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# Performance-driven, pulmonary delivery of systemically acting drugs

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Systemic drug delivery using inhalation aerosols presents requirements and challenges. To be well absorbed from the lung, a compound needs to be delivered to the alveolar region and recent high technology inhaler systems have allowed increased efficiency of drug administration to the deep lung. Yet, clearance mechanisms within the respiratory tissue operate effectively and considerably diminish bioavailabilities. Methods for enhancing drug absorption from the lung have been investigated. Viable and recent strategies to accelerate drug transport across respiratory epithelia or to decrease the rate of local degradation processes are reported.

## Introduction

Inhalation aerosols have been used for therapy of lung pathologies for thousands of years but have been developed for systemic therapeutic applications only since the 1990s [1]. This recent interest in systemic absorption from the lung results from the increasing number of drugs with a proteinous nature, the quest for their noninvasive administration as well as the recent comprehensive understanding of particle deposition within the respiratory system.

The first challenge in systemic delivery by inhalation has been to deliver with high efficiency and reliability the aerosol dose to the alveoli, the optimal site for systemic drug absorption [2]. This challenge has been successfully overcome [3]. This review aims at underlying parameters of the drug (e.g., biological stability within the pulmonary tissue) and lung

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(anatomy-physiology of the airways and alveoli) that affect systemic absorption. It aims at showing that clearance mechanisms within the lung compete with drug transport from the lung lumen to the bloodstream and at proposing viable strategies to overcome degradation pathways and to further increase the efficiency of systemic delivery by inhalation.

## Drugs currently investigated for systemic pulmonary delivery

The identification of a drug candidate appropriate for systemic delivery by inhalation is important in the process of developing successful systemic pulmonary applications. The criteria for selecting a drug for pulmonary systemic delivery include: (i) noninvasive alternatives to injection are not currently available or current noninvasive alternatives present limitations (e.g., an elevated metabolism by the liver following oral delivery and before the drug acts systemically); (ii) systemic absorption of the selected drug from the lung is regarded as high enough commercially, that is the bioavailability is sufficiently high and therefore, the dose delivered to the lung sufficiently low, so that production costs are not prohibitive; (iii) the pharmacokinetic profile obtained following pulmonary administration meets therapeutic needs.

Table 1 represents the drugs that are presently assessed in clinical trials for systemic pulmonary delivery, together with the products currently available for their administration via

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**Table 1. Drugs presently assessed in human trials for systemic pulmonary application, together with products currently available on the market for their administration**

Drug	Injection					Noninvasive routes of administration				
	Method	Onset <sup>a</sup>	T <sub>max</sub> <sup>b</sup>	Duration <sup>c</sup>	F <sup>d</sup>	Method	Onset	T <sub>max</sub>	Duration	F
<b>Epinephrine for anaphylaxis, 183 Da, t<sub>1/2</sub><sup>e</sup> = 2 min [39,40]</b>	IM	–	8 min	1 h	100%	Inhalation, Phase I clinical trials	–	1–2 min	1 h	4%
<b>Opioid analgesics, 300–500 Da, t<sub>1/2</sub> = 2–40 h [41,42]</b>	IV	1–2 min	–	2–6 h	100%	Oral	15–30 min	0.5–1.3 h	5–6 h	24–75%
	SC, IM and others	10–30 min	–	2–6 h	>90%	Oral sustained-release	30 min	3.7 h	12–24 h	40–70%
						Sublingual	30 min	20–40 min	7 h	30–50%
						Rectal	30 min	–	4–8 h	75%
						Nasal butorphanol	15 min	–	2.5–4.5 h	60–70%
						Inhalation, Phase I clinical trials	–	3–7 min	–	59%
<b>LHRH analogues, 1.2 kDa, t<sub>1/2</sub> = 1.2–63 h [8,41,43]</b>	IV or SC	–	0 or 38–90 min	–	100%	Nasal	–	10–45 min	12 h	3%
	SC or IM sustained-release	–	3 h–2 weeks	1–4 months	53%	Inhalation, Phase I clinical trials	–	1.1–2.3 h	–	18%
<b>Insulin, 6 kDa, t<sub>1/2</sub> = 5–6 min [23,44]</b>	SC lispro-aspart	5–15 min	50 min	4–6 h	–	Inhalation, Phases I and II clinical trials	30 min	0.7–1.6 h	6–7 h	10%
	SC regular	30–60 min	2 h	6–10 h	70%					
	SC intermediate acting	1–2 h	4–8 h	10–20 h	–					
	SC long acting	2–4 h	8 h	16–20 h	–					
	SC glargine	2–4 h	–	24 h	–					
<b>Interferon alpha-2b, 19.3 kDa, t<sub>1/2</sub> = 2–3 h [41,45]</b>	IV or SC	–	3–12 h	24 h	100%	Inhalation, Phase I clinical trials	–	8–10 h	–	10%
<b>Growth hormone, 22 kDa, t<sub>1/2</sub> = 22 min [41,46,47]</b>	SC or IM	–	3–6 h	–	65–90%	Inhalation, Phase I clinical trials	–	4 h	–	4–10%

Accessible pharmacokinetic and pharmacodynamic data are presented. Data are taken from the references indicated under the drug name. Bioavailability is expressed in terms of the nominal dose in the product or inhaler. IM: intramuscular injection; IV: intravenous injection; SC: subcutaneous injection.

<sup>a</sup> Onset of action.

<sup>b</sup> Time to peak plasma concentration.

<sup>c</sup> Duration of action.

<sup>d</sup> Bioavailability.

<sup>e</sup> Elimination half-life.

other routes. The potential benefits of inhaling these drugs might be delineated as follows. Epinephrine for anaphylaxis does not currently have a noninvasive alternative to intramuscular injection for its administration. Inhalation might be particularly appropriate for epinephrine delivery because it will allow patients self-administration using a compact inhaler. Prompt pre-hospital treatment is life saving in anaphylaxis and a short onset time of action (Table 1) will reduce delay in treatment. Opioid analgesics are delivered by several noninvasive routes of administration but inhalation has the advantage of providing an onset of action as short as that of injection. Analogues of luteinising hormone-releasing hormone (LHRH) are delivered nasally but with low efficiency; by contrast, absorption of the peptides from the lung is higher (Table 1). Although many noninvasive alternatives keep being intensely sought for insulin delivery, the hormone is still delivered by injection only, as is the case of interferon alpha-2b and growth hormone. Inhalation might present a particularly successful noninvasive route of administration for these macromolecules because their absorption into the systemic circulation is higher following inhalation than following any other noninvasive routes of administration assessed in absence of physical or chemical enhancers [4].

The following additional information can be drawn from Table 1. In contrast to the oral, nasal and transdermal routes of administration, the bioavailability of a drug delivered to the lung does not decrease with an increased molecular weight. However, similarly to other non-invasive routes of drug administration, the larger the molecular size, the slower the absorption rate and the later the time to peak plasma concentration ( $T_{\max}$ ). In this regard, epinephrine and interferon alpha-2b are worth comparing. The bioavailability of interferon alpha-2b following inhalation reaches 10% while that of the small molecule epinephrine is only 4%. Yet, epinephrine and interferon alpha-2b show a respective average  $T_{\max}$  of 1 min and 9 h, in correlation with their molecular size. Paracellular diffusion across respiratory epithelia regulates the rate of transport into the bloodstream for molecules  $\leq 40$  kDa and the rate of diffusion between epithelial cells decreases with increasing molecular size [5]. By contrast, the total amount of a drug absorbed from the lung depends on its biological stability during its residence within the pulmonary tissue. Large proteins cross the alveolar epithelium slowly and can remain within the alveolar space for several hours [6]. If they undergo limited degradation within the alveoli during this time, their systemic absorption can be high, as is the case of interferon alpha-2b. However, the comparisons of LHRH analogues and insulin, or interferon alpha-2b and growth hormone indicate that the correlation between molecular weight and  $T_{\max}$  is not perfect and that other parameters are involved in the pharmacokinetic profile *in vivo* as the elimination half-life (Table 1).

### Site of drug absorption from the lung

The amount of drug absorbed systemically from the lung is generally higher following deposition of drug particles in the lung periphery than after particle deposition in central regions of the lung. Deposition in the central and peripheral tract is attained by particles of 1–5  $\mu\text{m}$  aerodynamic diameter, which settle by gravity. The aerodynamic diameter of a particle,  $d_{\text{aer}}$ , is equivalent to the diameter of a sphere of 1  $\text{g}/\text{cm}^3$  density ( $\rho_0$ ), which has the same terminal velocity in still air as the particle:

$$d_{\text{aer}} = d \sqrt{\frac{\rho}{\rho_0 \chi}}$$

where  $d$  is the geometric diameter of the particle,  $\rho$  the particle density and  $\chi$  the particle dynamic shape factor denoting deviation of shape from sphericity [7]. Filtering of large particles ( $d_{\text{aer}} > 5 \mu\text{m}$ ) occurs in upper airways (mouth, trachea and main bronchi) by inertial impaction at sites of changes in airflow direction. Particles with  $d_{\text{aer}} < 1 \mu\text{m}$  are mostly exhaled or deposited by random Brownian motion in distal regions if  $d$ , as  $d_{\text{aer}}$ , is  $< 1 \mu\text{m}$  [7].

Increased bioavailability following drug administration to the lung periphery has been shown for molecules of varying molecular weight (0.6–54 kDa) and in various animal species and humans [2,8–11]. The studies by Pillai *et al.* in monkeys [11] and of Sangwan *et al.* in asthmatic patients [2] are perhaps the clearest demonstration. These authors simultaneously conducted pharmacokinetic studies and measurement of regional distribution of drug deposition within the respiratory tract using gamma camera scintigraphy. Pillai *et al.* obtained 38 and 100% insulin (6 kDa) absorption following inhalation using an ultrasonic and jet nebulizer, respectively. They expressed the percent absorbed in terms of the dose deposited in the lung and relative to subcutaneous injection. The ultrasonic and jet nebulizers generated particles with 4.2 and 0.8  $\mu\text{m}$  mass median aerodynamic diameter (MMAD), respectively, and a deposition ratio of lung to entire thoracic area of the insulin radio-colloid of, respectively, 71 and 89%. Sangwan *et al.* compared systemic absorption of aerosolized interleukin-4 receptor (54 kDa) in patients with asthma using two different aerosol delivery systems: a prototype AERx<sup>®</sup> delivery system and a standard jet nebulizer. The AERx<sup>®</sup> and jet nebulizer generated particles with MMAD of, respectively, 2.0 and 3.5  $\mu\text{m}$ . The ratio of area under the plasma concentration curve (AUC) for the AERx<sup>®</sup> device to AUC<sub>jet</sub> was 2.4 based on the amount of drug deposited in the lung. When normalized for central to peripheral regional distribution ratios, the ratio of AUC<sub>AERx</sub> to AUC<sub>jet</sub> decreased to 1.3, demonstrating that deposition in peripheral lung sites with the AERx<sup>®</sup> was involved in its improved systemic delivery.

However, some drugs (sodium cromoglycate and heparin) have exhibited a fraction of the delivered dose absorbed in

the bloodstream indifferent to site of particle deposition within the respiratory tract and of particle size [12,13]. For instance, Richards *et al.* [13] examined the effect of methacholine-induced bronchoconstriction on lung deposition and pharmacokinetics of inhaled sodium cromoglycate (512 Da) in human volunteers. Methacholine led to a 23% decrease in forced expiratory volume in 1 s, a 2.8 times increase in central:peripheral ratio of lung deposition but no major differences in pharmacokinetics of sodium cromoglycate.

### Fick's first law of diffusion

The above data might be explained by considering the anatomy-physiology of the different regions of the lung in the light of Fick's first law of diffusion:

$$\frac{dM}{dt} = \frac{DSKC_d}{h} = PSC_d$$

in which:

$$P = \frac{DK}{h}$$

$M$  is the amount of permeant flowing through the membrane,  $D$  its diffusion coefficient,  $K$  its partition coefficient,  $C_d$  its concentration on the luminal side and  $P$  its permeability coefficient.  $S$  is the cross-sectional area of the membrane and  $h$  its thickness and  $t$  is the time [14]. Paracellular diffusion is only considered here because compounds discussed in the previous section are essentially hydrophilic and  $\leq 40$  kDa [2,8–11], and therefore, principally transported across biological membranes via paracellular pathways [5]. However, transcytosis can become significant for macromolecules  $\geq 40$  kDa and specific receptor-mediated transcytosis mechanisms can be targeted for improving the pulmonary absorption of large macromolecules.

An increased quantity of drug absorbed following deposition in the deep lung probably results from higher absorption rate ( $dM/dt$ ) in alveoli as a consequence of the large surface area of the epithelium ( $S$ ) as well as short diffusion path between the alveolar epithelium and the capillary endothelium ( $h$ ). The epithelial surface area in human alveoli is  $100 \text{ m}^2$ , whereas that in the airways only  $2.5 \text{ m}^2$  ( $S_{\text{alveoli}} \gg S_{\text{airways}}$ ) [15]. The distance from the luminal air space to the capillaries is less than  $1 \text{ }\mu\text{m}$  in alveoli whereas it is an average of  $200 \text{ }\mu\text{m}$  in airways ( $h_{\text{alveoli}} \ll h_{\text{airways}}$ ) [9,15]. By contrast,  $P$  of conducting airway and alveolar epithelial cells in primary culture are astoundingly comparable ( $h$  in  $P$  in this case is the epithelial thickness and not the total distance between the luminal air space and the capillaries) indicating similar trans-epithelial flux per unit cross-section at both sites [16]. It is probable that the reduced tight junction diffusion area of the alveolar epithelium is offset by the very low diffusional distance compared to the airway epithelial cell monolayers. The total volume of epithelial surface fluid is pretty similar in human airways (4–25 mL) and alveoli (7–20 mL), indicating that the permeant will encoun-

ter a similar volume for dissolution at both epithelial surfaces (similar  $C_d$ ) [15]. More rapid alveolar than bronchial absorption has been demonstrated by several reports in the literature [17,18].

Fast absorption from the alveoli reduces the time of exposure to degradation processes occurring in the airspace and respiratory tissue, thereby increasing the drug fraction absorbed systemically. Slower absorption from the airways would then mean longer exposure to *in situ* clearance mechanisms, and thereby, smaller quantities of intact drug absorbed in the systemic circulation. From this point of view, drugs very stable in the lung environment could be fully absorbed from both the airways and alveoli, as is the case of sodium cromoglycate and heparin mentioned above [12,13]. The mucociliary clearance system transports the mucus layer that covers the airway epithelium towards the oropharynx by ciliary beating. Mucociliary clearance is probably not involved in the reduced absorption from the airways. In fact, systemic absorption from the lung occurs over less than 1 h for most investigated drugs [2,11], while half-time of mucociliary clearance is approximately 3 h [19].

### High technology inhaler systems

Conventional inhalers, as used by asthma patients, are unable to deliver a high fraction of the dose placed in the device to the lung periphery, which would be needed for systemic absorption. Fine particle fractions (FPF), defined as the fraction of aerosolised particles with  $d_{\text{aer}} \leq 5 \text{ }\mu\text{m}$ , are typically in the range of 5–20% and aerosols are delivered with poor reproducibility [20]. These limitations have led to the development of a new generation of inhalers, with the purpose of using the lung as a port of entry to the systemic circulation for conventional drugs, as well as therapeutic peptides and proteins (Table 1).

High technology inhalers have been developed since the 1990s and insulin has been the most investigated drug for inhalation delivery. Several high-technology inhaler systems have allowed increased efficiency and reliability of drug delivery to the lung. FPF has been typically increased to 60% [21,22]. One device is currently undergoing review by the Food and Drug Administration and the European Medicines Agency for noninvasive insulin delivery. It involves a dry powder insulin formulation packaged in individual blisters and an aerosol delivery system that disperses the powder using compressed air into an aerosol cloud in a holding chamber. The patient then inhales the aerosol bolus. Details on the technologies utilized in new generation inhalation systems can be found in the recent review of Valente *et al.* [3].

### Methods used for enhancing pulmonary absorption

Although delivery to the lung has been optimized, bioavailabilities of inhaled therapeutics remain limited (Table 1),

suggesting that uncontrolled biological losses in the respiratory tissue considerably diminish efficiency. Drug losses owing to passage through the lung can be quantified based on pharmacokinetic studies and pulmonary deposition data collected *in vivo*. For instance because inhaled insulin reaches 10% bioavailability in clinical trials [23] and given an average pulmonary deposition of 60% of the nominal dose in the device, it follows that only one–two molecules of insulin out of 10 deposited in the human lung reach the bloodstream.

Degradation of drug molecules within the lung competes with systemic absorption, and thereby, lowers bioavailability to the degree that the rate of degradation is near to or greater than the rate of systemic absorption. On the basis of this conclusion, two strategies could be undertaken to enhance pulmonary absorption. The first strategy would involve increasing the rate of molecular transport across airway and/or alveolar epithelia, while the second would consist in decreasing the rate of specific degradation pathways. Long-term safety concerns have limited the use of conventional penetration enhancers, such as bile salts. Some viable and recent approaches to accelerate transport are only reported below. Similarly, no attention will be given to protease inhibitors because of toxicity issues and selected methods to avoid local degradation will be described (Table 3).

#### *Increasing transport rate across lung epithelia*

Promising methods to accelerate transport across respiratory epithelia might involve alteration of the physicochemical properties of the permeant in a way that facilitates its transport across biological membranes. No effect on epithelia should be associated. Viable methods might also utilize specific active transport pathways.

Low molecular weight amino acid analogues or carriers have been shown to facilitate insulin transport across lung epithelia, thereby increasing its systemic bioavailability following pulmonary delivery in rats [24]. The carrier interacts with the protein and the interaction induces a reversible destabilization of its native state into a partially unfolded protein that is transported more easily across epithelia [25]. The amino acid analogues have no permeabilization and proteases inhibition properties and no toxicity to the lung was demonstrated in these experiments [24–26]. Yet, carrier doses were 100–500 times higher than the insulin dose and carriers effective at lower doses might need to be designed to obtain a load of material compatible with administration to the human lung [24].

Technosphere™, a new drug carrier for pulmonary administration, has been shown to increase absorption of insulin and of the (1–34) fragment of parathyroid hormone (4.1 kDa) from the lung in Phase I clinical trials [27,28]. Insulin bioavailability is increased to 30% relative to subcutaneous injection and  $T_{\max}$  is shortened to 13 min using Technosphere™

and a specifically developed breath-powered dry powder inhaler ([27]; see Table 1 for comparison of bioavailability and  $T_{\max}$  of inhaled insulin without enhancer). The system consists in a small organic molecule, 3,6-bis(*N*-fumaryl)-*N*-(*n*-Butyl)amino-2,5-diketopiperazine, that self-assembles in a mild acid environment into 2  $\mu\text{m}$  spheres and in the process encapsulates every molecule present in the solution. Following inhalation, dry particles dissolve in the neutral pH environment of the deep lung. However, the exact mechanism behind penetration enhancement is unknown and investigating the effects of the diketopiperazine derivative on respiratory epithelial cell monolayers *in vitro* should be very informative.

Fusion of proteins to the Fc domain of IgG can enable efficient transport of the proteins across the airway epithelium via neonatal Fc receptor (FcRn)-mediated transcytosis [29]. Erythropoietin–Fc conjugates delivered to the airways of monkeys were absorbed in the bloodstream with an average bioavailability almost twice that of unconjugated erythropoietin (30.4 kDa; 25% versus 15%) [29]. The higher level of expression of FcRn in epithelial cells of the conducting airways is the reason for targeting the airways and not the alveoli for optimal absorption [29]. Conjugation to Fc has the additional advantage of prolonging plasma elimination half-life significantly. However, verification of the biological activity of the protein following conjugation is crucial in this approach.

Targeting alveolar epithelium caveolae with a specific carrier might help therapeutic macromolecules cross the barrier to systemic absorption. Caveolae are flask-shaped invaginations of the plasma membrane that are open to the luminal space where molecules might enter them. They are mostly found in muscle cells, fibroblasts, capillary endothelium and type I pneumocytes and can provide a trafficking pathway for macromolecules across cells [30,31]. In support of this, an antibody directed against caveolae of the lung endothelium was shown to provide lung tissue accumulation of the antibody as high as 89% within 30 min of intravenous injection in rats. When conjugated to drugs or toxins, drug delivery by the antibody was selective to the lung and enhanced by up to 172-fold [31]. Albumin is transported across endothelia and the alveolar epithelium by caveolae following binding to the cell surface albumin-binding glycoprotein, gp60 [32]. It was recently shown that albumin enabled caveolae-mediated transcytosis of myeloperoxidase across endothelia because of binding of myeloperoxidase to albumin [33]. These suggest possibilities to use caveolae-specific carriers for effective transcytosis of proteins across the alveolar epithelium.

#### *Decreasing local degradation rate*

Improved understanding of transport and elimination pathways within the lung might help developing strategies to increase pulmonary absorption, as exemplified with FcRn-



**Table 2. Alveolar macrophages uptake of varying size macromolecules in various animal species**

Macromolecule	Molecular weight (kDa)	Animal species	Method of microscopy	Refs
Human growth hormone	22	Rat	Confocal	[48]
Superoxide dismutase	33	Baboon	Electron	[49]
Human chorionic gonadotropin	39.5	Rat	Confocal	[34]
Ovalbumin	45	Mouse	Confocal	[50]
FITC-albumin	67	Rat	Confocal	[51]
Albumin	67	Rabbit	Electron	[52]
Dextran	70	Rat	Electron	[53]
IgG	150	Rat	Confocal	[34]
Ferritin	600	Rat	Electron	[53]

mediated transport. Respiratory epithelia and local proteases have long been recognized barriers to systemic absorption following inhalation [15]. More recently, alveolar macrophages have been shown to be a primary barrier to pulmonary absorption of macromolecules [34].

Alveolar macrophages were found to comprise a major barrier to the transport of macromolecules from the lung into the bloodstream, particularly for moderate to large proteins. Using intratracheal instillation of liposome-encapsulated dichloromethylene diphosphonate, the rat lung was depleted of alveolar macrophages and several-fold enhancement in pulmonary absorption of IgG (150 kDa) and human chorionic gonadotropin (39.5 kDa) followed the elimination of alveolar macrophages. Lowering the rate of endocytic uptake by alveolar macrophages might, therefore, lead to increased pulmonary bioavailability. IgG transport across respiratory epithelia takes place via saturable FcRn-mediated transcytosis and decreasing the dose of IgG delivered to the lung was shown to favor transport of IgG from the lung lumen into the systemic circulation relative to local degradation [34–36]. Uptake of proteins by alveolar macrophages following delivery to the lung has been observed in species other than the rat (Table 2) and the universality of the phenomenon in the animal kingdom might open up novel approaches to inhibit protein uptake by alveolar macrophages.

PEGylating peptides and proteins in the proper position and with an appropriate sized polyethylene glycol (PEG) can protect the macromolecule from clearance mechanisms (probably proteolytic degradation) in the lung, and thereby increase bioavailability following inhalation [37]. Additionally, systemic activity can be prolonged owing to increased plasma half-life of the PEG derivative. Covalently attaching PEG of 750 or 2000 Da molecular weight to insulin increased bioavailability and duration of action several fold following dry powder inhalation in dogs [37]. Yet, attachment of larger PEGs (5–12 kDa) to insulin or to recombinant human gran-

ulocyte colony stimulating factor (18.8 kDa) impeded transport across respiratory epithelia and significantly decreased bioavailability following pulmonary delivery in rats, emphasizing the importance of optimizing PEG size [37,38].

### Summary and conclusions

Pulmonary delivery of systemically acting drugs presents requirements and challenges. The most adequate drugs for pulmonary systemic delivery need to be selected first. The therapeutic peptides and proteins represent particularly interesting candidates because inhalation is currently the only needle-free route of administration capable of delivering macromolecules with bioavailabilities as high as 10%, without the use of physical or chemical enhancers.

To be well absorbed from the lung, a compound needs to be delivered to the alveolar region. Increased fraction of drug absorbed from the alveoli results from their large epithelial surface area and from the very thin diffusion path to the bloodstream. Efficient deep lung deposition has been achieved with recent high technology inhaler devices that are able to overcome filtration of aerosols in the throat and upper airways.

Yet, in spite of the high efficiency of new generation inhaler systems, pulmonary bioavailabilities do not exceed 10% in general, indicating that clearance mechanisms within the respiratory tissue operate effectively. Many methods have been investigated to accelerate drug transport across respiratory epithelia and decrease exposure time to degradation processes (Table 3). Viable and recent strategies include the use of low molecular weight amino acid analogues that interact with proteins to convert them into partially unfolded structures, which are more easily transported across epithelia. Technosphere™, another small molecule carrier, is able to increase the pulmonary absorption of peptides as well. Fusion of proteins to the Fc domain of IgG increases transport efficiency of the proteins across airways via FcRn-mediated transcytosis. Caveolae are flask-shaped invaginations of the

**Table 3. Comparison table of the methods used for enhancing pulmonary absorption**

Methods	Pros	Cons	Refs
<b>For increasing transport rate across lung epithelia</b>			
<b>Amino acid analogues</b>	Application to protein drugs and highly charged compounds No effects on lung epithelia and proteases	High doses of the amino acid analogues needed	<a href="http://www.emisphere.com/">http://www.emisphere.com/</a> [24–26]
<b>Technosphere™</b>	Application to peptide drugs	Unknown effects on lung epithelia and no verification of long-term safety yet	<a href="http://www.mannkindcorp.com/">http://www.mannkindcorp.com/</a> [27,28]
<b>Drug conjugation to Fc</b>	Application to large protein drugs Prolongation of plasma elimination half-life	Possible decrease of biological activity of the protein after conjugation	<a href="http://www.syntnx.com/">http://www.syntnx.com/</a> [29]
<b>Caveolae-specific carriers</b>	Potential application to large protein drugs	No proof of concept available for enhancing pulmonary absorption	[30–33]
<b>For decreasing local degradation rate</b>			
<b>Diminishing uptake by alveolar macrophages</b>	Potential application to protein drugs	Challenging development of methods to inhibit alveolar macrophages uptake and no universal method available yet	[34]
<b>PEGylation</b>	Application to peptides and proteins Prolongation of plasma elimination half-life	Possible decrease of biological activity of the peptide and protein after conjugation	<a href="http://www.nektar.com/">http://www.nektar.com/</a> [37]

plasma membrane that shuttle macromolecules across endothelia and epithelia and might offer opportunities for carrier-mediated transport of proteins from the airspaces into the bloodstream.

Decreasing the rate of specific degradation processes occurring within the lung might represent an alternative option to increase pulmonary bioavailability (Table 3). Alveolar macrophages comprise a major barrier to pulmonary absorption of macromolecules and novel approaches to inhibit protein uptake by alveolar macrophages might be conceived. Proteolytic breakdown of macromolecules within the respiratory tissue can be avoided by PEGylation of proteins and polyethylene glycolated proteins have increased bioavailability and duration of systemic action.

Understanding the mechanisms of particle deposition within the respiratory tract has permitted to develop efficient high-technology inhaler systems. In the same way, understanding biological processes encountered by drug molecules within the lung has the potential to ameliorate pulmonary absorption, as it has been exemplified with the methods for enhancing pulmonary absorption presented in this review. Biological processes include transport mechanisms across airway and alveolar epithelia, as well as specific degradation pathways undergone by small molecules, peptides and proteins within the respiratory tissue. In addition to epithelia, enzymes and alveolar macrophages, it is probable that other barriers to absorption might be discovered and lead to novel methods to enhance absorption from the lung.

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