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NOVEL APPROACHES TO ENHANCE PULMONARY DELIVERY OF PROTEINS AND PEPTIDES

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In the last two decades, large efforts have been made to develop safe methods for the delivery of proteins and peptides via the lungs into blood circulation for treatment of systemic diseases. For this purpose, a number of biophysical and physiological parameters have to be considered, such as particle diameter, particle density, hygroscopicity, electrical charge, chemical properties of the substance and age, pulmonary diseases, breathing pattern, all of which affect the mechanisms of pulmonary drug deposition. Variations in these parameters result in a substantial change of particle deposition in the lung. For example, large particles (>10 µm) are not able to penetrate into the lung, because they are deposited by impaction in the upper respiratory tract. On the other hand, small particles (0.1-1.0 µm) are inspired into the alveoli but also expired without being deposited significantly. Particles of diameters 2-4 µm show the ideal pulmonary deposition behavior and are able to transport a substantial mass of pharmaceuticals into the lung. Modifications of breathing pattern allow an optimal particle deposition in the bronchial or the alveolar region. In addition, particle deposition in the alveolar region is the basis for treatment of systemic diseases by inhalant administration of drugs (e.g., insulin). This paper deals with the physical and physiological basics for inhalation therapy and demonstrates novel systems which were designed to optimize drug delivery into the lung periphery. The AKITA® inhalation system is an example for a system that guides the patient through the inhalation maneuver and ensures an optimized particle deposition and a minimized intersubject variability.

K e y   w o r d s: *aerosol, inhalation therapy, pulmonary drug delivery*

INTRODUCTION

The lung with its large surface area (80 m^2–120 m^2), good vascularization, low thickness of the alveolar epithelium (0.1 µm–0.2 µm) and the immense

capacity for solute exchange is an ideal target for the application of drugs for treatment of systemic disorders. However, it is not easy to get the drug into an appropriate formulation and into a delivery system which allows it to bring it so deep into the lung that it is able to penetrate into the blood circulation. The reason is that the respiratory system is designed as a series of filters to prevent environmental aerosols to get into the deep lung and to keep the lung surface clean. The first ones, the oropharyngeal region and the bronchial tree are excellent filters to eliminate aerosol particles from the inhaled air and particles deposited on the ciliated epithelium of the bronchial tree are subject of mucociliary transport to the gastrointestinal tract. Therefore, to deliver a drug into the deep lung one has to overcome these filters. The deposition behavior of aerosol particles in the respiratory tract depends on a number of physical (properties of the particle), chemical (properties of the drug), and physiological (breathing pattern, pulmonary diseases) factors. If these are not considered adequately, it is not possible to deposit a sufficient and reproducible amount of a drug in a predefined lung region by pulmonary administration. A low efficiency of commercially available inhalation systems and a large variability of the administered doses have been the major problems that prevented this administration route for so many years. However, since about 20 years there has been a substantial progress in aerosol research and pulmonary drug delivery. The underlying mechanisms of particle inhalation and pulmonary particle deposition have been studied extensively and are now understood. In consequence, an increasing number of studies were performed for the administration of drugs by means of various inhalation techniques even for treatment of extrapulmonary (i.e., systemic) diseases. It has been observed that due to the large alveolar surface and low thickness of the alveolar epithelium pharmaceuticals are rapidly absorbed after deep inhalation and deposition in the peripheral (i.e., alveolar) region of the lung. After deposition in the alveolar region of the lung, a number of mechanisms prevent the absorption of inhaled pharmaceuticals. Various absorption barriers (alveolar lining fluid layer, macrophages and other cells, alveolar epithelium) act to a different extent to inhibit drug permeation into the circulation, cellular uptake (e.g., by macrophages) and/or proteolytic degradation (1-4). In principle, absorption kinetics of inhaled substances deposited in the lung periphery depends on their molecular weight (small molecules are more rapidly absorbed than larger ones), pH-value, electrical charge, solubility, and stability of the inhaled substance (1-4). This review describes the physical and in part some physiological requirements for optimization of pulmonary drug delivery to target certain lung regions.

Parameters determining particle deposition in the deep lung

Some biophysical parameters determine regional pulmonary drug deposition. These are the aerodynamic particle behavior (e.g., particle size, density,

hygroscopicity, shape, electrical charge), the breathing pattern of the patients (e.g., flow rate, ventilation volume, end-inspiratory breath-holding), the time of aerosol pulse injection into the breathing cycle and the airway anatomy and morphometry of the patient (5). The aerodynamic particle diameter (d_{ae}) is the diameter of a sphere with a density of 1 g/cm³ that has the same aerodynamic behavior as the particle which shall be characterized. In that way, aerosol particles with different density and shape can be characterized depending on their aerodynamic properties. For a water droplet the geometric and aerodynamic particle diameter is identical. In contrast, large porous particles have a much smaller aerodynamic particle diameter compared to the geometric diameter.

Aerodynamic particle behaviour

Particles in the ambient air are transported by different physical mechanisms. The substantial mechanisms are diffusion by Brownian motion (particles <0.5 µm), sedimentation by the gravitational force (particles >0.5 µm) and impaction (size >3 µm). All three mechanisms determine the deposition of particles in human lungs and are described in detail elsewhere (5-8). The total deposition of inhaled particles as a function of their diameter is shown in *Fig. 1* (6, 7). One can see that particles between 0.1-1 µm in size are not well deposited in the lungs and a high fraction is usually exhaled. This is because neither Brownian motion nor sedimentation is very efficient in this size range. For therapeutic aerosols, which are usually >1 µm in size, the Brownian motion is not substantial. The impaction (inertial force during changes of direction of the inhaled air) is responsible for the fact that particles above about 10 µm cannot enter the lungs and are already deposited in mouth, throat, and larynx. This impaction deposition depends on the aerodynamic diameter of the particles and the ventilatory flow rate. The larger the particles and the higher the air flow, the more efficient is the deposition by impaction and in consequence the number of particles reaching lung periphery
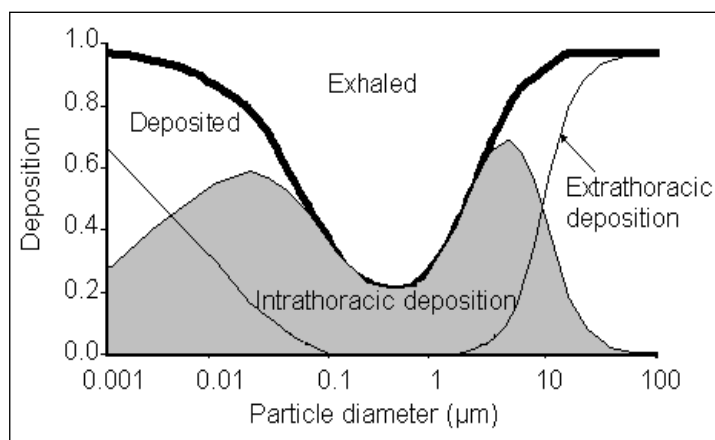

Fig. 1. Deposition efficiency in the respiratory system as function of the particle size.

decreases. However, the diameter is only one parameter that influences aerodynamic behavior. Electrical charge, hygroscopicity, aggregation, shape and temperature are other physical parameters that influence particle deposition in the lungs (1-3).

Breathing pattern of patients

Upper human airways have anatomical structures which are an efficient filter for inhaled aerosols (9). A fast inhalation (high flow rate, Q) leads to an increased deposition by impaction in the larynx. This prevents particle penetration into the deep lungs. Especially nose breathing prevents particle deposition in the lungs. On the other hand, this means that a slow inhalation through the mouth allows even larger particles (up to an aerodynamic diameter of 10 µm) to enter the lungs, while a fast inhalation maneuver reduces the lung deposition already for particles with diameters of about 2-3 µm significantly. This extrathoracic deposition efficiency has an extremely high intersubject variability because of large biological and anatomical differences of mouth and throat. For example, if a group of patients inhales 3-4 µm particles with an identical flow rate of about 500 ml/sec through the mouth, one can find subjects with almost no deposition of particles in the throat and others with almost 75% deposition in this region (5). A reduction of this variability can be achieved by a very slow inhalation and use of smaller particles with diameters of 1-3 µm. A combination of both slow respiratory flow and small particles leads to a strong reduction of particle impaction resulting in a minimal extrathoracic deposition in all subjects. Another important factor affecting the lung deposition is the residence time of inhaled particles (see also diffusion and sedimentation) which depends on the flow rate (slow flow rate = long residence time), the inhaled and exhaled volume (deep breath = longer residence time) and the end-inspiratory breath-holding of the patient. A deep and slow breathing maneuver gives the inhaled particles much more time to deposit by sedimentation and diffusion. Therefore, slow and deep breathing increases the deposition in the lung and especially in the lung periphery, while a fast and shallow breath increases deposition of particles in the extrathoracic airways by the impaction forces. The implementation of an end-inspiratory breath-hold in the breathing maneuver causes an increase of the pulmonary residence time of inhaled aerosol particles. However, such a breath-hold is only effective if the aerosol particles are still suspended in the air and are not yet deposited during the inhalation. Assuming that a normal breath takes about 5 s and that a 5 µm particle has a settling velocity of almost 1 mm/s, all particles which are located in airways <5 mm luminal diameter are already deposited during the tidal breath. In consequence, the implementation of a breath-hold cannot significantly increase deep lung deposition of such particles. On the other hand, a breath-hold can cause an increase of particle deposition in the lungs

especially in the lung periphery for small particles with diameters between 1 µm and 3 µm, where settling velocity is <100 µm/s.

Regional lung deposition for aerosolized drugs

As seen above, for particles with diameters larger than about 1 µm sedimentation and impaction are responsible for the lung deposition, whereas deposition by diffusion can be neglected. The remaining two mechanisms also account for the site of therapeutic particle deposition within the respiratory system, usually called 'regional deposition'. As already discussed before not only the aerodynamic behavior of the particles determine the probability of their deposition in a certain region, but also the breathing pattern. There are some mathematical models which are able to describe the deposition in different regions of the respiratory system and allow the estimation of regional deposition of inhaled aerosol particles (5, 8, 10).

Fig. 2 demonstrates the influence of aerodynamic particle diameters between 1 µm and 5 µm on alveolar deposition. Particles of this size are the main target for systemic drug delivery by means of aerosol inhalation (*Fig. 2*). Two different breathing patterns were used and the particles were assumed to be polydisperse with a standard deviation of the size distribution of about 2.0 (this is the size distribution that most aerosol systems deliver). One can see that a particle diameter of about 2 µm results in optimal alveolar deposition, but particles with diameters of 3 µm or 4 µm also deliver similar amounts to the deep lung. However, it should be considered that one 4 µm particle carries the mass of eight 2 µm particles and in that way can transport much higher doses of pharmaceuticals into the alveoli. Additionally, this figure shows the strong influence of the breathing pattern. The difference of the two distinct breathing patterns has a much stronger influence on the alveolar deposition than the particle size alone.
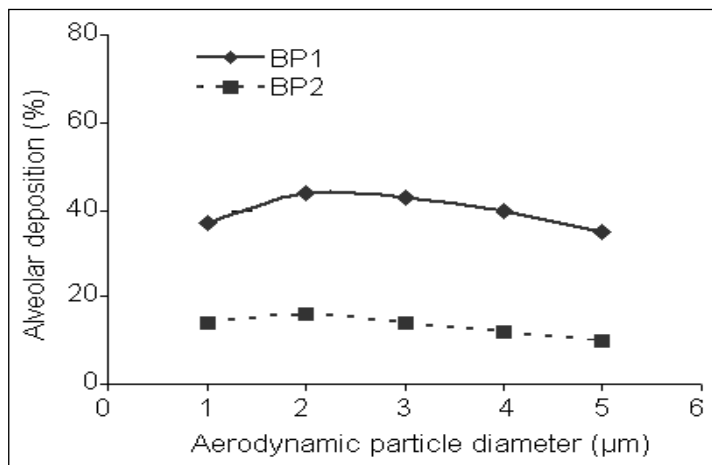

Fig. 2. Alveolar deposition as function of the particle diameter for two different breathing patterns (BP): BP1: flow rate Q=250 ml/sec, inhaled volume V=1250 ml. BP2: Q=1500 ml/sec and V=1000 ml.

Fig. 3 and *Fig. 4* demonstrate the effect of flow rate and inhaled volume on the alveolar deposition of aerosol particles. In detail, the effect from flow rate on alveolar deposition for a particle size of 3 µm (polydisperse particles and inhalation with a volume of 1000 ml) is shown in *Fig. 3*. One can see that the alveolar deposition of aerosol particles continuously decreases with increasing respiratory flow rate. *Fig. 4* illustrates the influence of the inhaled volume on the alveolar deposition of aerosol particles. The particle diameter was 3 µm and the flow rate was fixed at 250 ml/sec. One can see that the alveolar deposition continuously increases with the penetration depth of the aerosol into the lung.

In summary, *Figures 2–4* demonstrate that the inhalation maneuver of the patient is a strong predictor for the amount of drug that is really deposited in the deep lungs. Dependent on the patient´s specific data (e.g., age, size, lung function) it may be necessary to vary all these parameters for further optimization
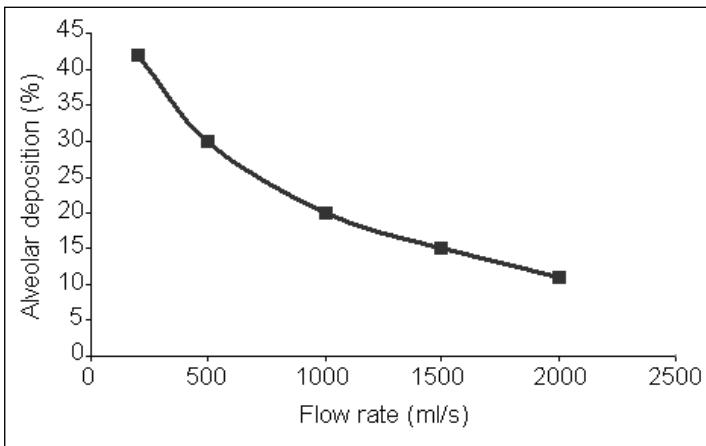

Fig. 3. Alveolar deposition as function of the flow rate (Q) for an aerosol with an aerodynamic particle diameter of 3 µm and an inhaled volume (V) of 1000 ml.

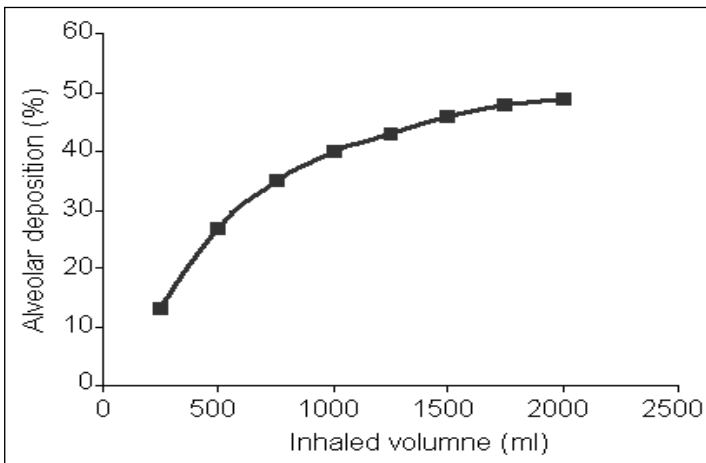*Fig. 4.* Alveolar deposition as function of the inhaled volume (V) for an aerosol with an aerodynamic particle diameter of 3 µm and an inhaled flow rate (Q) of 250 ml/sec.

of aerosol deposition in lung periphery to achieve a reliable deposition of sufficient drug doses for systemic treatment.

Aerosol bolus

Bolus inhalation technique is used since many years in aerosol medicine to study particle deposition and ventilation effects in lungs of animals and humans (11-15). An aerosol bolus (or pulse) is a small volume of aerosol sandwiched in clean (i.e., particle free) air. By changing the time of injection of the aerosol bolus in the inhaled volume one can determine the site of pulmonary aerosol deposition. Aerosols which enter the respiratory system first are penetrating deeper into the lungs as particles which are inhaled at the end of a breath. With this technique one can increase aerosol particle deposition in different regions of the human lungs which is a prerequisite for an efficient and save therapy by means of aerosol inhalation.

Novel devices to enhance pulmonary delivery

In many existing inhalation devices the bolus inhalation technique is already used (16). For example, metered dose inhalers (MDI) and dry powder inhalers (DPI) are supposed to deliver the aerosol cloud at the beginning of a breath. This leads to a more efficient lung deposition than an inhalation of an aerosol over the entire inspiration. The clean air that follows the aerosol cloud transports the particles deeply into the lungs and extends their pulmonary residence time. Another commercially available system, the AERx® Pulmonary Drug Delivery System of Aradigm uses a bolus of aerosol particles that can be activated during a certain time point during an inspiration. The bolus is produced by a piston that empties a small liquid reservoir into the inhalation air. Other devices, the AKITA® Inhalation System (Activaero, Germany) and the ProDose™ System (Profile Therapeutics) use standard liquid nebulizer systems which are operated only at a certain time during an inhalation cycle and therefore also use the bolus inhalation technique to increase particle deposition in the lungs. With this aerosol bolus technique one can even increase the particle deposition within the human alveolar region.

Brand et al (17) reported that when using the AKITA® technology one could get as much as 60% of the aerosolized drug into the lung periphery of patients with chronic obstructive pulmonary disease. Taking into account that in such patients the bronchial airways might be obstructed, one can imagine that in a healthy lung even more aerosol could be deposited in the alveolar region. Such aerosol boluses can be inhaled with constant or changing flow rates during the inhalation of the patient. The AKITA® technology is the most advanced aerosol delivery technology. Up to now it is the only technology that controls the entire inhalation maneuver of the patient. The latter is done by means of a positive pressure which is delivered by a computer controlled compressor. Corresponding data for the breathing pattern are transmitted to the computer by a SmartCard

technology. The SmartCard contains the information for any single individual patient and is based on the results of a prior lung function test. Griese et al (18) were able to demonstrate the high precision of this novel technology. These investigators reported a lung deposition of gluthathione aerosol in a group of CF patients of 85 ±2 %. In addition, none of their patients had a deposition of less than 80%. In another study, using the AKITA® technology, Scheuch et al (19) investigated the inhalant delivery of a low molecular weight heparin (Certoparin). These investigators observed a similar mean peak concentration of heparin after inhalation and after subcutaneous administration. However, a significantly lower intersubject variability was observed after inhalation, indicating that the bioavailability after inhalative administration of heparin was more precise than after subcutaneous administration (19).

A second generation of the AKITA (AKITA[2]) is operated with ultrasonic and ultrasonic mash nebulizers. These new nebulisers are able to nebulize up to 99% of the filled dose into particles with MMAD (mass median aerodynamic diameter) <4 µm. A high deposition rate of 85% of the AKITA technology means that about 85% of a filled medication makes it into the lungs of patients, compared with just 10-25% deposited in the lung by means of standard nebulizers. This technology with its high performance and low variability in pulmonary deposition allows to deposit high quantities of medication. Additionally, it enables the use of drugs with a small therapeutic window.

Conclusions

Aerosol therapy by means of nebulizers has been introduced into clinical therapy many years ago. However, the physical and physiological background of pulmonary aerosol deposition had not yet understood for many years. In consequence, many of the experimental studies of aerosol administration for systemic disorders often revealed a poor outcome in respect to the administration of sufficient doses in a reproducible manner. Other major problems were the availability of larger quantities of biotherapeutics (peptides, proteins) because many of these substances for a long time were only produced by isolation from biological materials as well as problems in respect of their stabilization within the nebulization process and after their deposition in the lung both also strongly affecting the bioavailability. Furthermore, for many years research in inhalation therapy mainly focussed on asthma which later on resulted in a breakthrough in the treatment of asthma by means of aerosols. However, the experimental settings and results for the achievement of an optimal bronchial deposition (as required for asthma therapy) could not be transferred for alveolar deposition and aerosol therapy for treatment of systemic disorders. Therefore, an intensive investigation of the mechanisms influencing the particle deposition especially in the alveolar region of the lung was necessary.

For example, in 1924 and 1925, i.e., only few years after begin of the therapeutic insulin era on January 11, 1922, the first studies on insulin inhalation were published. Laquer and Grevenstuk (20) published her investigation on intratracheal administration of insulin in 1924 and reported a more rapid onset of insulin action compared with its subcutaneous administration. A first study on inhalation of insulin in patients was performed by Heubner et al (21) also in 1924. These investigators reported a dose-dependent effect of insulin inhalation on blood glucose. However, they found a 30-times higher insulin dose for inhalation than for subcutaneous administration, and the requirement for high amounts of insulin was a problem, even though they also emphasized the advantage of this type of therapy for the patients (21). At about the same time, Gänsslen (22) performed an investigations in patients. The author also reported that inhalation of insulin was well tolerated, caused a significant decrease of the blood glucose concentration and that 30-times higher amounts of insulin were required for inhalation compared with subcutaneous application (22). Due to a large number of unresolved problems, it took another 46 years until Wigley et al (23) published their pivotal study of insulin inhalation offering proof of principle of this therapy. The authors investigated three subjects without diabetes mellitus and four patients with diabetes and were able to demonstrate that pork-beef insulin administered by a nebulizer caused a prompt increase in plasma immunoreactive insulin and that hypoglycaemia showed a temporal relationship with the increase in plasma immunoreactive insulin (23). However, even after that investigation inhalant insulin therapy was far from its introduction into clinical therapy and in the next two decades several studies ruled out the basics of insulin inhalation (24-27).

The situation now is fundamentally changed, not only for insulin which received its approval for the inhalant therapy in 2006, which is considered to be the milestone in inhalant administration of biomolecules for systemic treatment. As shown in this article, the effects of physical and physiological parameters on total and regional deposition of aerosol particles are, in the main, understood. A selective variation of these parameters can be used for an optimization of particle deposition dependent on specific conditions in some patient groups (e.g., children, small patients, patients with impaired lung function). In consequence, the reproducible administration of sufficient drug doses in the bronchi or alternatively in the alveoli will be possible for local or systemic treatment, respectively. In addition, a great number of biomolecules synthesized by means of molecular biology has been tested in animal experiments and in clinical studies, e.g., hormones (insulin, calcitonin, growth hormone, somatostatin, thyreoidea-stimulating hormone (TSH) and follicle-stimulating hormone (FSH)), growth factors (granulocyte colony stimulating factor (G-CSF), granulocyte-monocyte colony stimulating factor (GM-CSF), various interleukins (IL), and heparin (unfractionated and low molecular weight heparin) (1-3, 18, 19, 26, 28-30). For a number of these molecules, the problems of stability and alveolar absorption have been solved. It is likely that some of them will be introduced into

clinical therapy, because inhalation allows a non-invasive administration of pharmaceuticals. Further advantages depend on the structure of a molecule. For example, inhaled insulin is absorbed more rapidly than insulin after subcutaneous injection, whereas inhaled heparin shows a sustained release (19, 31). Variations of the pharmacokinetics may result from specific conditions in some patient groups (e.g., more rapid absorption of inhaled insulin in smokers than in nonsmokers) (26, 30, 31). In the future, many studies will be performed to ensure the large advantages of inhalant treatment in patients with various diseases. However, these studies must consider all the common physical and physiological and patient-specific biological factors for the optimization of the inhalant therapy.

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