Aerosol formulation and clinical efficacy of bronchodilators

CIP-DATA KONINKLIJKE BIBLIOTHEEK, DEN HAAG

Zanen, Pieter

Aerosol formulation and clinical efficacy of bronchodilators. Utrecht: Universiteit Utrecht, Faculteit Geneeskunde.
Thesis University Utrecht. With summary in Dutch
ISBN 90-393-2027-6
Subject headings: Aerosols / formulation / bronchodilators / deposition / efficacy / adverse effects

Aerosol formulation and clinical efficacy of bronchodilators

De formulering van luchtwegverwijdende aerosolen en de effectiviteit ervan

(met een samenvatting in het Nederlands)

Proefschrift

Ter verkrijging van de graad van doctor
aan de Universiteit Utrecht
op gezag van de rector Magnificus, Prof. Dr. H.O. Voorma
ingevolge het besluit van het College voor Promoties
in het openbaar te verdedigen
op dinsdag 6 oktober 1998 des namiddags te 4.15 uur
door

Pieter Zanen
Geboren op 6 november 1954 te Utrecht

Promotor: Prof. Dr. J-W. J. Lammers

The research described in this thesis were financially supported by Mr. J. Kelders.

Publication of this thesis was made financially possible by Genfarma BV and GlaxoWellcome BV, the Netherlands.

Voor Edith, Emmylou en Justin
het mooiste wat ik bezit

CONTENTS

Voor Edith, Emmylou en Justin
het mooiste wat ik bezit

Introduction

Aerosolised drugs are nowadays cornerstones in the treatment of pulmonary diseases. Although the use of inhaled drugs is known for some centuries, the break-through of this mode of administration was only a few decades ago. The metered dose inhaler (MDI) was the first apparatus, which is both reliable and practical. Previously, all kind of nebulisers were available, but their size prevented (and still prevents) outdoor use. The use of MDI's resulted in a decreased need for systemically administered bronchodilators or other anti-asthma drugs. This meant a considerable reduction of side-effects and an improvement of the quality of therapy[1]. The growing experience with MDI's revealed some drawbacks of the system: it appeared that a considerable number of patients were not able to use a MDI correctly[2]. Many authors pointed out that several mistakes were possible, like failing hand-lung co-ordination, stopping an inhalation after firing the MDI. These mistakes cause a suboptimal delivery of drugs to the airways and thereby a reduced therapeutic efficacy. To cope with these problems, spacers were introduced and somewhat later dry powder inhalers. At this moment a broad spectrum of aerosol generators is available, the latest development being a breath actuated MDI[3].

It became also known that only a small fraction (10-20%) of the emitted dose reaches the lower airways. The remainder deposits in the extrathoracic and upper airways, is swallowed and subsequently absorbed in the gastrointestinal tract[4]. This low availability is related to the formulation of the aerosols. All aerosol generators produce particles (or droplets) with a wide range of diameters. Such aerosols are defined as polydisperse, opposite to monodisperse aerosols, of which the diameter range is small. Large particles tend to deposit in the extrathoracic and upper airways; size measurements of the particles produced by all current generators reveal that the majority of the particles generated are these large particles[5].

To increase the efficacy of the administration, it is evident that the formulations of these aerosols have to be improved to increase lung deposition and to decrease extrathoracic and upper airway deposition. In general, the smaller a particle is the lower the extrathoracic and upper airway deposition will be and the deeper it penetrates the

lower airways. However, the tendency to deposit declines as particle size decreases. Therefore, a particle may not be too large or too small.

A confounding parameter is the diameter of the airways. In small tubes a smaller particle shall penetrate further due to its lower deposition tendency, while in larger tubes such a low tendency proves to be the wrong choice. An absence of deposition is equivalent to a lack of therapeutic effect. Transferred to therapeutic aerosols in asthmatic patients the ideal formulation may depend on the degree of constriction of the airways. The localisation of receptors may also be of influence. Barnes showed that more $ß_2$-receptors are present in small airways compared to large airways[6]. Hence it may be hypothesised that $ß_2$-mimetic aerosols should be directed towards the periphery of the lung and therefore should exhibit a small particle size.

The subject of this thesis is the definition of the most efficient aerosol formulation in terms of particle size or what is the most efficient formulation of a bronchodilator aerosol in patients exhibiting increasing degrees of bronchoconstriction.

This thesis will start with an overview of existing knowledge on deposition of aerosols. A review of the theoretical and physical backgrounds of deposition is offered in chapter 2 and on the measurements of lung deposition of non-therapeutic and therapeutic aerosols in chapter 3 and 4 respectively. In chapter 5 the goals of the experiments, described in this thesis will be explained in greater detail and in subsequent chapters the results.

Literature

[1] Timsina M.P., Martin G.P., Marriott C., Ganderton D. and Yianneskis M.
Drug delivery to the respiratory tract using dry powder inhalers.
Int J Pharm 101: 1-13 1994.

[2] Pedersen S., Frost L. and Arnfred T.
Errors in inhalation technique and efficiency in inhaler use in asthmatic children.
Allergy 41: 118-124 1986.

[3] Newman S.P., Weisz A.W.B., Talaee N. and Clarke S.W.
Improvement of drug delivery with a breath actuated pressurised aerosol for patients with poor inhaler technique.
Thorax 46: 712-716 1991.

[4] Biddiscombe M.F., Melchor R., Mak V.H.F., Marriott R.J., Taylor A.J., Short M.D. and Spiro S.G.
The lung deposition of salbutamol, directly labelled with technetium-99m, delivered by pressurised metered dose and dry powder inhalers.
Int J Pharm 91: 111-121 1993.

[5] Bouchikhi A., Becquemin M.H., Bignon J., Roy M. and Teillac A.
Particle size study of nine metered dose inhalers and their deposition probabilities in the airways.
Eur J Respir 1: 547-552 1988.

[6] Barnes P.J., Basbaum C.B., Nadel J.A. and Roberts J.M.
Localization of ß-adrenoreceptors in mammalian lung by light microscopic autoradiography.
Nature 299: 444-447 1982.

Predictive models of lung deposition

Introduction

In the last part of the 19th century mining companies started to realise that inhaled dust caused the frequently occurring pneumoconiosis in miners. Watkins-Pitchford and Muir reported that approx. 80% of all silica particles in the lungs of patients were <2 μm[1]. Mavrogordato launched the idea that the particles <5 μm, but >0.5 μm caused pneumoconiosis[2].

So a hypothesis emerged: a relation exists between particle size and penetration of dust into the lungs, but more research was needed to reject or accept this hypothesis. Unfortunately at that moment, measuring lung deposition of inhaled particles was technically impossible and researchers had to find an alternative: mathematical modelling. Physicists, studying the deposition of particles in straight tubes, revealed deposition-mechanisms and formulated some equations. To learn on the deposition in the human airways the ideal straight tube was replaced by a system of tubes, resembling the human airways. Knowing the complexity of the airways, the difficulty of these calculations is conceivable and simplifications unavoidable. In the sections below we will discuss one of the models in some detail, because it serves as good introduction into deposition mechanisms. Moreover, it is well established and elaborated.

Deposition model of Findeisen

In 1935 Findeisen published his 'Uber das Absetzen kleiner in der Luft suspendierter Teilchen in der mensliche Lunge bei der Atmung', in which he revealed his methods[3]. The model was based on the presence of four deposition-mechanisms: a] *impaction*, b] *sedimentation*, c] *Brownian movement* and d] *the so called 'rim-effect'*.

a] Impaction. Along its travel through the airways, a flow of air changes direction often and suddenly. Due to their inertia, particles shall not always be able to follow these changes, causing them to collide with the wall of the airways. The general character of deposition by impaction is: large particles travelling at high speed exhibit the highest probability of deposition. In the human airways the velocity of the air is

highest in the upper airways, therefore impaction is an important deposition mechanism in these parts. In other words, large particles are filtered out of the air in the upper airways.

b] Sedimentation. Due to gravity, particles will start to fall. At the moment the force of gravity equals the air resistance, the speed becomes constant: the so called terminal velocity. High terminal velocities are equivalent to high deposition-probabilities, because such particles travel larger distances in an unit of time. These higher velocities are found with the larger particles. Because the residence time is too short, sedimentation is of lesser importance in the upper airways. Sedimentation therefore causes particles, who 'escaped' impaction, to deposit in the smaller airways. An important aspect of sedimentation is, when particle-size reduces, the terminal velocity reduces too. So small particles need more time to reach the walls of the bronchi and when that time exceeds one complete breathing-cycle, the particles are exhaled. Increasing the volume of air inhaled and breath-holding are both important, because they both increase the residence time.

c] Brownian movement. Due to collisions with air molecules, very small particles (<0.01 μm) start to move in a random manner over random distances and a chance is present of a collision with the walls of the bronchi. It will be clear that only very small particles deposit efficiently by Brownian movement.

d] Rimeffect. By pure chance it is possible that a particle, in close vicinity of the wall touches it and deposits. Findeisen called that the 'rim-effect'. In the calculations the contribution of this effect to the total deposition turned out to be negligible.

To keep calculations at a practical level, the anatomy of the human airways was simplified considerably: it was restricted to only 9 airway generations. Findeisen also restricted his calculations to just one breathing volume and inhalation-flow. The outcome is presented in Fig. 1. The conclusions are clear: large particles deposit entirely (in the upper airways). Smaller particles do penetrate further, but the tendency to deposit reduces as they decrease in size. The deposition of 0.1-0.3 μm particles is minimal. These particles do not deposit by impaction any more, their terminal velocity is very low, while they are still too heavy for Brownian movement deposition.

From a protective point of view this is a desired effect. The alveoli do not possess a fast and effective clearance mechanism and must therefore be protected against (insoluble) inhaled particles. Large particles contain more mass then small ones and can be more toxic. It is therefore crucial that large particles are filtered out in those parts of the airways where they are quickly removed. Smaller particles are of less immediate danger: they deposit to a small extent and their mass is low. Due to their ability to penetrate deeper in the airways, they form a danger over a large period of time.

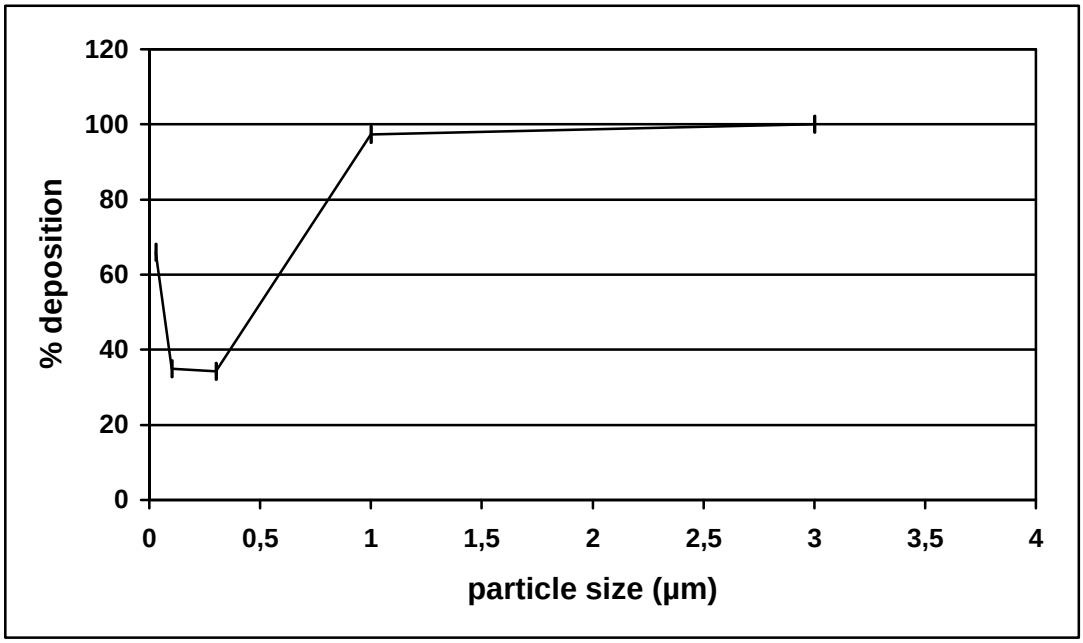


Fig. 1 Total lung deposition (= particles entering minus particles leaving the airways) as calculated by Findeisen[3]

In later years this first and crude model was refined. Landahl increased the number of bronchioli, alveolar ducts and alveoli considerably, redefined the deposition-equations and added to the model the influence of increasing breathing volumes[4]. The results of these calculations are presented in Fig. 2. Compared to Findeisen, the deposition rate for larger particles is somewhat lower, but the general character remains the same. A larger inhaled volume leads to an increased deposition (especially for small particles), while a fast inhalation favours proximal deposition.

A second improvement by Beeckmans was the incorporation of the Weibel-A lung-model[5 6]. The Weibel-A model is based on measurements of airways dimensions in casts of human airways and is more accurate. The higher accuracy of the Weibel-A model, however, did not improve the fit to the experimental data. Despite this minor improvement Beeckmans prefered the Weibel-A model on theoretical grounds and in later publications the model is commonly used.

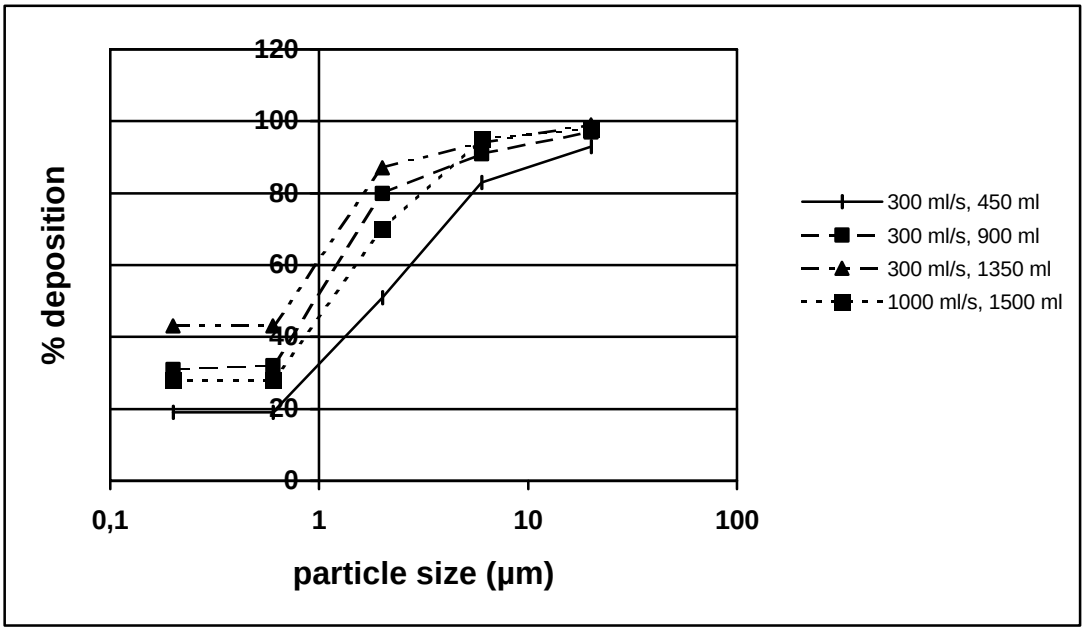


Fig. 2 Total deposition as function of inhalationflow and -volume, calculated by Landahl[4]

In 1966 a significant contribution to the modelling was published by the Task Group of the International Committee on Radiological Protection (IRCP)[7]. This Task Group had to formulate a model to estimate the deposition and especially the clearance from the lungs of radioactive dust. A very practical application for which it became famous. The Group did not design a new model, but modified/improved the Findeisen/Landahl model. First, one defined three compartments: 1] nasopharyngeal, 2] tracheobronchial and 3] alveolar or pulmonary. From a clearance point of view, these compartments behave differently. Compartment 1] and 2] possess ciliated epithelium, which can remove insoluble deposited particles quickly, while the alveolar compartment can not. A second improvement is the use of the so called *aerodynamic particle size*. In all calculations up to now, spherical particles were 'used', but these are rare in nature. The

terminal velocity of a particle also depends on its shape: needle shaped crystals show higher velocities. The definition of the aerodynamic size of a particle is: *the size of a spherical particle with a mass of 1, having the same terminal velocity of said particle*. In other words, every irregular shaped particle is transformed to a spherical one by comparing terminal velocities. The aerodynamic particle size is now standard in aerosol-sizing. The third change was the implementation of polydisperse aerosols in modelling. Natural aerosols exhibit a range of sizes (polydisperse) and as result, deposition therefore is expected to be widespread in the airways. In Table 1 results on the deposition of polydisperse aerosols are presented. From the data it is clear that the increasing polydispersity of 'large' aerosols poses a threat in terms of alveolar deposition. Large aerosols were not supposed to reach the alveoli and therefore they could not damage alveolar tissues. This is, however, only correct as long as the aerosols are monodisperse (=show a narrow size-distribution).

MAD (μm)	σ	Nasopharyngeal deposition	Tracheobronchial deposition	Pulmonary deposition
0.2	1.2	0.00	2.06	36.4
0.2	1.5	0.01	2.37	39.1
0.2	2.0	0.78	2.91	41.2
0.2	2.5	2.36	3.61	42.3
0.2	3.0	4.09	4.24	42.8
2	1.5	51.1	4.70	27.2
2	2.0	50.7	4.30	23.6
2	2.5	50.4	3.90	21.8
2	3.0	50.2	3.61	21.0
2	4.0	50.1	3.57	20.6
20	1.2	99.9	0	0
20	2.5	97.2	0.81	1.70
20	3.0	95.6	1.03	2.60

Table 1 Percentual deposition of polydisperse aerosols, according to the IRCP Task Group[7]. MAD = median aerodynamic diameter, σ = geometric standard deviation

Following the work of the Task Group deposition-modelling is characterised by a steady addition of parameters, thereby increasing the complexity of the Findisen/Landahl/ Beeckmans model. We will discuss some of these added parameters.

Deposition due to turbulence

All the previous models were based on the assumption that airflows are laminar. This is of course only true in small airways and Yeh feared an underestimation of the deposition due to negligence of turbulent deposition[8]. Yeh, however, states that only deposition by Brownian movement is affected by turbulence and formulates a set of equations, based on heat-transfer processes. Unfortunately no results are given, only the methods. A second attempt was made by Hamill, based on the studies on deposition under turbulent flow conditions by Friedlander and Johnstone[9 10]. Hamill's results indicate that turbulent deposition is a typical large particle phenomenon, but ten times less efficient as impaction itself.

Regional deposition

Findeisen attempted to calculate the deposition per individual airway-generation, but his rather crude lung-model prevented accurate conclusions. Based on the Landahl equations and the Weibel-A model Gerrity made a second attempt[11]. In Fig. 3 the results are presented. In general one can say that large particles deposit in the proximal airways and small ones in distal. Important to note is that deposition patterns are always widespread. Large particles do reach the distal airways in low numbers. So when an abundant number of large particles are inhaled, the burden to the alveoli can still be significant.

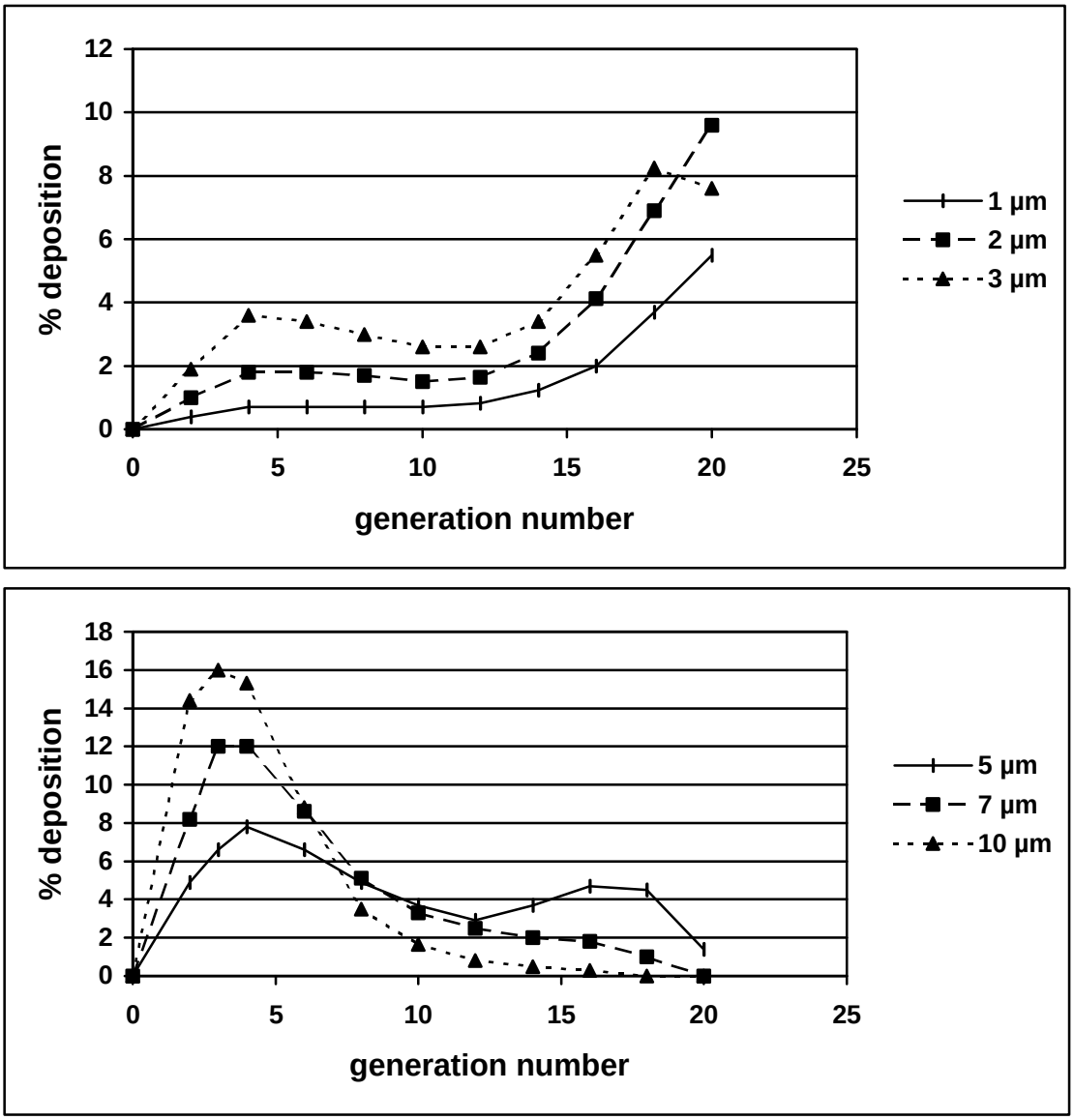


Fig. 3 Calculated regional deposition of 1 µm, 2 µm, 3µm, 5 µm, 7 µm and 10 µm non-hygroscopic particles[11]

Yeh and Schum measured the dimensions of human airways based on a cast of them. The advantage of this model is the possibility to calculate the deposition per lobe in stead of per airway generation (the Weibel-A model does not posses lung-lobes)[12]. In Table 2 the results are presented. It is clear that significant differences between the deposited amount of particles in the lobes exist. Anatomical differences are held responsible.

d(μm)	region	UR	MR	LR	UL	LL
1	TB	0.32	0.14	0.62	0.33	0.68
	P	1.91	1.01	3.40	1.69	0.32
2	TB	0.56	0.25	1.12	0.57	1.21
	P	2.85	1.50	5.17	2.57	5.02
3	TB	0.79	0.37	1.61	0.80	1.74
	P	3.09	1.63	5.66	2.83	5.50

Table 2 Percentual deposition per lung lobe, according to Yeh and Schum[12].
UR= upper right, MR = middle right, LR = lower right, UL = upper left, LL = lower left, TB = tracheo-bronchial, P = pulmonary, d = aerodynamic particle size

Hygroscopy

Particles that enter the airways, are exposed to an atmosphere of approx. 99% relative humidity. Insoluble particles shall be surrounded by a layer of water, while soluble particles will absorb water until the fluid is isotonic. The effect is particle-growth. Hypo-osmolar droplets will, however, decrease in size, because they give off water until an equilibrium is reached. Austin was one of the first to address this problem[13]. The changes in particle size, the place and the extent of deposition depends on the residence time and the speed at which the equilibrium is reached. A comparison with experimental data showed that Austin's model underestimates the deposition, most probably due to flawed estimates of the point, where the particle growth/evaporation equilibrium is reached.

A second attempt was made by Martonen[14]. His objectives were to calculate the deposition of hygroscopic H_2SO_4-aerosols, which are a part of the daily airpollution and occur as small particles. Based on the Weibel-A model and Landahls' equations, he calculated the deposition patterns of these droplets under varying airway humidity conditions (50% to 100%). The results indicate that, under 100% relative humidity conditions, 1 μm particles grow into 16 μm droplets, causing them to deposit much more quickly by impaction. If the relative humidity is 90% the equilibrium size is approx. 2.2 μm. Smaller particles (0.1 μm) grow to a final size of 0.7 μm and 0.2 μm

respectively. The changes in deposition are less extreme for these particles, as can be expected from the limited size changes.

Age

Children grow and growth is equivalent a continuous enlargement of the airways, both in length and diameter. These changes must have an effect on particle deposition. To find out how large the effects of ageing are, Schum scaled the Weibel-A model down to dimensions encountered in different age groups and calculated the deposition. In 2 year old children 23.8% of 1 μm particles deposit, while in 20 years old adults only 10.4% of them deposit. The general trend is that in larger airways particles deposit less well, as a function of increasing age. This is particularly true for particles >1 μm, which deposit by sedimentation or impaction[15].

Deposition in constricted airways

Up to now, the deposition of particles is modelled in a 'healthy' lung-model. Kim and co-workers developed a lung-model, based on the Weibel-A model, in which the airways were constricted to estimate the deposition in diseased airways[16]. They decreased the airway diameter with 25% or 40% in either peripheral or central airways. The next step was to calculate the increase in resistance and deposition. In Fig. 4 the results are presented. As can be concluded from the graphs, there is no simple relationship between the increase in deposition and resistance. Except when peripheral airways are constricted, the increase in resistance is always higher than in deposition, but not by a constant factor. This is to be expected, because the resistance relates to the airway-diameter by the fourth power and the deposition does not. The deposition increase occurs mainly in the large airways, while it decreases in the small airways (generation 14 and up). This can be explained by the higher filter efficiencies of the constricted upper airways.

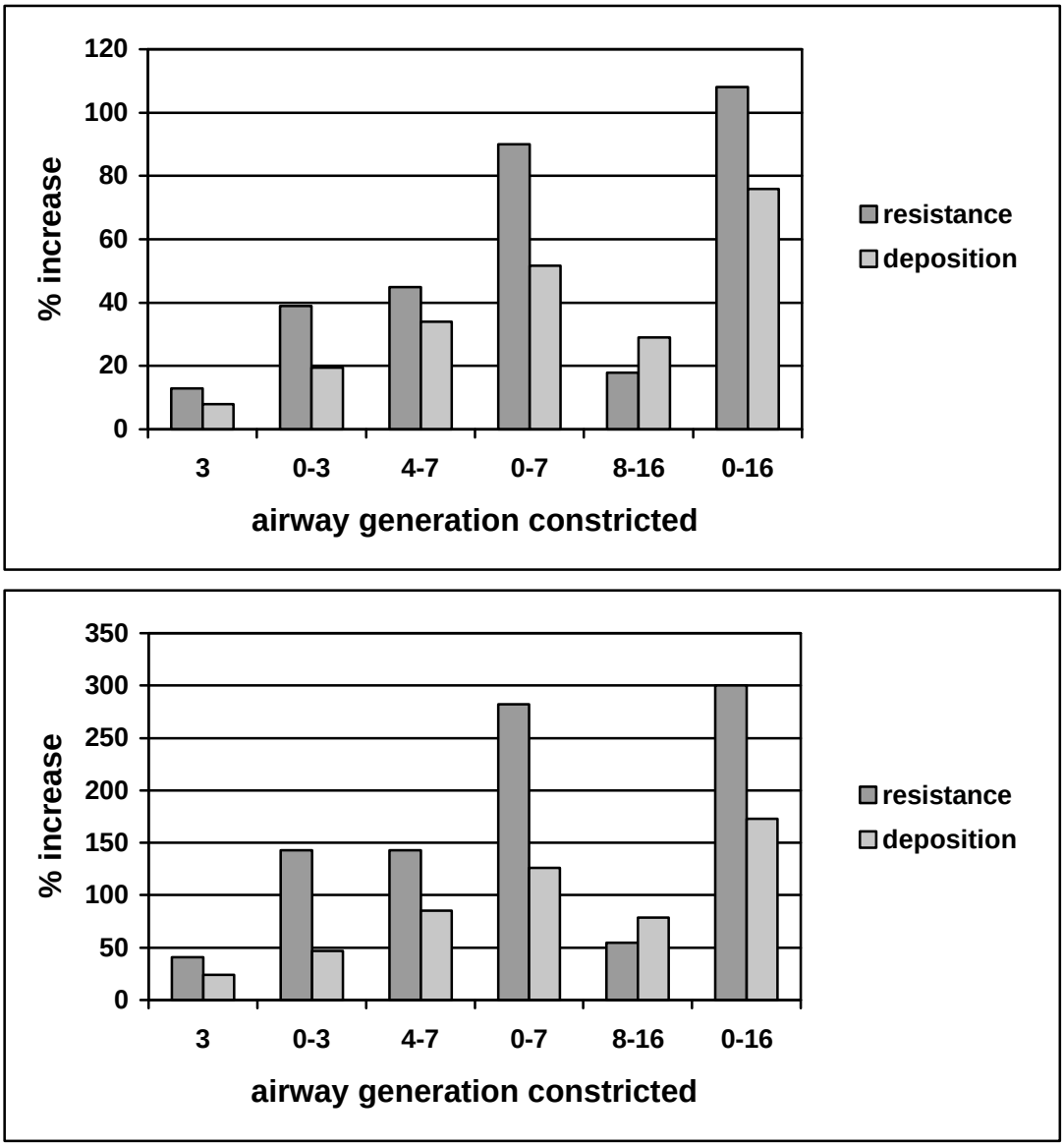


Fig. 4 Percentage increase of resistance and deposition of 3 µm particles after reducing airway diameter with 25% or 40%[16]. The upper graph represents the situation after a diameter reduction of 25%, the lower one after a 40% reduction

Literature

[1] Watkins-Pitchford W. and Muir J.
On the nature of the doubly refracting particles seen in the microscopic sections of silicotic lungs, and an improved method for disclosing siliceous particles in such sections.
S Afr Inst Med Res 1: 207-230 1916.

[2] Mavrogordato A.
Value of the koniometer, being an investigation into the method and dust sampling as at present practiced in the mines of Witwatersrand.
S Afr Inst Med Res 17: 224-246 1923.

[3] Findeisen W.
Uber das Absetzen kleiner in der Luft suspendierten Teilchen in der menslichen Lunge bei der Atmung.
Phlügers Arch Ges Physiol 236: 367-379 1935.

[4] Landahl H.D.
On the removal of airborne droplets by the human respiratory tract. I The lung.
Bull Math Biophys 12: 43-56 1950.

[5] Beeckmans J.M.
Correction factor for size-selective sampling results, based on a new computer alveolar deposition curve.
Ann Occup Hyg 8: 221-231 1965.

[6] Weibel E.R.
Morphometry of the human lung.
Springer Verlag 1963.

[7] Task Group on Lung Dynamics.
Deposition and retention models for internal dosimetry of the human respiratory tract.
Health Phys 12: 173-207 1966.

[8] Yeh H.C.
Use of a heat transfer analogy for a mathematical model of respiratory tract deposition.
Bull Math Biol 36: 105-116 1974.

[9] Hamill P.
Particle deposition due to turbulent diffusion in the upper respiratory system.
Health Phys 36: 355-369 1979.

[10] Friedlander S.K. and Johnstone H.F.
Deposition of suspended particles from turbulent gas streams.
Ind Engin Chem 49: 1151-1162 1957.

[11] Gerrity T.R., Lee P.S., Hass F.J., Marinelli A., Werner P. and Lourengo R.V.
Calculated deposition of inhaled particles in the airway generations of normal subjects.
J Appl Physiol 47: 867-873 1979.

[12] Yeh H.C. and Shum G.M.
Models of human lung airways and their application to inhaled particle deposition.
Bull Math Biol 42: 461-480 1980.

[13] Austin E., Brock J. and Wissler E.

A model for the deposition of stable and unstable aerosols in the human respiratory tract.
Am Ind Hyg Ass J 40: 1055-1064 1979.

[14] Martonen T.B. and Patel M.
Modelling the dose distribution of H_2SO_4 aerosols in the human tracheobronchial tree.
Am Ind Hyg Ass J 41: 453-460 1981.

[15] Schum G.M., Phalen R.F. and Oldham M.J.
The effect of dead space on inhaled particle deposition.
J Aerosol Med 4: 297-311 1991.

[16] Kim C.S., Brown L.K., Lewars G.C. and Sackner M.A.
Deposition of aerosol particles and flow resistance in mathematical and experimental airway models.
J Appl Physiol 55: 154-163 1983.

In-vivo measurements of lung deposition

Introduction

By using mathematical models, as described in the previous chapter, much knowledge was gained on the deposition of particles in the airways. However, models can not replace in-vivo experiments. As said before in-vivo experiments were hampered by the availability of reliable aerosol generators, as well as particle counting and -sizing techniques. At the moment these became available, nothing inhibited in-vivo experiments.
The design of the experiments was (and still is) heavily influenced by the state of the art of aerosol generators and measuring equipment. As result, one can see that the first experiments were confined to measurement of the total deposition. One measured the number of inhaled and exhaled number of particles, the difference being the number deposited. It is clear that data on the regional deposition can not be derived from these experiments. The γ-camera and radioactive labelled particles were needed to generate those data.
In this chapter the emphasis is on the deposition of non-therapeutic aerosols.

Deposition in healthy volunteers

Wilson was one of the firsts to measure the alveolar deposition in healthy volunteers. Using a so called Sinclair-LaMer generator he administered insoluble particles containing a nucleus of $Na^{24}Cl$, ranging from 0.2 μm to ±3 μm. The particles deposited in the alveoli will not be cleared within 24 hours and the radioactivity measured after 24 hours therefore represents the alveolar deposition. The alveolar deposition showed a bimodal distribution in almost all volunteers around 0.4 μm and 1 μm, not anticipated by the model studies reviewed in the previous chapter[1]. Three years later Landahl administered 0.11 μm to 6.3 μm particles and measured the difference between the in- and exhaled number of particles, as function of the inhaled volume. The results, presented in Table 1, are better in line with theoretical predictions: a deposition minimum is observed around 0.5 μm and increasing volumes of inhaled air cause additional deposition[2]. Landahl

compared these experimental data to his theoretical predictions and concluded that the experimental deposition for 1 µm particles was 'too low'[3].

particle size (µm)	inhalation mode (flow and volume)		
	300 ml/s, 450 ml	300 ml/s, 900 ml	300 ml/s, 1350 ml
0.11	34	36	46
0.25	32	32	41
0.55	17	23	33
1.4	26	53	65
2.9	52	69	82
3.8	59	72	89
6.3	86	93	96

Table 1 In-vivo percentual total deposition according to Landahl[2]

Lippmann and Albert measured the total deposition (=tracheobronchial plus alveolar) of radioactive 2.1 µm to 12.5 µm iron oxide particles immediately after administration. As known, these iron oxide particles are cleared from the tracheobronchial compartment by mucocilairy transport within 24 hours, so the alveolar deposition equals the radioactive count after 24 hours. The difference between the two measurements being the tracheo-bronchial deposition[4]. Their results indicate that the alveolar deposition decreases rapidly with particles >5 µm, while the tracheobronchial deposition related to the so-called impaction parameter $\sigma(d_m)^2Q_{avg}$ (σ=density, d_m= diameter and Q_{avg}= average inhalation flow). This indicates that the tracheobronchial deposition is governed by impaction and not by sedimentation.

Foord used the same technique to measure total and alveolar deposition, the results are presented in Table 2[5]. Foord also found a relationship between d_m^2 and the tracheobron-chial deposition, thereby strengthening the findings of Lippmann.

Site of deposition	particle size (µm)		
	2.5	5	7.5

exhaled	31	14	5
mouth/pharynx	5	17	40
tracheobronchial	10	24	39
alveolar	54	45	16

Table 2 Percentual deposition, according to Foord, of increasing particle sizes, broken down by site[1]

One of the main issues of the group of Heyder was a tight control of the inhalation manoeuvre: they changed the flow, keeping the volume constant or the volume, keeping the flow constant[6]. In general it was found that the deposition increases with larger inhalation volumes. An example: an increase of the inhaled volume from 250 ml to 2000 ml (flow 250 ml/s) causes 30% of all 0.5 μm particles to deposit in stead of 5%, while the deposition of 3 μm particles increases from 25% to 85%. The explanation offered is that these particles mainly deposit by sedimentation, the larger particles of course to a higher extent than the smaller ones. The increase in inhaled volume actually means an increase in residence time, favouring deposition. Contrary, an increase in inhalation flow leads to a decrease in deposition, because residence time reduces. So changing flow and volume in such a way that residence time is kept constant, would not change the deposition. In Table 3 the results of this experiment are presented, showing the validity of this thought. It means that the principal deposition mechanism for particles <3 μm is sedimentation, inertial impaction plays a less important role.

Inhaled volume (ml)	Flow (ml/s)	Residence time (s)	Particle size (μm)				
			0.2	0.62	1	2	3
250	250	2	8	6	7	13	21
1000	1000	2	8	7	8	24	44
500	250	2	14	11	14	27	42
1000	500	2	14	11	15	34	58
1000	250	4	33	25	40	69	79
2000	500	4	34	29	44	75	82

Table 3 Deposition as function of particle size, inhaled volume and breathing frequency as determined by Heyder[1]. The residence time comprises the inhalation and exhalation and is calculated as volume divided by flow multiplied by two.

In a next experiment the group used the technique described above of immediate and 24 hours measurements of the radioactive count after administering insoluble radioactive aerosols to estimate the total, tracheobronchial and alveolar deposition. Again, one controlled the inhalation manoeuvre tightly and the results clearly indicate that the alveolar deposition (by sedimentation) of the smaller particles is very high[7]. For particles <2 μm the total and alveolar deposition are the same. Extrathoracic and tracheobronchial deposition of the larger particles were governed by inertial deposition, because a good correlation between the impaction parameter and the deposition existed, confirming the results of Lippmann[4]. Years later an extension of these investigations was published: the total and regional deposition of a larger range of particles became known (Fig. 1)[8].

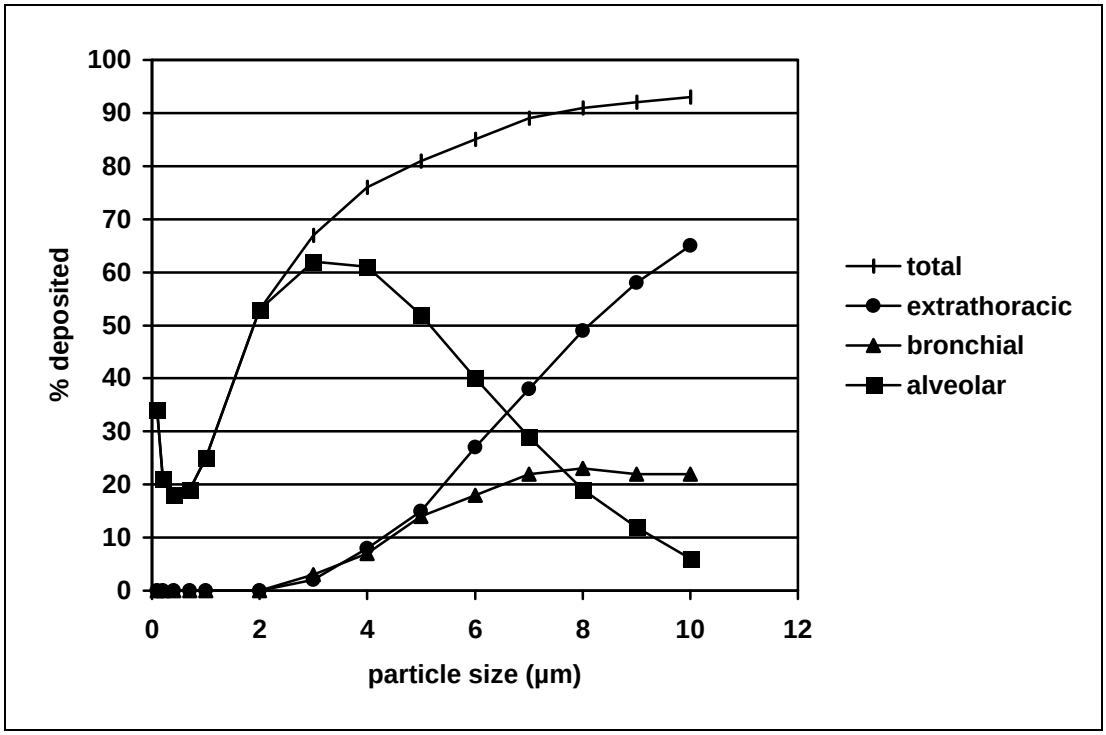


Fig. 1 In-vivo deposition of particles in the human airways[8].
(flow rate : 250 ml/s, tidal volume: 1000 ml)

In another experiment by the same group, one was able to prove that the total deposition is strongly governed by the aerodynamic particle size. Two types of aerosols were

administered: sebacate and iron oxide. Sebacate has a lower density than iron oxide and 23% of the 1 μm sebacate particles deposited compared to 50% of the heavier iron oxide particles. The aerodynamic particle size of iron oxide is by definition larger and when one corrected the deposition for these differences, the differences in deposition disappeared completely. Similar 'corrections' for the flow or residence time were calculated and the group was able to construct a so-called deposition parameter (X_m). This parameter normalises the total deposition in terms of particle size, particle density, residence time and inhalation flow. In other words, besides particle size, particle density, residence time and inhalation flow, there are no other factors governing total deposition[9].

$$X_m = \left(\lg \frac{Q}{Q_0} - 1.43 \right) \lg \left[\frac{r}{r_0} \left(\frac{d}{d_o} \right)^2 \left(\frac{t}{t_0} \right)^{24 / \sqrt{Q/Q_0}} \right]$$

in which: d= particle diameter (μm), d_0= 1 μm,

ρ= particle density (g/cm^3), ρ_0= 1 g/cm^3

V= inhalation flow (cm^3/s), V_0= 1 cm^3/s

F= breathing frequency (min^{-1}), F_0= 1 min^{-1}

The variability of the deposition in healthy volunteers

In most in-vivo experiments a considerable inter/intraindividual variability was noted. Tarroni tried to explain the variability by instructing volunteers to inhale 0.6 μm particles starting from different lung volumes, while keeping the vital capacity constant. He noted that lower starting volumes lead to an increased deposition[10]. Decreased airway dimensions at lower lung volumes no doubt caused this phenomenon.

Stahlhofen, using in-vivo deposition data and some deposition equations, was able to calculate the average airway diameter[11]. The results of these calculations point at a diameter coefficient of variation of approx. 23%, which according to Stahlhofen is caused by differences in extrathoracic deposition. His theory is based on the fact that a high extrathoracic deposition logically must result in a low airway deposition. So variability in the extrathoracic deposition causes highly different amounts of particles to penetrate into the lower airways. This theory was confirmed by two experiments of Svartengren, who found in two experiments that the mouth and throat deposition of 3.6

μm particles in asthmatics ranged from 7-66% resp. 9-76% of the dose administered[12] [13]. Heyder compared the variance of the total deposition using controlled and spontaneous breathing cycli[14]. He noted that the intraindividual variability was small, when the breathing cycle was controlled tightly. A second finding was a smaller interindividual variability after controlled compared to uncontrolled breathing. This lead Heyder to conclude that variability is caused by morphological *and* physiological factors. Controlling the breathing cycle eliminates the physiological factor and the interindividual variability left is due to the differences in airway anatomy. In later years Bennet also noted that the interindividual coefficient of variation of the alveolar deposition increased from 6% to 13% after switching from controlled to uncontrolled breathing[15].

Yeates and Svartengren, who both discovered a significant correlation between decreasing FEV_1, MMFR and MEF_{25} and a decreased deposition in the alveoli, substantiated the importance of morphological factors[16] [17]. Agnew, however, was unable to find such correlation: in his experiment only inhalation flow correlated inversely to the alveolar deposition. The volunteers, however, showed a small range of FEV_1's, making it harder to find a correlation[18]. Svartengren further examined the relation between airway morphology and deposition. He administered 4 μm Teflon particles, containing a radioactive label before and after metacholine-induced bronchoconstrictions in healthy volunteers. The airway resistance increased from 1.2 to 2.8 cm H_2O/l/s and at the same time the alveolar deposition decreased by 81%[19]. Repeating the experiment, but also measuring the deposition in the trachea showed that after bronchoconstriction the alveolar deposition decreased, while tracheal deposition increased[20].

Deposition in asthmatic patients

These kind of deposition experiments were soon extended to asthmatic patients, but from the start a different set up was chosen: many researchers administered radiolabelled particles of a small size range or only one size and visualised the deposition pattern of the particles by means of γ-cameras.

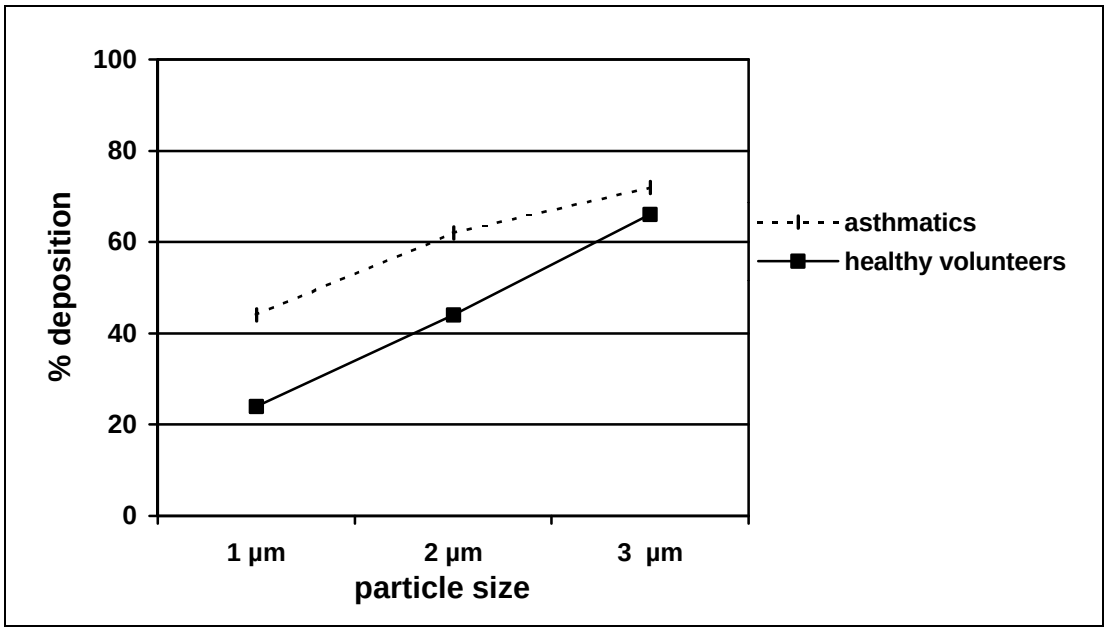


Fig. 2 Total airway deposition of non-hygroscopic particles in healthy and asthmatic subjects[22]

Only a few experiments were published in which the total deposition of a range of particle sizes was measured in the classic way, that is counting the difference between in- and exhaled number of particles. Anderson asked 5 asthmatic volunteers (mean FEV_1 40% of predicted) to inhale NaCl-particles in the range of 0.02 to 0.24 µm and compared the airway deposition to normal subjects. The results indicate that the deposition in asthmatics is increased. Anderson concluded that this is partly due to the constrictions of the airways, but also due to the longer residence time of particles in the asthmatic airways. The asthmatic volunteers were said to breath slower than the healthy volunteers[21]. Schiller-Scotland measured the total deposition of 1-3 µm particles in healthy and asthmatic volunteers. The results are shown in Fig. 2 and it is clear that differences in the deposition are almost nil as particle sizes equalled 3 µm (inspiratory flow 250 ml/sec, no breath holding). A second finding was that the deposition of 1 µm particles was inversely correlated to the FEV_1/IVC ratio and to the MEF_{50} values. These correlation's could not be demonstrated for the larger particles. Breath holding proved to be highly important, because the differences depicted in Fig. 2 disappeared when breath holding was changed to 6 sec, the increase in residence time being responsible for this effect[22].

Ramanna, using the γ-camera technique, visually scored γ-camera images of 70 volunteers, either healthy or asthmatic. He noticed that the 'asthmatic images' were characterised by a spotty and/or centrally orientated deposition. In a number of asthmatic volunteers these abnormal findings were present despite normal lung function tests. Ramanna interpreted this as a higher sensitivity of the aerosol penetration to small changes of the airway-anatomy compared standard lung function testing[23]. Lin, Taplin, and Santolicandro confirmed these observations[24 25 26].

Dolovich attempted to quantify the γ-camera images by counting the radioactivity in two areas: a central and peripheral one. The ratio of the central over the peripheral activity (C/P-ratio) bears a relation to the penetration of particles: a high ratio indicates a low penetration[27]. In a small group of patients with an average FEV_1 32.5% of predicted, Smaldone found that the mean C/P-ratio (for 1.5 μm particles) was 1.98 compared to 1.02 in healthy volunteers[28]. Chung and co-workers used this technique to demonstrate that a linear relationship between the conductance of the airways and the C/P ratio exists in asthmatics ($r=0.69$, $p=0.001$), while the changes due to dilation by salbutamol in the conductance and C/P ratio were also related ($r=0.68$, $p<0.001$)[29]. A disadvantage of the 'Dolovich-method' is that no rule exists how to define the central and peripheral fields in the scan and mostly it is done subjectively. This can bias researchers and several attempts were made to exclude this subjective element. Pavia scanned the thorax from the central to peripheral fields. By doing so he obtained a curve relating the distance from the midline and the intensity[30]. Laube scored the intensity of every pixel (= the smallest picture element) in the scan and constructed a frequency histogram. The more atypical the distribution of the aerosol is, the greater the deviation from the normal distribution will be and the histogram will show skewness and kurtosis. In asthmatic patients both parameters are highly significant, indicating strong deviations from the normal distribution. A relation between baseline FEV_1 and the degree of skewness/kurtosis was shown by Laube (confirmed by Olseni) and in cystic fibrosis patients[31 32 33].

Literature

[1] Wilson I.B. and LaMer V.K.
The retention of aerosol particles in the human respiratory tract as a function of particle radius.
J Ind Hyg Tox 30: 265-280 1948.

[2] Landahl H.D., Tracewell T.N. and Lassen W.H.
On the retention of air-borne particulates in the human lung.
Ind Hyg Occ Med ??: 359-366 1951.

[3] Landahl H.D.
On the removal of airborne droplets by the human respiratory tract: I. The lung.
Bull Math Biophys 12: 43-56 1950.

[4] Lippmann M. and Albert R.E.
The effect of particle size on the regional deposition of inhaled aerosols in the human respiratory tract.
Am Ind Hyg Ass J 30: 257-275 1969.

[5] Foord N., Black A. and Walsh M.
Pulmonary deposition of inhaled particles with diameters in the range 2.5 to 7.5 µm
In: Inhaled particles IV
Ed: Walton W.H.
Pergamon Press, Oxford, 1975

[6] Heyder J., Armbuster L., Gebhart J., Grein E. and Stahlhofen W.
Total deposition of aerosol particles in the human respiratory tract for nose and mouth breathing.
J Aerosol Sci 6: 311-328 1975.

[7] Stahlhofen W. Gebhart J. and Heyder J.
Experimental determination of the regional deposition of aerosol particles in the human respiratory tract.
Am Ind Hyg Ass J 41: 385-398 1980.

[8] Heyder J., Gebhart J., Rudolf G., Schiller C.F. and Stahlhofen W.
Deposition of particles in the human respiratory tract in the size range 0.005-15 µm.
J Aerosol Sci 17: 811-825 1986.

[9] Heyder J., Gebhart J., Rudolf G. and Stahlhofen W.
Physical factors determining particle deposition in the human respiratory tract.
J Aerosol Sci 11: 505-551 1980.

[10] Tarroni G., Melandri C., Prodi V., De Zaiacomo T., Formignani M. and Bassi P.
An indication on the biological variability of aerosol total deposition in humans.
Am Ind Hyg Assoc J 41: 826-831 1980.

[11] Stahlhofen W., Gebhart J. and Heyder J.
Biological variability of regional deposition of aerosol particles in the human respiratory tract.
Am Ind Hyg Assoc J 42: 348-353 1981.

[12] Svartengren M., Anderson M., Bylin G., Philipson K. and Camner P.
Mouth and throat deposition of 3.6 µm radiolabelled particles in asthmatics.
J Aerosol Med 4: 313-321 1991.

[13] Svartengren K., Lindestad P-Å, Svartengren M., Bylin G., Philipson K. and Camner P.
Deposition of inhaled particles in the mouth and throat of asthmatic subjects,

Eur Respir J 7: 1467-1473 1994.

[14] Heyder J., Gebhart J., Stahlhofen W. and Stuck B.
Biological variability of particle deposition in the human respiratory tract during controlled and spontaneous mouth-breathing.
Ann Occup Hyg 26: 137-147 1982.

[15] Bennet W.D. and Smaldone G.C.
Human variation in the peripheral air-space deposition of inhaled particles.
J Appl Physiol 62: 1603-1610 1987.

[16] Yeates D.B., Gerrity T.R. and Garrard S.
Characteristics of tracheobronchial deposition and clearance in man.
Ann Occup Hyg 26: 245-257 1982.

[17] Svartengren M., Hassler E., Philipson K. and Camner P.
Spirometric data and penetration of particles to the alveoli.
Br J Ind Med 43: 188-191 1986.

[18] Agnew J.E., Pavia P. and Clarke S.W.
Factors affecting the alveolar deposition of 5 μm inhaled particles in healthy subjects.
Clin Phys Physiol Meas 6: 27-36 1985.

[19] Svartengren M., Philipson K., Linnman L. and Camner P.
Airway resistance and deposition of particles in the lung.
Exp Lung Res 7: 257-269 1984.

[20] Svartengren M., Philipson K., Linnman L. and Camner P.
Regional deposition in human lung of 2.5 µm particles.
Exp Lung Res 12: 265-279 1987.

[21] Anderson P.J., Wilson D.J. and Hiller F.C.
Respiratory tract deposition of ultrafine particles in subjects with obstructive or restrictive lung disease.
Chest 97: 1115-1120 1990.

[22] Schiller-Scotland C.F., Gebhart J., Hochrainer D. and Siekmeier R.
Deposition of inspired aerosol particles within the respiratory tract of patients with obstructive lung disease
Toxicology Letters 88: 255-261 1996.

[23] Ramanna L., Tashkin D.P., Taplin G.V., Elam D., Detels R., Coulson A. and Rokaw S.N.
Radioaerosol lung imaging in chronic obstructive pulmonary disease. Comparison with pulmonary function tests and roentgenography.
Chest 68: 634-640 1975.

[24] Lin M.S. and Goodwin D.A.
Pulmonary distribution of an inhaled radioaerosol in obstructive pulmonary disease.
Radiology 118: 645-651 1976.

[25] Taplin G.V., Tashkin D.P., Chopra S.K., Anselmi O.E., Elam D., Calvarese B., Coulson A., Detels R. and Rokaw S.N.
Early detection of chronic obstructive pulmonary disease using radionuclide lungimaging procedures.
Chest 71: 567-575 1977.

[26] Santolicandro A. and Giuntini C.
Patterns of deposition of labelled monodisperse aerosols in obstructive lung disease.

J Nucl Med 23: 115-127 1979.

[27] Dolovich M.B., Sanchis J., Rossman C. and Newhouse M.T.
Aerosol penetrance: a sensitive index of peripheral airways obstruction.
J Appl Phys 40: 468-471 1976.

[28] Smaldone G.C. and Messina M.S.
Flow limitation, cough and patterns of aerosol deposition in humans.
J Appl Physiol 59: 515-520 1985.

[29] Chung K.F., Jeyasingh K. and Snashall P.D.
Influence of airway calibre on the intrapulmonary dose and distribution of inhaled aerosol in normal and asthmatic subjects.
Eur J Respir 1: 890-895 1988.

[30] Pavia D., Thomson M. and Shannon H.S.
Aerosol inhalation and depth of deposition in the human lung.
Arch Environ Health 32: 131-136 1977.

[31] Laube B.L., Limks J.M., Wagner H.N., Norman P.S., Koller D.W., LaFrance N.D. and Adams G.K.
Simplified assessment of fine aerosol distribution in human airways.
J Nucl Med 29: 1057-1065 1988.

[32] Olseni L., Palmer J. and Wollmer P.
Quantitative evaluation of aerosol deposition patterns in the lung in patients with chronic bronchitis.
Physiol Meas 15: 41-48 1994.

[33] Laube B.L., Limks J.M., LaFrance N.D., Wagner H.N., and Rosenstein B.J.
Homogeneity of bronchopulmonary distribution of ^{99m}Tc aerosol in normal subjects and in cystic fibrosis patients.
Chest 95: 822-830 1989.

The deposition of therapeutic aerosols

Introduction

The character and way of administration of therapeutic aerosols differs profoundly from the non-therapeutic aerosols described above. Most 'non-therapeutic' experiments were performed with non-hygroscopic particles, while aerosols were inhaled from still air. Therapeutic aerosols frequently contain hygroscopic drugs and are not delivered from still air. Therefore, the deposition characteristics of therapeutic systems can (and will) differ heavily and is reviewed below, with special emphasis on the influence of the formulation on the deposition. Subsequently the relation between particle size distribution and therapeutic effect is discussed.

Deposition of metered dose inhalers

Numerous experiments have been carried out to determine the deposition efficacy of the metered dose inhaler (MDI) by measuring the percentual deposition. Although from study to study, the outcome varies somewhat, overall results indicate that a small amount of the dose emitted actually enters the lungs. Most experiments report that 10-20% of the dose delivered, ends up in the airways and alveoli[1 2 3 4]. The reasons for this inefficacy lies in the way patients use MDI's and in the formulation. Aerosols generated by MDI's are characterised by large primary droplets showing high velocities. Early measurements of the droplet sizes indicate a MMAD of 2.8-4.3 μm, depending on the brand tested[5]. The aerosols measured, however, resided in a flask for 1 to 4 min before measurement, so the results indicate the size *after* evaporation of the liquid propellants: the primary size must have been larger. Later, slightly larger sizes (MMAD 2.3 to 8.3 μm) were reported, but again these aerosols were dried before measurement[6]. The initial droplet size of a MDI aerosol is said to be approximately 30 μm and the droplets leave the inhaler with high speeds (±120 km/h)[7]. These circumstances make it very likely that the majority of the droplets impact in the oropharynx, because there is not sufficient time for evaporation. The result is only 10-20% effective deposition in the lower airways.

Decreasing the primary droplet size seems to be the logical solution. Unfortunately, this can only be achieved by increasing the pressure in the canister of the MDI. By increasing the canister-pressure from 374 kPa to 502 kPa, Morén was able to decrease the mouth deposition from 12.7% to 8.3%. This effect was, however, only noticeable at a low metered volume, which is equivalent to a low emitted dose. Increasing the metered volume increased the mouth deposition, despite the pressure used[8]. In later experiments, using technetium labelled aerosols, it was found that a high pressure MDI delivered 17.6% of the dose to the airways and alveoli, compared to 13.6% of the low-pressure inhaler. The oropharyngeal deposition decreased by a small 3.9% to 72.8%[9]. The advantages of a reduced primary droplet size seem to be minimal: the higher pressure necessary increases the inertia of the droplets and this factor counterbalances the beneficial effects of size reduction.

A remark has to be made: the results above were obtained by using so called suspension aerosols. The drug crystals in these aerosols do not dissolve in the propellants, but form a suspension. This means that in a primary droplet several crystals will be included, so the secondary size is dependent on the number (and size) of the crystals present in the primary droplet. The gain of reducing the primary droplet is therefore on forehand limited. If one dissolves the drug in the propellants one can avoid the above mentioned problems: experiments, using a model drug, showed that a lung deposition of 39% is achievable with propellant soluble drugs. Improving the administration using a spacer further increased the lung deposition to 57%[10]. Again, as with suspension MDI's, an increase in canister pressure (from 255 kPa to 488 kPa) causes smaller primary droplets and an increase in lung deposition (from 50.9% to 65%)[11]. Only few drugs are dissolvable in the propellants.

spacers

An effective way to improve drug delivery from MDI's is to slow down the high initial droplet speed and to remove any liquid propellant by evaporation. This can be achieved by firing the aerosol into a temporary container, a spacer device. Currently many of these devices are available and although based on the same principle it has become clear that different brands of spacers can elicit a profound change in the aerosol cloud characteristics.

Dolovich reported that a small holding chamber lowered the oropharyngeal deposition from 71% of the emitted dose to 4%, while the total lung deposition remained unaltered. The deposition pattern, however, changed to a more peripheral profile: 25% of the total lung mass was recovered from the outer lung zone following the MDI compared to 34% of the combination[12]. Newman reported the same for another spacer device, the oropharyngeal deposition decreased form 81% to 17% and the lung deposition increased form 8.7% to 21%. In the spacer 56% of the emitted dose deposited[13]. These data illustrate the general character of spacers: a considerable amount of drug deposits within the spacer itself and that mass is of course not available for inhalation. Judging from the lowered oropharyngeal deposition, the retained mass must consist of large particles. In this way a spacer acts as a separating device taking out the bulk of the large particles, while offering sufficient evaporation time for smaller droplets. These droplets will decrease in size, enabling them to enter the lower airways in higher numbers.

That separating action of spacers is related to their construction. Vidgren showed that the spacer deposition can vary enormously between different brands. A sodium cromoglycate MDI elicited an oropharyngeal deposition of 81%, lowered to 56% by a spacer called Inhalet® and to 22% by an Inspirease®. The lung deposition ranged from between 16% to 20%[14]. Many others confirmed these data. Holzner reported that the dose deposited in the spacer depends on the spacer design, but also the MDI brand (see Table 1). It therefore seems hard to predict the characteristics of a spacer-MDI combination[15].

Spacer	Metered dose inhaler A	Metered dose inhaler B
Inhacort (telescope)	29	25.4
Inhacort (normal)	40.2	29.2
Viarox	37.9	19.3
Aru	35.7	37.5
Rondo	48.6	35.9
Fisonair	44.5	31.8
Nebulator	66.6	61.5
Beclomet	83.6	74.4

Spacer	Metered dose inhaler A	Metered dose inhaler B
Volumatic	77	71.9

Table 1 Percentage of the emitted dose retained in the spacer of several spacer-MDI combinations[15]

Barry and O'Callaghan reported an identical 'unpredictable' behaviour of several combinations[16, 17]. It is no surprise that Hindle subsequently showed that the systemically absorbed amount of salbutamol highly differed between several combinations: the total 24^{h} salbutamol urinary recovery varied between 44% of the emitted dose and 27%[18]. Later on it was shown that the mass output of spacers is negatively influenced by electrostatic charges or by multiple actuations[19]. Removing the electrostatic charges increased the mass of drug delivered to the lungs[20].

The conclusion of 'unpredictablility' can also be drawn with respect to the particle size distribution. In the same experiment Holzner also measured the aerosol mass consisting of particles <6.4 µm (Table 2)[15].

Spacer	Metered dose inhaler A	Metered dose inhaler B
Inhacort (telescope)	26.6	36.5
Inhacort (normal)	26	32.1
Viarox	27	41.7
Aru	29	25.2
Rondo	25.5	32.7
Fisonair	23.9	27.6
Nebulator	16.8	21.4
Beclomet	4.2	6.7
Volumatic	4.4	8.3

Table 2 Percentual particle mass <6.4 µm inhaled from several MDI-spacer combinations[15]

Similar findings have been reported by others, all showing widely different aerosol particle size distributions[16 17 21]. The reason for these differences mostly remains unexplained. Some blame the design of an exhalation valve in the spacer (they too

much act as an impactor for inhaled particles), while others stress the electrostatic properties. More work needs to be done to elucidate this problem.
The conclusion is that, in general, a spacer improves the deposition characteristics of a MDI-aerosol cloud from by removing large particles, but the extent of this removal depends on many (unknown) factors. Interchangeability of spacers is an issue not solved.

Dry powder inhalers

Dry powder inhalers lack the hand/lung co-ordination problems of MDI's and are easier to use. The powder formulation comprises frequently a blend of lactose and micronised drug. The lactose acts as a bulking agent, while it prevents the micronised drug from agglomeration. During inhalation the inspired air aerosolises the blend and turbulent air separates the drug crystals from the lactose. Inhalers do exist, which contain lactose free formulations, but even with these a separation process is present, rendering them not basically different from lactose based inhalers. The efficacy in terms of lung deposition depends on several factors, like the drug itself, the design of the inhaler, the height of the inspiratory flow[22]. In the following paragraphs the effects of some of these factors will be discussed.

Inhaler design

Dry powder inhalers (DPI) are generally believed to show lower deposition percentages as MDI's. Melchor radiolabelled salbutamol raw material and incorporated it into both a MDI and a DPI, subsequently measuring the total lung deposition as percentage of the nominal dose. In healthy volunteers 22% of the MDI dose was recovered form the lungs opposed to 12.5% of the DPI dose, in asthmatics these figures were, respectively, 18.2% and 11.4%[4]. Others found similar results[23]. The actual deposition is, however, highly dependent on the DPI itself: comparing the deposition of a Turbuhaler with that of a MDI lead Thorsson to conclude that the Turbuhaler's deposition was twice as high. They measured the systemic uptake of budesonide after a Turbuhaler of MDI and reported a systemic availability of 32% for the Turbuhaler and 15% for the MDI[24]. Later this was also shown for a terbutaline Turbuhaler[25]. However, one has to remember that measuring the systemic availability does not necessarily reflect the total deposition of drug in the lung: part of the drug mass

might not be absorbed. This is most likely to occur in the upper airways where mucociliary transport is fast and the mucosa relatively thick. So absorption might be dependent on the deposition pattern. The latter would have been measured by scintigraphy.

Vigdren manufactured a radiolabelled sodium cromoglycate/lactose blend and this 'standard' formulation was inhaled through four inhalers (Spinhaler®, ISF®, Ingelheim® and Rotahaler®). The deposition within the (healthy) lungs differed considerably: 16.4% of the dose after the ISF-inhaler® compared to 6.2% after the Rotahaler[26]. The reason given for this large difference is the (in)efficacy to aerosolise the drug. When powder is insufficiently aerosolised, it will be retained in the inhaler. The direct effect being a low dose delivered to the patient and too much drug/lactose complexes too large to penetrate into the airways.

Inhalation flow

The powder in a DPI is to be aerosolised by the air inhaled by the patient and intuitively the more air inhaled the better the aerosolisation. The latter indicates that the aerosol created is effort-dependent and much research has gone into this area. A possible approach is to measure the resistance of the inhaler and peak inspiratory flow through that inhaler. This approach is based on the assumption that to obtain a good dispersion of the powder, a minimum amount of air at a minimum flow must be inhaled through the DPI. Should a patient not be able to meet these requirements, he or she should use another and more suitable DPI. Clark has determined the resistance of several DPI's and the data are depicted in Table 3[27].

Device	Resistance (cm $H_2O^{1/2}$/l/min)
Rotahaler®	0.040
Spinhaler®	0.051
ISF inhaler®	0.055
Diskhaler®	0.067
Turbuhaler®	0.100
Inhalator Boehringer Ingelheim®	0.180

Table 3 Resistance of six commercial inhalers[27]

They also measured the maximum pressure drop over the inhaler volunteers could generate and noted that, even at maximum effort, a maximum pressure-drop when the inhaler resistance was larger than 0.1 cm H_2O/(l/min). Apparently the diaphragm and intercostal muscles strength limits the pressure drop over the inhaler one can generate. Later Ross showed that an increase of the flow through an inhaler is equivalent with a reduction of the MMAD and an increase of the mass < 5µm[28]. A relation between a low inspiratory flow, dispersion and suboptimal clinical effects is apparent.

Engel, who taught patients to inhale at increasing flows through a Turbuhaler, investigated the clinical effect of these varying characteristics[29]. The highest flow was 84 l/min, releasing 86% of a 5 mg terbutaline dose, the lowest flow 34 l/min resulting in inhaling 58% of the same dose. No significant differences in bronchodilation were found, nor were they present with respect to side effects. Zanen et al found the same when testing the ISF inhaler with a salbutamol/lactose blend at 40 l/min or 80 l/min[30]. The drug itself, however, is of importance: Bisgaard showed inhalation flow dependence when testing the same ISF device but now with formoterol[31].

The conclusion of all data above is that the characteristics of the aerosol cloud from a DPI are highly dependent on the inhaler itself and no general statements are possible.

The effects of particle size distribution on the clinical effect

From the earlier chapters it is clear that size has a profound effect on the penetration of particles into the lower airways. It is logical to expect that the degree of penetration and deposition patterns of therapeutic aerosols influences the beneficial effects of an inhaled drug. In the sections below the relationship between size and clinical effect is elucidated.

Bronchodilators and sodium cromoglycate

The first report, linking the effect of an aerosol to particle size distribution, comes from Godfrey. Using a spinning disc nebuliser he generated 2 µm and 11.7 µm sodium cromoglycate particles, in both cases approximately 30 mg of drug were inhaled. Subsequent exercise testing showed that the therapeutic effect of the 11.7 µm aerosol was not significantly different from placebo, only the smaller 2 µm aerosol was effective in reducing EIB. The probable explanation for this finding is that 11.7

µm particles of a highly hygroscopic drug will grow into very large droplets in the humid airways, so it can be doubted whether these particles ever reached the lower airways in an effective quantity. It appears that two placebo's were compared to one 'active' preparation[32]. Later Ruffin administered 3.3 µm and 1.5 µm isoproterenol aerosols and noted that the cumulative dose-response curve of the 1.5 µm aerosol was shifted to the right, indicating a lower potency. Whether this shift was significant, is not mentioned, however[33].

Rees milled terbutaline crystals down to three different size ranges. The mass median diameters were 5.6 µm, 9.1 µm and 13.6 µm respectively, but the distribution showed strong polydispersity with considerable overlap. These crystal sizes were incorporated into standard MDI's and it was shown that only the smallest aerosol improved the FEV_1, sG_{aw} and MEF_{50} significantly. The two remaining aerosols had no dilating effect at all and the investigators concluded that effective particles should be smaller than 5 µm[34]. Using three different nebulisers, Clay generated terbutaline aerosols with a mass median diameter of 1.8 µm, 4.6 µm and 10.3 µm. All aerosols elicited significant changes with respect to baseline. Except in case of the $MEF_{50/25}$, where the smallest aerosol was most potent, none of the aerosols differed significantly amongst each other[35]. A year later Clay showed that 80% of the 1.8 µm aerosol deposited in the lung compared to 60% and 44% of the 4.6 µm and 10.3 µm aerosols[36].

Again using different types of nebulisers, Mitchell generated 1.4 µm and 5.5 µm salbutamol aerosols. The lung deposition was studied by radiolabelling and no significant difference was found between the deposition patterns. Mitchell constructed cumulative dose-FEV_1 curves and these curves showed to be superimposable[37]. These conclusions, which are only based on the FEV_1-improvement, are in line with those of Clay, t.i. no clear cut relationship between particle size distribution and clinical efficacy.

The data above suggest that the clinical effect of bronchodilator aerosols is not strongly influenced by the size distribution, but some evidence emerged that particles below 5 µm are more potent. The importance of that mass <5 µm was shown by Persson, who modified the mouthpiece of terbutaline Turbuhalers in such a way that the inhalers delivered either 90 µg, 40 µg or 5 µg as <5 µm particles. The increase in

FEV_1 with the 5 µg mouthpiece was significantly lower than with the higher dose mouthpieces. The latter two did not differ from each other[38].

Johnson reported that 3.3 µm particles were more potent than 7.7 µm particles[39]. Two different nebulisers generated these particles and both for ipratropium bromide and salbutamol cumulative dose-response curves were constructed. The ipratropium curves were not significantly different for all lung function parameters measured, while the salbutamol curves did show a higher potency for the smallest aerosol. Radiolabelling studies did not show significant changes in deposition patterns of the aerosols, but a higher total lung dose of smaller aerosol. This indicates that the oropharyngeal passage of the 3.3 µm aerosol is better, which is apparently the explanation for the higher potency. Patel and co-workers used a spinning top generator to manufacture monodisperse 2.5 µm and 5 µm isoproterenol aerosols[40]. The advantage of this approach is that it has an higher discriminative power: nebuliser aerosols do generate highly polydisperse aerosols and the overlap in the particle size distributions tend to obscure differences in size-effects. The 2.5 µm dose-response curves of Patel for all parameters measured were shifted to the left, indicating a higher potency. He calculated that on a weight basis the 2.5 µm aerosol was 2 to 4 times more potent compared to the 5 µm aerosol. This conclusion is however questionable because one did not control the inhaled dosage accurately.

The parallel designed study by Hultquist comparing 1.5 µm and 4.8 µm particles generated by two different nebulisers could not show an advantage for the smaller aerosol[41]. Neither the cumulative FEV_1 dose response curves, nor parameters for gas-exchange did show significant differences.

Histamine/methacholine

Only a few experiments relating particle size and histamine/methacholine efficacy have been performed. Ruffin administrated 1.5 µm or 3 µm methacholine aerosols by means of two nebulisers. Like the bronchodilator experiments, Ruffin noted a considerable right shift of the 1.5 µm dose-response curves. He calculated that the average potency-ratio was approx. 15:1 in favour of the 3 µm aerosol[42]. Contrary to these findings, Ryan could not find any influence of the particle size distribution[43]. He compared 1.32 µm and 3.6 µm methacholine aerosols using (as usual) different

nebulisers and corrected for differences in the mass output of the nebulisers. He noted that the weight loss per minute per nebuliser differed and that the ratio of the PC_{20} and the mass output per minute was very similar. Based on that observation he concluded that nebuliser mass output is highly important, whereas particle is size not. To complicate interpretation, Laube could show that a central deposition of methacholine was equivalent to lower PD_{20}-values[44]. She used only one type of nebuliser, but asked volunteers to inhale at high flows (60 l/min) to induce a central deposition pattern or at low (12 l/min) flows for a more peripheral pattern. The first was equivalent with a significantly lower PD_{20}: log PD_{20} 0.77 versus 1.20. This preference for a central methacholine distribution was confirmed by Schmekel et al. by comparing a 2 µm and 9 µm aerosol[45].

Next to the above experiments into the aerosol size and histamine/methacholine relationship, several researchers tried to explain the interindividual variability of the responsiveness to inhaled histamine/methacholine by correlating it to the inhaled dose of these drugs. Donna concluded that in a group of 10 healthy volunteers and asthmatics that differences in methacholine responsiveness are not due to different masses inhaled from the nebuliser, but to the variability of the intrinsic abnormality[46]. One also concluded that, in healthy volunteers, the degree of bronchoconstriction is correlated to the inhaled dose, which was not found in asthmatics. This can be expected to a certain extent. Any correlation between inhaled dose and responsiveness will disappear as the interindividual variability of the latter increases. The geometric SD of the methacholine responsiveness was 6.7 in the asthmatic group and 2.6 in the healthy volunteers. This alone makes it hard to find significant correlations in such small samples.

Gillet, too, was unable to discover a correlation between inhaled dose and methacholine responsiveness much for the same reason as above[47]. However, one did find a significant correlation between the inhaled atropine dose and the elicited decrease of the methacholine responsiveness. Atropine is a methacholine antagonist, so the higher the dose deposited, the stronger the antagonism: a correlation between atropine dose and methacholine responsiveness seems logical. Anderson again noted that the interindividual methacholine responsiveness variability exceeded that of the aerosol deposition, so deposition variability can not explain it[48].

Literature

[1] Newman S.P., Pavia D., Morén F., Sheahan N.F. and Clarke S.W.
Deposition of pressurised aerosols in the human respiratory tract.
Thorax 36: 52-55 1981.

[2] Morén F.
Drug deposition of pressurised inhalation aerosols.
Eur J Respir Dis 63 (suppl 119): 51-55 1982.

[3] Biddiscombe M.F., Melchor R., Mak V.H.F., Marriot R.J., Taylor A.J. Short M.D. and Spiro S.G.
The lung deposition of salbutamol, directly labelled with technetium-99m, delivered by pressurised metered and dry powder inhalers.
Int J Pharm 91: 111-121 1993.

[4] Melchor R., Biddiscombe M.F., Mak V.H.F., Short M.D. and Spiro S.G.
Lung deposition patterns of directly labelled salbutamol in normal subjects and in patients with reversible airflow obstruction.
Thorax 48: 506-511 1993.

[5] Hiller C., Mazumder M., Wilson D. and Bone R.
Aerodynamic size distribution of metered dose bronchodilator aerosols.
Am Rev Resp Dis 118: 311-317 1978.

[6] Bouchikhi A., Becquemin M.H., Bignon J. Roy M. and Teillac A.
Particle size study of nine metered dose inhalers and their deposition probabilities in the airways.
Eur Respir J 1: 547-522 1988.

[7] Morén F.
Aerosols dosage forms and formulations.
in: Morén F., Newhouse M.T., and Dolovich M.B.
Aerosols In Medecine
Amsterdam, Elsevier, 1985.

[8] Morén F.
Drug deposition of pressurised inhalation aerosols II. Influence of vapour pressure and metered volume.
Int J Pharm 1: 213-218 1978.

[9] Newman S.P., Morén F., Pavia D., Corrado O. and Clarke S.W.
The effects of changes in metered volume and propellant vapour pressure on the deposition of pressurised inhalation aerosols.
Int J Pharm 11: 337-344 1982.

[10] Ashworth H.L., Wilson C.G., Sims E.E. and Hardy J.J.
Delivery of propellant soluble drugs from a metered dose inhaler.
Thorax 46: 245-247 1991.

[11] Harnor K.J., Perkins A.C., Wastie M., Wilson C.G., Sims E.E., Feely L.C. and Farr S.J.
Effect of vapour pressure on the deposition pattern from solution phase metered dose inhalers.
Int J Pharm 95 111-116 1993.

[12] Dolovich M., Ruffin R. and Newhouse M.T.
Clinical evaluation of a simple demand inhalation MDI aerosol delivery device.

Chest 84: 36-41 1983.

[13] Newman S.P., Millar A.B., Lennard-Jones T.R., Morén F. and Clarke S.W.
Improvement of pressurised aerosol deposition with Nebuhaler spacer device
Thorax 39: 935-941 1984.

[14] Vidgren M.T., Paronen T.P., Karkkainen A. and Karjalainen P.
Effect of extension devices on the drug deposition from inhalation aerosols
Int J Pharm 39: 107-112 1987.

[15] Holzner P.M. and Müller B.W.
An in vitro evaluation of various spacer devices for metered dose inhalers using the Twin Impinger
Int J Pharm 106: 69-75 1994.

[16] Barry P.W. and O'Callaghan C.
In vitro comparison of the amount of salbutamol available for inhalation from different formulations used with different spacer devices.
Eur J Respir 10: 1345-1348 1997.

[17] Barry P.W. and O'Callaghan C.
Inhalational drug delivery from seven different space devices
Thorax 51: 835-840 1996.

[18] Hindle M. and Chrystyn H.
Relative bioavailability of salbutamol to the lung following inhalation using metered dose inhalation methods and spacer devices.
Thorax 49: 549-553 1994.

[19] Wildhaber J.H., Devadason S.G., Eber E., Hayden M.J., Everard M.L., Summers Q.A. and LeSouëf P.N.
Effect of electrostatic charge, flow, delay and multiple actuation's on the in vitro delivery of salbutamol from different small volume spacers for infants.
Thorax 51: 985-988 1996.

[20] O'Callaghan C., Lynch J. and Robertson C.
Improvement in sodium cromoglycate delivery from a spacer device by use of an antistatic lining, immediate inhalation and avoiding multiple actuations of drug.
Thorax 48: 603-606 1993.

[21] Kenyon C.J., Dewsbury N. J. and Newman S.P.
Differences in aerodynamic particle size distributions of innovator and generic beclomethasone dipropionate aerosols used with and without a large volume spacer.
Thorax 50: 846-850 1995.

[22] Timsina M.P., Martin G.P., Marriott C., Ganderton D. and Yianneskis ?.
Drug delivery to the respiratory tract using dry powder inhalers.
Int J Pharm 101: 1-13 1994.

[23] Zainudin B.M.Z., Biddiscombe M., Tolfree S.E.J., Short M. and Spiro S.G.
Comparison of bronchodilator responses and deposition patterns of salbutamol inhaled from pressurised metered dose inhaler, as dry powder inhaler and as a nebulised solution.
Thorax 45: 469-473 1990.

[24] Thorsson L., Edsbäcker S. and Conradson T-B.
Lung deposition of budesonide from Turbuhaler is twice that from a pressurized metered dose inhaler P-MDI.

Eur Respir J 7: 1839-1844 1994.

[25] Borgström L., Derom E., Ståhl E., Wåhlin-Boll E. and Pauwels R.
The inhalation device influences lung deposition and bronchodilating effect of terbutaline.
Am J Respir Crit Care Med 153: 1636-1640 1996.

[26] Vidgren M., Kärkäinen A., Karjalainen P., Paronen P. and Nuutinen J.
Effect of powder inhaler design on drug deposition in the respiratory tract.
Int J Pharm 42: 211-216 1988.

[27] Clark A.R. and Hollingworth A.M.
Th relationship between powder inhaler resistance and peak inspiratory conditions in healthy volunteers-implications for in-vitro testing.
J Aerosol Med 6: 99-110 1993.

[28] Ross D.L. and Schultz R.K.
Effect of inhalation flow rate on the dosing characteristics of dry powder inhaler and metered dose inhaler products.
J Aerosol Med 9: 215-226 1996.

[29] Engel T., Scharling B., Skovsted B. and Heinig J.H.
Effects, side effects and plasma concentrations of terbutaline in adult asthmatics after inhaling from a dry powder inhaler device at different inhalation flows and volumes.
Br J Clin Pharmac 33: 439-444 1992.

[30] Zanen P., van Spiegel P.I., van der Kolk H., Tushuizen E. and Enthoven R.
The effect of the inhalation flow on the performance of a dry powder inhalation system.
Int J Pharm 81: 199-203 1992.

[31] Nielsen K.G., Skov M., Klug B., Ifversen M.and Bisgaard H.
Flow-dependent effect of formoterol dry-powder inhaled from the Aerosizer.
Eur J Respir 10: 2105-2109 1997.

[32] Godfrey S., Zeidifard E., Brown K. and Bell J.H.
The possible site of action of sodium cromoglycate assessed by exercise challenge.
Clin Sci Mol Med 46: 265-272 1974.

[33] Ruffin R.E., Dolovich M.B., Oldenburg F.A. and Newhouse M.T.
The preferential deposition of inhaled isoproterenol and propanolol in asthmatic patients.
Chest 80: 904-907 1981.

[34] Rees P.J., Clark T.J.H.a nd Morén F.
The importance of particle size in response to inhaled bronchodilators.
Eur J Respir Dis suppl 119: 73-78 1982.

[35] Clay M.M., Pavia D. and Clarke S.W.
Effect of aerosol particle size on bronchodilation with nebulised terbutaline in asthmatic subjects.
Thorax 41: 364-368 1986.

[36] Clay M.M. and Clarke S.W.
Effect of nebulised aerosol size on lung deposition in patients with mild asthma.
Thorax 42: 190-194 1987.

[37] Mitchell D.M., Solomon M.A., Tolfree S.E.J., Short M. and Spiro S.G.
Effect of particle size of bronchodilator aerosols on the lung distribution and pulmonary function in patients with chronic asthma.

Thorax 42: 457-461 1987.

[38] Persson G. and Wirén J.E.
The bronchodilator response from inhaled terbutaline is influenced by the mass of small particles: a study on a dry powder inhaler (Turbuhaler).
Eur Respir J 2: 253-256 1989.

[39] Johnson M.A., Newman S.P., Bloom R., Talaee N. and Clarke S.W.
Delivery of albuterol and ipratropium bromide from two nebulizer systems in chronic stable asthma.
Chest 96: 1-10 1996.

[40] Patel P., Mukai D. and Wilson A.F.
Dose-response effects of two sizes of monodisperse isoproterenol in mild asthma.
Am Rev Respir Dis 141: 357-360 1990.

[41] Hultquist C., Wollmr P., Eklund G. and Jonson B.
Effect of inhaled terbutaline sulphate in relation to its deposition in the lungs.
Pulm Pharm 5: 127-132 1992.

[42] Ruffin R.E., Dolovich M.B., Wolff R.K. and Newhouse M.T.
The effects of preferential deposition of histamine in the human airway.
Am Rev Respir Dis 117: 485-492 1978.

[43] Ryan G., Dolovich M.B., Obminski G., Cockcroft D.W., Juniper E., Hargreave F.E. and Newhouse M.T.
Standardization of inhalation provocation tests: influence of nebulizer output, particle size and method of inhalation.
J Allergy Clin Immnuol 76: 156-161 1981.

[44] Laube B.L., Normann P.S. and Adams G.K.
The effect of aerosol distribution on airway responsiveness to inhaled methacholine in patients with asthma.
J Allergy Clin Immunol 89: 510-518 1992.

[45] Schmekel B., Hedenström H., Kämpe M., Lagerstrand L., Stålenheim G., Wollmer P. and Hedenstierna G.
The bronchial response, but not the pulmonary response to inhaled methacholine is dependent on the aerosol deposition pattern.
Chest 106: 1781-1787 1994.

[46] Donna E., Danta I., Kim C.S. and Wanner A.
Relationship between deposition of and responsiveness to inhaled methacholine in normal and asymptomatic subjects.
J All Clin Immunol 83: 456-461 1989

[47] Gillett M.K., Briggs B.A. and Snashall P.D.
The influence of aerosol retention and pattern of deposition on bronchial responsiveness to atropine and methacholine in humans.
Am Rev Respir Dis 140: 1727-1733 1989

[48] Anderson P.J., Garshick E., Blanchard J.D., Feldman H.A. and Brain J.D.
Intersubject variability in particle deposition does nor explain variability in responsiveness to methacholine.
Am Rev Respir Dis 144: 649-654 1991

Aim of the study

The data on a relationship between particle size and clinical efficacy, as reviewed in the previous chapter, are hard to interpret. Results of bronchodilators studies are contradictory: early trials suggest that particles simply should be smaller than 5 µm and that the actual particle size below that 5 µm is irrelevant[1 2 3]. Other researchers, however, do show differences in the degree of bronchodilation in the <5 µm size range[4 5 6]. Unfortunately, an optimal particle size for a bronchodilator aerosol cloud can not be derived from the latter experiments. Frequently one compared just two particle sizes *and* amongst the studies the range of sizes varied.

The contradictions on the importance of the below 5 µm size may find their origin in the experimental set up of the studies. Most experiments show a common fundamental weakness: in order to generate the aerosols, one uses different nebulisers. The nebulisers generate smaller of larger *polydisperse* aerosols, which renders it hard to define the effect of a changing particle size due to the considerable overlap in size distributions[7 8]. The stronger that overlap, the lower the chance to find a particle size effect. So, whether one reports a significant size/efficacy relationship or not, seems to be nebuliser dependent. Studies, using nebulisers with narrow particle size distributions, have the highest probability is to find a particle size efficacy relationship. Information on that distribution is lacking in the reports.

Second confounders are the differences in nebuliser output. It is well known that the different types nebulisers used show widely different mass outputs[7 8 9]. It is conceivable that a high mass output of a large particle nebuliser counterbalanced the low particle size efficacy. This effect of course lowers the discriminative power of the study, favouring the conclusion of non-significant differences. So an accurate estimation of the mass output is highly advisable, but most experiments did not control this confounder.

To summarise, based the data in chapters 2 and 3, a relationship between the particle size of bronchodilators aerosols and clinical effect seems highly probable, but not confirmed due to experimental problems. Overlapping distributions of polydisperse aerosols should be avoided by generating aerosols with a very narrow size distribution. Only then it is possible to discriminate between particle sizes. At the same time the inhaled dose should be controlled tightly by correcting differences in mass output. The study by Patel addressed the issue of overlapping size distributions: one

used a spinning top generator to generate monodisperse aerosols, but unfortunately in that study a control of the dose was lacking[4].

The aims of our studies are therefore:

1. to define the optimal particle size for bronchodilator drugs. The range of particle sizes studied may not be too small: based on the modelling and in-vivo deposition studies, a range between 1 µm and 5 µm seems to be a reasonable choice. Bronchodilators, as monodisperse aerosols, will be administered to asthmatic patients under tight control of the dose. The selection of the best particle size is then straightforward because the strongest dilation will be elicited by the optimal aerosol size. These experiments will be carried out in both mild and severe asthmatics, because the actual airway diameter can influence the choice of the optimal particle size. (To ensure sufficient penetration in constricted airways a smaller particle size might be warranted). Mutatis mutandis this also goes for different types of bronchodilators: ß$_2$-mimetics may require another aerosol formulation than parasympathicolytics.
2. Having defined an optimal particle size, one than can determine how much mass of such an optimal aerosol formulation one has to administer to obtain a bronchodilation equivalent to MDI's. It is expected that the optimal aerosol dose is lower. This then informs on the extent of the so called non-active (=non-dilatory) part of MDI aerosols (the latter the result of generating non-penetrating particles).
3. Directly linked to that experiment one can ask oneself what then is the role, in terms of adverse effects, of that non-dilatory part of the emitted dose is.

Literature

[1] Rees P.J., Clark T.J.H. and Morén F.
The importance of particle size in response to inhaled bronchodilators.
Eur J Respir Dis suppl 119: 73-78 1982.

[2] Clay M.M., Pavia D. and Clarke S.W.
Effect of aerosol particle size on bronchodilation with nebulised terbutaline in asthmatic subjects.
Thorax 41: 364-368 1986.

[3] Mitchell D.M., Solomon M.A., Tolfree S.E.J., Short M. and Spiro S.G.
Effect of particle size of bronchodilator aerosols on the lung distribution and pulmonary function in patients with chronic asthma.
Thorax 42: 457-461 1987.

[4] Patel P., Mukai D. and Wilson AF.
Dose-response effects of two sizes of monodisperse isoproterenol in mild asthma.
Am Rev Respir Dis 141: 357-360 1990.

[5] Ruffin R.E., Dolovich M.B., Oldenburg F.A. and Newhouse M.T.
The preferential deposition of inhaled isoproterenol and propanolol in asthmatic patients.
Chest 80: 904-907 1981.

[6] Johnson M.A., Newman S.P., Bloom R., Talaee N. and Clarke S.W.
Delivery of albuterol and ipratropium bromide from two nebulizer systems in chronic stable asthma.
Chest 96: 1-10 1996.

[7] Loffert D.T., Ikle D.and Nelson H.S.
A comparison of commercial jet nebulizers.
Chest 106: 1788-1793 1994.

[8] Smith E Dnyer J. and Kendrick A.H.
Comparison of twenty three nebulizer/compressor combinations for domiciliary use.
Eur J Respir 8:1214-1224 1995.

[9] Thomas S.H.L., O'Doherty M.J. Page C.J. and Nunan T.O.
Variability in the measurement of nebulized aerosol deposition in man.
Clin Sci 81: 767-775 1991.

The optimal particle size for beta-adrenergic aerosols in mild asthmatics*

Introduction

The treatment of asthma and chronic obstructive pulmonary diseases (COPD) has improved considerably with the introduction of drugs by inhalation. As compared to oral administration, dosages could be decreased substantially and the incidence of side effects was diminished considerably. Unfortunately, the currently available inhalation preparations show one major disadvantage: only a small quantity of the administered mass reaches the airways[1]. Part of the problems is caused by the fact that only highly polydisperse aerosols are available. Such aerosols contain large particles, which are not effective, because they deposit extrathoracical.

The site of deposition of the particles in the airways depends strongly on the way of inhalation and the size of the particles. Targeting of deposition can be achieved by adjusting the inhalation manoeuvre and the particle size. A way to improve the efficacy therefore is to determine the optimal particle size, since adequately targeted ß$_2$-mimetic agents will induce the strongest decrease in airway obstruction[2]. The deposition patterns of aerosols in the lung will be influenced, however, by the degree of constriction of the airways, so one is forced to stratify patients. Only few studies have focused on the relationship between particle size of a ß$_2$-mimetic aerosol and its efficacy[3 4 5 6 7]. However, it is impossible to conclude from these studies, the optimal particle size, since the results of the various studies are contradictory.

To determine the optimal particle size we compared the effects of salbutamol aerosols with variable diameters on the degree of lung function improvement in a group of asthmatic patients with mildly impaired lung function. To do so we use monodisperse aerosols because polydisperse aerosols contain overlapping particle size distributions, which will obscure differences between larger and smaller particles.

---

* published in: Int J Pharm 107:211-217 1994

Materials and methods

Patients

Eight mild asthmatic patients participated in the trial (3 women and 5 men). The average age (sd) was 40 (10) years, the mean FEV_1 (sd) was 72.3 (6.8) percent of the predicted value. In all patients a bronchodilator response of >15% after inhalation of 200 µg salbutamol had been measured just before the trial. None of the patients were smokers. All patients used corticosteroids by inhalation, cromoglycate or long-acting $ß_2$-mimetic agents were not used. Oral anti-asthma medication was not allowed. Except the corticosteroids, their regular medication was discontinued 6-8 hours before the start of the trial. All patients gave their written consent before the entry of the trial, which was approved by the hospital ethics committee.

Aerosol generation

Monodisperse aerosols (geometric SD <1.2) were produced by a spinning top generator[8]. A spinning top generator consists of a small disk, rotating at 12000 rpm. Liquid is fed to the centre of the disk and the high centrifugal force causes droplets to leave the rim of the disk. These droplets are all of the same size. These droplets are dried by hot air and led to a small tank, from which the patients inhaled. The concentration of the drug in the solution and the viscosity of it governs the diameter of the resulting dry particles. Salbutamol solutions (50% water/50% ethanol) of 0.1%, 1% and 10% were used to yield aerosols with a mass median aerodynamic diameter (MMAD) of 1.5 µm, 2.8 µm, and 5 µm, respectively. Each time a patient was due to start aerosol inhalation, the mass of salbutamol per litre of air and the particle diameter of the dry aerosol particles in the tank were measured by an Aerodynamic Particle Sizer 33 (TSI, St. Paul, Min). For each dose the volume of air inhaled was calculated by dividing the dose by the mass of salbutamol per litre of air. If sufficient aerosol-containing air had been inhaled, switching over to non-aerosol containing air discontinued the aerosol inhalation.

Procedure

Each patient was studied at the lung function laboratory with intervals of one week. The baseline FEV_1 during each session was not allowed to vary more than 10%. Each

session consisted of 4 cycles, which consisted of measurement of the lung function 15 and 30 minutes after administration of the aerosol. A next cycle started within 5 minutes after the previous one. First, 5 µg salbutamol was administered, followed by 5 µg, 10 µg and 20 µg during the second, third and fourth cycle, respectively, resulting in cumulative doses of 5 µg, 10 µg, 20 µg and 40 µg salbutamol. (All dosages are expressed as µg delivered to the mouth). The inhalation manoeuvre consisted of inhalation of the slow vital capacity with a flow of 40-60 L/min, followed by a breath-holding period of 10 seconds and a slow exhalation. A hot wire anemometer placed close to the mouth of the patient measured the inhalation flow and volume. The amount of aerosol deposited in the anemometer was neglectable. Before the aerosol inhalation the patients were taught to inhale and they had the opportunity to correct the inhalation flow by watching an indicator connected to the anemometer. Administration of the aerosols was done in a randomised single-blind manner. On the first day a placebo aerosol was administered in order to facilitate early detection of any adverse reactions of the patient to equipment or solvents. In addition, the placebo measurement served to determine the spontaneous variability in airway obstruction during the measuring period.

Lung function assessment

The lung function was assessed 15 and 30 minutes after inhalation of the aerosol. The lung function assistant was not informed about the type of aerosol administered. The R_{tot} was measured with a body plethysmograph, the FEV_1, FVC, and VC by means of spirometry, and the PEF and $MEF_{75/50/25}$ were derived from maximal expiratory flow-volume curves.

Statistics

The change in lung function was expressed as a percentage of the predicted value. Four dose-response curves were generated, one for each type of aerosol. These dose-response curves were analysed for effects related to the type of aerosol (aerosol-size effect), effects of increasing dosages (dose effect), and interaction between size and dosage using repeated measurements anova[9]. Any differences between the measurements at t = +15 and t = +30 minutes were evaluated with the paired T-test.

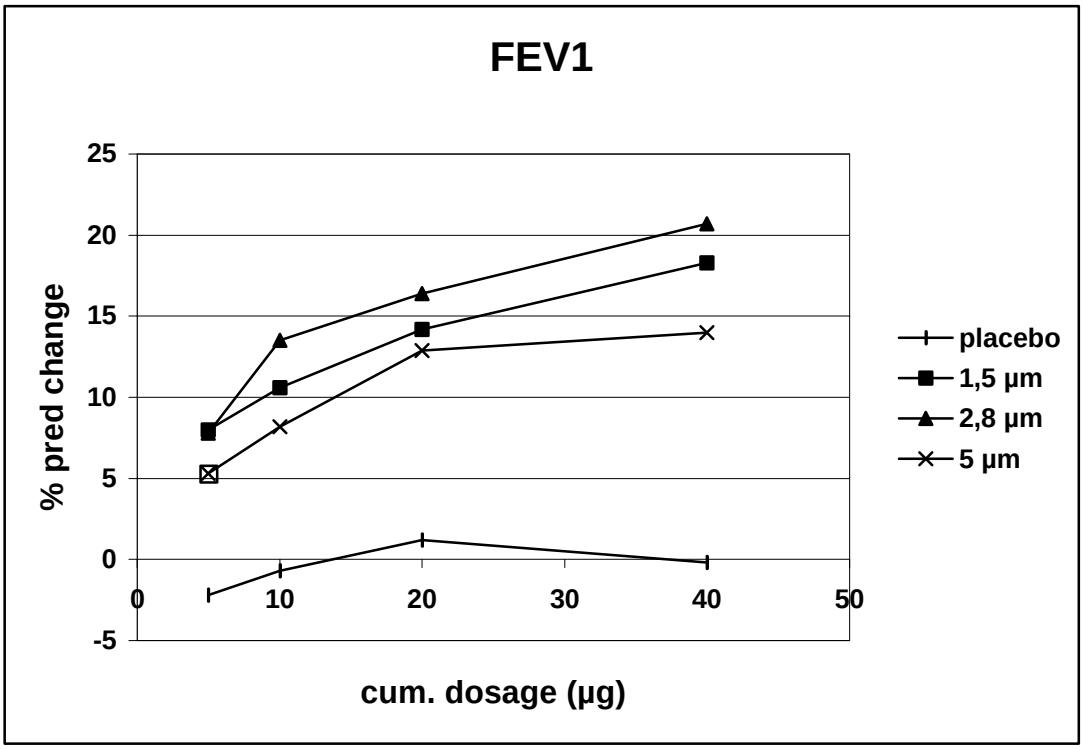
The mean lung function improvement over all four dosages will be higher for the most potent aerosol as compared to the less potent aerosols. In order to find out whether a less potent aerosol deviates significantly from the most potent aerosol, it was calculated how large the deviation between these means should be before it was fair to speak of significance. In this respect the method of Schuirmann was applied, this method is comparable to the LSD-test[10]. In all calculations an α-value of 0.05 was considered to be significant.

Results

All 8 patients completed the four sessions. None of the values of the lung function parameters, measured 30 minutes after administration of the aerosol differed significantly from those measured 15 minutes after administration. Therefore, only an evaluation is given of the measurements conducted 15 minutes after aerosol administration.

In Fig. 1 the dose-response curve for the FEV_1 and peak flow are represented. In any of the parameters no change was measured during the inhalation of placebo. The evaluation of the dose effects demonstrated that for all lung function parameters statistically significant differences existed between dosages, with the higher dosages causing a stronger bronchodilation ($p<0.05$). The interaction between the dose and the effects of the three types of salbutamol aerosols was non-significant for all lung function parameters ($p>0.1$), which indicates that the dose-response curves run parallel.

In evaluating the aerosol-size effect, the analysis of variance demonstrated significant differences with reference to placebo for the FEV_1 ($p<0.01$), the PEF ($p<0.01$), de FVC ($p<0.01$), the MEF_{75} ($p<0.01$), the MEF_{50} ($p<0.01$) and the MEF_{25} ($p<0.01$). This implies that all the dose-response curves of the salbutamol aerosols are located higher than the placebo curves. For the R_{tot} ($p=0.116$) and the VC ($p=0.068$) no significant differences due to the different aerosol-sizes were demonstrable. The reason for this is to be found in the strong spontaneous variability of the R_{tot} and/or the minor improvement of the VC.



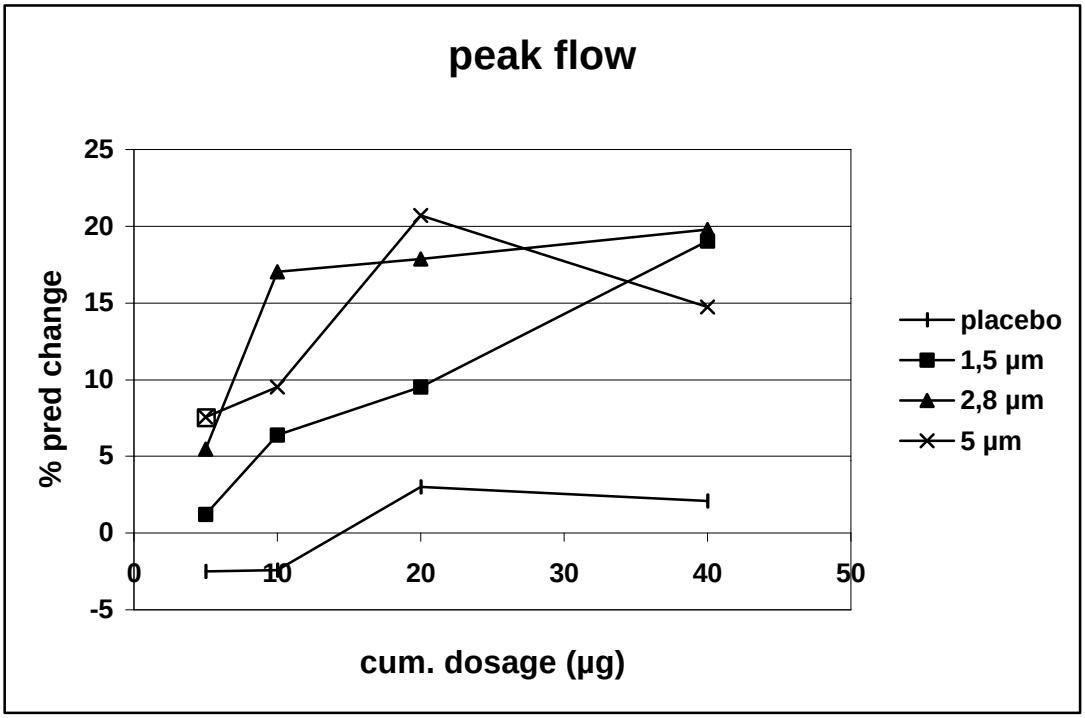


Fig. 1 Dose-response curves for the FEV_1 and peak flow

Lung function parameter	Particle size of the aerosol (µm)			Significant different aerosols
	1.5	2.8	5	
FEV1	12.8(4.3)	14.6(6.5)	10.1(4)	2.8 vs. 5
FVC	8.9(5.6)	9.6(9.3)	7.9(6.6)	NS
MEF25	12.1(6.6)	14.9(9.5)	9.2(7.9)	2.8 vs. 5
MEF50	15.2(6.7)	18.5(9.4)	12.1(6.2)	2.8 vs. 5
MEF75	17(4.4)	21.4(9)	14.6(5.6)	2.8 vs. 5
PEF	9(7.1)	15(7)	13.1(7.9)	2.8 vs. 1.5
Rtot	-27.6(93)	-84.3(92)	-52.7(62.1)	NS
VC	7(7.8)	8.1(9.4)	6(8.9)	NS

Table 1 Mean (sd) improvement in lung function (%predicted) over all four dosages per type of aerosol (NS= non significant)

A significant difference with reference to 2.8 µm aerosol will occur for the FEV_1 if the deviation between the means exceeds 2.9%, for the PEF this deviation should be at least 5.9%, for the $MEF_{75/50/25}$ at least 5.4%, 4.3% and 4.9%, respectively, and, finally, for the FVC at least 9.4%. In the case of the FEV_1 and the $MEF_{75,50,25}$ a statistically significant difference occurred between the 5 µm aerosol and the 2.8 µm aerosol. For the PEF a significant difference was found between the 1.5 µm and the 2.8 µm aerosol. For the FVC the differences were too small to be significant.

Table 2 lists the mean improvements as %predicted with 95% confidence intervals after administration of 40 µg salbutamol for all lung function parameters. Despite the low dosage a significant improvement of the lung function can be observed. The improvement in the VC was not significant.

None of the patients reported any adverse effects as a result of the experiment.

Lung function parameter	Improvement (% predicted)	95% confidence interval	
FEV_1	20.7	13.5	28.0
FVC	12.6	2.4	22.7
MEF_{25}	26.9	20.6	36.9
MEF_{50}	26.7	18.5	34.8

Lung function parameter	Improvement (% predicted)	95% confidence interval	
MEF_{75}	28.8	06.4	37.6
R_{tot}	-102.2	-29.0	-175.0
PEF	19.8	12.4	27.1
VC	9.3	-0.11	18.7

Table 2 Mean improvement in lung function (%predicted) following 40 µg salbutamol administered as a 2.8 µm aerosol

Discussion

The increase in FEV_1 and the $MEF_{75/50/25}$ after the 5 µm aerosol differed significant from the 2.8 µm aerosol, while there were no significant differences between the 1.5 µm and the 2.8 µm aerosol. The increase in the PEF was highest after the 2.8 µm aerosol, not being significantly different from the 5 µm aerosol. No size-effect was present in case of the VC, FVC and the R_{tot}. We were able to show these differences in a relatively small group of volunteers. This is due to a low intrasubject variability and the use of repeated measurement anova, which eliminates the interindividual variability. Patel et al. also showed comparable differences in a small group of volunteers[3].

The lung has the capacity to intercept a large portion of the inhaled particles rapidly and effectively by several mechanisms that cause particles to deposit on the mucous membrane. Two important processes in this context are impaction and sedimentation[11]. Impaction means that particles are not able to follow changes in the direction of the air stream and deposit. This mechanism is of particular relevance for large or heavy particles. Sedimentation is a time-dependent process related to the velocity with which particles fall down under the influence of gravitation. The speed of fall becomes constant at the moment the resistance of the air is equal to gravitation. These two mechanisms cause large particles to deposit in the upper airways, whereas smaller particles escape from impaction and penetrate the airways more deeply. So a deposition pattern in the airways is evident. Targeting the deposition towards a segment of the airways can be achieved by selecting the right particle-size of the aerosol or by adjustment of the breathing-technique[2].

It is possible that in the efficacy of ß$_2$-mimetic agents a significant role is played by the fact that the ß$_2$-receptors are not uniformly distributed in the airways. In a number of publications an increase in the number of receptors is reported in association with distances further into the periphery of the lung[12 13]. Assuming that a greater effect is obtained when the concentration at the receptor is higher, there is a ratio for matching the deposition pattern of ß$_2$-mimetics to the beta-adrenoceptor distribution.

In the present study we have based ourselves on the assumption that a more peripheral deposition was desirable. One way to achieve this is by a slow and deep inhalation of the aerosols. In addition, the particles were reduced in size. Reduction in size, however, cannot be continued without impunity. "Too small" particles are known to have a terminal velocity that is so small that they hardly deposit. This implies that there is an ideal particle size: not too large and not too small. The optimal particle size will depend on a number of factors, i.e. the preferred deposition pattern, the condition of the airways - in this context their diameter - and the inhalation technique. We decided on an upper limit of 5 µm, because various studies have demonstrated that particles with a MMAD above 5 µm only reach the airways to a limited extent[14]. The lower limit of 1.5 µm was chosen on technical grounds (since both the spinning top generator and the aerodynamic particle sizer are characterised by a functional lower limit of 0.5 - 1 µm), and it has been documented that particles with a MMAD below 0.5 µm hardly deposit in the airways[11].

In asthmatics Clay found that a 1.8 µm terbutaline aerosol induces a stronger $MEF_{50/25}$ improvement than a 4.6 µm or a 10.3 µm aerosol, whereas Patel found that a 2.5 µm isoproterenol aerosol is more potent than a 5 µm aerosol[3 7]. Johnson observed a significant difference between a 3.3 µm and a 7.7 µm salbutamol aerosol, as did Ruffin between a 1.5 µm and a 3.2 µm isoproterenol aerosol; the outcome of both studies was in favour of the smaller aerosol[15 16]. However, from all these data it is not feasible to derive an optimal aerosol diameter. Moreover, the matter is complicated by the negative findings of Hultquist and Mitchell: neither of these investigators found any differences in potency between 1.5 µm and 4.8 µm aerosols and 1.4 µm and 5.5 µm aerosols, respectively[4 5].

The results of our study demonstrate that in asthma patients with a mild airway obstruction an aerosol with a MMAD of around 2.8 µm is to be preferred. The results

of our study confirm the conclusion drawn by Patel that a 2.5 µm aerosol is more potent than a 5 µm aerosol, but add to it that smaller aerosols are of no benefit. At the same time an explanation is found for the negative findings of Hultquist and Mitchell[4] [1]. Both investigators have selected aerosol diameters that lead to minor differences in potency. The discrepancy between these and other investigators thus is merely an apparent one, to be attributed to the choice of particle sizes. The results obtained by Clay are not easy to explain. However, this study the aerosols were administered by means of various nebulisers[7]. It is possible that these nebulisers have released divergent dosages, which might be interpreted as differences in potency.

The results of our study can be explained as follows: particles of 5 µm will be deposited extrathoracical to a greater extent than the smaller particles, which are able to penetrate the airways deeply. (In case of the 1.5 µm aerosol the smallest amount can be expected extrathoracical). So small particles are to be preferred for a deep penetration. However these particles deposit in minute quantities, so a deep penetration is at the expense of a lower mass deposited[11]. As for the PEF, it is striking that the 5 µm particles perform better than the 1.5 µm particles: the bulk of the 1.5 µm particles pass the central and the extrathoracic compartment. Here we see a contrast with the more peripherally oriented lung function parameters: the 5 µm aerosol is inferior to the two others. The 5 µm aerosol reaches the peripheral compartment to a lower degree. The lower potency of the 1.5 µm aerosol can be ascribed to its limited tendency to deposit. The fact that in all cases 2.8 µm particles induce a better effect than 5 µm particles can be attributed to the difference in extrathoracic deposition[17]. We did not measure the deposition patterns of these aerosols within the lung. So we are not sure whether the differences in potency are to be ascribed to a better matching between the ß$_2$-receptor distribution and the deposition. One can state however that the results of this investigation are in line with theoretical predictions of deposition patterns[17]. The deposition of particles is never confined to a small segment of the airways: one always encounters wide patterns. The calculations of Gerrity show that in many segments of the airways comparable number of particles will deposit, while the changes in the patterns due to differences in particle-size are not overwhelming[18]. In asthmatics the same conclusions were drawn[19]. We therefore feel that the high extrathoracical deposition of large particles and a low one for smaller particles

combined with an inherent low deposition for very small particles offers a good explanation for our results. Without taking the receptor distribution into account.

In agreement with Patel and Mitchell we conclude that it is possible to induce adequate bronchodilation with very small dosages[3] [4]. In Table 2 we have included the improvement of lung function after 40 µg salbutamol. This dosage is only one fifth of frequently used MDI-dosages and one tenth of the dosages usually administered by dry-powder inhalation (DPI). These low dosages lead to such a distinct bronchodilation because they are monodisperse, contrary to the aerosols administered by metered dose or dry-powder inhalers. In the usual polydisperse aerosols only a minor fraction (depending on the formulation) of the mass will consist of particles <2.8 µm.

We conclude that in mild asthmatics the mean particle diameter of a ß$_2$-mimetic aerosol should be around 2.8 µm for optimal improvement of the lung function. The dosage of salbutamol can be reduced for such aerosols.

Literature

[1] Clarke S.W.
Aerosols as a way of treating patients.
Eur J Respir Dis 69(suppl 146): 525-533 1986.

[2] Byron P.R.
Pulmonary targeting with aerosols.
Pharm Tech: 11 42-56 1987.

[3] Patel P., Mukai D. and Wilson, A.F.
Dose-response effects of two sizes of monodisperse isoproterenol in mild asthma.
Am Rev Respir Dis 141: 357-360 1990.

[4] Mitchell D.M., Solomon M.A., Tolfree S.E.J. and Spiro, S.G.
Effect of particle size of bronchodilator aerosols on lung distribution and pulmonary function in patients with chronic asthma.
Thorax 42: 457-461 1987.

[5] Hultquist C., Wollmer P., Eklund G. and Jonson B.
Effect of inhaled terbutaline sulphate in relation to its deposition in the lungs.
Pulm Pharm 5: 127-132 1992.

[6] Rees P.J. and Clark T.J.H.
The importance of particle size in response to inhaled bronchodilators.
Eur J Respir Dis suppl 119: 73-78 1982.

[7] Clay M.M, Pavia D. and Clarke S.W.
Effect of aerosol particle size on bronchodilation with nebulised terbutaline in asthmatic patients.
Thorax 41: 364-368 1986.

[8] Cheah P.K.P. and Davies C.N.
The spinning top aerosol generator: improving the performance.
J Aerosol Sci 15: 741-751 1984.

[9] Girden E.R.
Anova: repeated measures.
Sage, Newbury Park, 1992.

[10] Schuirmann D.J.
A comparison of the two one-sided tests procedure and the power approach for assessing the equivalence of average bioavailability.
J Pharmacokin Biopharm 15: 657-680 1987.

[11] Lippmann M., Yeates D.B. and Albert R.E.
Deposition, retention and clearance of inhaled particles.
Br J Ind Med 37: 337-362 1980.

[12] Barnes P.J., Basbaum C.B., Nadel J.A. and Roberts J.M.,
Localization of ß-receptors in mammalian lung by light microscopic auto-radiography.
Nature 299: 444-447 1982.

[13] Barnes P.J., Basbaum C.B. and Nadel J.A.

Autoradiographic localization of autonomic receptors in airway smooth muscle.
Am Rev Respir Dis 127: 758-762 1983

[14] Svartengren M., Anderson M., Bylin G., Philipson K. and Camner P.
Mouth and throat deposition of 3.6 μm radiolabelled particles in asthmatics.
J Aerosol Med 4: 313-321 1991.

[15] Johnson M.A., Newman S.P., Bloom R., Talee N. and Clarke S.W.
Delivery of albuterol and ipratropiumbromide from two nebulizer systems in chronic stable asthma.
Chest 96: 6-10 1989.

[16] Ruffin R.E., Dolovich M.B., Oldenburg F.A. and Newhouse M.T.
The preferential deposition of inhaled isoproterenol and propanolol in asthmatic patients.
Chest 80: 904-907 1986.

[17] Gonda I.
A semi-empirical model of aerosol deposition in the human respiratory tract for mouth inhalation.
J Pharm Pharmacol 33: 692-696 1981.

[18] Gerrity T.R., Lee P.S., Hass F.J., Marinelli A., Werner P. and Lourenco R.V.
Calculated deposition of inhaled particles in the airway generations of normal subjects.
J Appl Physiol 47: 867-873 1979.

[19] Kim C.S., Brown L.K., Lewars G.G. and Sackner A.A.
Deposition of aerosol particles and flow resistance in mathematical models and experimental airway models.
J Appl Physiol 55: 154-163 1983.

The optimal particle size for parasympathicolytic aerosols in mild asthmatics*

Introduction

In a previous report we described the optimal particle-size of a ß$_2$-mimetic aerosol[1]. We found that a salbutamol aerosol consisting of particles with a MMAD of 1.5/2.8 µm elicited a higher degree of lung function improvement than a 5 µm aerosol. These results were explained by adopting the hypothesis that the degree of penetration of particles into the airways is an important factor. Particles need to pass the extrathoracic/upper airways to reach the lower airways. In the extrathoracic/upper airways large particles are filtered out of the inhaled air to a greater extent than smaller ones. Therefore the deposited amount of particles in the lower airways is lower in case of large particles. In mild asthmatics it appeared that this dose-reduction is large enough to cause a less intense bronchodilatation. It is not certain whether this outcome is transferable to other types of bronchodilators, such as ipratropium bromide. The distribution of the receptors may play a role. The ß$_2$-adrenoreceptors are located mainly in the periphery of the airways, the muscarinic receptors are preferentially encountered in the central airways[2 3]. One can conceive that for a salbutamol particle it is harder to reach the receptor than for an ipratropium bromide particle, therefore it must be smaller. On the other hand such small particles will pass the upper airways better than large ones, missing the muscarinic receptor. Our hypothesis is that the optimal particle-size of an ipratropium bromide-aerosol will be larger than of a salbutamol-aerosol. Due to the resulting centrally orientated deposition-pattern, the match to the receptor-distribution is better. To reject or accept this hypothesis we determined the relationship between the particle size of an ipratropium bromide-aerosol and the lung function improvement in asthmatic patients with a mild reduction of the FEV_1.

Materials and methods

---

* published in: Int J Pharm 114:111-115 1995

Patients

Eight mild asthmatic patients participated in the trial (5 women and 3 men). The average age (sd) was 39.6 (14.4) years, the mean FEV_1 (sd) was 72 (14.3) percent of the predicted value. In all patients a bronchodilator response of ≥15% after inhalation of 200 µg salbutamol had been measured just before the trial. None of the patients were smokers. All, but one, used corticosteroids by inhalation, cromoglycate or long-acting ß$_2$-mimetic agents were not used. Oral anti-asthma medication was not allowed. Except the corticosteroids, their regular medication was discontinued 6-8 hours before the start of the trial. All patients gave their written consent before the entry of the trial, which was approved by the hospital ethics committee.

Aerosol generation

Monodispersee aerosols (geometric SD < 1.2) were produced by a spinning top generator[4]. A spinning top generator consists of a small disk, rotating at 12000 rpm. Liquid is fed to the centre of the disk and the high centrifugal force causes droplets to leave the rim of the disk. These droplets are all of the same size. These droplets are dried by hot air and led to a small tank, from which the patients inhale. The diameter of the resulting dry particles is governed by the concentration of the drug in the solution and the viscosity of it. Ipratropium bromide solutions (50% water/50% ethanol) of 0.1%, 1% and 10% were used to yield aerosols with a mass median aerodynamic diameter (MMAD) of 1.5 µm, 2.8 µm, and 5 µm, respectively. Each time a patient was due to start the aerosol inhalation, the mass of ipratropium bromide per litre of air and the particle diameter of the dry aerosol particles in the tank were measured by an Aerodynamic Particle Sizer 33 (TSI, St. Paul, Min). For each dose the volume of air inhaled was calculated by dividing the dose by the mass of ipratropium bromide per litre of air. If sufficient aerosol-containing air had been inhaled, the aerosol inhalation was discontinued by switching over to non-aerosol containing air.

Procedure

Each patient was studied at the lung function laboratory with intervals of one week. The baseline FEV_1 during each session was not allowed to vary more than 10%. Each session

consisted of measurement of the lung function 30 minutes after administration of the aerosol. From the previous study we learned that using monodispersee aerosols of these size-range small dosages of drug (5-10% of a MDI-dosage) are needed, we therefore decided to administer only 8 μg ipratropium bromide (dosage expressed as μg delivered to the mouth). The inhalation manoeuvre consisted of inhalation of a slow vital capacity with a flow of 40-60 L/min, followed by a breath-holding period of 10 seconds and a slow exhalation. The inhalation flow and volume were measured by a hot wire anemometer placed close to the mouth of the patient. The amount of aerosol deposited in the anemometer was neglectable. Before the aerosol inhalation the patients were taught to inhale and they had the opportunity to correct the inhalation flow by watching an indicator connected to the anemometer. Administration of the aerosols was done in a randomised single-blind manner. On the first day a placebo aerosol was administered in order to facilitate early detection of any adverse reactions of the patient to equipment or solvents. In addition, the placebo measurement served to determine the spontaneous variability in airway obstruction during the measurement period.

Lung function assessment

The lung function was assessed 30 minutes after inhalation of the aerosol. The lung function assistant was not informed about the type of aerosol administered. The R_{tot} was measured with a body plethysmograph, the FEV_1, FVC, and VC by means of spirometry, and the PEF and $MEF_{50/25}$ were derived from maximal expiratory flow-volume curves.

Statistics

The change in lung function was expressed as a percentage of the predicted value. These changes were analysed for effects related to the type of aerosol (aerosol-size effect) using repeated measurements anova[5]. In order to discover whether a less potent aerosol deviates significantly from the most potent aerosol, it was calculated how large the deviation between these means should be before it was fair to speak of significance. In this respect the method of Schuirmann was applied, which method is comparable to the LSD-test [6]. In all calculations an α-value of 0.05 was considered to be significant.

Results

All 8 patients completed the four sessions. In Table 1 the change in all lung function parameters is represented. No significant change was measured in any of the parameters during the inhalation of placebo.

In evaluating the aerosol-size effect, the analysis of variance demonstrated significant differences with reference to placebo for the FEV_1 ($p<0.01$), the PEF ($p=0.001$), the FVC ($p=0.034$), the MEF_{50} ($p<0.001$) and the MEF_{25} ($p=0.001$). For the R_{tot} ($p=0.202$) and the VC ($p=0.052$) no significant differences were demonstrable.

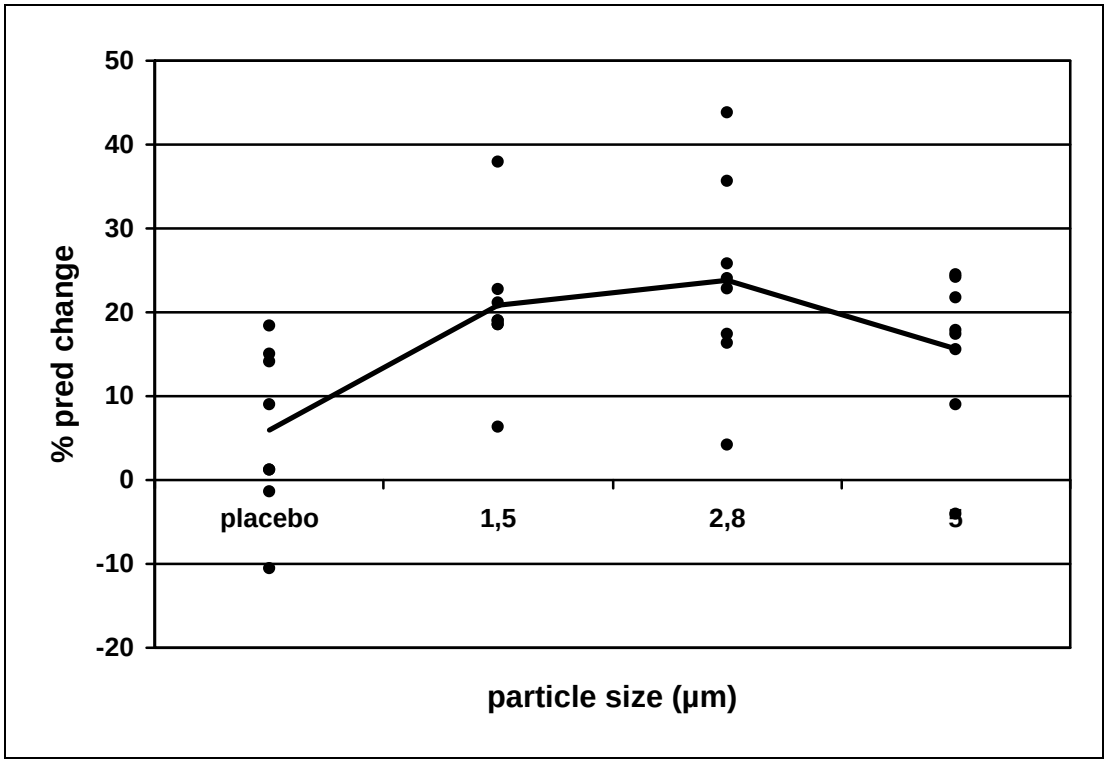


Fig. 1 Scatterplot of FEV_1 improvement after administration of the different aerosols, the mean improvement is depicted by the solid line

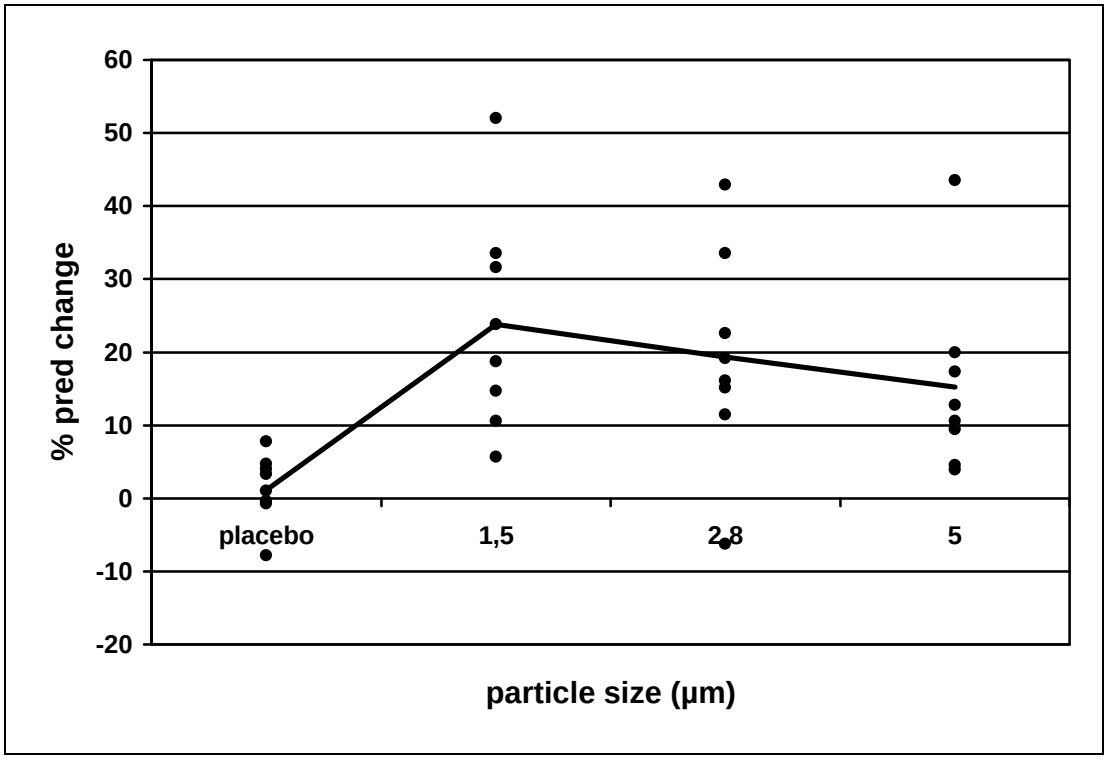


Fig. 2 Scatterplot of MEF_{25} improvement after administration of the different aerosols, the mean improvement is depicted by the solid line

Lung function parameter	Particle size of the aerosol 1.5 µm	2.8 µm	5 µm	Significant different aerosols
VC	13.6(13)	11.5(12.5)	7.7(7.8)	NS
R_{tot}	-74.4(73.2)	-114.8(97.1)	-104.5(135.9)	NS
FVC	12.4(12.4)	13(12.5)	9.4(10.8)	NS
FEV_1	20.3(8.7)	23.7(12.1)	15.6(9.8)	1.5/2.8 vs. 5
MEF_{25}	23.7(15)	19.3(14.7)	15.3(12.7)	1.5/2.8 vs. 5
MEF_{50}	24.7(10.7)	24.7(12.1)	17.7(10.3)	1.5/2.8 vs. 5
PEF	21(7.2)	22(13.2)	16.6(12.4)	NS

Table 1 Mean (sd) improvement in lung function (% predicted) after inhalation of ipratropium bromide aerosols with different particle sizes (NS = non-significant)

A significant difference with reference to the best aerosol will occur for the FEV_1 if the deviation between the means exceeds 5.7%, for the PEF this deviation should be at least 7.5%, for the $MEF_{50/25}$ at least 6.3% and 8.2%, respectively, and, finally, for the FVC at

least 5.5%. In the case of the FEV_1 and the $MEF_{50,25}$ a statistically significant difference occurred between the 5 µm aerosol and the 1.5/2.8 µm aerosol. For all other parameters the differences were too small to be significant.

None of the patients reported any adverse effect as a result of the experiment.

Discussion

The increase in FEV_1 and the $MEF_{50/25}$ after the 5 µm aerosol was significantly less than after the 1.5/2.8 µm aerosol. No differences due to changing aerosol sizes were detectable in case of the other lung function parameters. These findings strongly resemble our earlier findings with salbutamol: in those experiments the optimal particle size was also ≤2.8 µm[1]. In contrast to the previous study no aerosol-size effect was noticeable in case of the PEF. Our initial hypothesis pointed in another direction. To achieve an optimal match between the deposition-pattern and muscarinic receptor-distribution a central deposition-pattern seemed logical. Centrally orientated patterns will occur after inhaling large particles. Contrary to our thoughts the results of this study indicate that the optimal particle size (=deposition patterns) for $ß_2$-mimetics and parasympathicolytic drugs are similar. Hence the distribution of the receptors, as reported in the literature, do not seem to play an important role. The explanation we offer for these results is of a physical nature. In our previous publication we suggested that the way particles penetrate into the lower airways, combined with a local effect, explains all findings. All, but especially large, particles are filtered out quickly in the central airways due to a high impaction-probability. Only small particles will escape from extrathoracical/central deposition[7]. The dose in the lungs or lower airways therefore heavily depends on the filter characteristics of the extrathoracic/upper airways. The "lung-dose" will be higher in case of small particles. This is reflected by the increased bronchodilation after administration of the 1.5/2.8 µm aerosols.

In contrast to the earlier study we could not find a better PEF-improvement after the 5 µm compared to the 1.5 µm aerosol. If the theory is correct one expects that the smallest particles pass the central airways and only a small amount deposit. As a result the improvement of the PEF, which is highly influenced by the condition of the central airways, should be lower. This not the case (although the statistical power to detect

differences was comparable to the previous experiment). Svartengren showed that in some patients the cut-off point of the oropharynx, due to local anatomical structures, is very low[8]. Two of the volunteers showed rather low lung function changes after inhaling the 5 µm aerosol. Thereby reducing the mean improvement of all lung function parameters and obscuring the differences between the effects of the 5 µm and 1.5 µm aerosols of the PEF, as found before. These volunteers must "suffer" from a low oropharyngeal cut-off point. This explanation underlines the importance of choosing an aerosol with a small particle size and low geometric standard deviation (GSD).

Our results are in in accordance with those of Padfield, but not with those of Johnson[9] [10]. Padfield showed that an aerosol with 35% of the particles ≤6.4 µm elicited a better bronchodilatation than an aerosol with only 10% ≤6.4 µm. Johnson could not detect any differences between aerosols with a median mass diameters (MMD) of 3.3 resp. 7.7 µm. The author reported a lower whole lung (and local airway) dose after administration of the largest aerosol with much of the coarse aerosol deposited extrathoracical. The explanation for this negative finding was that the percentual higher central deposition of the 7.7 µm aerosol compensated for the lower total and local dose. In other words a low central dose is to be preferred over a high peripheral one. We do not think that this is plausible explanation. It suggests that the dose-response curve for centrally deposited ipratropium bromide is steeper. However the reported dose-response curves of the MEF_{25} are much steeper than of the FEV_1. This fact is not consistent with the explanation given.

Ipratropium bromide and salbutamol (or other $ß_2$-mimetics) are frequently combined in metered dose inhalers or nebulisers. It is believed that the combination induces larger and/or longer bronchodilations than the single components. By definition both drugs will be administrated in the same droplet or dry particle, thus showing the same particle-size distribution. If ipratropium bromide would need a coarse aerosol to be most active and salbutamol a fine one, the combination would be rendered less effective as the two drugs delivered independently. Our finding that both drugs exhibit the same optimal particle-size, makes such a combination a rational one.

We conclude that in mild asthmatics the mean particle diameter of a parasympathicolytic aerosol should be 2.8 µm for optimal improvement of the lung function. As for $ß_2$-

mimetic aerosols the dosage, compared to conventional polydisperse aerosols can be reduced for such aerosols.

Literature

[1] Zanen P., Go T.L. and Lammers J-W.J.
The optimal particle size for ß-adrenergic aerosols in mild asthmatics.
Int J Pharm 107: 211-217 1994.

[2] Barnes P.J., Basbaum C.B., Nadel J.A. and Roberts J.M.
Localization of ß-receptors in mammalian lung by light microscopic auto-radiography.
Nature 299: 444-447 1982.

[3] Barnes P.J., Basbaum C.B. and Nadel J.A.
Autoradiographic localization of autonomic receptors in airway smooth muscle.
Am Rev Respir Dis 127: 758-762 1983.

[4] Cheah P.K.P. and Davies C.N.
The spinning top aerosol generator-improving the performance.
J Aerosol Sci 15: 741-751 1984.

[5] Girden E.R.
Anova: repeated measures.
Sage, Newbury Park, 1992.

[6] Schuirmann D.J.
A comparison of the two one-sided tests procedure and the power approach for assessing the equivalence of average bioavailability.
J Pharmacokin Biopharm 15: 657-680 1987.

[7] Lippmann M., Yeates D.B. and Albert, R.E.
Deposition, retention and clearance of inhaled particles.
Br J Ind Med 37: 337-362 1980.

[8] Svartengren M., Anderson M., Bylin G., Philipson K. and Camner, P.
Mouth and throat deposition of 3.6 µm radiolabelled particles in asthmatics.
J Aerosol Med 4: 313-321 1991.

[9] Padfield J.M., Winterborn I.K., Pover G.M. and Tattersfield A.
Correlation between inertial impactor performance and clinical performance of a bronchodilator aerosol.
J Pharm Pharmac 35: 10p 1983.

[10] Johnson M.A., Newman S.P., Bloom,R., Talee N. and Clarke S.W.,
Delivery of albuterol and ipratropium bromide from two nebulizer systems in chronic stable asthma.
Chest 96: 6-10 1989.

The optimal particle size for ß$_2$-agonist and anticholinergic aerosols in patients with severe airflow obstruction*

Introduction

In two previous publications we reported on the optimal particle-size of ß$_2$-agonist and anticholinergic aerosols[1 2]. We demonstrated that in asthmatics, with a forced expiratory volume in one second (FEV_1) >70% of predicted, salbutamol and ipratropium bromide aerosols consisting of particles with a median mass aerodynamic diameter (MMAD) <2.8 µm elicited statistically significant higher degrees of lung function changes than a 5 µm aerosol. These findings were explained by taking into account the filter characteristics of the airways. Airways filter particles out of the inhaled air: 5 µm particles deposit rapidly in the extrathoracic/upper airways, while smaller particles escape rapid deposition and reach the dilatable parts of the airways better than the 5 µm particles, resulting in higher local doses, assuming equal doses administered[3].

The narrower the airways are, the higher the tendency to deposit and particle deposition patterns shift to the central airways[3]. Heyder showed that 3.5 µm particles deposit preferentially in the alveoli of normal subjects, while in our previous study smaller particles caused the greatest bronchodilatation[4]. To reach the smaller airways in such cases, it maybe necessary that the particle size of the inhaled aerosol should be decreased. In patients with severe airflow obstruction, aerosols with a smaller particle size maybe more suitable.

We therefore carried out experiments to determine the most suitable particle size for bronchodilator aerosols in patients with severe airflow obstruction.

Materials and methods

Patients

Eight patients started the trial (two women and six men). Due to personal reasons,

---

* published in Thorax 51: 977-980 1996

one patient dropped out, so seven completed. The average age (sd) of those seven was 55 (4) years, the mean FEV_1 (sd) was 37.9 (7.3) percent of the predicted value. In all patients a more than 15% increase of baseline FEV_1 after inhalation of 200 µg salbutamol had been measured just before the trial. None of the patients were smokers. All used corticosteroids by inhalation, disodium cromoglycate and oral anti-asthma medication were not used. Except for corticosteroids, their regular medication was discontinued six to eight hours before the start of the trial, while long acting $ß_2$-agonists were stopped 15 hours prior to the start. All patients gave their written consent before entry into the study, which was approved by the hospital ethics committee.

Aerosol generation

Monodisperse aerosols (geometric SD <1.2) were produced by a spinning top generator[5] .A spinning top generator consists of a small disk, rotating at 12000 rpm. Liquid is fed to the centre of the disk and the high centrifugal force causes identical sized droplets to leave the rim of the disk. These droplets are dried by hot air and led to a small tank, from which the patients inhale. The diameter of the resulting dry particles is governed by the concentration of the drug in and the viscosity of the solution. Salbutamol and ipratropium bromide solutions (50% water/50% ethanol) of different concentrations were used to yield aerosols with a MMAD of 1.5 µm, 2.8 µm, and 5 µm, respectively. Each time a patient was due to start the aerosol inhalation, the mass of salbutamol or ipratropium bromide per litre of air and the particle diameter of the dry aerosol particles in the tank were measured by an Aerodynamic Particle Sizer 33 (TSI, St. Paul, Min). This is a so called time of flight particle sizer and informs on both the particle size distribution and the mass contained in the aerosol in µg per litre of air. To calculate the volume of air to be inhaled, we divided the dose to be administered by the mass of salbutamol or ipratropium bromide per litre of air rendering the litres to be inhaled. If sufficient aerosol-containing air had been inhaled, the aerosol inhalation was discontinued by switching over to non-aerosol containing air.

Procedure

Each patient was studied at the lung function laboratory with intervals of one week.

The baseline FEV_1 during the sessions was not allowed to vary more than 10%. Each session consisted of measurement of the baseline lung function and 30 minutes after administration of the aerosol. Based on previous experience, we administered 20 µg salbutamol and 8 µg ipratropium bromide (dosage expressed as µg delivered to the mouth). The inhalation manoeuvre consisted of inhalation of a slow vital capacity with a flow of 40-60 L/min, followed by a breath-holding period of ten seconds and a slow exhalation. The aerosol was administered during the entire inhalation manoeuvre. The inhalation flow and volume were measured by a hot wire anemometer placed close to the mouth of the patient. The amount of aerosol deposited in the anemometer was negligible. Before the aerosol inhalation the patients were taught the inhalation technique and they had the opportunity to correct the inhalation flow by watching an indicator connected to the anemometer. Administration of the aerosols was done in a randomised single-blind manner. On the first day a placebo aerosol was administered in order to facilitate early detection of any adverse reactions of the patient to equipment or solvents. In addition, the placebo measurement served to determine the spontaneous variability in airway obstruction during the measurement period.

Lung function assessment

The lung function was assessed 30 minutes after inhalation of the aerosol. The lung function assistant was not informed about the type of aerosol administered. The specific airway conductance (sG_{aw}) was measured with a body plethysmograph, the FEV_1, forced vital capacity (FVC), and vital capacity (VC) by means of spirometry, and the peak flow (PEF) and maximum expiratory flow at 75/50/25% of the forced vital capacity ($MEF_{75/50/25}$) were derived from maximal expiratory flow-volume curves.

Statistics

The change in lung function was expressed as a percentage of the predicted value[6]. Using repeated measurements analysis of variance (ANOVA) it was determined whether significant differences were present due to a) the particle size of the aerosol, b) the type of drug and c) the interaction between drug and aerosol-size[7]. A significant interaction, in this case, means that the difference between salbutamol and ipra-

tropium bromide is not constant and depends on the particle size of the aerosol administered. When a statistically significant change was observed, the within-group mean sum of squares was used to calculate the least significant difference. In all calculations an α-value of 0.05 was considered to be significant.

Results

All patients completed the study without noticeable side-effects. No significant change was measured in any of the lung function parameters during the inhalation of placebo. Nor could we show significant differences between the bronchodilator response of salbutamol and ipratropium bromide. Comparing identical particle sizes there were no significant differences between salbutamol and ipratropium bromide.

Lung function parameter	Particle size of the aerosol			
	placebo	1.5 µm	2.8 µm	5 µm
sG_{aw}	-2.88*	-14.46	-21.93	-12.86
FEV_1	0.08*	3.57*	8.87	3.84*
MEF_{75}	-0.43*	1.44*	4.85	1.95
MEF_{50}	-0.30*	0.58*	4.23	1.17*

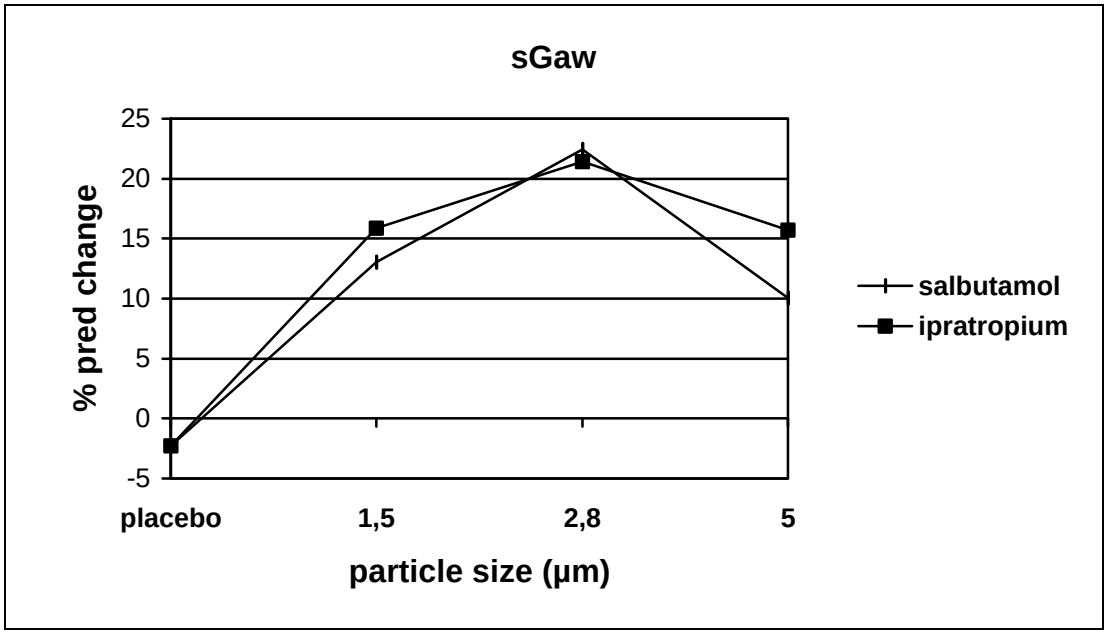
Table 1 Mean improvement in lung function (% predicted) after inhalation of aerosols with different particle sizes or placebo. The improvements shown reflect the net effects of the different particle sizes, not confounded by the type of bronchodilator used. An asterisk indicates a significant difference versus the 2.8 µm aerosol.

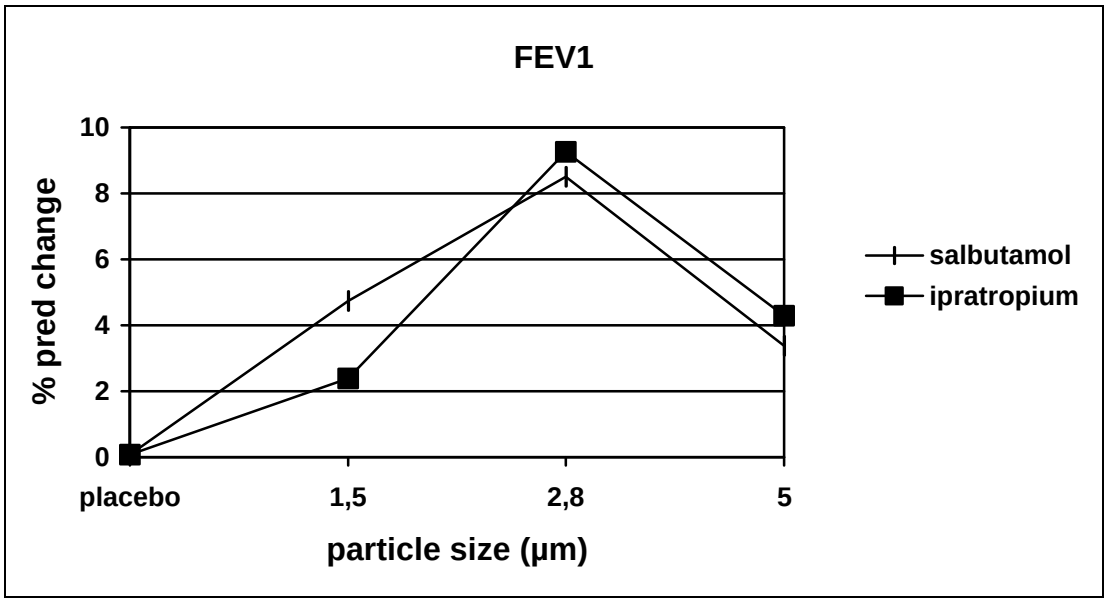
Lung function parameter	Improvement (sd)	
	after 20 µg salbutamol	after 8 µg ipratropium bromide
sG_{aw}	-22.4% (17.3%)	-21.4% (16.9%)
FEV_1	8.5% (7%)	9.3% (4.1%)
MEF_{75}	3.4% (4.6%)	6.3% (4.1%)
MEF_{50}	3.4% (4.8%)	5% (3.2%)

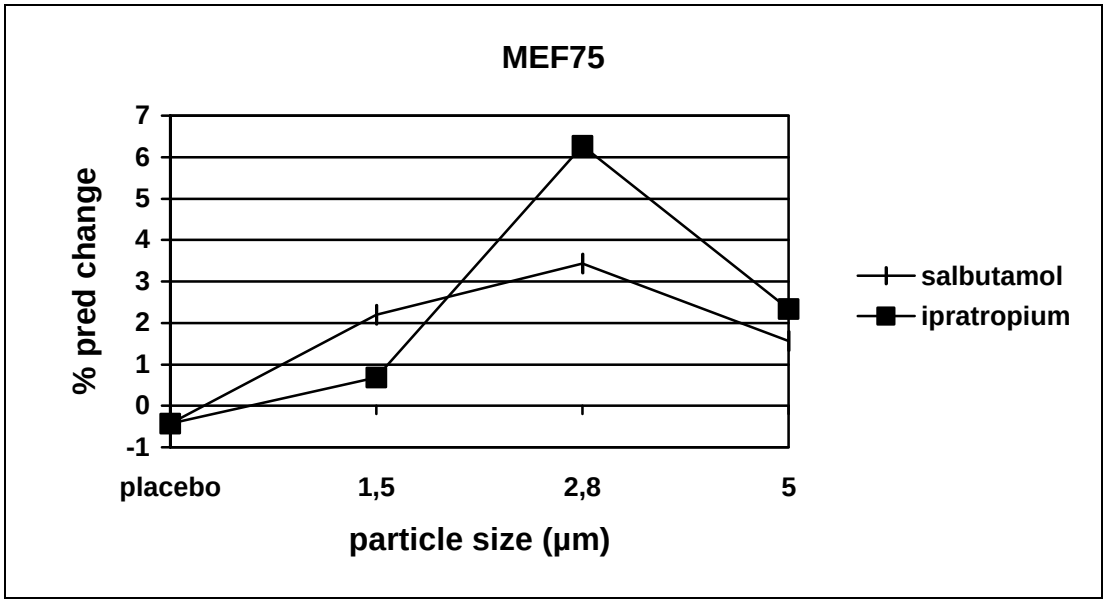
Table 2 Mean improvement in lung function (sd, as % predicted) after inhalation of a 2.8 µm salbutamol or ipratropium bromide aerosol.

With respect to the effects of different particle sizes of the aerosol administered, we

found significant differences in case of the FEV_1 ($p<0.001$), the MEF_{75} ($p=0.02$), the MEF_{50} ($p<0.001$) and sG_{aw} ($p=0.01$) (see Fig. 1). In Table 1 we depict for each lung function parameter between which particle sizes these significant differences were present. In Table 2 we list the improvements of various lung function parameters after administration of 20 µg salbutamol and 8 µg ipratropium bromide respectively as 2.8 µm aerosols.







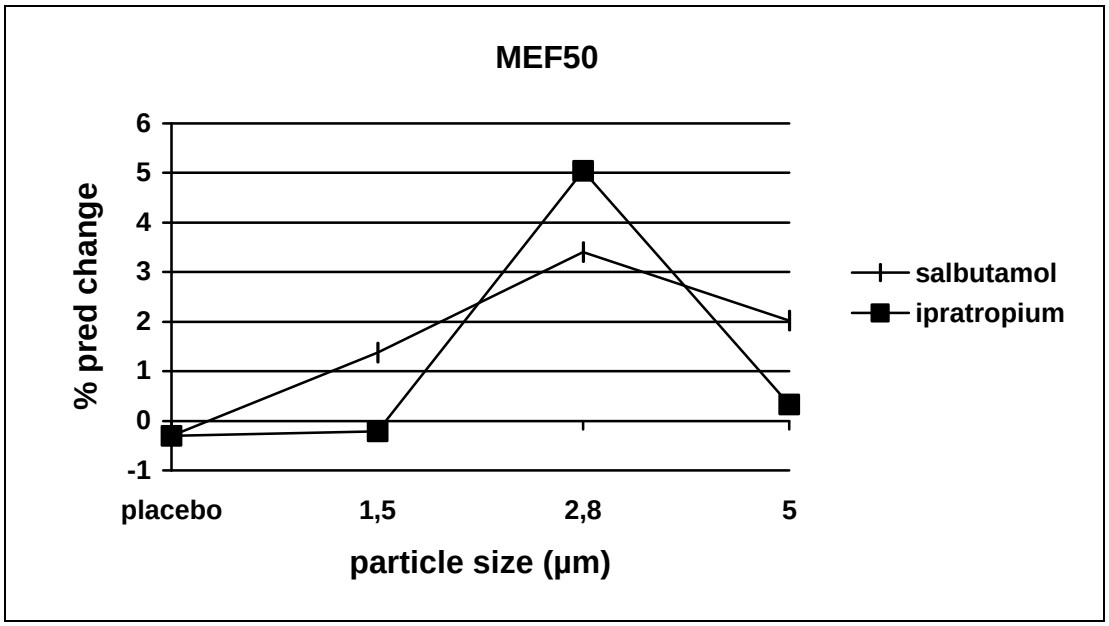


Fig. 1 Improvement of the, SG_{aw}, FEV_1, $MEF_{75/50}$ due to placebo or salbutamol and ipratropium bromide aerosols of increasing particle sizes. The improvement is presented as percentage change of the predicted value.

Discussion

In patients with a severe airflow obstruction the particle size of choice for an aerosol is approximately 3 µm, both for ipratropium bromide and salbutamol. These results are comparable to our earlier findings with salbutamol and ipratropium bromide in mild asthmatics. In those experiments the greatest bronchodilation was elicited by aerosols with a MMAD <2.8 µm[1 2].

We believe that these findings are explained by filtering by the extrathoracic/upper airways. Large particles are filtered out quickly in the central airways due to a high impaction-probability. Only small particles will escape from extrathoracic/upper airway deposition[3]. The dose of inhaled drugs in the lungs or lower airways therefore heavily depends on the filter characteristics of the extrathoracic/upper airways. Due to the fact that particles <2.8 µm pass the extrathoracic/upper airways better than the larger 5 µm particles, the actual dose in the airways is higher.

The observation that 2.8 µm aerosol induced greater bronchodilation than the 5 µm aerosol both in patients with mild and severe airway obstruction, suggests that the degree of airflow obstruction in the lower airways is not the most important factor determining response. We feel that the most likely explanation for the difference are the filtering characteristics of the non constrictable extrathoracic/upper airways.

In contrast to the earlier studies we now found that the 1.5 µm aerosol induced significantly less bronchodilation than the 2.8 µm aerosol. A likely explanation can be found in differences of deposition patterns. Smaller particles will always pass the central airways better than larger particles, so even in severely constricted patients a more peripheral orientated deposition pattern of 1.5 µm particles can be expected. Changes in lung function parameters are composed of changes in both central and peripheral airways. It is conceivable that in severely constricted patients the peripheral airways are less able to dilate. Therefore, deposition of bronchodilators in these parts of the airways results in less total dilatation. The smaller improvement of the peripheral lung function parameters strengthen this hypothesis. We have not visualised the deposition patterns of our aerosols in the airways, therefore we can not prove this explanation. The alternative explanation would be a low lung dose: small particles deposit less well and are exhaled to a higher degree causing low pulmonary deposition. However, due to the severe constriction one expects a higher deposition probability, leading to less particles exhaled and higher doses[8].

As in our previous experiments we used low dosages and obtained significant bronchodilation. We based the choice of administering only 20 µg salbutamol and 8 µm ipratropium bromide on the assumption that the lung deposition of monodispers aerosols is very high compared to polydispers aerosols delivered by a metered dose in-

haler. Many studies have shown that approximately 10-20% of the actuated dose reaches the airways and is effective[9]. 10% of a standard 200 µg salbutamol dose is 20 µg as is 8 µg for a standard ipratropium bromide MDI-dose. We do not know the relative efficacy of our formulations compared to MDI's, because this study was not set up as a direct comparison. The prestudy-check of the patients, however, included measurement of the reversibility after 200 µg salbutamol via MDI and it was found that the mean (sd) FEV_1-improvement was 7.6% (2.7%), compared to 8.5% (7%) improvement after 20 µg salbutamol as a 2.8 µm aerosol. We feel therefore that it is possible to induce clinically significant bronchodilation using low dosages of correctly formulated bronchodilators.

We conclude that in patients with a severe airflow obstruction the most suitable particle size of a $ß_2$-agonist and anticholinergic aerosol should be approximately 3 µm. Significant bronchodilation is obtainable with 10% of standard MDI dosages.

Literature

[1] Zanen P., Go T.L. and Lammers J-W.J.
The optimal particle size for ß-adrenergic aerosols in mild asthmatics.
Int J Pharm 107: 211-217 1994.

[2] Zanen P., Go T.L. and Lammers J-W.J.
The optimal particle size for parasympathicolytic aerosols in mild asthmatics.
Int J Pharm 114: 111-115 1995.

[3] Lippmann M, Yeates D.B. and Albert R.E.
Deposition, retention and clearance of inhaled particles.
Br J Ind Med 37: 337-362 1980.

[4] Heyder J, Gebhart J, Rudolf G, Schiller C.F. and Stahlhofen W.
Deposition of particles in the human respiratory tract in the size range 0.005-15 µm.
J Aerosol Sci 17: 811-825 1986.

[5] Cheah P.K.P and Davies C.N.
The spinning top aerosol generator-improving the performance.
J Aerosol Sci 15: 741-751 1984.

[6] Weir D.C. and Sherwood Burge P.
Measures of reversibility in response to bronchodilators in chronic airflow obstruction: relation to airway calibre.
Thorax 46: 43-45 1991.

[7] Girden E.R.
Anova: repeated measures.
Sage Newbury Park, 1992

[8] Anderson P.J., Wilson D.J. and Hiller F.C.
Respiratory tract deposition of ultrafine particles in subjects with obstructive or restrictive lung disease.
Chest 97: 1115-1120 1990.

[9] Zaninudin B.M.Z., Biddiscombe M, Tolfree S.E.J., Short M. and Spiro S.G.
Comparison of bronchodilator responses and deposition patterns of salbutamol inhaled from a pressurized metered dose inhaler, as a dry powder inhaler and as a nebulised solution.
Thorax 45: 469-473 1990.

The efficacy of a low dosed, monodisperse parasympathicolytic aerosol compared to a standard aerosol from a metered dose inhaler*

Introduction

In both mild and severe obstructed patients, monodisperse bronchodilator aerosols (geometric standard deviation <1.2) with a median mass aerodynamic diameter (MMAD) <2.8 µm induced a greater bronchodilation as compared to aerosols consisting of particles with larger MMAD's[1] [2] [3]. In these experiments a bronchodilator dose equal to 8 µg ipratropium bromide or 20 µg salbutamol was administered to patients, lead to clinically relevant bronchodilatations. These dosages constitute approximately 10-20% of the emitted dose of a standard metered dose inhaler (MDI)[4]. However, due to a lack of a direct comparison with the bronchodilator effects of a MDI, these experiments could not answer the question whether 8 µg ipratropium bromide or 20 µg salbutamol will elicit therapeutically equivalent effects. To base further research, these dosage choices needed validation. Therefore, we designed a study to compare the bronchodilator effects of a low dosed, monodisperse 2.8 µm bronchodilator aerosol with those of a conventional metered dose inhaler plus spacer, which is generally considered to be the most optimal way to administer an aerosol.

Materials and methods

Patients

Ten outpatients (3 women and 7 men) with at least partially reversible airflow obstruction volunteered. The average age (sd) was 49.5 (14.3) years, the mean forced expiratory volume in one second (FEV_1) (sd) was 58.1 (17.3) percent of the predicted value. Eligible patients showed a stable airflow obstruction and a more than 15% increase of baseline FEV_1 after inhalation of 200 µg salbutamol measured shortly before the trial. None were allowed to be smokers. Corticosteroids by inhalation were al-

* published in Eur J Clin Pharmacol 54: 27-30 1998

lowed and the dose kept stable, sodium cromoglycate and oral anti-asthma medication were not allowed. Some patient characteristics are given in table 1. Except for corticosteroids, their regular medication was discontinued six to eight hours before the start of the trial, while long acting $ß_2$-mimetics were stopped 15 hours prior to the start. All patients gave their written consent before entry into the study, which was approved by the hospital ethics committee.

Aerosol generation

Monodisperse aerosols (geometric sd <1.2) were produced by a spinning top generator[5]. A spinning top generator consists of a compressed air driven disk, rotating at 12000 rpm. Liquid is fed to the centre of the disk by a pump identical sized droplets are expelled from the rim of the disk. By blowing hot air over the disk these droplets are dried and subsequently transported to a small tank, from which the patients inhale. The concentration of the drug in and the viscosity of the solution govern the diameter of the resulting dry particles. An ipratropium bromide solution (1% in 50% water/50% ethanol) was used to obtain an aerosol with a MMAD of 2.8 µm. Each time a patient was due to start the aerosol inhalation, the mass of ipratropium bromide per litre of air and the particle diameter of the dry aerosol particles in the tank were measured by an Aerodynamic Particle Sizer 33 (TSI, St. Paul, Min). This is a so called time of flight particle sizer and gives information on both the particle size distribution and the mass contained in the aerosol in µg per litre of air (an APS 33 can not measure particles of a size exceeding 15 µm). To calculate the volume of air to be inhaled, we divided the dose to be administered by the mass of ipratropium bromide per litre of air rendering the litres to be inhaled. If 8 µg ipratropium bromide (dosage expressed as µg delivered to the mouth) had been inhaled, the aerosol inhalation was discontinued by switching over to non-aerosol containing air.

An ipratropium bromide containing metered dose inhaler (Atrovent®, Boehringer Ingelheim, batchno.: 95B28) was primed and connected to a spacer (Aerochamber®, Trudell Medical, London, Canada). One dose of 20 µg was fired into the spacer and to ensure maximal evaporation of the aerosol, 5 seconds later the patient inhaled from the spacer. Subsequently a second dose was administered in the same way within 10

seconds after the first one. A dose of 40 µg is the standard dose approved by regulators and does not reside on the top of the dose-response curve[6]. Between dosing the inhaler was shaken and care was taken to reduce electrostatic charging of the spacer. The particle size distribution and the mass of the aerosol as it leaves the spacer was measured by the above mentioned APS 33 (n=5).

Procedure

Each patient was studied three times at the lung function laboratory with intervals of one week. The baseline FEV_1 during the sessions was not allowed to vary more than 10%. Each session consisted of measurement of the lung function at baseline and 30 minutes after administration of the aerosol. A double-dummy technique was used to avoid bias due to differences in the inhalation equipment. In one session we administered 8 µg ipratropium bromide from the spinning top generator and a placebo from the MDI, in another session a monodisperse placebo aerosol and 40 µg ipratropium bromide from the MDI, and in a third session just placebo's. Administration of the aerosols was done in a randomised manner.

The inhalation manoeuvre from the spinning top generator consisted of inhalation of a slow vital capacity with a flow of 40-60 L/min, followed by a breath-holding period of ten seconds and a slow exhalation. The aerosol was administered during the entire inhalation manoeuvre. The inhalation flow and volume were measured by a hot wire anemometer placed close to the mouth of the patient. The amount of aerosol deposited in the anemometer was negligible. Before the aerosol inhalation the patients were taught the inhalation technique and they had the opportunity to correct the inhalation flow by watching an indicator connected to the anemometer. The inhalation manoeuvre from the MDI plus spacer also consisted of an inhalation of a slow vital capacity with a flow of 40-60 L/min, followed by a breath-holding period of ten seconds and a slow exhalation. An Aerochamber® contains a flow indicator that gives of a warning when the inhalation flow exceeds 40 L/min.

Lung function assessment

The lung function was assessed 30 minutes after inhalation of the aerosol. The lung function assistant was not informed about the type of aerosol administered. The spe-

cific airway conductance (sGaw) was measured with a body plethysmograph, the FEV_1, forced vital capacity (FVC), and vital capacity (VC) by means of spirometry, and the peak flow (PEF) and the maximum expiratory flows at 75/50/25% of the forced vital capacity ($MEF_{75/50/25}$) were derived from maximal expiratory flow-volume curves. Per determination three readings were taken and the best of three selected.

Statistics

The change in lung function was expressed as a percentage of the predicted value[7]. Using a pre-post test repeated measurements analysis of variance (ANOVA) it was determined whether significant differences, induced by the three types of aerosols, were present between the bronchodilatations[8]. If a significant difference was present, the mean square error of the ANOVA-procedure was used to calculate the least significant difference. The statistical power was calculated. In all calculations an α-value of 0.05 was considered to be significant.

Results

The MMAD (gsd) of the monodisperse aerosol was 2.8 (1.1) µm, that of the 'MDI+spacer' aerosol cloud 1.8 (2) µm. This means that respectively 84.4%, 50% and 15.6% of the mass of the 'MDI+spacer' aerosol consists of particles ≤3.8 µm, ≤1.8 µm and ≤0.9µm. The same data for the monodisperse aerosol are: ≤3 µm, ≤2.8 µm and ≤2.5 µm. The mass (sd) delivered to the mouth of the patient by the MDI+spacer combination proved to be in total 11.6 ± 2.3 µg.

All patients completed the study without noticeable side-effects. Administration of placebo did not induce significant bronchodilatations. With regard to the effects of different aerosols administered, we found significant differences versus placebo in case of the FVC, the FEV_1, the sGaw, the PEF and the MEF_{75}, but not for the $MEF_{50/25}$. No statistical differences between the monodisperse- and the MDI-aerosols were found for any of the lung function parameters measured (Table 2). The statistical power to detect a difference was, in case of the FEV_1, 99.8%, while any difference between the mean FEV_1-improvement exceeding 2.3% (equal to 107 ml) would lead to a statistical significant difference.

Patient no	Age (years)	Sex	Baseline FEV_1 (% predicted)	FEV_1-reversibility vs. baseline (%)
1	41	female	80	18.9
2	59	male	29.2	23.6
3	57	male	54.4	17
4	60	male	68.8	21.4
5	50	female	76.4	16.1
6	56	male	34.1	27.6
7	26	male	75.3	16,5
8	24	female	55.7	41
9	62	male	52,8	19.7
10	60	male	54,8	18.8

Table 1 Patient characteristics

	Improvement (sd)			
Lung function parameter	after placebo	after 40 µg ipratropium bromide from a MDI+spacer	after 8 µg monodisperse ipratropium bromide	p-value of the difference versus placebo
sGaw	10.4(19)	35.7(26.8)	29.3(19.6)	0.036
FVC	1.1(7.7)	10.7(7.9)	9.1(10.7)	0.004
FEV_1	0.3(5.6)	7.2(5.5)	8.4(5.1)	<0.001
peak flow	-3.9(11.2)	7.1(6.9)	8.9(10.3)	0.003
MEF_{75}	0(4.7)	9.8(7.8)	10.1(12.7)	0.005
MEF_{50}	0.1(3.8)	6.7(10.9)	7.1(6.9)	0.061
MEF_{25}	-0.8(3.9)	4.1(9.1)	9.8(7.8)	0.154

Table 2 Mean (sd) lung function improvement as % predicted after administration of placebo, 40 µg ipratropium from a MDI or 8 µg ipratropium as 2.8 µm monodisperse aerosol.

Discussion

We showed that bronchodilatations after administration of an 8 µg, 2.8 µm ipratropium bromide aerosol did not differ from the higher emitted dose of a metered dose inhaler. The number of patients studied was rather small. Therefore, the absence of significant differences may be due to a low discriminative power. Enright reported an intra-individual FEV_1-variability of 119 ml in women and 162 ml in men and we used these data for sample size calculations[9]. 8-10 patients appeared to be sufficient Being able to detect differences smaller than the intra-individual variability, we feel we are able to claim equivalence between the monodisperse and MDI-aerosol, because the differences are smaller than the intra-individual variability and therefore clinical irrelevant.

Aerosols generated by metered dose inhalers all show a wide distribution of particle sizes. A major part of the cloud consists of large particles which deposit in the extrathoracic/upper airways[10 11]. The drug-availability is therefore very low[12]. The high loss of drug in the upper airways may cause (systemic) side-effects, because the drug still can be absorbed from the upper airway mucosa (especially important in case of inhaled steroids). In general terms: an aerosol from a MDI is composed of an effective and a non-effective part, the latter consisting of large particles.

That non-effective part of a MDI-aerosol is unavoidably present. Aerosol generation by MDI's is based on pressure and thus results in polydispers aerosols. The high starting pressure delivers sufficient energy to break-up the fluid in small particles. Towards the end of the generation cycle, the pressure in the metering valve will have decreased, which is equivalent to large particles. Particle sizes can be reduced by increasing the pressure in the metered dose inhaler or by decreasing the metered volume, but the effect of these measures is reported to be limited[13]. We have administered an aerosol we consider to be optimal: it was monodisperse and contains those particles previously shown to be most effective[1 2 3]. Compared to a MDI aerosol one can say that we 'removed' the non-effective part and as a result the dose could be lowered substantially. The only further improvement we see possible, is an even stronger reduction of the dose. We think that the latter endangers an equivalent degree of bronchodilatation. In previous experiments we have determined dose-

response curves and noted that dosages lower than the one we administered now induced less marked dilatations[1]. Noting the current equivalence between our low monodisperse and the full MDI dose we feel that the 80% reduction of the latter is the maximum one possibly can do without the danger of decreased efficacy. Rephrasing this argument we can say that only 20% of an emitted MDI dose elicits a clinical effect. We have compared fixed dosages in stead of dose-response curves. One might remark that another light can be shed on our conclusion of equivalence when the 40 µg ipratropium bromide MDI dosage resides on the plateau of the dose-response curve. In other words when 20 µg and 40 µg are therapeutically equivalent, the finding of a 80% dose reduction is not universally applicable. In this respect we like to point at the fact that 40 µg ipratropium bromide is currently the *recommended* dose used by patients. That dose contains 80% of non-efficacious material due to its formulation. The formulation of 20 µg is an exact copy of the 40 µg, is seems logical to expect that of a 20 µg dose 80% is also non-efficacious.

Another possibility for improvement of aerosol characteristics is the use of spacers. Large droplets impact on the walls of the spacer due to their high inertia and in this way a spacer acts as a selecting device for aerosol particles. The result of both evaporation and impaction is a smaller sized aerosol. The drawback is that a considerable portion of the emitted MDI dose is lost within the spacer, which becomes relevant in case of (very) high priced drugs. In case of the Aerochamber® a loss of approximately 70% of the emitted dose was reported, which is in line with our findings[14]. The selection of smaller droplets by impaction and evaporation by an Aerochamber® leads to an aerosol with a MMAD of 1.9 µm, which complies with the recent findings of Barry[15]. An Aerochamber® delivers to the mouth of a patient an aerosol, which resembles in mass output and MMAD, our monodisperse 2.8 µm aerosol. In that sense an Aerochamber® seems adequate. This finding underlines our conclusion that it is possible to reduce the emitted MDI dose by improving aerosol characteristics: MDI + spacer combinations are generally considered to be equivalent to a MDI, while the ‘emitted dose’ of MDI + spacer combination is lower.

Still a spacer is not a standard solution to the problem how to reduce the emitted dose. Barry has also investigated the characteristics of other spacers and found less

favourable selection characteristics. Some spacers had a much higher mass output and/or coarser aerosols. A 80% reduction is not always possible with spacers and therefore they do not constitute a standard solution. A hallmark for an efficient spacer seems to be a 80% reduction of the emitted MDI dose and a MMAD of approximately 2-3 μm.

Our conclusion is that by administrating monodisperse aerosols of sufficiently small size, the total inhaled bronchodilatator dose can be reduced without loss of efficacy.

Literature

[1] Zanen P., Go T.L. and Lammers J-W.J.
The optimal particle size for ß-adrenergic aerosols in mild asthmatics.
Int J Pharm 107: 211-217 1994.

[2] Zanen P., Go T.L. and Lammers J-W.J
The optimal particle size for parasympathicolytic aerosols in mild asthmatics.
Int J Pharm 114: 111-115 1995.

[3] Zanen P., Go T.L. and Lammers J-W.J.
The optimal particle size for $ß_2$-mimetic and parasympathicolytic aerosols in patients with severe airway constrictions.
Thorax 51: 977-980 1996

[4] Zaninudin B.M.Z., Biddiscombe M, Tolfree S.E.J., Short M and Spiro S.G.
Comparison of bronchodilator responses and deposition patterns of salbutamol inhaled from a pressurised metered dose inhaler, as a dry powder inhaler and as a nebulised solution.
Thorax 45: 469-473 1990.

[5] Cheah P.K.P. and Davies C.N.
The spinning top aerosol generator-improving the performance.
J Aerosol Sci 15: 741-751 1984.

[6] Britton J, Hanley S.P., Garrett H.V., Hadfield J.W., Tattersfield A.E.
Dose related effects of salbutamol and ipratropium bromide on airway calibre and reactivity in subjects with asthma.
Thorax 48: 300-305 1988.

[7] Weir D.C. and Sherwood Burge P.
Measures of reversibility in response to bronchodilators in chronic airflow obstruction: relation to airway calibre.
Thorax 46: 43-45 1991.

[8] Girden E.R.
Anova: repeated measures.
Sage Newbury Park 1992.

[9] Enright P.L., Johnson L.R., Connett J.E., Voelker H. and Buist A.S.
Spirometry in the Lung Health Study 1 Methods and quality control.
Am Rev Respir Dis 143: 1215-1223 1991.

[10] Bouchikhi A., Becquemin M.H., Bignon J., Roy M. and Teillac A.
Particle size study of nine metered dose inhalers and their deposition probabilities in the airways.
Eur Respir J 1:547-552 1988.

[11] Hiller C., Mazumder M., Wilson D. and Bone R.
Aerodynamic particle size distribution of metered dose bronchodilator aerosols.
Am Rev Respir Dis 118: 311-317 1978.

[12] Newman P., Clarke S.W.
Inhalation devices and techniques
In: Asthma, 1992,
ed Clark T.J.H., Godfrey S. and Lee T.H.

[13] Morén F.
Drug deposition of pressurised inhalation aerosols II. Influence of vapour pressure and metered volume.
Int J Pharm 1: 213-218 1978.

[14] Rau J.L., Restrepou R.D., Deshpande V.
Inhalation of single vs. multiple metered-dose bronchodilator actuations from reservoir devices. An in-vitro study.
Chest 109: 969-974 1996.

[15] Barry P.W., O'Callaghan C.
Inhalational drug delivery from seven different spacer devices
Thorax 51: 835-840 1996.

Reducing adverse effects of inhaled fenoterol through optimisation of the aerosol formulation

Introduction

In a previous experiment we showed that it is possible to reduce the dose of inhaled bronchodilators by an optimisation of the aerosol formulation. The latter means generating only those particles, which proved to be most efficient i.e. 2.8 µm particles[1]. In that experiment inhalation of 8 µg of a 2.8 µm monodisperse ipratropium bromide aerosol proved to be therapeutically equivalent to 40 µg from a conventional metered dose inhaler plus spacer[2]. Aerosols generated by conventional metered dose inhalers all show a wide distribution of particle sizes (polydisperse aerosols). Most mass delivered to the patient consists of large particles, which mainly deposit in the extrathoracic or upper airways and as we have shown, that mass does not contribute to the beneficial effect of the drug[2 3]. On the other hand, some of the delivered mass consists of very fine particles, i.e. <2.8 µm, which penetrate the airways up to alveolar levels, where absorption is, most probably, quick and complete. The bronchodilator effects of these parts of the aerosol cloud will be small, while our hypothesis is that they still may contribute to the occurrence of adverse effects. We conceived that the ineffective part of the aerosol cloud will deposit on a mucosal surface where it is prone to absorption with systemic adverse effects as possible result.

In this report we describe the results of a placebo-controlled study to the adverse effects of a 2.8 µm monodisperse fenoterol aerosol as compared to a conventional fenoterol metered dose inhaler in a dose ratio 1: 5.

Materials and methods

Volunteers

Twelve healthy volunteers (8 women and 4 men) without pulmonary diseases participated in this study. The average age (sd) was 23.5 (2.6) years. None were allowed to be smokers or to use any medication, expect from oral anticonceptives. All volunteers gave their written consent before entry into the study, which was approved by the hospital

ethics committee.

Dosages administrated

The dose-response curve of fenoterol adverse effects has been described repeatedly[4, 5]. Therefore, to minimise volunteer load, we decided to use only fixed doses of fenoterol i.e. 800 µg from the MDI and 160 µg in the form of the monodisperse aerosol. The 800 µg MDI dose was presumed to induce measurable though acceptable adverse effects.

Aerosol generation

Monodisperse aerosols (geometric sd <1.2) were produced by a spinning top generator[6]. A spinning top generator consists of a compressed air driven disk, rotating at 12000 rpm. Liquid is fed to the centre of the disk by a pump identical sized droplets are expelled from the rim of the disk. By blowing hot air over the disk these droplets are dried and subsequently transported to a small tank, from which the volunteers inhale. The concentration of the drug in and the viscosity of the solution govern the diameter of the resulting dry particles. An fenoterol solution (1% in 50% water/50% ethanol) was used to obtain an aerosol with a MMAD of 2.8 µm. Each time a volunteer was due to start the aerosol inhalation, the mass of fenoterol per litre of air and the particle diameter of the dry aerosol particles in the tank were measured by an Aerodynamic Particle Sizer 33 (TSI, St. Paul, Min). This is a so called time of flight particle sizer and gives information on both the particle size distribution and the mass contained in the aerosol in µg per litre of air. To calculate the volume of air to be inhaled, we divided the dose to be administered by the mass of fenoterol per litre of air rendering the litres to be inhaled. If 160 µg fenoterol (dosage expressed as µg delivered to the mouth) had been inhaled, the aerosol inhalation was discontinued by switching over to non-aerosol containing air.

A fenoterol containing metered dose inhaler (Berotec®, Boehringer Ingelheim, the Netherlands, batchno.: 96D23) was primed and connected to a non-static spacer device (Aerochamber®, Trudell Medical, London, Canada). One dose of 200 µg was fired into the spacer and the volunteer inhaled from the spacer. Subsequently three additional doses were administered in the same way within 20 seconds after the first one. Between dosing the inhaler was shaken. Between sessions the spacer was immersed in a water/detergent solution and just before the sessions air-dried to prevent build of electro-

static charges.

Parameters measured

We selected the decrease in serum potassium level, the increase in finger-tremor, changes in heart rate and blood-pressure as primary end-points. A secondary end-point was the increase in specific airway conductance as a measure of the beneficial effect of fenoterol.

The specific airway conductance (sGaw) was measured with a body plethysmograph (Jaeger Masterlab, Wurzburg, Germany). Serum potassium was obtained through non occluded venapuncture and subsequently determined by means of flame photometry and reported in mmol/litre. Finger tremor was determined by using a displacement transducer (Philips, the Netherlands, PR 9310)[7]. This transducer gives off a voltage related to the actual tremor. Because tremor induces both positive and negative voltages, it was squared and subsequently averaged over a period of one minute. Blood pressure was obtained by the Riva-Rocci method and heart rate by manual counting over a period of 60 sec's. The systolic and diastolic blood pressure were subtracted to obtain the so called pulse pressure.

Procedure

Each volunteer was studied three times at the lung function laboratory with intervals of one week. Each session consisted of measurement of all parameters at baseline and 15 minutes after administration of the aerosol. A double-dummy technique was used to avoid bias due to differences in the inhalation equipment. In one session we administered 160 µg fenoterol from the spinning top generator and a placebo from the MDI plus spacer, in another session a monodisperse placebo aerosol and 800 µg fenoterol from the MDI plus spacer, and in a third session just placebo's. Administration of the aerosols was done in a randomised manner.

The inhalation manoeuvre from the spinning top generator consisted of inhalation of a slow vital capacity with a flow of 40-60 L/min, followed by a breath-holding period of ten seconds and a slow exhalation. The aerosol was administered during the entire inhalation manoeuvre. The inhalation flow and volume were measured by a hot wire anemometer placed close to the mouth of the volunteer. The amount of aerosol deposited in

the anemometer was negligible. Before the aerosol inhalation the volunteers were taught the inhalation technique and they had the opportunity to correct the inhalation flow by watching an indicator connected to the anemometer. The inhalation manoeuvre from the MDI plus spacer also consisted of an inhalation of a slow vital capacity with a flow of 40 L/min, followed by a breath-holding period of ten seconds and a slow exhalation. An Aerochamber® contains a flow indicator that gives of a warning when the inhalation flow exceeds 40 L/min.

Statistics

Using a pre-post test repeated measurements analysis of variance (ANOVA) it was determined whether significant differences, induced by the three types of aerosols, were present[8]. If a significant difference was present, the mean square error of the ANOVA-procedure was used to calculate orthogonal contrasts between the formulations to determine which formulations differed from each other. In all calculations an α-value of 0.05 was considered to be significant. All data are presented as mean values with their standard deviation.

Results

The results of the measurements of all parameters before and after administration of placebo, the monodisperse and the metered dose inhaler aerosol are depicted in Fig. 1. The differences in serum potassium changes between the monodisperse and MDI-aerosol on the one hand and placebo on the other were highly significant ($p=0.004$ resp. $p<0.001$), but also between the two active formulations ($p=0.001$). The monodisperse aerosol elicited a mean (sd) fall of 0.27 (0.27) mmol/l, compared to 0.67 (0.18) mmol/l after the MDI-aerosol. The tremor response showed an identical picture: between placebo and actives a significant difference ($p=0.001$ resp. $p=0.01$) and between the actives ($p=0.029$). The monodisperse aerosol elicited a mean (sd) increase of 0.07 (0.07) volt, compared to 0.29 (0.34) volt after the MDI-aerosol. The changes in pulse pressure and heart rate did not differ significantly from placebo or between the actives. The specific airway conductance changes were significantly different between placebo and the actives ($p<0.001$), but not between the two drug aerosols ($p=0.87$). An 0.31 (0.24) resp. 0.29(0.49) $kPa^{-1}sec^{-1}$ increase was noted after the monodisperse or MDI-aerosol.

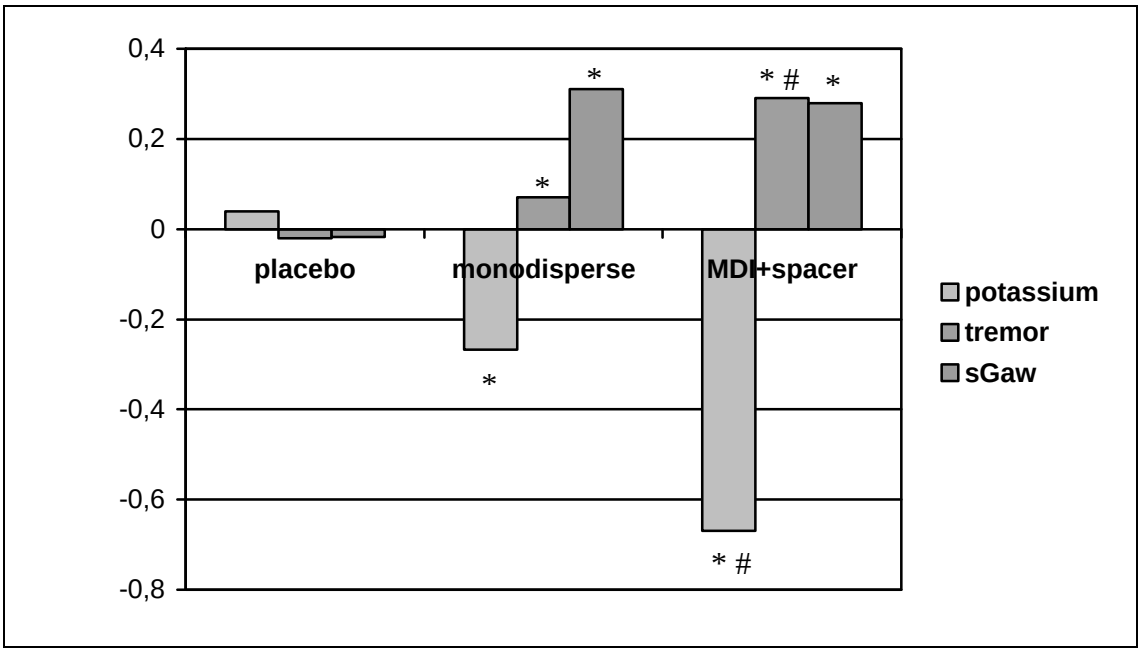


Fig. 1 Changes in serum potassium (mmol/l), tremor response (volt) and sGaw ($kPa^{-1}s^{-1}$) due to placebo, 160 µg fenoterol as 2.8 µm monodisperse aerosol or 800 µg fenoterol from a MDI + spacer. * resp. # indicates significant changes versus placebo or the monodisperse aerosol.

Discussion

We have shown that administrating an optimised aerosol formulation, while preserving the bronchodilating effects, can reduce systemic adverse effects of inhaled fenoterol. Our group defined 2.8 µm particles as the best choice for bronchodilator aerosols: larger and smaller particles showed a reduced efficacy[1]. Unfortunately these 2.8 µm particles constitute only a small percentage of the emitted dose of a MDI: the bulk of the emitted dose consists of less or even non-efficacious particles[3]. These less efficacious particles are unavoidable due to the way MDI's aerosols are generated, but increased dosages are needed to compensate for the lower particle efficacy. By generating and administration of the most efficacious particles we succeeded to lower dosages by 80% relative to the emitted dose of a MDI[2].

However, that less efficient part of the aerosol cloud is still prone to absorption after deposition on mucosal surfaces in the upper airways and the potency to elicit systemic adverse effects is of course not altered. The ratio of local efficacy over systemic potency is therefore not identical for each particle size: larger and smaller particles will show a

more unfavourable ratio than the 2.8 µm particles. This means that the adverse effect eliciting potency of such particles is stronger compared to the 2.8 µm particles.

The results of this study show that the larger part of the adverse effects of inhaled fenoterol are elicited by that less efficacious part of the aerosol cloud. At the same time it is evident that a complete abolishment of adverse effects of inhaled drugs is not a realistic goal. In our volunteers a decrease of the potassium serum level and an increase of the finger tremor is still noticeable after the monodisperse aerosol. These changes were statistically significant, but clinically less important. The mean reduction of the serum potassium level after the monodisperse aerosol was only 0.27 mmol/l at a dose equivalent to 800 µg from a MDI. It is to be expected that higher dosages of any monodisperse aerosol, as administered here, will elicit stronger adverse effects.

It will be evident that both large and small particles will show unfavourable local efficacy over systemic potency ratios. The question now is which of these particles are most to blame for eliciting adverse effects. This might be dependent on the drug administered. Spacers are generally known to reduce the presence of large particles, which often comprise the majority of the mass delivered[9]. Using large volume spacers Meeran and coworkers showed that the osteocalcin levels fell less when a beclomethason MDI/spacer combination was used, while Brown reported similar conclusions[10 11]. This indicates that large particles are to blame for systemic adverse effects. As said before these large particles constitute the bulk of the mass and an efficient absorption from the upper airways and/or the gastro-intestinal tract results in a high systemic load. When gastro-intestinal absorption is prevented or the drug inactivated through pre-systemic elimination, the adverse effects eliciting capacity of large steroid particles is of course diminished and the focus then changes to smaller particles.

Lipworth showed that lung bioavailability of salbutamol in severe asthmatics is reduced compared to mild patients or healthy volunteers[12]. In severe asthmatics peripheral deposition will be reduced and the latter experiment indicates that absorption from the peripheral airways is more efficient compared to central compartment absorption. The above suggests that small bronchodilator particles mainly contribute to systemic absorption and thereby to adverse effects. The use of an Aerochamber® spacer device strengthens this assumption, because the adverse effects due to the MDI/spacer combination are

significant. Dolovich showed both in healthy volunteers and in bronchitis patients that a spacer considerably reduced the throat-deposition of a fenoterol MDI. In healthy volunteers the deposition in the lung periphery slightly increased, but in patients it did not change[13]. This means that the effect of an Aerochamber® is an efficient removal of the larger particles, which now can not enter the airways or the gastro-intestinal tract. Therefore the small particle fraction is the most likely candidate for eliciting adverse effects.

In this respect is seems miraculous that a small 2.8 µm aerosol still elicits significantly less adverse effects as the small particle fraction of a MDI. The latter, however, is a polydisperse aerosol even now containing smaller and larger particles. Especially the smaller particles are efficiently absorbed because they deposit in the airway regions with the thinnest mucosa. Recent data on HFA-BDP MDI's show that small changes in MMAD from 2.5 to 1.1 µm increased the lung bioavailability by a factor 2 [14]. Apparently 2.8 µm particles show a deposition pattern which is a better compromise between penetration, effect and absorption as other particles.

Spacers are known to alter the particle size distribution of MDI aerosol clouds. However, the data reported by Dolovich do show that the use of an Aerochamber® does not invalidate the results of this experiment: the mass depoisted in the lung and the deposition pattern are similar relative to the MDI alone, so our results will be valid for fenoterol MDI's too[13]. Furthermore we ensured that build up of electrostatic charges during the use of the Aerochamber® was minimal. Even, if some of the respirable fraction would be lost due to electrostatic phenomena, the results stay valid. The adverse effects noted are then due to a somewhat 'emitted' dose from the MDI/spacer, only showing the potency of the drug to elicit adverse effects.

Previously we showed therapeutic equivalence of an 8 µg, 2.8 µm ipratropium bromide aerosol to a 40 µg MDI aerosol. This equals a five times higher potency of the monodisperse aerosol (based on the nominal dose) and we adopted the same dosage ratio for this experiment, although we now used fenoterol. We believe this is a valid approach, because 1] the optimal particle size for a salbutamol and ipratopium bromide aerosol proved to be the same, 2] the particle size distributions of a fenoterol and ipratropium bromide MDI aerosols will not differ much and 3] aerosol deposition patterns are gov-

erned by the aerodynamic characteristics of particles, which are not significantly influenced by the chemical nature of the drug itself[1 15]. Therefore our choice of a 1:5 ratio in dosages is a valid one.

Cardiovascular parameters, like blood pressure and heart rate, did not change much. We feel that these parameters react in a more blunt way than tremor or serum potassium. Earlier studies by Newhouse showed that cardiovascular parameters changed significantly versus baseline at dosages exceeding 1200 µg fenoterol[5]. In two studies in asthmatic patients the cardiovascular dose-response curves were flat up to 1000 µg resp. 800 µg dosages and only become steeper at higher dosages[16, 17]. Having administered 800 µg, it is no surprise to us that we did not encounter significant changes in these parameters.

We have carried out these experiments in healthy volunteers, who did not use $ß_2$-mimetics and, by definition, were not tolerant to the action of fenoterol. As known, long term users of $ß_2$-mimetics might show tolerance, resulting in diminished beneficial or adverse effects. The relevance of this study is, therefore, changed when long term users due to tolerance do not suffer from adverse effects. In the literature tolerance has been debated repeatedly, but a consensus on its impact has not been reached[18, 19]. Fenoterol has been reported not to induce tolerance[20]. When tolerance is an issue, compared to long term users, healthy volunteers will be better discriminators and they will resemble to short users of $ß_2$-mimetics. Besides that, we believe that our results can be extrapolated to long term bronchodilators users. Development of tolerance to fenoterol is a pharmacodynamic phenomenon and not influenced by the formulation of the aerosol. So it is unlikely that the difference, noted now between the adverse affects of the two active preparations, will change on the long run.

In conclusion we can say that a change in aerosol formulations towards monodisperse aerosols will reduce systemic adverse effects of $ß_2$-mimetics, but it is unlikely that they will disappear entirely, especially at higher dosages.

Literature

[1] Zanen P., Go T.L. and J-W.J. Lammers.
The optimal particle size for ß$_2$-mimetic and parasympathicolytic aerosols in patients with severe airway constrictions.
Thorax 51: 977-980 1996.

[2] Zanen P., Go T.L. and J-W.J. Lammers.
The efficacy of a low dosed, monodisperse parasympathicolytic aerosol compared to a standard aerosol from a metered dose inhaler.
Eur J Clin Pharmacol 54: 27-30 1998.

[3] Zaninudin B.M.Z., Biddiscombe M., Tolfree S.E.J., Short M. and Spiro S.G.
Comparison of bronchodilator responses and deposition patterns of salbutamol inhaled from a pressurised metered dose inhaler, as a dry powder inhaler and as a nebulised solution.
Thorax 45: 469-473 1990.

[4] Bremner P., Siebers R., Crane J., Beasley R. and Burgess C.
Partial vs full ß-receptor agonism. A clinical study of inhaled albuterol and fenoterol.
Chest 109: 957-962 1996.

[5] Newhouse M.T., Chapman K.R., McCallum A.L., Abboud R.T., Bowie D.M., Hodder R.V., Paré P.D., Mesic-Fuchs H. and Molfino N.A.
Cardiovascular safety of high doses of inhaled fenoterol and albuterol in severe asthma.
Chest 110: 595-603 1996.

[6] Cheah P.K.P. and Davies C.N.
The spinning top aerosol generator-improving the performance.
J Aerosol Sci 15: 741-751 1984.

[7] Fokkens J.K., van Loenen V.L. and van den Berg W.
Tremor measurement in asthma.
Eur J Respir Dis 63: 388-391 1982.

[8] Girden E.R.
Anova: repeated measures. Sage Newbury Park 1992.

[9] Barry P.W. and O'Callaghan C.
Inhalational drug delivery from seven different spacer devices
Thorax 51: 835-840 1996.

[10] Meeran K, Burin J.M., Noonan K.A., Price C.P. and Ind P.W.
A large volume spacer significantly reduces the effect of inhaled steroids on bone formation.
Postgrad Med J 71: 156-159 1995.

[11] Brown P.H., Greening A.P. and Crompton G.K.
Large volume spacer devices and the influence of high dosed beclomethasone dipropionate on hypothalamo-pituitary-adrenal axis function.
Thorax 48: 233-238 1993.

[12] Lipworth B.J. and Clark D.J.
Effects of airway calibre on lung delivery of nebulised salbutamol.
Thorax 52: 1036-1039 1997.

[13] Dolovich M., Ruffin R., Corr D. and Newhouse M.T.
Clinical evaluation of a simple demand inhalation MDI aerosol delivery device.
Chest 84: 36-541 1983.

[14] Harrison L, Soria I., Machaek J., Jacobson J., Purrington A. and Cline A...
Beclomethasone pharmacokinetics in asthmatics given CFC and CFC-free inhalers.
Eur Resp J suppl 23 256S 1996.

[15] Lippmann M., Yeates D.B. and Albert R.E.
Deposition, retention and clearance of inhaled particles.
Br J Ind Med 37: 337-362. 1980.

[16] Lipworth B.J., Newnham D.M., Clark R.A., Dhillon D.P., Winter J.H. and McDevitt D.G..
Comparison of the relative airways and systemic potencies of inhaled fenoterol and salbutamol in asthmatic patients.
Thorax 50: 54-61 1995.

[17] Burgess C.D., Windom H.H., Pearce N., Marshall S., Beasley R., Siebers R.W. and Crane J.
Lack of evidence for beta-2 receptor selectivity: a study of metaproterenol, fenoterol, isoproterenol and epinephrine in patients with asthma.
Am Rev Respir Dis 143: 444-446 1991.

[18] Teale C. and Pearson S.B.
Regular nebulised terbutaline in chronic obstructive airways disease: dose-response studies fail to detect tolerance.
Br J Clin Pharmacol 32: 111-114 1991.

[19] Bhagat R., Kalra S., Swystun V.A. and Cockcroft D.W.
Rapid onset of tolerance to the bronchoprotective effect of salmeterol
Chest 108: 1235-1239 1995.

[20] Angelici E, Delfino M., Carlone S., Serra P., Fineberg N.S. and Farber M.O.
Tolerance to inhaled fenoterol
Am Rev Respir Dis 129: 1014-1016 1984.

Summary, discussion and future research

Summary

The aim of our research was to determine the optimal aerosol formulation for inhaled bronchodilators and subsequently to what extent dose and adverse effects could be lowered.

In chapter 2 of this thesis the most relevant deposition mechanisms (impaction, sedimentation and Brownian movement) are presented and elaborated. These mechanisms, which all show their own characteristics, are related to the size of the inhaled particle. *Impaction* is caused by the inertia of large particles, which cause them to leave the airstream and to collide with the mucosa. The particles are too heavy or travel too fast to follow bends in the bronchial tree. *Sedimentation* is a time-dependent process: due to gravity particles fall and the velocity reached is related to their size. The smaller the particle, the lower their terminal velocity will be and therefore small particles need more time to reach the mucosa. They have the capacity to penetrate the smaller airways together with low deposition rates. When they are 'too small', the sedimentation time frequently exceeds that of a complete breathing-cycle and these particles will be exhaled almost completely. *Brownian movement* is only of importance for very small particles and of less interest for therapeutic aerosols, due to their low total mass. Deposition is a physical process and each mechanism is can be expressed in mathematical equations enabling researchers to build models. The most elaborated model, that of Findeisen and followers, is reviewed. Consensus exists on the general pattern of deposition: large particles impact in the upper or extrathoracic airways, while smaller particles penetrate the airways further. The number of depositing particles is related to their size: the smaller the size, the lower the number. A deposition minimum is present with particles of 0.5 μm. Data on the so-called regional deposition indicate that deposition patterns within the airways are not circumscript, but widespread. The amount of spread is related to the width of the size distribution of an aerosol, the stronger that width, the stronger the spread. Obstruction of airways leads to an increased central deposition.

Chapter 3 deals with aerosol deposition in healthy subjects. The deposition pattern, derived from modelling, is confirmed by these in-vivo experiments. The extratho-

racic deposition increases rapidly as particle size increases. The major part of particles over 6 µm deposit in extrathoracic airways, while smaller particles may end in the alveoli. The bronchial deposition is relatively low with a maximum of 20%. Next to size, the inhalationflow and volume heavily influence deposition. An increase in volume increases deposition, while the reverse is true for the flow. From particle size, inhalationflow and volume a researcher can predict the total deposition. Conceivably, lack of control of these parameters in an experiment increases the variance of the deposition pattern.
In asthmatic subjects particle deposition is increased and related to the extent of the bronchobstruction. In these patients the deposition patterns, as measured by γ-cameras are patchy and not uniformly distributed.

Deposition of therapeutic aerosols is reviewed in chapter 4. The deposition characteristics are strongly dependent on the inhaler or nebuliser used. Metered dose inhalers are capable to deliver 10-20% of the emitted dose to the lower airways. The aerosols they generate, are polydisperse and the bulk of the emitted mass consists of large particles, which deposit in the upper airways. Spacers decrease upper airway deposition, although this effect varies between the different devices. Dry powder inhalers lack some of the drawbacks of metered dose inhalers, but the efficacy may negatively be influenced by the inspiratory effort of the patient.
Only in few experiments the relationship between particle size and therapeutic effect has been investigated and unfortunately the outcome is contradictory. Some researchers do not show differences in efficacy for particle sizes below 5 µm, the only important factor is to be smaller than 5 µm. However, others do show differences in that region: a 2.5 µm aerosol appeared to be more potent than 5 µm aerosols. Many factors can be responsible for these different results. For instance the use of different nebulisers can lead to differences in administered dosages and (highly) polydisperse aerosols. Depending on the severity of these confounding factors a size-efficacy relationship can be missed.

In chapters 6 to 10 the results of our experiments are presented, which (in summary) showed that:

1. in mild and severe obstructed patients the strongest bronchodilation is elicited by 2.8 µm monodisperse salbutamol or ipratropium aerosols,

2. 8 µg ipratropium bromide as 2.8 µm monodisperse aerosol elicits an equivalent bronchodilation as 40 µg from a standard metered dose inhaler,
3. adverse effects of such a low dosed monodisperse aerosol are significantly less compared to a standard metered dose inhaler.

In the first experiment 8 stable asthmatics with a FEV_1 of 72% of the predicted value inhaled 3 types of monodisperse salbutamol aerosols, with particle sizes of 1.5 µm, 2.8 µm and 5 µm, respectively, and a placebo aerosol (chapter 6). The volunteers inhaled cumulative dosages of 5 µg, 10 µg, 20 µg and 40 µg salbutamol, followed by lung function measurements. The dose-response curves were analysed with repeated measurements anova. For the FEV_1 and the $MEF_{75/50/25}$ the 2.8 µm aerosol induced a significant better bronchodilation than the 5 µm aerosol. In case of the PEF the 1.5 µm aerosol elicited a significantly smaller improvement than the 2.8 µm aerosol. No particle size effects were noticeable in case of the VC, FVC and the R_{tot}. From these results it was concluded that in mild asthmatics the particle size of choice for a $ß_2$-mimetic aerosol should be around 2.8 µm.

In the second experiment mild asthmatics (FEV_1>70% of predicted) inhaled monodisperse ipratropium bromide aerosols, with the same particle sizes as above (chapter 7). The volunteers inhaled only 8 µg ipratropium bromide, followed by lung function measurements and evaluated. Again, according to the changes in FEV_1 and $MEF_{50/25}$ the 1.5/2.8 µm aerosol induced a significant larger bronchodilation than the 5 µm aerosol. No particle size effects were noticeable with regard to changes in R_{tot}, VC, FVC and PEF. In mild asthmatics the particle size of choice for a parasympathicolytic aerosol should also be ≤2.8 µm.

Logically the next experiment was carried out in patients with more severe airflow limitations (chapter 8). Seven stable patients with a mean FEV_1 of 37.9% of the predicted value inhaled monodisperse salbutamol and ipratropium bromide aerosols, with particle sizes of 1.5 µm, 2.8 µm and 5 µm, respectively, and a placebo aerosol. The volunteers inhaled 20 µg salbutamol and 8 µg ipratropium bromide, followed by lung function determination and evaluation was performed as mentioned above. The 2.8 µm aerosol induced greater improvements in FEV_1, sGaw and $MEF_{75/50}$ than the other particle sizes. In patients with severe airflow obstruction the particle size of choice for a $ß_2$-agonist or anticholinergic aerosol should also be approximately 3 µm.

To discover whether the bronchodilator effects of these low dosed monodisperse aerosols differed from those of standard dosages delivered by metered dose inhalers, we carried out a comparative trial (chapter 9). 10 stable outpatients, with a mean FEV_1 of 58.1 % of the predicted value, inhaled a placebo aerosol, 8 µg of a 2.8 µm monodisperse ipratropium bromide aerosol and 40 µg from a metered dose inhaler plus spacer, followed by lung function measurements and analysis by repeated measurements analysis of variance. Greater improvements compared to placebo were evident for the FVC, the FEV_1, the sGaw, PEF and the MEF_{75}. In these cases the low dosed 2.8 µm aerosol proved to be equivalent to the higher dosed metered dose inhaler. So, by changing the polydisperse characteristic of inhaled aerosols to a monodisperse pattern, the dose of the drug administered can be reduced by 80% without a loss of efficacy.

Subsequently we compared the adverse effects of 160 µg fenoterol in the form of a 2.8 µm monodisperse aerosol to those of 800 µg inhaled as a conventional metered dose inhaler aerosol + spacer (chapter 10). Healthy volunteers (8 women and 4 men) participated in this study and inhaled in random order placebo, the monodisperse and the MDI aerosol. Changes in serum potassium, finger tremor, blood pressure, heart rate and specific airway conductance were measured before and 15 min after administration. Compared to placebo the active aerosols elicited a significant similar improvement of airway conductance and adverse effects. Potassium levels decreased by 0.27 mmol/l after the monodisperse aerosol, while the MDI lowered them by 0.67 mmol/l ($p=0.001$). Finger tremor also increased less. Changes in the cardiovascular parameters were not significantly different from placebo. From these results it can be concluded that changing the formulation of MDI aerosols enables reduction of adverse effects.

Discussion

The first main conclusion from these experiments is that in patients with mild and severe airflow limitation, both for $ß_2$-mimetics and parasympathicolytic drugs the optimal particle size is ±3 µm. It also appears is that administration of these optimal sized aerosols can lead to a significant reduction of adverse effects.

The first conclusion can be explained by the existence of an upper airway filter. The extrathoracic and upper airways function as an effective filter for large particles,

protecting the lower airway against a high load of dust or other possibly harmful materials. Administration of large particles is therefore inefficient, because these are lost in the upper airways filter leading to low dosages in the lower airways. This may result in a low therapeutic effect. This phenomenon occurs both for ß$_2$-mimetics and parasympathicolytic aerosols and offers an explanation for the lower efficacy of the 5 µm aerosols, which we found in all experiments. In this respect the calculations of the regional deposition by Gerrity et al. are highly informative[1]. For 5 µm particles they show a predominant upper airway deposition, while the lower airway deposition is lower compared to the 2 and 3 µm particles. The experimental data by Svartengren et al. on the cut-off characteristics of the oropharynx underline the importance of the upper airway filter[2]. Moreover, it has to be taken into account that ß$_2$-mimetics and parasympathicolytic aerosol particles will be hygroscopic to a certain extent. In a moisture environment, their size will grow and thereby the upper airway deposition of the larger particles. 5 µm particles grow into much larger particles than 2.8 µm, favouring their upper airway deposition.

Having explained the reasons for the low 5 µm efficacy, the differences between the 2.8 µm and the 1.5 µm aerosols need explanation. We expected that for ß$_2$-mimetics and parasympathicolytic aerosols, due to the differences in receptor distributions, different optimal particle sizes would appear. A more centrally orientated deposition pattern for parasympathicolytic drugs seemed logical and our results confirm this hypothesis (2.8 µm more potent than 1.5 µm). Maybe the deposition pattern of 5 µm particles would be better, in the sense of an even better fit to the receptor distribution. However, if this would be the case, the low penetration and dose apparently out-weighs the closer fit to the receptor distribution. In other words, an optimal fit be-tween deposition pattern is of relatively low value when the deposited mass is (very) low. An ill-fitting pattern with higher local masses is then preferable.

For ß$_2$-mimetics it might have been expected or argued that 1.5 µm aerosols are a better choice, because of a better fit to the receptor distribution. We feel that a com-bination of effects can explain the ß$_2$-mimetics results. First, more smaller particles are exhaled than larger particles, even in asthmatics. The experiments by Schiller-Scotland et al. clearly show an almost twice as high deposition for 3 µm particles compared to 1 µm particles. The differences in deposition rates are less pronounced in asthmatics compared to healthy volunteers, but they are present[3]. So it is expected

that 2.8 µm aerosols are equivalent to higher dosages and hence more potent. Apparently, the dose outweighs the deposition pattern.
It has to be realised that depositions patterns are never circumscript. The data by Gerrity et al. indicate that the deposition patterns of 1 µm, 2 µm and 3 µm particles in the lower airways do not differ strongly. The spread of particles is more or less comparable, but the mass deposited is higher for the 3 µm particles. Therefore when the deposited dose is higher with comparable deposition patterns, the dose definitely outweighs the deposition patterns.

The results in patients with severe airflow limitations can be explained by the same principles. 5 µm particles hardly will enter the lower airways. The function of the upper airway filter will not be highly different from that of mild asthmatics or healthy subjects. One might have expected that the penetration of 1.5 µm particles into constricted airways would be the most optimal, but this proved not to be true. The deposition of 1.5 µm particles will always be lower than that of 2.8 µm particles and apparently the latter reach the ß$_2$-receptor in sufficient numbers. Again dose outweighs the deposition pattern.

In the explanations above the nature of the drug inhaled hardly plays a role. Inhaled particle deposition is a physical process, not linked to the chemical nature of the drug. The aerodynamic particle size is not directly influenced by the chemical structure, except for the density and hygroscopy. Density only plays a minor role, because a density increase by a factor of eight increases the aerodynamic size only by a factor two. So the above relationship between size and efficacy is most probably true for all inhaled drugs, the most conceivable exceptions being receptor distributions peaking in the upper airways or in the alveoli.

Metered dose and dry powder inhalers both generate polydisperse aerosol clouds. Therefore it is predicted that the lower airway deposition (as percentage of the emitted dose) is low and that the dose can be reduced substantially if the deposition patterns can be improved. Most of the larger particles deposit in the upper airway filter and unfortunately is absorbed from the local mucosa or from the gastro-intestinal tract after being swallowed, which may result in systemic adverse effects. Elimina-

tion of the large particles reduces the systemic load and adverse effects and offers a reasonable explanation for our findings.

However, any drug deposited in the lower airways is also prone to absorption o the systemic circulation, which may lead to adverse effects. The severity and frequency of adverse effects will be lower with optimal formulated aerosols. Nevertheless, rapid deactivation of absorbed drug is necessary to eliminate this contribution to systemic adverse effects. Such deactivation must occur before the drug occupies receptors in the body, which may result in requirements of a deactivation within seconds, regarding the circulation time of the blood.

Future research

If it were true that the character of the drug plays an unimportant role for corticosteroids a similar optimal particles size would be valid as for bronchodilators and a significant reduction of steroid adverse effects would be possible. There is however, one parameter, which is different for steroids. Bronchodilators are all water-soluble and therefore hygroscopic. Steroids are hydrofobic and therefore growth to moisture absorption can be minimal or absent. This may lead to a less prominent particle size vs. efficacy relationship. This will need further investigations.

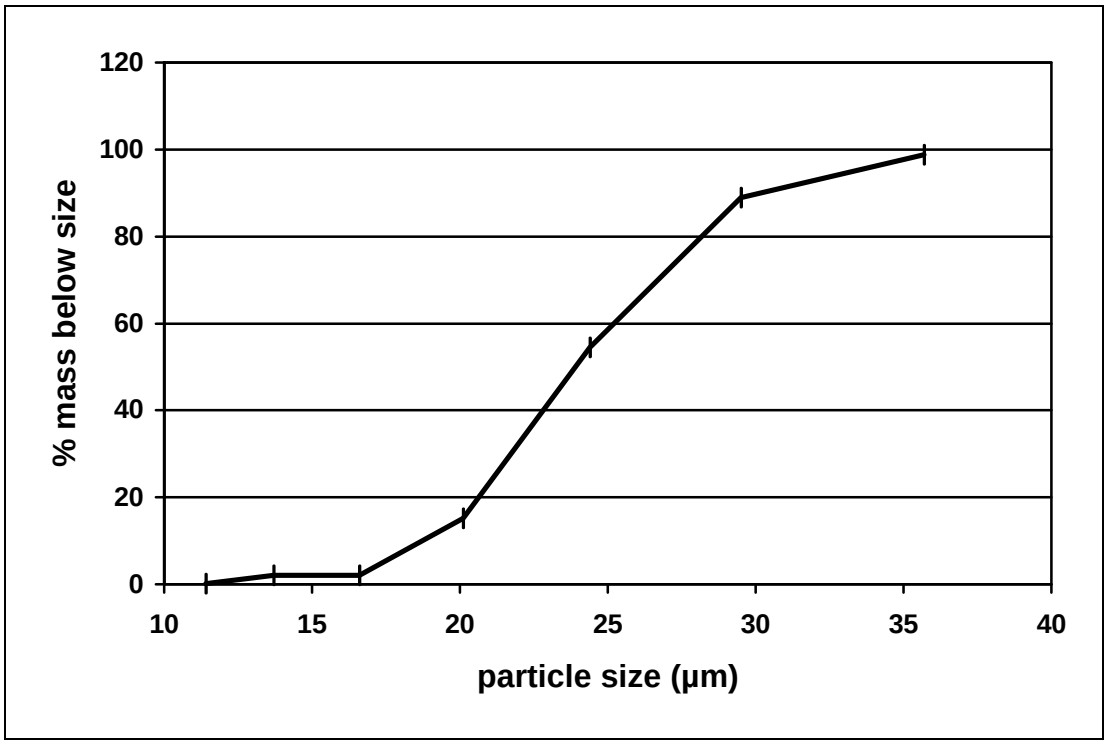


Fig. 1 Distribution of droplets produced by a piezo-electric inkjet printhead.
The Y-axis depicts the cumulative mass, 50 % of the aerosol mass being smaller than 24.5 µm and 100 % smaller than 36 µm.

Generating narrow distributed (=monodisperse) aerosols may be an important step forward in improving therapeutic quality. Currently several options being investigated to produce a portable monodisperse aerosol generator. Preliminary experiments with the head of inkjet printers show that these devices produce monodisperse aerosols, although the droplets are still too large for inhalation. These heads need therefore to be redesigned to produce smaller droplets.

Aerosols based on the Taylor cone principle are another option[4]. When a low conductive fluid is pumped through a steel needle, which is connected to a 10 kV source, fluid will not drip from the tip of the needle, but form a cone. The high electrical charges now forces many identical shaped droplets to leave the tip of the cone. The size of these droplets is governed by only a few parameters, which can be easily controlled. The high 10 kV voltage can be supplied by batteries because currents are very, very low.

In the near future we expect that technical problems will be solved and that inhalers with a better defined aerosol output come available to the patient. Therefore it is expected that through these measures the quality of antiasthma therapy will improve.

Literature

[1] Gerrity T.R., Lee P.S., Hass F.J., Marinelli A., Werner P. and Lourengo R.V.
Calculated deposition of inhaled particles in the airway generations of normal subjects.
J Appl Physiol 47: 867-873 1979.

[2] Svartengren M., Anderson M., Bylin G., Philipson K. and Camner P.
Mouth and throat deposition of 3.6 μm radiolabelled particles in asthmatics.
J Aerosol Med 4: 313-321 1991.

[3] Schiller-Scotland C.F., Gebhart J., Hochrainer D. and Siekmeier R.
Deposition of inspired aerosol particles within the respiratory tract of patients with obstructive lung disease.
Toxicology Letters 88: 255-261 1996.

[4] Meesters G.M.H., Vercoulen P.H.W., Marijnissen J.C.M. and Scarlett B.
Generation of micron-sized droplets from the Taylor cone.
J Aerosol Sci 23: 37-49 1992.

Samenvatting, bespreking en toekomstig onderzoek

Samenvatting

Het doel van ons onderzoek was de optimale formulering van luchtwegverwijdende aerosolen en in hoeverre doseringen en nevenwerkingen gereduceerd konden worden. In die vraagstelling speelt de depositie en de grootte van de geïnhaleerde deeltjes een cruciale rol.

In hoofdstuk 2 van dit proefschrift worden de meest relevante depositiemechanismen, zijnde impactie, sedimentatie en Brownse beweging geïntroduceerd en nader uitgewerkt. Deze mechanismen, welke alle een eigen karakter bezitten, zijn gerelateerd aan de grootte van het geïnhaleerde deeltje. Zo is *impactie* gebaseerd op de inertie van grote deeltjes. Deze deeltjes zijn simpelweg te zwaar of bezitten een te hoge snelheid om de bochten van de bronchiaalboom te kunnen volgen. Zij verlaten de luchtstroom en botsen tegen het slijmvlies. *Sedimentatie* is daarentegen een tijdsafhankelijk proces. T.g.v. de zwaartekracht vallen deeltjes naar beneden en de snelheid, die zij daarbij bereiken, is afhankelijk van de afmetingen: des te kleiner het deeltje is, des te lager die terminale valsnelheid. Zij dringen diep door in de luchtwegen met een geringe neiging om neer te slaan. Wanneer zij ‘te klein’ zijn, is de tijd nodig voor sedimentatie zo groot dat die uitgaat boven de duur van een in- en uitademingscyclus. Zij worden daarom vrijwel geheel weer uitgeademd. *Brownse beweging* is alleen van belang bij zeer kleine deeltjes en daarmee niet interessant voor therapeutische aerosolen, omdat de totale massa van deze deeltjes erg laag is.

Depositie is een fysisch proces en elk mechanisme kan worden beschreven middels mathematische vergelijkingen, hetgeen het onderzoekers mogelijk maakt om modellen te bouwen. Het meest uitgewerkte model, dat van Findeisen en navolgers, wordt in dit proefschrift verkend. Er bestaat overeenstemming over het algemene karakter van de berekende neerslag. Grote deeltjes impacteren in de extrathoracale of bovenste luchtwegen, terwijl kleinere deeltjes verder in de luchtwegen doordringen. Het aantal neergeslagen deeltjes is wederom gerelateerd aan de grootte: des te kleiner het deeltje, des te lager de neerslagkans. Een minimum wordt geobserveerd bij deeltjes van 0.5 μm. Gegevens m.b.t. de regionale depositie laten zien dat depositiepatronen in de

luchtwegen nooit tot een smal gebied gelokaliseerd, maar altijd sterk gespreid zijn. Hierbij geldt dat een brede aërosolgrootte verdeling, gelijk staat met een sterkere verspreiding van de depositie over de luchtwegen. Vernauwing van de luchtwegen leidt altijd tot een toename van de depositie.

Hoofdstuk 3 van dit proefschrift behandelt de luchtwegdepositie van de aerosolen in gezonden. Het karakter, bekend uit de modelstudies, wordt door in-vivo experimenten bevestigd. De extrathoracale depositie neemt snel toe wanneer de deeltjesgrootte oploopt. Het grootste gedeelde van 6 µm deeltjes deponeert extrathoracaal, terwijl de kleinere deeltjes vooral in de alveoli neerslaan. De depositie in de bronchi is relatief laag met een maximum van ongeveer 20%. Naast de deeltjesgrootte zijn het inhalatievolume en de inhalatieflow van groot belang. Een toename van het volume doet de depositie stijgen, terwijl het omgekeerde het geval is voor de inhalatieflow. Wanneer men deeltjesgrootte, inhalatieflow en -volume kent, is het mogelijk om de totale depositie te voorspellen. Gelet op het belang van deze factoren, is het noodzakelijk deze parameters in elk experiment nauwkeurig te standaardiseren, om de depositie-variabiliteit te beperken. In astmatici blijkt de deeltjesneerslag te zijn toegenomen, afhankelijk van de mate van bronchusobstructie. De depositiepatronen, zoals gemeten met gammacamera's zijn vlekkerig en verre van uniform.

De depositie van therapeutische aerosolen wordt beschouwd in hoofdstuk 4. De neerslagkarakteristieken zijn sterk afhankelijk van het toegepaste type inhaler of vernevelaar. Dosis-aerosolen worden gekenmerkt door een lagere luchtwegdepositie van 10 tot 20% van de nominale dosis. Deze aerosolen blijken polydispers te zijn en het grootste deel van de massa bestaat uit zeer grote deeltjes, welke in de bovenste luchtwegen neerslaan. Voorzetkamers verlagen deze bovenste luchtwegdepositie, hoewel hun effect sterk afhangt van het type kamer. Droge poeder inhalatoren missen een aantal nadelen van de dosis-aerosolen, maar de effectiviteit ervan kan negatief beïnvloed worden door lage inspiratoire krachten, die een patiënt soms op kan brengen.
Slechts weinig experimenten hebben de relatie tussen de deeltjesgrootte en het therapeutisch effect onderzocht en helaas zijn de uitkomsten ook nog met elkaar in

tegenspraak. Sommige onderzoekers tonen géén verschil aan in effectiviteit voor deeltjes <5 µm. Men concludeert dat de meest belangrijke factor een deeltjesgrootte <5 µm is. Andere onderzoekers laten daarentegen zien dat 2.5 µm aerosolen sterker werkzaam zijn dan 5 µm aerosolen. Vele factoren kunnen worden aangewezen voor dit gebrek aan consensus. Zo gebruikte men diverse soorten vernevelaars, wat leidt tot sterke verschillen in de toegediende doseringen en daarnaast waren de aerosolen sterk polydispers. Beide factoren verlagen het onderscheidend vermogen van het experiment en afhankelijk van de ernst van deze factoren rapporteerde men wel of geen relatie tussen deeltjesgrootte en effectiviteit.

De resultaten van onze experimenten zijn opgenomen in hoofdstuk 6 t/m 10. In het eerste experiment hebben 8 stabiele astmatici met een gemiddelde FEV_1 van 72% van de voorspelde waarde 3 monodisperse salbutamol-aerosolen geïnhaleerd (hoofdstuk 6). De deeltjesgrootten waren respectievelijk 1.5, 2.8 en 5 µm. Daarnaast werd een placebo geïnhaleerd. De vrijwilligers inhaleerden een cumulatieve reeks van 5, 10, 20 en 40 µg salbutamol, waarna de toename van de longfunctie werd gemeten. De dosis-respons curven zijn geanalyseerd d.m.v. repeated measurements anova. In geval van de FEV_1 en de $MEF_{75/50/25}$ liet het 2.8 µm aërosol een sterkere luchtwegverwijding zien dan het 5 µm aërosol, terwijl bij de piekstroom het 1.5 µm aërosol significant zwakker was dan het 2.8 µm aërosol. In geval van de VC, de FVC en de ademweerstand was er geen verschil in de luchtwegverwijding merkbaar t.g.v. van de verschillende aerosol-typen. De conclusie van dit experiment was dat in deze milde astmatici de optimale deeltjesgrootte voor $ß_2$-mimetica aerosolen 2.8 µm is.
In het tweede experiment inhaleerden astmatici met een FEV_1 >70% van de voorspelde waarde, monodisperse ipratropium bromide aerosolen met dezelfde deeltjesgrootte als hierboven vermeld (hoofdstuk 7). De vrijwilligers inhaleerden nu 8 µg ipratropium bromide, waarna op identieke wijze de longfunctietoename werd bepaald en geëvalueerd. Wederom bleek bij de FEV_1 en de $MEF_{50/25}$ het 5 µm aërosol de minst sterke luchtwegverwijding te zien te geven. In geval van de VC, FVC, ademweerstand en de piekstroom was geen deeltjesgrootte effect merkbaar. Ook voor parasympatholytische aerosolen is de optimale deeltjesgrootte 2.8 µm.

Logischerwijze werd het volgende experiment uitgevoerd in patiënten met een sterkere bronchusobstructie (hoofdstuk 8). Zeven stabiele patiënten met een gemiddelde FEV_1 van 37.9% van de voorspelde waarde hebben monodisperse salbutamol en ipratropium bromide aerosolen geïnhaleerd met deeltjesgrootte variërend van 1.5 tot 5 µm en wederom een placebo aerosol. De vrijwilligers inhaleerden respectievelijk 20 µg salbutamol en 8 µg ipratropium bromide, waarna de longfunctietoename werd bepaald en geëvalueerd op de gebruikelijke manier. Het 2.8 µm aerosol leidde tot een sterkere toename van de FEV_1, de specifieke luchtwegconductantie en de $MEF_{75/50}$ dan bij de andere deeltjesgrootten het geval was. In patiënten met een sterke luchtwegobstructie blijkt de optimale deeltjesgrootte voor zowel een ß$_2$ mimeticum als een parasympatholytisch aërosol 2.8 µm te zijn.

Om te bezien of de luchtwegverwijdende effecten van deze laag gedoseerde monodisperse aerosolen verschillen van de standaarddoseringen, zoals toegediend via een dosis-aërosol, hebben wij een vergelijkende studie uitgevoerd (hoofdstuk 9). Tien stabiele patiënten met een gemiddelde FEV_1 van 58.1% van de voorspelde waarde hebben een placebo aërosol, 8 µg ipratropium bromide als 2.8 µm monodispers aërosol en 40 µg via een dosis-aërosol met voorzetkamer geïnhaleerd. Daarna is de longfunctietoename bepaald en geanalyseerd middels repeated measurement anova. Significante verbeteringen t.o.v. placebo werden waargenomen voor de FVC, de FEV_1, de specifieke luchtwegconductantie, de piekstroom en de MEF_{75}. In al deze gevallen was het laag gedoseerde 2.8 µm aerosol therapeutisch equivalent aan de hoger gedoseerde dosis-aerosol. Het blijkt dus dat, wanneer men het polydisperse karakter van geïnhaleerde aerosolen wijzigt naar een monodispers patroon, de dosering van inhalatie-middelen kan worden gereduceerd met minstens 80% zonder effectiviteitsverlies.
Daarop hebben wij de bijwerkingen van 160 µg fenoterol als 2.8 µm monodispers aerosol vergeleken met die van 800 µg fenoterol afkomstig uit een conventioneel dosis-aërosol met voorzetkamer (hoofdstuk 10). Gezonde vrijwilligers, 8 vrouwen en 4 mannen, hebben deelgenomen aan deze proef en hebben in willekeurige volgorde placebo, het monodisperse en het dosis-aerosol preparaat geïnhaleerd. De wijzigingen in het plasmakaliumgehalte, vingertremor, bloeddruk, hartslag en specifieke

luchtwegconductantie zijn gemeten voor en 15 minuten na de toediening van de aerosolen. De beide actieve aerosolen lieten een significante toename zien van de luchtwegconductantie *en* de bijwerkingen. Het serumkaliumgehalte nam na het monodisperse aërosol af met 0.27 mmol/l, terwijl het dosis-aërosol een verlaging liet zien van 0.67 mmol/l ($p = 0.001$). De vingertremor nam significant minder toe na toediening van het monodisperse aërosol in vergelijking met het dosisaerosol. Wijziging in de cardiovasculaire parameters waren niet significant t.o.v. placebo. Er waren geen significante verschillen tussen de toename van de specifieke luchtwegconductantie tussen de twee actieve preparaten. De conclusie is daarom dat een wijziging van de formulering van dosis-aërosolen het mogelijk maakt bijwerkingen te reduceren.

Bespreking

De eerste hoofdconclusie van deze experimenten is dat bij patiënten met zowel milde als sterkere luchtwegvernauwingen de optimale deeltjesgrootte voor ß$_2$-mimetische en parasympatholytische aerosolen 2.8 µm is. De tweede conclusie is dat toediening van deze optimaal geformuleerde aerosolen een forse reductie van de dosering en het aantal bijwerkingen mogelijk maakt.

De eerste conclusie kan worden verklaard door het bestaan van een ‘bovenste luchtwegfilter’. De extrathoracale en bovenste luchtwegen functioneren als een effectief filter voor grote deeltjes, waardoor de lagere luchtwegen beschermd worden tegen een hogere belasting door stof of andere potentieel schadelijke stoffen. Toediening van grote deeltjes is inefficiënt, omdat deze verloren gaan in dat bovenste luchtwegfilter en lage doseringen in de lagere luchtwegen tot gevolg hebben. Dit laatste staat gelijk aan een gering therapeutisch effect. Dit geldt zowel voor ß$_2$-mimetica als parasympatholytica en geeft de verklaring voor de lagere effectiviteit van de 5 µm aerosolen. De berekening van de regionale depositie door Gerrity en medewerkers zijn in dit geval van belang. Voor 5 µm aerosolen laten zij een voornamelijk bovenste luchtweg depositie zien, terwijl deze in de lagere luchtwegen veel lager is in vergelijking met 2 of 3 µm aerosolen. De experimentele gegevens van Svartengren m.b.t. de afsnijpunten van de oropharynx onderstrepen het belang van dit bovenste luchtwegfilter. Daarnaast dient men te realiseren dat zowel ß$_2$-mimetische als parasympatholytische aerosolen in een zekere mate hygroscopisch zijn. Door

vochtopname zal de grootte toenemen en daarmee de depositie in de bovenste luchtwegen. Deeltjes van 5 µm nemen door hygroscopie veel sterker in grootte toe dan 2.8 µm deeltjes, hetgeen de bovenste luchtwegdepositie alleen maar bevordert.

Na deze verklaring voor de lage effectiviteit van 5 µm aerosolen dienen de verschillen tussen de 2.8 en 1.5 µm aerosolen nader verklaard te worden. Wij verwachtten dat voor ß$_2$-mimetische en parasympatholytische aerosolen, door verschillen in receptor-verdeling, de optimale deeltjesgrootte zou verschillen. Voor parasympatholytische aerosolen leek een centraal georiënteerd depositiepatroon logisch en de resultaten van onze experimenten bevestigen dit, omdat 2.5 µm aerosolen sterker werkzaam zijn dan 1.5 µm aerosolen. Wellicht zou het depositiepatroon van 5 µm aerosolen nog beter zijn in die zin, dat er een betere overeenstemming bestaat met de verdeling van de receptoren. Echter de lage penetratie en daarmee dosering is kennelijk van groter gewicht dan een betere overeenstemming met de receptorverdeling. Met andere woorden een optimale afstemming tussen depositiepatroon en receptorverdeling is van gering belang wanneer de gedeponeerde massa geneesmiddel erg laag is. Een minder goed passend patroon met hogere massa's valt te prefereren.
Voor ß$_2$-mimetica zou men kunnen verwachten dat 1.5 µm aerosolen een betere keuze zouden zijn geweest, gelet op de betere overeenstemming van het depositiepatroon met receptorverdeling. Dit bleek niet het geval te zijn en een aantal verklaringen zijn hiervoor mogelijk. Ten eerste worden kleinere deeltjes altijd sterker uitgeademd dan grotere deeltjes, ook bij astmatici. De experimenten door Schiller-Scotland laten dit duidelijk zien. Haar gegevens toonden een tweemaal zo hoge depositie van 3 µm deeltjes in vergelijking tot 1 µm deeltjes. 2.8 µm aerosolen leveren altijd hogere lage luchtweg doseringen op en zijn daarmee sterker werkzaam. Dosering lijkt van groter belang te zijn dan het depositiepatroon.
De gegevens van Gerrity tonen aan dat de depositiepatronen van 1, 2 en 3 µm deeltjes in de lagere luchtwegen niet zo sterk verschillen. De verspreiding van de deeltjes over de luchtwegen is min of meer vergelijkbaar, maar de massa van 3 µm deeltjes is veel hoger. Wanneer depositiepatronen vergelijkbaar zijn, is de hoeveelheid massa die deponeert van veel groter belang.

De resultaten in sterk obstructieve patiënten kunnen worden verklaard door dezelfde mechanismen. 5 µm deeltjes zullen de lagere luchtwegen niet bereiken. De effectiviteit van het bovenste luchtwegfilter zal niet veel verschillen van die in minder obstructieve astmatici of gezonde personen. Sterker nog, zelfs de geringste vernauwing in deze regio zal het afsnijpunt van het filter verlagen, hetgeen betekent dat grote deeltjes in nog mindere mate de lagere luchtwegen zullen bereiken. Men had kunnen verwachten dat de 1.5 µm deeltjes in 'versterkte' mate in de vernauwde luchtwegen zouden doordringen en tot een groter effect zouden induceren, maar dit bleek niet het geval te zijn. De depositie van 1.5 µm aerosolen zal altijd lager zijn dan die van 2.8 µm aerosolen en blijkbaar bereiken de 2.8 µm aerosolen in voldoende mate de $ß_2$-receptor. De hogere neerslag van de 2.8 µm aerosolen is dan belangrijker.

In alle bovenstaande verklaringen speelt de chemische samenstelling van het geneesmiddel nauwelijks een rol. Depositie van geïnhaleerde deeltjes is een fysisch proces, welke niet verbonden is met de chemische structuur. De aërodynamische deeltjesgrootte wordt niet beïnvloed door de chemische structuur, met een uitzondering voor de hygroscopiciteit en de dichtheid van het deeltje. De dichtheid speelt een ondergeschikte rol, omdat een toename van de dichtheid met een factor 8 de aërodynamische diameter slechts met een factor 2 doet toenemen. Dientengevolge zal de bovenstaande relatie tussen deeltjesgrootte en effectiviteit waarschijnlijk voor alle typen geneesmiddelen gelden, waarbij mogelijkerwijze een uitzondering gemaakt moet worden voor receptorverdelingen die gelokaliseerd zijn in de bovenste luchtwegen of in de alveoli.

Dosis-aerosolen en droge poeder inhalatoren genereren beide sterk polydisperse aerosolen, waarbij er in de aërosolwolk veel grote deeltjes aanwezig zijn. Het is daarom niet vreemd dat de lage luchtwegdepositie zo laag is en dat de dosering zo sterk kan worden gereduceerd. Het overgrote deel van de afgegeven massa is simpelweg te groot om effectief te zijn. Die massa deponeert in de bovenste luchtwegen en wordt daar of geabsorbeerd dan wel doorgeslikt en in het maag/darmkanaal geabsorbeerd. Het gevolg van deze absorptie zijn systemische bijwerkingen. Eliminatie van deze grote deeltjes reduceert de systemische belasting en daarmee

bijwerkingen, hetgeen een redelijke verklaring is voor onze resultaten. Echter, elk geneesmiddel, dat deponeert in de lage luchtwegen zal ook geabsorbeerd worden en daarmee zijn enige systemische bijwerkingen onvermijdelijk. De ernst en de frequentie van deze bijwerkingen zullen lager zijn, dan bij onze optimaal geformuleerde aerosolen. De enige mogelijkheid om deze systemische bijwerkingen verder te elimineren is een snelle deactivatie van het geabsorbeerde geneesmiddel. Deze deactivatie moet echter geschieden voordat het geneesmiddel de receptor bezet, hetgeen kan resulteren in een eis van een zeer snelle deactivatie.

Toekomstig onderzoek

Als de chemische structuur van de geneesmiddelen een weinig belangrijke rol voor wat betreft de depositiepatronen speelt, zal voor corticosteroïden een optimale deeltjesgrootte gelden, gelijksoortig aan luchtwegverwijders. Een sterke reductie van de steroïdbijwerkingen ligt dan ook binnen de mogelijkheden. Er is echter één parameter die verschilt van luchtwegverwijders. Luchtwegverwijders zijn alle wateroplosbaar en daardoor hygroscopisch, terwijl steroïden hydrofoob zijn, waardoor deeltjesgroei ten gevolge van wateropname minimaal of zelfs afwezig is. Het is daarom mogelijk dat de relatie tussen deeltjesgrootte en effectiviteit minder uitgesproken is bij steroïden. Dit vereist nader onderzoek.

Het zal duidelijk zijn dat het genereren van monodisperse aerosolen een vooruitgang betekent in termen van therapeutische kwaliteit. Op dit moment zijn er diverse mogelijkheden beschikbaar om een draagbare monodisperse aërosol generator te vervaardigen. Enkele experimenten met de koppen van inkjet printers tonen aan dat deze koppen in staat zijn monodisperse aerosolen te genereren, alhoewel het bleek dat de druppels op dit moment nog te groot zijn voor inhalatie. Een wijziging in het ontwerp van deze koppen moet het mogelijk maken kleinere druppels te genereren.

Aerosolen gebaseerd op het zogenaamde "Tayler cone" principe zijn een andere mogelijkheid. Wanneer een slecht geleidende vloeistof door een stalen naald wordt gepompt en deze naald verbonden is met een 10 kV bron, zal de vloeistof niet van de punt van de naald afdruppelen maar een conus vormen. De hoge elektrische ladingen dragen er nu zorg voor dat even grote druppeltjes de top van deze conus verlaten. De grootte van deze druppeltjes wordt bepaald door een gering aantal parameters, welke

alle eenvoudig gecontroleerd kunnen worden. Het hoge 10 kV voltage kan worden opgebracht door simpele batterijen, omdat de stroom die verbruikt wordt erg laag is.
In de nabije toekomst moet het mogelijk zijn de technische problemen op te lossen en te komen tot generatoren, die een beter gedefinieerd aerosol afgeven. De kwaliteit van de anti-astma therapie kan hierdoor verhoogd worden.

Curriculum Vitae

Pieter Zanen is op 6 november 1954 geboren in Utrecht. Na achtereenvolgens de ULO, HAVO en Atheneum doorlopen te hebben, werd in 1974 met de studie Geneeskunde aan de Universiteit Utrecht begonnen. Na het behalen van het artsexamen en het voltooien van de militaire verplichtingen, werd aangevangen met de huisartsenopleiding.

Na een korte periode op het Consultatiebureau voor Alcohol en Drugs te Hilversum, kwam hij te werken bij Pharbita te Zaandam. Deze fabrikant van generieke geneesmiddelen specialiseerde zich in droge poeder inhalatie-preparaten. Aldaar werd de basis gelegd voor dit onderzoek, hetgeen werd uitgevoerd naast de normale werkzaamheden. Vervolgens is hij overgestapt naar Genfarma te Maarssen, in welke periode het onderzoek kon worden afgesloten.

Sinds 1 januari 1998 is hij als longfysioloog werkzaam op het Hart Long Centrum Utrecht, een samenwerkingsverband van het Academische Ziekenhuis Utrecht (prof. dr. J-W. J. Lammers) en het St. Antonius Ziekenhuis te Nieuwegein (dr. J.J.M. van den Bosch)

Dankwoord

De promovendus past bescheidenheid als men beziet hoeveel mensen er onontbeerlijk bleken te zijn voor het voltooien van een proefschrift. De onderstaande kring van mensen verdienen een deel van de eer.

Drs. Ch. F. Gusdorf, beste Charles, weinigen zullen beseffen welke cruciale rol jij hebt gespeeld in dit onderzoek. Het komt volgens mij zelden voor dat binnen de generieke farmaceutische industrie de kans gegeven wordt een onderzoek als dit uit te voeren. Jij hebt mij, zowel bij Pharbita als bij Genfarma, ruimhartig de gelegenheid gegeven dit te doen. Het getuigt van groot inzicht, zonder dat was ik niet zover gekomen.

Dhr J. Kelders, beste Jan, wat voor Charles geldt, geldt in dubbele zin voor jou. Je achtergrond en werkzaamheden hebben weinig relatie tot aerosol-onderzoek. Toch heb je al die jaren het mogelijk gemaakt dat ik door kon gaan en niet omdat er een ‘gat in de markt zat’. Je grote Brabantse hart was het belangrijkst. Ik hoop dat jij nog vele jaren mag genieten van een goede gezondheid.

Prof. Lammers, beste Jan-Willem, jij kwam naar Utrecht en ik was er al (eens per week, beetje een buitenbeentje). Die enigszins curieuze positie heb je laten bestaan en er kwam vertrouwen. Ik kon doorgaan en uitbouwen. Heel veel dank voor die wijsheid.

Dhr. A Plomp, beste Adri, nog zo’n onbekend maar belangrijk figuur op de achtergrond. Was jij er niet geweest dan was dit onderzoek nooit van de grond gekomen. Jouw kennis van aerosolen en het geduld om mij de eigenschappen van de spinnig top generator te leren, vormden de basis onder dit alles.

Dhr. T.L. Go, beste Liam, kamergenoot al deze jaren. Samen aan de opstelling gewerkt en hem aan de gang gekregen en gehouden. Ik zal niet vergeten hoe je als er iets tegen zat, je de telefoon greep en begon te organiseren. Dames om te prikken kwamen te voorschijn, technische diensten begonnen te werken en de biologen

moesten maar even wachten. Dank voor het er zijn en veel geluk in je verdere loopbaan.

Drs. R. Otten, beste Rene, goede vriend. Literatuur is zo belangrijk, maar het is vaak zo goed verstopt. Doch waar het ook was, jij wist het wel te achterhalen. Samen uren achter die computer gezeten en ik hoop er nog vaak met je achter te zitten. Dank daarvoor en ook voor de vele aangename uren met Monique aan de eettafel.

Henny en Peter, de metingen van de longfunctie waren jullie toevertrouwd. Toen wist ik er (nog) niet zoveel van af en vertrouwde op jullie vakkennis en inzet. Die laatste zijn groot en ik zie uit naar de volgende onderzoeken met jullie.

Lieve Edith, de eerste jaren met Justin zijn niet gemakkelijk geweest en vele zorgen komen nog. Weinigen zullen beseffen hoe het ingrijpt om te weten dat je altijd voor je kind zult moeten zorgen, ook als wij er niet meer zijn. Dat wij dat samen doen, schept een band van staal tussen ons. Laten wij heel oud worden, dat zou mooi zijn.
Lieve Emmy, lieve Justin, stoere Friese dame en heerlijke zoon. Ik geniet van elke dag dat jullie er zijn, ik zal jullie nog heel lang naar bed brengen en nog langer voor jullie zorgen.