in patients with mild, stable asthma compared to healthy subjects. When expressed as a percentage of initial counts as a function of time (whole lung clearance curve), the clearance pattern is very similar to ours in healthy subjects and in mild asthmatics. In these studies, the mean whole lung clearance of radiolabelled polystyrene particles (MMAD 5 µm) was in asthmatic patients in remission faster than in healthy subjects. . When the clearance was expressed as a tracheobronchial clearance curve, where the alveolar deposition (24-hour retention) reading was subtracted from the whole lung retention curve of each subject, the result was quite the opposite. Tracheobronchial clearance of the radiolabelled particles was slower in patients with asthma than in healthy subjects.

Effect of inhaled formoterol on lung functions and aerosol distribution in the lungs (Study III)

Study III consisted of a group of asthmatic patients on inhaled steroids. The group was quite heterogenous in their baseline lung values the mean FEV₁ (% of the predicted) ranging from 56 % to 96 %. The mean daily dose of inhaled steroid in use was 680 µg beclomethasone or 500 µg fluticasone. All patients considered their asthma to be “well controlled” or “completely controlled”. Still, one-week usage of inhaled formoterol improved all measured lung functions. In scintigraphic analyses after medical treatment, enhanced peripheral lung deposition of beclomethasone liposomes were seen and thus diminished the central/peripheral deposition ratio.

The bronchodilating effect of formoterol is well documented in several clinical studies (Palmqvist et al. 1997, Pauwels et al. 1997, Akpinarli et al. 1999, Lötvall et al. 1999) whereas pulmonary deposition studies evaluating the dilatation of the bronchial tree due to β_2 -agonists have, as far as we know, been done only by Isawa and his colleagues (1987). They performed lung scintigraphy and lung function tests to ten patients with bronchial asthma on remission before and after inhalation of salbutamol following intravenously administered aminophylline. Patients were on minimal maintenance medication consisting of oral bronchodilators, mucolytic agents, sodium cromoglycate, and/or xantine derivatives. None of them had inhaled or oral corticosteroid treatment. The results were accordance of our present study. The bronchodilating effect was clearly shown in the lungs by more homogenous and less central deposition of the radioaerosol; lung function parameters were also significantly improved.

Inhaled steroids have been shown to be the most effective anti-inflammatory medication available for long-term control of persistent asthma. Dose/response studies of ICS have demonstrated that in patients with mild to moderate asthma there is a relative flat dose/response curve, with most of the benefit obtained at the lowest doses (Barnes et al. 1998, Holt et al. 2001, Adams et al. 2003a,b, and c). The greatest efficacy of inhaled fluticasone in adult asthma is achieved at a dose of 150-250 µg/day (Holt et al. 2001) while a dose-response study of beclomethasone administered by metered dose inhaler reported that the top of the dose-response curve, in terms of efficacy, was 400-800 µg/day (Busse et al. 1999). The findings of a large dose-response study of budesonide delivered by a Turbuhaler have been very similar in terms of effective daily doses and clinical outcomes (Busse et al. 1998).

These dose-response evidences of inhaled corticosteroids indicate that when asthma is not under control, other classes of drugs given as add-on therapy should be considered instead of increasing daily doses of corticosteroids. Since

both bronchoconstriction and inflammation play a role in the pathogenesis of asthma, addressing both components would provide the most efficacious treatment for patients with persistent asthma. Long-acting β_2 -agonists are currently considered as most effective add-on therapy (Barnes 2002) which suggests that β_2 -agonists must exert some additional action on airways that complements the effect of corticosteroids. A true synergy effect is also suggested, since LABA may prime the inactive glucocorticoid receptors (Baraniuk et al. 1997) as do corticosteroids the β_2 -receptors (Li et al. 1999). We may conclude that an asthmatic patient, although symptomless, would be thus undertreated regardless of a moderate daily dose of inhaled steroids, and therefore would benefit from the bronchodilating effect of formoterol.

The ability of β_2 -agonists including formoterol to accelerate ciliary beat and mucus secretion has been demonstrated in several *in vitro* and *in vivo* studies (Van As 1974, Irvani et al. 1974, Phipps 1979). A similar effect of enhanced mucociliary clearance in healthy non-smokers has been verified (Yates et al. 1976, Foster et al. 1974). However, in asthmatic patients very little influence by the treatment has been seen in previous studies by Isawa et al. (1986 and 1987). In our study, the mucociliary clearance pattern of Bec-DLPC liposome was faster in all asthma patients compared to healthy subjects before and after treatment. However, no influence by the treatment was seen in clearance values; at the 4-h measurement point, retention of the liposome-attached radioactivity was 76 % and 75 %, respectively. Perhaps in asthma, the bronchial epithelial inflammation or the viscosity of the mucus paralyze the ciliary function and thus prevent the stimulative effects of β_2 adrenoceptor agonists.

Inhaled corticosteroids and novel asthma (Study IV)

In the current study, steroid-naïve asthmatic patients with normal or slightly reduced lung functions were treated for 4 months with inhaled corticosteroids. As a sign of the damping of inflammatory process in airways, clinical asthma symptoms vanished as well as serum ECP values declined. There is a growing knowledge that asthma is an inflammatory disorder in which small airways of the lung play an important role (Hamid et al 1997, Howarth 1998). Structurally the small airways are those bronchial passages < 2 mm in diameter. In normal subjects, the small airways provide only 10 % of the total airway resistance. This has led to the small airways being termed the 'silent zone' since airflow obstruction within them causes little change in the conventional tests of pulmonary function (Hogg et al. 1968, Green 1998). A statistically significant improvement in FVC values can be a sign of the gas trapping which frequently accompanies small airway disease, but will also be modified by changes in lung

volume and may thus be misleading. Only a relatively modest trend of increased values was seen in FEV₁, which mainly measures large airway function. FEF_{25-75%} (flow rate in mid and low lung volume) is perhaps a more sensitive measure of the caliber and function of small airways (Lipworth et al. 1997). Unfortunately in our spirometric reports FEF_{25-75%} values were not available.

In small airway obstruction, ventilation scans can provide an alternative method of assessing obstruction, since in asthma, much greater aerosol deposition occurs in central airways relative to peripheral airways, suggesting a defect in small airways ventilation. In our lung scintigraphic studies, no evidence of changes in pulmonary deposition patterns were seen during follow-up period. The central/peripheral deposition ratio after inhalation of radiolabelled Bec liposome was 0.77 before and 0.78 after anti-inflammatory therapy, while in healthy subject the same ratio was 0.66, indicating a still remaining obstruction in asthmatic airways. Only two other studies similar than ours has been published, namely Changlai et al. (1995) and Kao et al (1995) which in fact used the same asthmatic patient material. In these studies, a 1-week course of inhalation therapy of 500 µg beclomethasone propionate four times daily significantly increased the deposition of the radiolabelled aerosols in the intermediate lung parts and decreased in the central and peripheral portions. Unfortunately, neither the severity of asthma nor the inhaler device (particle size) of the inhaled corticosteroids drug was mentioned, which allows only for a speculative comparison of results.

It was reasonable to believe, that 4-months inhaled steroid treatment would affect mucociliary function, as two weeks' oral corticosteroids therapy demonstrated favourable effects on peripheral mucus clearance (Agnew et al 1984). However in our material, statistically significant changes in clearance pattern were not observed. The underlying explanation may be the analysis method used to determine whole lung retention, as discussed earlier in this thesis. Measuring tracheobronchial clearance might be more sensitive tool in determining particle clearance. On the other hand, the lung scintigraphy as an imaging method allows rather large intersubject and intrasubject variability, which diminishes its sensibility.

The efficacy of inhaled corticosteroids for the treatment of asthma is well documented (Barnes 1995) with national and international treatment guidelines unanimous recommending their use as first-line therapy except for very mild asthma. Successful clinical management of asthma depends on achieving adequate delivery of inhaled drugs to the lung, with anti-inflammatory agents such as corticosteroids appearing to be most effective when deposited throughout the airways (Laube 1996). However until recently, even with the optimal inhaler technique, the proportion of the released dose which actually reaches the airways is generally less than 30 % (Newman et al 1989, Melchor et al 1993, Borgström et al. 1994). Metered dose inhaler (MDI) with the Volumatic spacer containing

salbutamol deliver aerosol particles a MMAD 3.5-4.0 μm with the total lung deposition of 19 % in asthmatics (Melchor et al. 1993).

Lung scintigraphy can be a useful tool for evaluating the passage of inhaled steroids into the respiratory tract. While it is well recognized, that particle size of inhaled steroid is critical at the delivery site in airways, the data of the evidence relating corticosteroids particle size to site of the action and efficacy is still lacking (Howarth 2001). The molecular action of corticosteroids occurs at intracellular glucocorticoid receptors, which are widely distributed in cells throughout the airways. (Howarth 1997). The reformulation of asthma medication with non-ozone depleting propellants such hydrofluoroalkaline-134a (HFA) has provided the opportunity to apply new knowledge and inhaler technology to significantly improve the delivery of aerosol drugs to the respiratory tract. Leach et colleagues (1998) compared deposition patterns of the chlorofluorocarbon (CFC) and HFA aerosols of beclomethasone dipropionate with an average particle size of 3.5 μm and 1.1 μm , respectively. In healthy volunteers, 55 %-60 % of the HFA formulation was deposited in the lungs in contrast of CFC formulation which was deposited mostly in the oropharynx; the lung proportion being only 4 %-7 %. Thus our results may indicate that relatively large particle size of inhaled corticosteroids using conventional (CFC)-MDI might leave the small airways partly untreated and thereby obstructed, even in mild asthma. Recent findings with extra-fine hydrofluoroalkaline (HFA)-propelled corticosteroids aerosol suggest that these newer small particle formulations, with better peripheral lung deposition, better reduce asthma exacerbations - even at lower doses of inhaled steroids – than CFC-propelled steroids (Leach 1998, Nelson et al. 2001).

VIII Conclusions

The most important conclusions to be drawn are:

1. Both beclomethasone liposomes (DLPC and DPPC) were suitable for nebulization, although aerosol cloud were more efficiently made from the DLPC liposome suspension. In a gamma scintigraphy no significant differences were demonstrated in the central/peripheral lung deposition between the DLPC and DPPC formulation. Progressive clearance of both Tc-labelled Bec liposomes was seen. 4-hours after inhalation, 87 % of the originally deposited radioactivity of DLPC liposomes and 89 % of the DPPC liposomes remained in the lungs.
2. After inhalation of DLPC liposome, the aerosol particles are deposited more centrally in the lungs of patients with severe asthma than in those with mild form of the disease. In 24-hour follow-up period, clearance in both asthmatic groups proved to be strikingly slow. However, due to the more peripheral penetration of inhaled liposomes in patients with mild asthma, the clearance in this group was slower than in those with severe disease. At the 4-h measurement, 82 % of the initial pulmonary dose was detected in the lungs of mild asthmatics while in those with severe disease the dose was 69 %.
3. A 1-week medical treatment period of long-acting β_2 -agonist formoterol in asthmatic patients on inhaled corticosteroids enhanced the peripheral lung deposition of beclomethasone liposomes and thus diminished central/peripheral deposition ratio. A systemic positive connection was seen between enhanced lung functions and greater lung deposition measured as $AUC_{(0-24h)/24}$. The beclomethasone liposome formulation maintained its long-lasting effect in connection with formoterol treatment. At the 4-h measurement, 76 % of the liposome-attached radioactivity still remained in the lungs before and 75 % after the medication period.

4. A 4-month inhaled corticosteroids treatment period in steroid-naïve novel asthmatics did not change the deposition or clearance pattern of beclomethasone liposomes in lung scintigraphy. However, all lung function were enhanced, although only the improvement of FVC values reached statistical significance as well as the association between changes in improved FVC values and reduced serum ECP levels.

IX Acknowledgements

This study was carried out at the Department of Respiratory Medicine and the Department of Clinical Physiology and Nuclear Medicine in Tampere University Hospital during the years 1996-1999. The study was financially supported by grants from The Ida Montin Foundation, The Tampere Tuberculosis Foundation, The Tampere University Foundation and The Medical Research Fund of Tampere University Hospital.

I express my sincere thanks to:

Professor Markku M. Nieminen, MD, the supervisor of my thesis, for his encouragement and help throughout the work. Without his emphatic and long-suffering attitude this thesis would never have been finished.

Docent Mika Vidgren, PhD, the co-supervisor of my thesis, for offering me the opportunity to conduct deposition studies with liposomes. His experience in lung deposition studies was of great value while the labelling and imaging methods of liposomes were designed. I am particularly grateful of his guidance while planning the study design and protocol.

Professor Aarne Lahdensuo, MD, and Professor Väinö Turjanmaa, MD, the former Heads of the Department of Respiratory Medicine and the Clinical Physiology and Nuclear Medicine, and Docent Seppo Saarelainen, MD, the Head of the Department of Respiratory Medicine, for providing me with ideal working conditions and opportunity to perform my research.

Docent Matti Koskinen, PhD, who ingeniously designed the ^{133}Xe ventilation chamber. His serene way of approaching matters and his excellent skill of explaining the mysteries of isotope to a novice like me left a permanent impression.

Professor Hannu Tukiainen, MD, and Docent Esko Vanninen, MD, as the official reviewers, for their thoroughness, positive criticism and valuable comments on the manuscript.

The expert staff of Clinical Physiology and Isotope laboratories, namely Helena Ahonen, Anne Hietikko, Mirva Roos and Tiina Säilä for their technical

assistance. This project would have been impossible without your voluntary and enthusiastic work.

Tapani Ahola, MSc, for most helpful co-operation in designing the labelling process of liposomes as well as providing all the equipment needed in these processes.

Tuija Poussa, MSc, in the statistical planning and analysis of the data, Mr Greg Kernagan in the English revision of the text and Mrs Marjut Ihantola for performing the spirometrics.

All my colleagues and co-workers at the Department of Respiratory Medicine for their positive attitudes during the years of the studies. My special thanks belong to the staff of the Sleep Laboratory. Working with you ladies has been a pleasure during these years.

“The Witch circle girls”, Maarita, Sari, Anne and Hanski, for our unique friendship for over 30 years. The marathon girls, Marja and Pirjo, for sharing endless sweaty kilometres. With your companion even a marathon feels like enjoyment. TSK squash players for offering hard squash matches and good fellowship. Especially Jaana, dear friend, sharing the precious moments of friendship in the squash court and on the sailing boat, and living beside me the ups and downs of my life for many years. And the one and only Relapsed band members, Jouni, Aarne and Minna; for bringing music into my life and having numerous happy moments playing with you. Mamas and Papas, let’s keep on rocking!

Finally my dearest family, the cornerstone of my life. My father Roope and step-mother Kaarin, my brother Sami, sister-in-law Martiina and Saga for loving and supporting atmosphere, and endless “jaappaus-nights which have been balsam to my wounds”. My sons Juuso and Janne and my little princess Viliina for just being around. You are all at the same adorable and awful, and therefore I love you all.

Tampere, July 2003

Marisanna Saari

X References

- Abu-Dahab R, Schäfer UF and Lehr C-M. (2001): Lechitin-functionalized liposomes for pulmonary drug delivery: effect of nebulization on stability and bioadhesion. *Eur J Pharm Science* 14: 37-46.
- Adams N, Bestall J and Jones P (2003a): Inhaled fluticasone at different doses for chronic asthma. (Cochrane Review). *Cochrane Database Syst Rev* 1: 02479.
- Adams N, Bestall J and Jones P (2003b): Inhaled budesonide (BUD) is available in a range of doses for treating chronic asthma. (Cochrane Review). *Cochrane Database Syst Rev* 1: 02373.
- Adams N, Bestall J and Jones P (2003c): Inhaled beclomethasone at different doses for chronic asthma. (Cochrane Review). *Cochrane Database Syst Rev* 1: 01934.
- Agertoft L and Pedersen S (1997): A randomized double-blind dose reduction study to compare the minimal effective dose of budesonide Turbuhaler and fluticasone propionate Diskhaler. *J Allergy Clin Immunol* 99: 773-780.
- Agnew JE (1984): Aerosol contribution to the investigation of lung structure and ventilatory function. In: *Aerosols and the Lung*, pp. 92-126. Eds. Clarke SW and Pavia D, London, Butterworth.
- Agnew JE (1991): Characterizing lung aerosol penetration. *J Aerosol Med* 4 (3): 237-249.
- Agnew JE, Bateman JRM, Watts M, Paramananda V, Pavia D and Clarke SW (1981): The importance of aerosol penetration for lung mucociliary clearance studies. *Chest* 80 (Suppl): 843-846.
- Agnew JE, Bateman JRM, Pavia D and Clarke SW (1984): Peripheral airways mucus clearance in stable asthma is improved by oral corticosteroids therapy. *Bull Eur Physiopatol Respir* 20: 295-301.
- Agnew JE, Sutton PP, Pavia D and Clarke SW (1986): Radioaerosol assessment of mucociliary clearance: towards definition of normal range. *British J Radiol* 59: 147-151
- Alafandy M, Goffinet G, Umbrain V, D'Haese J, Camu F and Legros FJ (1996): ^{99m}Techetium-stannous oxinate as marker of liposome formulations. *Nucl Med Biol* 23: 881-887.
- Albert RE, Lippmann M, Peterson HT, Berger J, Sanborn K and Bohning D (1973): Bronchial deposition and clearance of aerosols. *Arch Intern Med* 131: 115-127.
- Alving CR (1983): Delivery of liposome-encapsulated drugs to magrophages. *Pharm Ther* 22: 407-424.
- American Thoracic Society (1987): Guidelines to the diagnosis and treatment of asthma. *Am Rev Respir Dis* 136: 224-244.
- Avery RM, Mead J (1959): Surface properties in relation to atelectasis and hyaline membrane disease. *Am J Dis Child* 97: 517-523.
- Bangham AD (1968): Membrane models with phoposholipids. *Progress in Biophysics & Molecular Biology* 18: 29-95.

- Bangham AD (1987): Lung surfactant: How it does and does not work. *Lung* 165: 17-25.
- Bangham AD (1992): Liposomes: Realizing their promise. *Hosp Pract*: 27 (12): 51-62.
- Bangham AD, Standish MM and Watkins JC (1965): Diffusion of univalent ions across the lamellae of swollen phospholipids. *J Mol Biol* 13: 238-252.
- Baraniuk JN, Ali M, Brody D, Maniscalco J, Gaumont E, Fitzgerald T, Wong G, Yuta A, Mak JC, Barnes PJ, Bascom R and Troost T (1997): Glucocorticoids induce β 2-adrenergic receptor function in human nasal mucosa. *Am J Respir Crit Care Med* 155: 704-710.
- Barker SA, Taylor KMG and Short MD (1994): The deposition and clearance of liposome entrapped ^{99m}Tc -DTPA in human respiratory tract. *Int J Pharm* 102: 159-165.
- Barnes PJ (1995): Inhaled glucocorticoids for asthma. *N Engl J Med* 332: 868-875.
- Barnes PJ (1998a): Efficacy of inhaled corticosteroids in asthma. *J Allergy Clin Immunol* 102: 531-538.
- Barnes PJ (1998b): Anti-inflammatory actions of glucocorticoids: molecular mechanisms. *Clin Sci* 94: 557-572.
- Barnes PJ (2002): Scientific rationale for inhaled combination therapy with long-acting β 2-agonist and corticosteroids. *Eur Respir J* 19: 182-191.
- Barnes PJ and Godfrey S (1998): *Asthma therapy*. Martin Dunitz Ltd. London.
- Barnes PJ, Pedersen S and Busse WW (1998): Efficacy and safety of inhaled corticosteroids: an update. *Am J Respir Crit Care Med* 157: S1-S53.
- Bartow RA and Brogden RN (1998): Formoterol. An update of its pharmacological properties and therapeutic efficacy in the management of asthma. *Drugs* 55: 303-322.
- Bateman JRM, Pavia D, Sheahan NF, Newman SP and Clarke SW (1983a): Effects of terbutaline sulphate aerosol on bronchodilators response and lung mucociliary clearance in patients with mild stable asthma. *Br J Clin Pharmacol* 15: 695-700.
- Bateman JRM, Pavia D, Sheahan NF, Agnew JE and Clarke SW (1983b): Impaired tracheobronchial clearance in patients with mild asthma. *Thorax* 38: 463-467.
- Bendas G. (2001): Immunoliposomes. A promising approach to targeting cancer therapy. *Bio Drugs* 15 (4): 215-224.
- Bennett WD and Smaldone GC (1987): Human variation in the peripheral air-space deposition of inhaled particles. *J Appl Physiol* 62: 1603-1610.
- Boerman OC, Oyen WJG, Corstens FHM and Storm G (1998): Liposomes for scintigraphic imaging: optimisation of in vivo behavior. *Q J Nucl Med* 42: 271-279.
- Boerman OC, Dams ETM, Oyen WJG, Corstens FHM and Storm G. (2001): Radiopharmaceuticals for scintigraphic imaging of infection and inflammation. *Inflamm. Res.* 50: 55-64.
- Bonte F and Juliano RL (1986): Interaction of liposomes with serum proteins. *Chemistry and Biophysics of Lipids* 40: 359-372.
- Borgström OL, Bondesson E, Moren F, Trofast E and Newman SP (1994): Lung deposition of budesonide inhaled via Turbuhaler: a comparison with terbutaline sulphate in normal subjects. *Eur Respir J* 7: 69-73.
- Brain JD and Valberg PA (1979): Deposition of aerosol in the respiratory tract. *Am Rev Respir Dis* 120: 1325-1373.
- Brain JD, Valberg P and Sneddon S. (1985): Mechanism of aerosol deposition and clearance. In: *Aerosols in Medicine: Principles, Diagnosis and Therapy*, pp 123-147. Eds. Moren F, Newhouse MT and Dolovich MB, Elsevier, Amsterdam.
- British Thoracic Society (1997): The British guidelines on asthma management. *Thorax* 52 (suppl 1): S1-21.

- Brogden RN and Faulds D (1991): Salmeterol xinafoate. A review of its pharmacological properties and therapeutic potential in reversible obstructive airway disease. *Drugs* 42: 895-912.
- Busse WW, Chervinsky P, Condemni J, Lumry WR, Petty TL, Rennard S and Townley RG (1998): Budesonide delivered by Turbuhaler is effective in a dose-dependent fashion when used in the treatment of adult patients with chronic asthma. *J Allergy Clin Immunol* 101: 457-463.
- Busse WW, Brazinsky S, Jacobson K, Stricker W, Schmitt K, Vanden Burgt J, Donnell D, Hannon S and Colice GL (1999): Efficacy response of inhaled beclomethasone dipropionate in asthma to dose and is improved by formulation with a new propellant. *J Allergy Clin Immunol* 104: 1215-1222.
- Buvat I, Benali H, Todd-Pokropek A and Di Paola R (1994): Scatter correction in scintigraphy: the state of the art. *Eur J Nucl Med* 21: 675-694.
- Camner P, Philipson K and Arvidsson T (1973): Withdrawal of cigarette smoking. A study on tracheobronchial clearance. *Arch Environ Health* 26: 90-92.
- Camner P, Mossberg B, Philipson K and Strandberg K. (1979): Elimination of test particles from the human respiratory tracheobronchial tract by voluntary coughing. *Scand J Respir Dis* 60: 56-62.
- Changlai S-P, Kao C-H, Wang S-J, Lin W-Y and Lan J-L (1995): The change in the distribution of Tc-99m human serum albumin radioaerosol in asthma after a 1-week course of corticosteroids inhalation treatment. *Clin Nucl Med* 7: 626-629.
- Cho MJ, Scieczka JF, Cramer CT, Thompson DP and Vidmar TJ (1989): Neutrophil-mediated transport of liposomes across Madin Darby canine kidney epithelial cell monolayer. *Pharm Res* 6: 78-84.
- Chopra SK, Taplin GV, Tashkin DP, Trevor E and Elam D (1979): Imaging sites of airway obstruction and measuring functional responses to bronchodilator treatment in asthma. *Thorax* 34: 493-500.
- Chung KF and Barnes PJ (1999): Cytokines in asthma. *Thorax* 54: 825-857.
- Clarke SEM and Secker-Walker RH (1991): Lung scanning. In: *Clinical Nuclear Medicine*, pp. 47-74. Eds. Maisey M, Britton KE and Gilday DL, London. Chapman og Hall Medical.
- Clarke SW (1989): Rationale of airway clearance. *Eur Respir J* 2 (Suppl 7): 599-604.
- Clarke SW and Yates DB (1994): Deposition and clearance. In: *Textbook of respiratory medicine: 2nd edition*, pp. 345-369. Eds. Murray JF and Nadel JA. WB Saunders Company, Philadelphia, Pennsylvania.
- Clements JA (1957): Surface tension of lung extracts. *Proc Soc Exp Biol Med* 95: 170-172.
- Cullis PR, Mayer LD, Bally MB, Madden TD and Hope MJ (1989): Generating and loading of liposomal systems for drug-delivery applications. *Advanced Drug Delivery Reviews* 3: 267-282.
- Dahl R and Bjermer L (2000): Nordic consensus report on asthma management. *Respir Med* 94: 299-327.
- De Jongh R, Ing A and Ruthland J (1992): Mucociliary function, ciliary ultrastructure, and ciliary orientation in Young's syndrome. *Thorax* 47: 184-187.
- Derom EY and Pauwels RA (1992): Time course of bronchodilating effect of inhaled formoterol, a potent and long-acting sympatomimetic. *Thorax* 47: 30-33.
- Djukanovic R, Roche WR, Wilson JW, Beasley CR, Twentyman OP, Howarth RH and Holgate ST (1990): Mucosal inflammation in asthma. *Am Rev Respir Dis* 142: 434-457.

- Djukanovic R, Wilson JW, Britten KM, Wilson SJ, Walls AF, Roche WR, Howarth PH and Holgate ST (1992): Effect of an inhaled corticosteroids on airway inflammation and symptoms in asthma. *Am Rev Respir Dis* 145: 669-674.
- Dolovich MB, Sanchis J, Rossman C and Newhouse MT (1976): Aerosol penetrance: a sensitive index of peripheral airways obstruction. *J Appl Physiol* 40: 468-471.
- Dolovich M, Eng P, Ryan G and Newhouse MT (1981): Aerosol penetration into the lung. *Chest* 80 (6): 834-836.
- Dolovich MA (2000): Influence of inspiratory flow rate, particle size, and airway calibre on aerosolised drug delivery to the lung. *Respir Care* 45 (6): 597-608.
- Dompeling E, Van Schayck CP, Molema J, Folgering H, van Grusven Pm and van Weel C (1992): Inhaled beclomethasone improves the course of asthma and COPD. *Eur Respir J* 5: 945-952.
- Dupond B (2002): Overview of the lipid formulations of amphotericin B. *JAC* 49 (Suppl S1): 31-36.
- Dvorak J, Zoul Z, Melichar B, Jandik P, Mergancova J, Motyckova I, Kalousova D and Petera J (2002): Pegylated liposomal doxorubicin in combination with hyperthermia in the treatment of a case of advanced hepatocellular carcinoma. *J Clin Gastroenterol* 34 (1): 96-98.
- Farr SJ, Kellaway IW, Parry-Jones DR and Woolfrey SG (1985): 99m-Tcnetium as a marker of liposomal deposition and clearance in the human lung. *Int J Pharm* 26: 303-316.
- Fielding RM (1991): Liposomal drug delivery. Advantages and limitations from a clinical pharmacokinetic and therapeutic perspective. *Clin Pharmacokinet* 21 (3): 155-164.
- Fleming JS (1979): A technique for the absolute measurement of activity using a gamma camera and computer. *Phys Med Biol* 24 (1): 176-180.
- Forbes AR (1976): Halothane depresses mucociliary flow in the trachea. *Anesthesiology* 45: 59-63.
- Forbes AR and Horrigan RW (1977): Mucociliary flow in the trachea during anesthesia with enflurane, ether, nitrous oxide, and morphine. *Anesthesiology* 46: 319-321.
- Forbes AR and Gamsu G (1979): Depression of lung mucociliary clearance by thiopental and halothane. *Anesth Analg* 58: 387-389.
- Foster WM, Langenback E, Bergofsky EH (1980): Measurement of tracheal and mucus velocities in man: relation to lung clearance. *J Appl Physiol* 48: 965-971.
- Foster WM, Langenbach EG and Bergofsky EH (1982): Lung mucociliary clearance in man: interdependence of bronchial and tracheal mucus transport velocities with lung clearance in bronchial asthma and healthy subjects. *Ann Occup Hyg* 26: 227-244.
- Foster WM, Langenbach EG and Bergofsky EH. (1985): Disassociation in the mucociliary function of central and peripheral airways of asymptomatic smokers. *Am Rev Respir Dis* 132: 633-639.
- Gabizon A, Huberty J, Straubinger RM, Price DC and Papahadjopoulos D (1988): An improved method for in vivo tracing and imaging of liposomes using a gallium-67-deferoxamine complex. *J Liposome Res* 1: 123-135.
- Gabizon A, Sulkes A, Peretz T, Druckmann S, Goren D, Amselem S and Barenholz Y (1989): Liposome-associated doxorubicin; preclinical pharmacology and exploratory clinical phase. In: *Liposomes in the therapy of infectious diseases and cancer*, pp. 391-402. Eds. Lopez-Berenstein and Fidler, Alain R. Liss Inc., New York.
- Garg A, Gopinath PG, Pande JN and Guleria JS (1983): Role of radio-aerosol and perfusion lung imaging in early detection of chronic obstructive lung disease. *Eur J Nucl Med* 8: 167-171

- Gill PS, Espina BM, Muggia F, Cabriaes S, Tulpule A, Esplin JA, Liebman HA, Forssen E, Ross ME and Levine AM (1995): Phase I/II clinical and pharmacokinetic evaluation of liposomal daunorubicin. *J Clin Oncol* 13: 996-1003.
- GINA, Global Initiative for Asthma. (2002): Pocket Guide for asthma management and prevention. Washington: National Heart, Lung and Blood Institute. NIH-Publication, N:o 02-3659.
- Goins BA and Phillips WT (2001): The use of scintigraphic imaging as a tool in the development of liposome formulations. *Progress in Lipid Research* 40: 95-123.
- Gonda I (1992): Absolute and relative quantitation measurement of deposition and clearance by scintigraphy. *J Biopharm Sci* 191-198.
- Gonzalez-Roth RJ, Suarez S, Hochhaus G, Schreier H, Lukyanov A, Derendorf H and Dalla Costa T (1996): Pulmonary targeting of liposomal triamcilonone acetone phosphate. *Pharm Res* 13: 1699-1703.
- Goodman RM, Yergin BM, Landa JF, Golinvax MH and Sackner MA (1978): Relationship of smoking history and pulmonary function tests to tracheal mucous velocity in nonsmokers, young smokers, ex-smokers, and patient with chronic bronchitis. *Am Rev Respir Dis* 117: 205-214.
- Goto R, Kubo H and Okada S (1989): Liposomes prepared from synthetic amphiphiles. I. Their technetium labelling and stability. *Chem Pharm Bull Tokyo* 37: 1351-1354.
- Green M (1998): Treating large and small airways. *Int J Clin Pract* 96 (Suppl): 2-4.
- Greening AP, Miniati M and Fazio F (1980): Regional deposition of aerosols in health and in airways obstruction: a comparison with krypton-81m ventilation scanning. *Bull Eur Psychopathol Respir* 16: 287-98.
- Greening AP, Ind PW, Northfield M and Shaw G (1994): Added salmeterol versus higher-dose corticosteroids in asthma patients with symptoms on existing inhaled corticosteroids. *Lancet* 344: 219-224.
- Gregoriadis G, Florence AT (1993): Liposomes in drug delivery. Clinical, diagnostic and ophthalmic potential. *Drugs* 45 (1): 15-28.
- Gupta PK and Hickey AJ (1991): Contemporary approaches in aerosolised drug delivery to the lung. *J Control. Rel* 17: 127-147.
- Hahtela T, Järvinen M, Kava T, Kiviranta K, Koskinen S, Lehtonen K, Nikander K, Persson T, Reinikainen K, Selroos O, Sovijärvi A, Stenius-Aarniala B, Svahn T, Tammivaara R and Laitinen LA (1991): Comparison of beta-2-agonist, terbutaline, with an inhaled corticosteroid, budesonide, in newly detected asthma. *N Engl Med* 325: 388-392.
- Hahtela T, Järvinen M, Kava T, Kiviranta K, Koskinen S, Lehtonen K, Nikander K, Persson T, Selroos O, Sovijärvi A, Stenius-Aarniala B, Svahn T, Tammivaara R and Laitinen LA (1994): Effects of reducing or discontinuing inhaled budesonide in patients with mild asthma. *N Engl J Med* 331: 700-705.
- Hamid Q, Song Y, Kotsimbos TC, Minshall E, Bai TR, Hegele RG and Hogg JC (1997): Respiratory pathophysiology responses. Inflammation of small airways in asthma. *J Allergy Clin Immunol* 100: 44-51.
- Hasani A, Spiteri MA, Pavia D, Lopez-Vidriero MT, Agnew JE and Clarke SW (1992): Effect of temazepam on tracheobronchial mucus clearance. *Thorax* 47: 298-300.
- Herrala J, Puolijoki H, Impivaara O, Liippo K, Tala E and Nieminen MM (1994): Bone mineral density in asthmatic women on high-dose inhaled beclomethasone dipropionate. *Bone* 15: 621-623.
- Heyder J. (1981): Mechanism of aerosol particle deposition. *Chest* 80 (Suppl.): 820-823.
- Hnatowich DJ, Friedman B, Clancy B and Novak M (1981): Labelling of preformed liposomes with Ga-67 and Tc-99m by chelation. *J Nucl Med* 22: 810-814.

- Holt S, Suder A, Weatherall M, Cheng S, Shirtcliffe P and Beasley R (2001): Dose-response relation of inhaled fluticasone propionate in adolescents and adults with asthma: meta-analysis. *BMJ* 323: 253-256.
- Hogg JC, Macklem PT and Thurlbeck WM (1968): Site and nature of airway obstruction in chronic obstructive lung disease. *N Engl J Med* 278: 1355-1360.
- Hope MJ, Bally MB, Mayer LD, Janoff AS and Cullis PR (1986): Generation of multilamellar and unilamellar phospholipid vesicles. In: *Chemistry and Physics of Lipids. Special Issue: Liposomes*, pp.89-107. Eds. Paltauf F and Schmid HHO, Elsevier.
- Howart PH (1998): Small airways and asthma – an important therapeutic target? *Am J Respir Crit Care Med*: 157 (Suppl): S188-190.
- Howarth PH (2001): Why particle size should affect clinical response to inhaled therapy. *J Aerosol Med*: S27-34.
- Hwang KJ, Merriam SE, Beaumier PL and Luk KE (1982): Encapsulation, with high efficiency, of radioactive metal ions in liposomes. *Biochim Biophys Acta* 716: 101-109.
- Ilowite JS, Gorwoy JD and Smaldone GC (1987): Quantitative deposition of aerosolised gentamicin in cystic fibrosis. *Am Rev Respir Dis* 136: 1445-1449.
- Ilowite JS, Smaldone GC, Bennett WD and Foster WM. (1989): Relationship between tracheobronchial particle clearance rates and sites of initial deposition in man. *Arch Environ Health* 44: 267-273.
- ISAAC (1998): The International Study of Asthma and Allergies in Childhood Steering committee. Worldwide variations in prevalence of symptoms of asthma, allergic rhinoconjunctivitis and atopic eczema. *Lancet* 351: 1225-1232.
- Ivey H, Roth S and Kattwinkel J (1976): Nebulization of sonicated phospholipids for respiratory distress syndrome of infancy. *Pediatric Research* 10: 462.
- Jobe A and Ikegami M. (1987): Surfactant for treatment of respiratory distress syndrome. *Am Rev Respir Dis* 136: 1256-1275.
- Johnson M (1995a): Pharmacology of long-acting β -agonists. *Ann Allergy Asthma Immunol* 75: 177-179.
- Johnson M (1995b): Salmeterol. *Med Resp Rev* 15: 225-257.
- Johnson PC, Wheat LJ, Cloud GA, Goldman M, Lancaster D, Bamberger DM, Powderly WG, Hafner R, Kauffman CA and Dismukes W (2002): Safety and efficacy of liposomal amphotericin B compared with conventional amphotericin B for induction therapy of histoplasmosis in patients with AIDS. *Ann Intern Med* 137: 105-109.
- Jones JG (1984): Clearance of inhaled particles from the alveoli. In: *Aerosols and the Lung*, pp 170-197. Eds. Clarke SW and Pavia D, Clinical and Experimental Aspects. Butterworths, London,
- Juliano RL (1984): Controlled delivery of the drugs to the respiratory system. *Pharmac Ther* 24: 355-365.
- Juniper EF, Kline PA, Vanzieleghem MA, Ramsdale EH, O'Byrne PM and Hargreave FE (1990): Effect of long-term treatment with inhaled corticosteroids (budesonide) on airway hyperresponsiveness and clinical asthma in non-steroid-dependent asthmatics. *Am Rev Respir Dis* 142: 832-836.
- Kao C-H, Lin H-T, Yu S-L, Wang S-J and Lan J-L (1995): Effect of corticosteroids inhalation therapy on the deposition pattern of Tc-99m human serum albumin radioaerosol in asthma. *Lung* 173: 291-296.
- Kaneda Y (2001): Improvements in gene therapy technologies. *Molecular Urology* 5 (2): 85-89.

- Kellaway IW and Farr SJ (1990): Liposomes as drug delivery systems to the lung. *Advanced Drug Delivery Reviews* 5: 149-161.
- Kestens S, Chapman KR, Broder I, Cartier A, Hyland RH and Knight A (1991): A three-month comparison of twice daily inhaled formoterol versus four times daily inhaled albuterol in the management of stable asthma. *Am Rev Respir Dis* 144: 622-625.
- Kraft M (1999): The distal airways: are they important in asthma. *Eur Respir J* 14: 1403-1417.
- Kwan BA, daSilva J, Vural I, Narula J and Torchilin VP (2001): Intracytoplasmic gene delivery for in vitro transfection with cytoskeletal-specific immunoliposomes. *J Controlled Release* 75: 199-210.
- Laube B (1996): In vivo measurements of aerosol dose and distribution: clinical relevance. *J Aerosol Med* 9: S77-S91.
- Laverman P, Dams ETM, Oyen WJG, Storm G, Koenders E, Prevost R, van der Meer JW, Corstens FH and Boerman OC (1999): A novel method to label liposomes with Tc-99m via the hydrazine nicotinylic derivatives. *J Nucl Med* 40: 192-197.
- Leach CL (1998): Improved delivery of inhaled steroids to the large and small airways. *Respir Med* 92 (Suppl): 3-8.
- Leach CL, Davidson PJ and Boudreau RJ (1998): Improved airway targeting with the CFC-free HFA-beclomethasone metered-dose inhaler compared with CFC-beclomethasone. *Eur Respir J* 12: 1346-1353.
- Li X, Ward C, Thien A, Bish R, Bamford T, Bao X, Bailey M, Wilson JW and Haydn Walters E (1999): An anti-inflammatory effect of salmeterol, a long-acting β_2 -agonist, assessed in airway biopsies and bronchoalveolar lavage in asthma. *Am J Respir Crit Care Med* 160: 1493-1499.
- Lin MS and Goodwin DA (1976): Pulmonary distribution of inhaled radioaerosol in obstructive pulmonary disease. *Radiology* 118: 645-651.
- Lindberg S, Khan R and Runer T (1995): The effect of formoterol, a long-acting β_2 -adrenoceptor agonist, on mucociliary activity. *Eur J Pharm* 285: 275-280.
- Linden A, Rabe KF and Löfdahl C-G (1996): Pharmacological basis for duration of effect: formoterol and salmeterol versus short-acting β_2 -adrenoceptor agonists. *Lung* 174: 1-22.
- Lippmann M. (1977): Regional deposition of particles in the human respiratory tract. In: *Handbook of physiology: reaction to environmental agents*, pp. 213-232. Eds. Lee DHK, Falk HL, Murphy SD and Bethesda MD. The American Physiology Society.
- Lipworth BJ (1999): Systemic adverse effects of inhaled corticosteroids therapy. *Arch Intern Med* 159: 941-955.
- Lipworth BJ and Clark DJ (1997): Effects of airway calibre on lung delivery of nebulized salbutamol. *Thorax* 52: 1036-1039.
- Lokling K-E, Fosshem SL, Skurtveit R, Bjornerud A and Klaveness J. (2001): PH-sensitive paramagnetic liposomes as MRI contrast agents: in vitro feasibility studies. *Magnetic Resonance Imaging* 19: 731-738.
- Lourenco RV, Klimek MF and Borowski CJ (1971): Deposition and clearance of 2 μ m particles in the tracheobronchial tree of normal subjects – smokers and nonsmokers. *J Clin Invest* 50: 1411-1420.
- Love RG and Muir DCF (1976): Aerosol deposition and airway obstruction. *Am Rev Respir Dis* 114: 891-897.
- Love WG, Amos N, Williams BD and Kellaway IW (1989): Effect of liposome surface charge on the stability of technetium (^{99m}Tc) radiolabelled liposomes. *J Microencapsul* 6: 105-113.

- May KR (1973): The collision nebulizer: description, performance and application. *Aerosol Science* 4: 235-243.
- McCabe JL, Grossmann SJ and Joyce JM (1991): Ventilation-perfusion scintigraphy. *Emerg Med Clin North America* 4: 805-25.
- Melchor R, Biddiscombe MF, Mak VHF, Short MD and Spiro SG (1993): Lung deposition pattern of directly labelled salbutamol in normal subjects and in patients with reversible airflow obstruction. *Thorax* 48: 506-511.
- Melloni B and Germouty J (1992): Influence sur la fonction muco-ciliaire d'un nouveau beta-agoniste: formoterol. *Rev Mal Resp* 9: 503-507.
- Messina MS, O'Riordan TC and Smaldone GC (1991): Changes in mucociliary clearance during acute exacerbation of asthma. *Am Rev Respir Dis* 143: 993-997.
- Mihalko PJ, Schreier H and Abra RM (1988): Liposomes: A pulmonary perspective. In: *Liposomes as a drug carriers*, pp. 679-94. Ed. Gregoriadis G. John Wiley & Sons, New York.
- Miller RF and O'Doherty MJ (1992): Pulmonary nuclear medicine. *Eur J Nucl Med* 19: 355-368.
- Morgan JR, Williams KE, Davies RL, Leach K, Thomson M and Williams LAP (1981): Localisation of experimental staphylococcal abscesses by ^{99m}Tc-technetium-labelled liposomes. *J Med Microbiol* 14: 213-217.
- Morrow PE (1973): Alveolar clearance of aerosols. *Arch Intern Med* 131: 101-108.
- Mortensen J (1998): Bronchoscintigraphy and pulmonary clearance of ^{99m}Tc-albumin colloid in the study of mucociliary clearance. *Danish Medical Bulletin* 45 (2): 195-209.
- Mortensen J, Groth S, Lange P and Rossing N (1989): Bronchoscintigraphic visualization of the acute effect of tobacco exposure and terbutaline on mucociliary clearance in smokers. *Eur Respir J* 2: 721-726.
- Mortensen J, Groth S, Lange P and Hermansen F (1991): Effect of terbutaline on mucociliary clearance in asthmatics and healthy subjects after inhalation from pressured inhaler and a dry powder inhaler. *Thorax* 46: 817-823.
- Mortensen J, Engel AM Milman N, Nissen F and Faurshou P (1992): Inhaled terbutaline improves mucociliary clearance in patients with bronchiectasis. *Eur Respir J* 5 (suppl 15): 385.
- Mortensen J, Lange P, Nyboe J and Groth S (1994): Lung mucociliary clearance. *Eur J Nucl Med* 21: 953-961.
- Mossberg B, Strandberg K, Philipson K and Camner P (1976a): Tracheobronchial clearance in bronchial asthma: response to beta-adrenoceptor stimulation. *Scand J Resp Dis* 57: 119-128
- Mossberg B, Strandberg K, Philipson K and Camner P (1976b): Tracheobronchial clearance and beta-adrenoceptor stimulation in patients with chronic bronchitis. *Scand J Resp Dis* 57: 281-289.
- Mufson D, Guo LSS and Fielding RM (1990): Amphotericin B-cholesteryl sulfate complex (ABCD): stability and tissue distribution of a novel dosage form. *Proceedings of the International Symposium on the Controlled Release of Bioactive Materials* 17: 81-82.
- Nelson HS (1995): Beta-adrenergic bronchodilators. *N Engl J Med* 333: 499-506.
- Nelson H and Newman K (2001): Prevention of asthma exacerbations by HFA flunisolide in adults and adolescence with mild-to-moderate asthma. *J Allergy Clin Immunol* 107: abstract 350.
- Newman SP (1993): Scintigraphic assessment of therapeutic aerosols. *Critical Reviews in Therapeutic Drug Carrier Systems* 10 (1): 65-109.

- Newman SP and Pavia D (1985): Aerosol deposition in man. In: *Aerosols in Medicine: Principles, Diagnosis and Therapy*, pp. 193-217. Eds. Moren F, Newhouse MT and Dolovich MB, Elsevier Amsterdam.
- Newman SP, Pavia D, Garland N and Clarke SW (1982): Effects of various inhalation modes on the deposition of radioactive pressurized aerosols. *Eur J Respir Dis* 63 (Suppl 119): 57-65.
- Newman SP, Clark AR, Talae N and Clarke SW (1989): Pressured aerosol deposition in the human lung with and without an "open" spacer device. *Thorax* 44: 706-710.
- Nieminen MM, Holli H, Lahdensuo A, Muittari A and Karvonen J (1987): Aerosol deposition in automatic dosimeter nebulization. *Eur Respir Dis* 71: 145-152.
- Nieminen MM, Lahdensuo A, Kellomäki L, Karvonen J and Muittari A (1988): Methacholine bronchial challenge using dosimeter with controlled tidal breathing. *Thorax* 43: 896-900.
- Niven RW and Schreier H (1990): Nebulization of liposomes. I. Effects of lipid composition. *Pharm Res* 7: 1127-1133.
- Niven RW, Carvajal TM and Schreier H (1992): Nebulization of liposomes. III. The effects of operating conditions and local environment. *Pharm Res* 9 (4): 515-520.
- O'Riordan TG, Zwang J and Smaldone GC (1992): Mucociliary clearance in adult asthma. *Am Rev Respir Dis* 146: 598-603.
- O'Riordan TG, Waldrep JC, Abraham WM, Mao Y, Sabater JR, Sielzak M and Knight V (1997): Delivery of nebulized budesonide liposomes to the respiratory tract of allergic sheep. *J Aerosol Med* 10: 117-128.
- Pallasaho, P, Lundbäck P, Läspä SL, Jonsson E, Kotaniemi J, Sovijärvi AR and Laitinen LA (2000): Increasing prevalence of asthma but not chronic bronchitis in Finland? Report from the FinEsS-Helsinki Study. *Resp Med*: 93: 728-809.
- Palmqvist M, Persson G, Lazer L, Rosenberg J, Larsson P and Lötvall J (1997): Inhaled dry-powder formoterol and salmeterol in asthmatic patients: onset of action, duration of effect and potency. *Eur Respir J* 10: 2484-2489.
- Pauwels RA, Löfdahl C-G, Postma DS, Tattersfield AE, O'Byrne P, Barnes PJ and Ullman A (1997): Effect of inhaled formoterol and budesonide on exacerbations of asthma. *N Engl J Med* 337: 1405-1411.
- Pavia D, Short MD and Thomson ML (1970): No demonstrable long term effects of cigarette smoking on the mucociliary mechanism of the human lung. *Nature* 226: 1228-1231.
- Pavia D, Thompson ML and Shannon HS (1977a): The effect of airway obstruction and tidal volume of aerosol inhaled on the depth of deposition in the human lung. *Arch Environment Health* 32 (3): 131-137.
- Pavia D, Thomson ML, Clarke SW and Shannon HS. (1977b): Effect of lung function and mode of inhalation on penetration of aerosol into the human lung. *Thorax* 32: 194-197.
- Pavia D, Sutton PP, Agnew JE, Lopez-Vidriero MT, Newman SP and Clarke SW (1983): Measurement of bronchial mucociliary clearance. *Eur J Respir Dis* 64 (Suppl 127): 41-56.
- Pavia D, Bateman JRM, Sheahan NF, Agnew JE and Clarke SW (1985): Tracheobronchial mucociliary clearance in asthma: impairment during remission. *Thorax* 40: 171-175.
- Pavia D, Agnew JE, Sutton PP, Lopez-Vidriero MT, Clay MM, Killip M and Clarke SW (1987): Effect of terbutaline administered from metered dose inhaler (2 mg) and subcutaneously (0.25 mg) on tracheobronchial clearance in mild asthma. *Br J Dis Chest* 81: 361-70.

- Pavia D, Vora H, Hasani A, Agnew JE and Clarke SW (1989): Is there a difference in lung mucociliary clearance between males and females. *Eur Respir J* 2 (Suppl 5): 284s.
- Perkins WR, Dause RB, Parente RA, Sharma RM, Neuman KC, Gruner SM, Taraschi TF and Janoff AS (1996): Role of lipid polymorphism in pulmonary surfactant. *Science* 273: 330-332.
- Phillips WT, Rudolph AS, Goins B, Timmons JH, Klipper R and Blumhardt R (1992): A simple method for producing a technetium-99m-labelled liposome which is stable in vivo. *Nucl Med Biol* 19: 539-547.
- Pircher FJ, Temple JR and Kirsch WJ (1965): Distribution of pulmonary ventilation determined by radioisotope scanning. *Am J Roentgenol* 94: 807-814.
- Priehard JN, Jefferies SJ and Black A (1986): Sex differences in the regional deposition of inhaled particles in the 2.5 – 7.5 μm size range. *J Aerosol Sci* 17 (3): 385-389.
- Quintero OA and Wright JR (2000): Metabolism of phosphatidylglycerol by alveolar macrophages in vitro. *Am J Physiol Lung Cell Mol Physiol* 279 (2): 399-407.
- Ramanna L, Taskin DP, Taplin GV, Elam D, Detels R, Coulson A and Rokaw SN (1975): Radioaerosol lung imaging in chronic obstructive pulmonary disease: Comparison with pulmonary function tests and roentgenography. *Chest* 68: 634-640.
- Rees PJ and Clarke TJH (1982): The importance of particle size in response to inhaled bronchodilators. *Eur J Respir Dis* 63 (Suppl 119): 73-78.
- Reid L (1973): Bronchial secretion. *Bull Physiopathol Respir* 9:15-30.
- Reid L, Bhaskar K and Coles S (1982): Clinical aspects of respiratory mucus. In: *Advances in experimental medicine and biology*, pp. 369-391. Eds. Chantler EN, Elder JB and Elstein M, Plenum Press, New York.
- Roy A, Leblanc C, Paquette L, Ghezze H, Cote J, Cartier A and Malo JL (1996): Skin bruising in asthmatic subjects treated with high doses of inhaled steroids: frequency in association with adrenal function. *Eur Respir J* 9 (2): 226-231.
- Sackner MA, Yergin BM, Brito M and Januszkiewicz A (1979): Effect of adrenergic agonist on tracheal mucous velocity. *Bull Europ Physiopath Resp* 15: 505-511.
- Sanchis J, Dolovich M, Chalmers R and Newhouse M (1972): Quantitation of regional aerosol clearance in the human lung. *J Appl Physiol* 33: 757-62.
- Santa Cruz R, Landa J, Hirsch J and Sackner MA (1974): Tracheal mucous velocity in normal man and patients with obstructive lung disease; effects of terbutaline. *Am Rev Respir Dis* 109: 458-463.
- Santolicoandro A and Giuntini C (1979): Patterns of deposition of labelled monodisperse aerosols in obstructive lung disease. *J Nucl Med Allied Sci* 23: 115-127.
- Schreurs AJM, Sinnighe Damste HEJ, de Graaff CS and Greefhorst APM (1996): A dose-response study with formoterol Turbuhaler as maintenance therapy in asthmatic patients. *Eur Respir J* 9: 1678-1683.
- Schroit AJ, Hart IR, Madsen J and Fidler IJ (1983): Selective delivery of drugs encapsulated in liposomes: natural targeting to macrophages involved in various states. *Journal of Biological Response Modifiers* 2: 97-100.
- Schreier H, Gonzalez-Rothi RJ and Stecenko AA (1993): Pulmonary delivery of liposomes. *J Control Release* 24: 209-223.
- Schwarz LA, Johnson JL, Black M, Cheng SH, Hogan ME and Waldrep JC (1996): Delivery of DNA-cationic liposome complexes by small-particle aerosol. *Human Gene Therapy* 7: 731-741.
- Schweibert LM, Stellato C and Schleimer RP (1996): The epithelium as a target for glucocorticoid action in the treatment of asthma. *Am J Respir Crit Care Med* 154: S16-20.

- Seberove E and Andersson A (2000): Oxis (formoterol given by Turbuhaler) showed as rapid an action as salbutamol given by a pMDI. *Respir Med* 94: 607-611.
- Senior JH (1987): Fate and behavior of liposomes in vivo: a review of controlling factors. *Crit Rev Ther Drug Carrier Syst* 3: 123-193.
- Shek PN, Suntres ZE and Brooks JI (1994): Liposomes in pulmonary applications: physicochemical considerations, pulmonary distribution and antioxidant delivery. *J Drug Target* 2: 431-442
- Short MD, Dowsett DJ, Heaf PJD, Pavia D and Thomson ML (1979): A comparison between monodisperse Tc-99m-labelled aerosol particles and krypton-81m for assessment of lung function. *J Nucl Med* 20: 194-200.
- Short MD, Singh CA, Few JD, Studdy PT, Heaf PJD and Spiro SG (1984): The labelling and monitoring of lung deposition of an inhaled synthetic anticholinergic bronchodilating agent. *Chest* 80 (Suppl): 918-921.
- Smaldone GC and Messina MS. (1985): Enhancement of particle deposition by flow limiting segments in humans. *J Appl Physiol* 59: 509-14.
- Smaldone GC, Perry RJ, Bennett WD, Messina MS, Zwang J and Ilowite J. (1988): Interpretation of "24 hour lung retention" in studies of mucociliary clearance. *J Aerosol Med* 1: 11-20.
- Smaldone GC, Walser L, Perry RJ, Ilowite JS, Bennett WD and Greco M (1989): Generation and administration of aerosols for medical and physiological research studies. *J Aerosol Med* 2: 81-87.
- Smaldone GC, Foster WM, O'Riordan TG, Messina MS, Langenback EG and Perry RJ (1993): Regional impairment of mucociliary clearance in chronic obstructive pulmonary disease. *Chest* 103: 1390-1396.
- Snell NJC and Ganderton D (1999): Assessing lung deposition of inhaled medications. *Respir Med* 93: 123-133.
- Sparano JA and Winer EP (2001): Liposomal anthracyclines for breast cancer. *Semin Oncol* 28 (suppl 12): 32-40.
- Suarez S, Gonzalez-Roth RJ, Schreier H and Hochhaus G (1998): Effect of dose and release rate on pulmonary targeting of liposomal triamcilonone acetone phosphate. *Pharm Res* 15: 461-465.
- Sutton PP, Pavia D, Bateman JRM and Clarke SW (1981): The effect of oral aminophylline on lung mucociliary clearance in man. *Chest* 80: 889-892.
- Svartengren M, Mossberg B, Philipson K and Camner P (1986): Mucociliary clearance in relation to clinical features in patients with bronchiectasis. *Eur J Respir Dis* 68: 267-278.
- Taniguchi K, Yamazawa N, Itakura K, Morisaki K and Hayashi S (1987): Partition characteristics of anti-inflammatory steroids in liposomal ophthalmic preparation. *Chem Pharm Bull* 35: 1214-1222.
- Taplin GV and Poe ND. (1965): A dual lung scanning technique of pulmonary function. *Radiology*, 85, 363-8.
- Taplin GV and Chopra SK (1978): Inhalation lung imaging with radioactive aerosol and gases. *Prog Nucl Med* 5: 119-143.
- Taplin GV, Poe ND, Dore EK and Greenberg A. (1967): Bronchial patency and aerated space assessment by scintiscanning. *Lahey Clin Found Bull* 16: 297-312.
- Taylor KMG and Farr SJ (1993): Liposomes for drug delivery to the respiratory tract. *Drug Dev and Ind Phar* 19: 123-142.
- Taylor KMG, Taylor G, Kellaway IW and Stevens J (1989): The influence of liposomal encapsulation on sodium cromoglycate pharmacokinetics in man. *Pharm Res* 6 (7): 633-636.
- Taylor KMG, Taylor G, Kellaway IW and Stevens J (1990): The stability of liposomes to nebulization. *Int J Pharm* 58: 57-61.

- Thomas DA, Myers MA, Wichert B, Schreier H and Gonzalez-Rothi RJ. (1991): Acute effects of liposome aerosol inhalation on pulmonary function in healthy human volunteers. *Chest* 99: 1268-1270.
- Thomas SHL, O'Doherty MJ, Page CM, Nunan TO and Bateman NT (1991a): Which apparatus for inhaled pentamidine? A comparison of pulmonary deposition via eight nebulizers. *Eur Respir J* 4: 616-612.
- Thomas SHL, O'Doherty MJ, Page CJ and Nunan TO (1991b): Variability in the measurement of nebulized aerosol deposition in man. *Clinical Science* 81: 767-775.
- Urban RC and Cotlier E (1986): Corticosteroid induced cataracts. *Surv Ophthalmol* 31: 102-110.
- Van deer Baan S, Veerman AJP, Weltevreden EF and Feenstra L (1983): Keragen's syndrome: clinical symptoms and laboratory studies. *Eur J Respir Dis* 64 (Suppl 127): 91-95.
- Van der Molen T, Postma DS, Turner MO Jong BM, Malo JL, Chapman K, Grossman R, de Graaff CS, Riemersma RA and Sears MR (1997): Effects of the long-acting β -agonist formoterol on asthma control in asthmatic patients using inhaled corticosteroids. *Thorax* 52: 535-539.
- Vidgren M, Waldrep JC, Arppe J, Black M, Rodarte JA, Cole W and Knight V (1995): A study of 99m technetium labelled beclomethasone dipropionate dilaurylphosphatidylcholine liposome aerosol in normal volunteers. *Int J Pharm* 115: 209-216.
- Vignola AM and Bousquet J (2000): Tissue remodelling as a feature of persistent asthma. *J Allergy Clin Immunol* 10: 1041-1053.
- Viljanen A (1982): Reference values for spirometric, pulmonary diffusing capacity and body plethysmographic studies. *Scan J Clin Lab Invest* 42: 1-50.
- Waldrep JC (1998): New aerosol drug delivery systems for the treatment of immune-mediated pulmonary diseases. *Drugs of Today* 34 (6): 549-561.
- Waldrep JC, Scherer PW, Black M and Knight V (1993): Cyclosporin A liposome aerosol: particle size and calculated respiratory deposition. *Int J Pharm* 97: 205-212.
- Waldrep JC, Scherer PW, Hess GD, Black M and Knight V. (1994a): Nebulized glucocorticoids in liposomes: aerosol characteristics and human dose estimates. *J Aerosol Med* 7: 135-144
- Waldrep JC, Keyhani K, Black M and Knight V. (1994b): Operating characteristics of 18 different continuous-flow jet nebulizers with beclomethasone dipropionate liposome aerosols. *Chest* 105: 106-110.
- Waldrep JC, Gilbert BE, Knight CM, Black MB, Scherer PW and Knight V (1997): Pulmonary delivery of beclomethasone liposome aerosol in volunteers. Tolerance and safety. *Chest* 111: 316-323.
- Wanner A (1977): Clinical aspects of mucociliary transport. *Am Rev Respir Dis* 116: 73-125.
- Wanner A, Salathe M and O'Riordan TG (1996): Mucociliary clearance in the airways. *Am J Respir Crit Care Med* 154: 1868-1902.
- Weibel ER (1963): Morphometry of the human lung. Academic Press, New York.
- Weiner N and Chiang C-M (1988): Gastrointestinal uptake of liposomes. In: *Liposomes as drug carriers, recent trends and progress*, pp. 599-607. Ed. Gregoridianis G, John Wiley & Sons Ltd.
- Wilkey DD, Lee PS, Hass FJ, Gerrity TR, Yeates DB and Lourenco RV (1980): Mucociliary clearance of deposited particles from the lung: intra- and inter-subject reproducibility, total and regional lung clearance, and model comparison. *Arch of Environ Health* 35: 294-303.

- Willey RF, Godden DJ, Carmichael J, Preston P, Frame M and Crompton GK (1982): Comparison of twice daily administration of a new corticosteroids budenoside with beclomethasone dipropionate four times daily in the treatment of asthma. *Br J Dis Chest* 76: 61-68.
- Wilson AM and Lipworth BJ (1997a): Systemic dose-response relationship with oral and inhaled corticosteroids in asthmatics. *Thorax* 52 (Suppl 6): A57.
- Wilson AM, McFarlane LC and Lipworth BJ (1997b): Dose response effect for adrenal suppression with repeated twice daily inhaled fluticasone priopionate and triamcilone acetoneide in adult asthmatics. *Am J Respir Crit Care Med* 156: 1274-1277.
- Woolcock A, Lundback B, Ringdahl N and Jacques LA (1996): Comparison of addition of salmeterol to inhaled steroids with doubling of the dose of inhaled steroids. *Am J Respir Crit Care Med* 153: 1481-1488.
- Wright JR and Clements JA (1987): Metabolism and turnover of lung surfactant. *Am Rev Respir Dis* 135: 426-444.
- Yates DB, Aspin N, Levison H Jones MT and Bryan AC (1975): Mucociliary tracheal transport in man. *J Appl Physiol* 39: 487-495.
- Yates DB, Gerrity TR and Garrard CS (1982): Characteristics of tracheobronchial deposition and clearance in man. *Ann Occup Hyg* 26: 245-257.