Drug Delivery to the Lung: Permeability and Physicochemical Characteristics of Drugs as the Basis for a Pulmonary Biopharmaceutical Classification System (pBCS)

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Abstract: The respiratory tract is currently considered as an alternative to gastrointestinal and dermal drug delivery systems and is used to deliver drugs for respiratory diseases as well as the treatment of non-pulmonary disorders. The first step in drug profiling for delivery *via* the respiratory tract needs to address intrinsic physicochemical parameters and their impact on or correlation with absorption. Moreover, the more the pulmonary drug delivery shall find acceptance, the greater will be the need for validated test systems, methods, and guidelines for regulatory purposes. The Biopharmaceutical Classification System (BCS) remains the simplest and most common guiding principle for predicting drug absorption, but it is limited to the gastrointestinal tract. This review suggests an extension, the *pulmonary Biopharmaceutical Classification System (pBCS)*, that will take into consideration the specific biology of the lung as well as particle deposition, aerosol physics, and the subsequent processes of drug absorption and solubility. We will describe the steps to be taken to develop a pBCS as well as the compounds that will be used to establish this classification. Furthermore, we will introduce two cellular models with which drug permeability across the pulmonary barrier will be determined as an alternative to the currently and widely used studies with animals.

Keywords: Absorption, BCS, pBCS, solubility, permeability, *in vitro-in vivo* correlation, Calu-3, pAEpC.

INTRODUCTION

Many drugs are currently delivered directly to the respiratory system. They are mainly aimed at treating diseases of this organ. Main advantages of the delivery of drugs *via* the respiratory tract are a reduction of side effects and an immediate onset of action. In addition, inhalation is attracting much interest as an alternative route of systemic drug administration. This can be understood considering the large surface area for drug absorption, the high blood flow, and the absence of first pass metabolism characteristic for the lung. This leads to a rapid clearance after administration and thus in high absorption rates and a rapid onset of action. Delivery systems such as nasal sprays or inhalers have already proved as useful. For instance, intranasal administration of human growth hormone intended for systemic delivery resulted in a bioavailability of 8.11% in rats [1]. Intranasal spray administration of drugs turned also out to be successful in the reduction of intraocular pressure in glaucoma patients [2]. During the process of drug development aimed for systemic delivery as well as directed to the respiratory system deep knowledge of the physicochemical and biological properties are necessary. The solubility and lipophilicity of drugs may affect its behaviour in the bronchial and deep lung spaces (e.g. a resulting long residence time in the respiratory system versus fast permeation across epithelial cell barriers). Additionally, it should be considered that the properties of inhaled aerosolised drug particles such as size, shape, density, hygroscopicity, velocity and pressure of delivery, and charge as well as the physiological state of the respiratory tract will have an impact on the correct point of delivery inside the respiratory tract (upper airways versus deep lung) [3]. Harmonization of all these factors would provide a powerful tool for predicting and accelerating the drug development process. We suggest therefore that a drug classification system for the respiratory system comparable to the Biopharmaceutics Classification System (BCS) currently used for gastrointestinal tract drug absorption should be established.

The Biopharmaceutics Classification System (BCS)

In 1995, Amidon and co-workers established the bases for the BCS by categorizing drugs into four types according to their solubility and permeability [4]. The objective was to predict the *in vivo* pharmacokinetic performances of drugs from measurements of permeability and solubility. During the drug development process, the BCS provides an opportunity to optimize the structures or physicochemical properties of lead candidates thereby achieving better deliverability by displaying the features of BCS class I (high solubility, high permeability) without compromising their pharmacodynamics. The Food and Drug Administration (FDA) adopted the BCS in several of its guidelines thereby simplifying and improving the drug approval process [5]. Since its introduction, the BCS has undergone many revisions [6], and many alternatives have been proposed. For instance, the Biopharmaceutics Drug Disposition Classification System (BDDCS) proposes a modest revision of the BCS criteria. That might be useful in predicting routes of enzymatic drug elimination, the effects of efflux and absorptive transporters, drug-drug interactions, and drug-food interactions that could influence bioavailability following oral and intravenous dosing [7]. In turn, the Permeability-Based Classification System (PCS)

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has been proposed as an alternative to the BDDCS [8]. This system suggests that compounds could belong to different *in vitro* and *in vivo* PCS classes depending on the involvement and magnitude of active transport. In addition, routes of elimination, drug-drug interactions, and drug and metabolite organ toxicity are also taken into consideration. Alternatively, the Quantitative Biopharmaceutics Classification System emphasises the dose/solubility ratio for absorption phenomena in conjunction with mean time concepts for dissolution, transit, and uptake of drug in the intestines [9].

THE PULMONARY BIOPHARMACEUTICS CLASSIFICATION SYSTEM (pBCS)

It is the purpose of this review to establish the basis for a pBCS that will consider the impact of the unique physiology of the respiratory system on drug absorption. Fig. (**1**) schematically summarizes factors that potentially influence the absorption process and how they can be integrated into the pBCS. They are grouped into biological factors, related to the organ's physiology, and formulation and physicochemical factors dependent on the drug's properties.

Lung Biology

Comparison of the biological properties of the bronchi and the alveoli (Fig. **2**) clearly shows that each space will respond differently to drug administration, and that drug absorption might be differently affected. Upper airways and deep lung have different absorption areas (1-2 m^2 for the conducting airways and 140 m^2 for the alveolar surface). The airway epithelium is covered by a thick viscous mucus gel like layer (up to 10 μm thick), in contrast to the thin film of alveolar surfactant (approximately 0.07 μm on average) found on the surface of the alveolar epithelium. This results in different final volumes of dissolution in these two compartments [10]. The presence of this mucus or surfactant lining might therefore not only affect compound solubility, but also diffusion processes towards the epithelium as well as the interaction of drugs with cell surfaces and receptors. Furthermore, cellular thickness in both tissues (up to 58 μm for bronchial cells in comparison with the perinuclear 0.3 μm of alveolar pneumocytes) and cellular population are dissimilar [11]. Mucus is responsible for drug clearance in the bronchi while macrophages have a very active role in clearance in the deep lung. Drug metabolism and drug-drug interactions should also be considered as well as efflux transporters. For instance, functional P-Glycoprotein (P-gp) has been detected in bronchi and in human alveolar type I cells that largely constitute the absorptive surface in the deep lung, whereas alveolar type II cells were negative for this protein [12, 13].

Unfortunately, the proportion of drug deposited in either bronchi or deep lung after inhalation can still not be accurately measured *in vivo*. Therefore, an exact calculation of the contribution of each physiological space to the final drug absorption is not possible. For this reason, a single pBCS will be adopted that will consider characteristics of drugs at both sites.

Drug Formulation / Physicochemical Properties

The drug delivery system will clearly affect the whole absorption process: particle deposition, aerosol physics, as well as the impact of quantity and quality of excipients on the metabolic stability, and solubility kinetics should be quantified.

The drug physicochemical properties to be considered include molecular size, lipophilicity (log P), solubility, pKa, protein binding, polar surface area, and charge or rotatable bonds. These properties will in the end influence the permeability of a compound across the lung epithelial barrier.

ESTABLISHMENT OF THE pBCS

Several pBCS classes should be defined to classify drugs according to their ability to reside in the lung or to be transferred to the bloodstream. For this, the actual impact of the above mentioned factors in pulmonary drug absorption should be established with the help of model compounds. By this means, and once the system is established, knowledge of

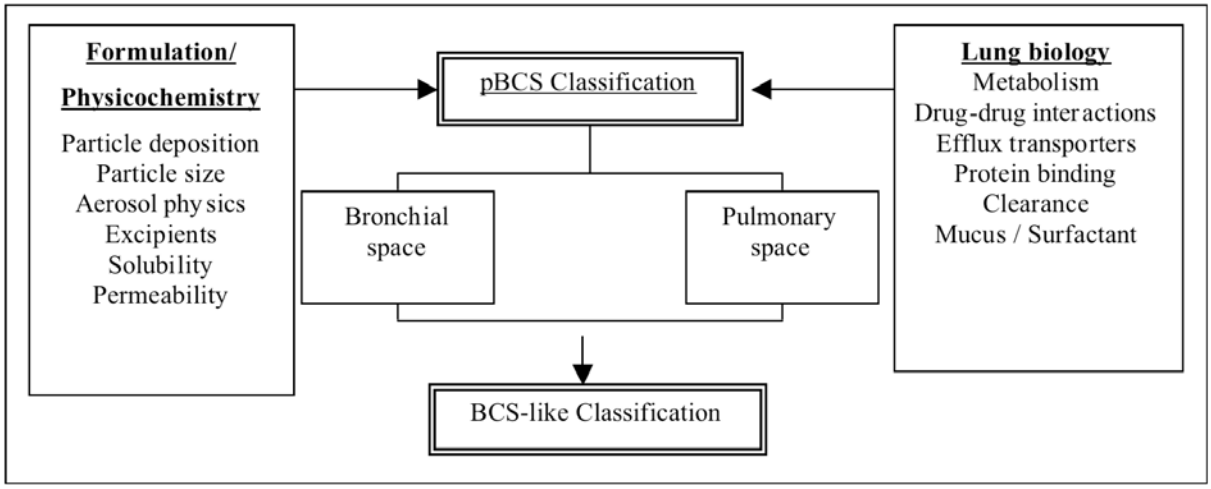

Fig. (1). Proposal for the pulmonary BCS (pBCS). Compounds will be classified according to their properties similar to the well established BCS. As the exact proportion of drug deposited in the bronchial compared to the pulmonary space is not measurable, a single pBCS will be applied for each compound which will be a compromise. As mucus and surfactant lining volumes are different in the bronchial and pulmonary spaces, it is inferred that solubility for a given compound might also vary. In addition, different epithelial cells thickness and cellular types will influence the drug absorption process.

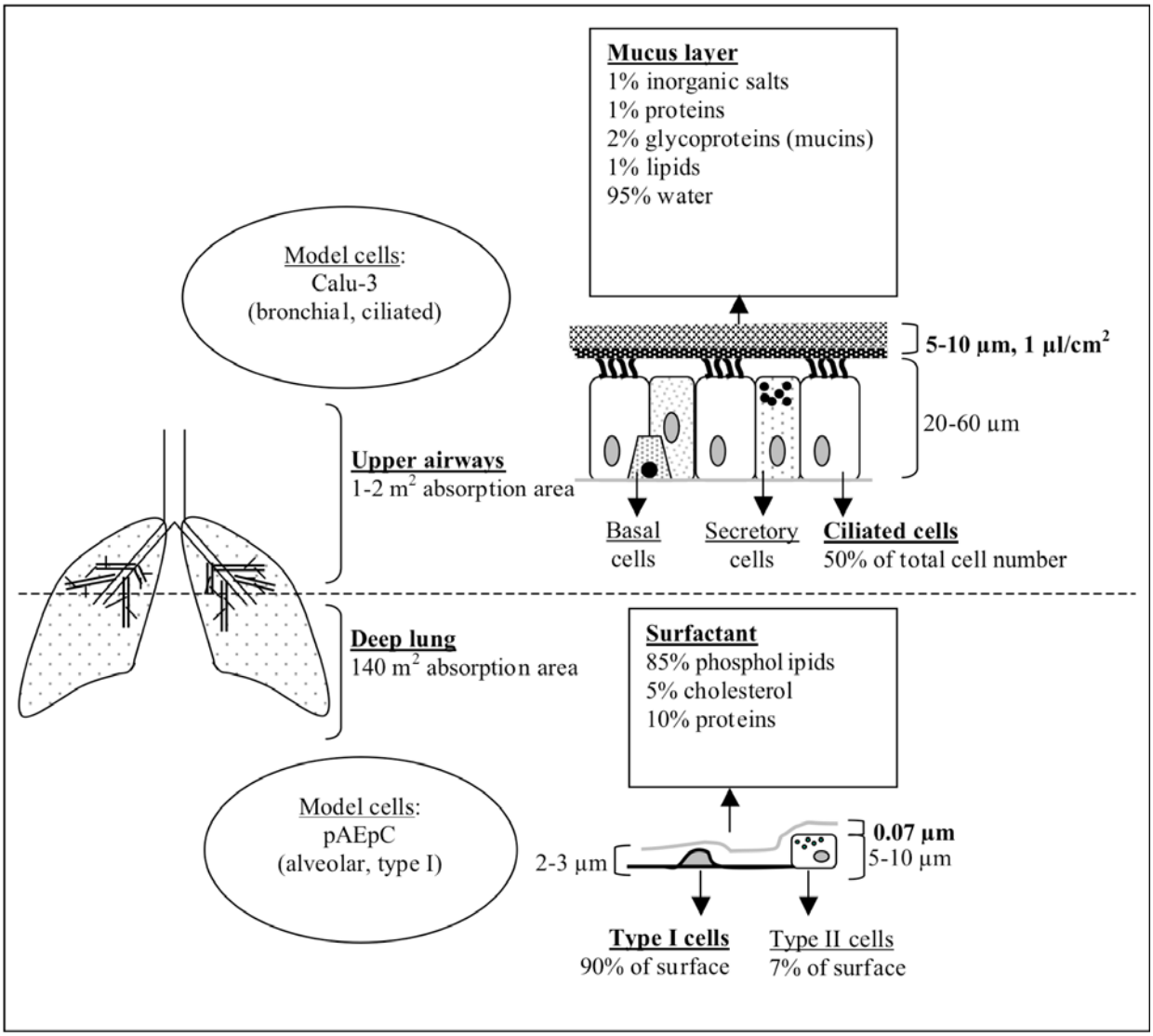

Fig. (2). Comparison of the upper airways and the deep lung. Differences are found in the absorption area, cellular variety and height, lining composition (mucus vs. surfactant), thickness, and total volume.

the physicochemical properties of a drug in development would allow a prediction of its behaviour in the pulmonary tissue.

First Step: Pulmonary Drug Permeability Determination

A set of chemical entities is selected for the study of drug permeability in the respiratory tissue (Tab. 1). The experimental permeability of this factors will be determined with the help of two *in vitro* cellular systems: Calu-3 cells representing a bronchial epithelial model and alveolar primary cells as a deep lung model (discussed below). The obtained results will be evaluated and correlated with data available from the literature such as solubility, lipophilicity, and *in vivo* models of pulmonary absorption. This evaluation will give an idea how physicochemical properties such as solubility and permeability affect drug absorption in the lung and will be the base of the pBCS. It should be taken into account that mucus and surfactant might influence solubility characteristics of a given compound. Potential effects should be further tested and considered in the classification system. Experimental permeability values may also correlate with known log P values for each compound and provide an additional prediction tool.

In order to check the validity and robustness of the used *in vitro* pulmonary models, the experimental permeability values will be compared with permeability data of other test systems such as gastrointestinal models using Caco-2 cells (data of our group and from literature). This will allow to assess any similarities or dissimilarities between the models and will give insights into characteristics of the lung that are unique for this organ. Indeed, others have already shown that *in vitro – in vivo* correlations can be established for the pulmonary system [14]. Furthermore, preliminary data (own unpublished data) have highlighted striking permeability differences between gastrointestinal and respiratory models as well as between the upper and lower respiratory tract. This underlines the need for a pulmonary biopharmaceutical classification system. There is clear evidence that the different biology between the lung and the gastrointestinal system affects drug absorption.

Next Steps: Integration of Additional Factors

Once the base of the pBCS is established according to solubility and permeability values additional parameters will be integrated: active metabolic pathways in the lung that could influence drug pharmacology, drug-drug interactions that could affect these metabolic pathways, active transporters that could influence drug pharmacokinetics, and aerosol particle size, that could be decisive for drug localization. Further improvement of the cellular models could include co-cultures to examine the effects of macrophages on drug metabolism and bioavailability in the distal airways.

SELECTED CHEMICAL ENTITIES FOR pBCS ESTABLISHMENT

The compounds chosen for establishing the pBCS have a molecular weight below 1000 Da and cover, according to the BCS classification, a wide range of permeabilities and solubilities as well as pharmacodynamic effects. In addition, they are candidates for delivery *via* the respiratory tract: some of them are already administered *via* the respiratory tract, others are aimed at treating pulmonary diseases, but are currently orally administered. Bypassing the secondary effects characteristic of an oral administration would be beneficial for all of the chosen compounds. The presented chemical entities have been classified into six categories: steroids, compounds with existing *in vitro-in vivo* correlation, small active pharmaceutical ingredients, consumer products, efflux transporter substrates, and quality control compounds. The classification is open as many compounds fit into more than one category at a time.

Tables **1** to **3** summarize some properties of the compounds that will be useful for pBCS establishment (data taken from the literature, where indicated, own data). Among others, log P and solubility values, known apparent permeabilities (obtained from different experimental models), and known metabolic pathways are shown.

Steroids

Steroids have been used for over 40 years as anti-inflammatory, immunosuppressive, and antiproliferative agents. They are available in numerous formulations, including nasal formulations or inhalers. Inhaled corticosteroids are the current choice for the treatment of persistent asthma. Their efficiency and safety is dependent on pharmacokinetic and phamacodynamic aspects such as particle size, delivery system, lung residence, oral bioavailability, or protein binding. *In vitro* measurements consistently demonstrate differences among the various corticosteroids [15, 16].

In Vitro – In Vivo Correlation

Knowledge of the physicochemical properties of a drug candidate certainly accelerates the process that leads to its final use as a medicine. During the last steps of this development process, animal studies have to be performed to assess toxicity, absorption, metabolism, and clearance of the compound, among others. For ethical reasons it is desirable to perform as few as possible of these tests in animal studies. Thus, *in vitro* assays need to be developed that are appropriate to substitute *in vivo* studies or, at least, reduce their number by, for instance, predicting the best starting doses of a drug for an *in* vivo assay. For the selected compounds in this group extensive data are available from *in vivo* experiments that will allow to establish correlations with their physicochemical properties (*in vitro* data) and to check the robustness of the *in vitro* models.

Small APIs

APIs (active pharmaceutical ingredient) are the active chemicals used in the manufacturing of drugs. Entities with a molecular weight of less than 1000 Da and a diversity of pharmaceutical applications are considered. All APIs mentioned here have the potential to be used as inhalative drugs, either because they target respiratory diseases or because the upper airways are close to their site of action, e.g. the central nervous system.

Consumer – Life Style Related Products

During everyday's life people are in contact with a series of products that being so usual and common do not properly get our attention. Some of these products, nevertheless, might have unexpected effects on concomitantly taken drugs such as diminished or enhanced absorption and/or effectiveness, and altered metabolism. In addition, some of these products have the potential to become inhaled drugs.

Efflux Substrates

Protein transporters such as P-gp (also called MDR), Breast Cancer Resistance Protein (BCRP), Multidrug Resistance associated Protein (MRP), and Lung Resistance Protein (LRP) are responsible for the efflux transport of many drugs. This results in absorption and bioavailability values lower than expected. In fact, the presence of these proteins is generally an indicator of bad treatment outcome and poor prognosis, e.g. in cancer patients [17, 18]. It is important, thus, to characterize a drug in terms of possible efflux transport and to properly calculate the administered dose in order to assure the correct final pharmacological effectiveness. Inhibitors of these transporters are useful tools for activity studies. The compounds classified in this group are known targets of these proteins.

Quality Control Products

When establishing an *in vitro* cellular model for drug permeability studies, it is desirable that it mimics as good as possible the *in vivo* situation. This includes the establishment of a cellular barrier able to discriminate compounds according to their permeability (low/high) and the expression of the efflux transporters that are known to be present *in vivo*. The compounds in this group can be used as markers for these properties and as quality control products that help determine the appropriateness of an epithelial *in vitro* model.

CELLULAR MODELS FOR DRUG PERMEABILITY DETERMINATION

A wide range of possibilities exists to asses pulmonary drug absorption [19]. Although *in vivo* assays are currently widely used, the availability of cellular models that reproduce the pulmonary system is a major concern for researchers. Assessment of drug permeability and screening of formulations by this means can speed up the drug development process and reduce the number of animals used as well as the costs. Therefore, we have focused our attention on *in vitro*

Table 1. List of Selected Compounds and their Physicochemical Properties

Category	Name	Molecular Formula	MW (g/mol)	[a]Exp. Log P	[b]Exp. water Solubility (mg/l)	[c]BCS	[d]Delivery	[e]Application
Steroids	Hydrocortisone	$C_{21}H_{30}O_5$	362.5	0.5	320	I	I	Resp, Inf
	Testosterone	$C_{19}H_{28}O_2$	288.4	3.6	23.4	IV	C	Card, Horm
	Dexamethasone	$C_{22}H_{29}FO_5$	392.5	1.1	89	II-IV (*)	C	CNS, Resp, Inf
	Budesonide	$C_{25}H_{34}O_6$	430.5	1.9	Insoluble	II	I	Resp, Inf
	Betamethasone	$C_{22}H_{29}FO_5$	392.5	1.1	Insoluble	II	C	Inf, Resp
IVIVC	Cromolyn	$C_{23}H_{16}O_{11}$	468.4	1.6	210	I	I/S	Resp, Inf
	Formoterol	$C_{19}H_{24}N_2O_4$	344.4	2.2	Slight	II	I	Resp
	Imipramine	$C_{19}H_{24}N_5$	280.4	3.9	18.2	IV	C	CNS
	Losartan	$C_{22}H_{23}CIN_6O$	422.9	6.1	0.82	III (*)	C	Card, Resp
	Terbutaline	$C_{12}H_{19}NO_3$	225.3	1.4	213000	I	I	Resp
	Salbutamol	$C_{13}H_{21}NO_3$	239.3	1.4	3	II	I	Resp
Small APIs	Zopiclone	$C_{17}H_{17}CIN_6O_3$	388.8	0.8	151	I	C	CNS
	Zaleplon	$C_{17}H_{15}N_5O$	305.3	0.9	N.A.	N.A.	C	CNS
	Zolpidem	$C_{19}H_{21}N_3O$	307.4	1.2	23000	I(*)	C	CNS
	Leflunomide	$C_{12}H_9F_3N_2O_2$	270.2	2.8	21	II	C	Resp
	Oxymetazoline	$C_{16}H_{24}N_2O$	260.4	3.4	N.A.	N.A.	S	Resp, Inf
Consumer products	Nicotine	$C_{10}H_{14}N_2$	162.2	1.1	10^6	I	I/S	CNS, Inf
	Menthol	$C_{10}H_{20}O$	156.3	3.2	490	III	C	DD
	Mannitol	$C_6H_{14}O_6$	182.2	-3.9	216000	I	S	CNS, DD, Resp
	Caffeine	$C_8H_{10}N_4O_2$	194.2	-0.5	22000	I	C	CNS, Can
Efflux substrates	Methotrexate	$C_{20}H_{22}N_8O_5$	454.4	-2.2	2600	III(*)	T	Inf, Can
	Vinblastine	$C_{46}H_{58}N_4O_9$	811.0	3.9	Negligible	IV	C	Can
	Topotecan	$C_{23}H_{23}N_3O_5$	421.4	0.8	1000	I	C	Can
	Sulfasalazine	$C1_8H_{14}N_4O_5S$	398.4	2.5	N.A.	II(*)	T	Inf
Quality control markers	Digoxin	$C_{41}H_{64}O_{14}$	780.9	2.2	Insoluble	I-III(*)	C	Card, Can
	Rhodamine123	$C_{28}H_{30}N_2O_3$	442.5	N.A.	N.A.		C	M, Can
	Fluorescein	$C_{20}H_{12}O_5$	332.3	3.4	600000	III	I	M
	Propranolol	$C_{16}H_{21}NO_2$	259.3	3	70	I(*)	T	Card
	Atenolol	$C_{14}H_{22}N_2O_3$	266.3	0.5	13500	III(*)	T	Card
	Talinolol	$C_{20}H_{33}N_3O_3$	363	N.A.	N.A.	III-IV	C	Card
	Metoprolol	$C_{15}H_{25}NO_3$	267.4	1.6	16900	I(*)	C	Card, Resp

Source: Drug Bank www.drugbank.ca
[a]Experimental log P / hydrophobicity.
[b]Experimental solubility in water.
[c]Source of entries marked with (*): www.tsrlinc.com/services/bcs/search.cfm
[d]Type of delivery to the lung (if available).
[e]Current clinical applications.

N.A.: Not available
I: Inhaled
S: Spray
C: Candidate for pulmonary delivery
T: Toxic effects in the lung
Can: Cancer
Card: Cardiovascular diseases
CNS: Central Nervous System diseases
Horm: Hormone therapy
DD: Drug Delivery enhancer
Inf: Inflammatory diseases
Resp: Respiratory diseases
M: Marker

Table 2. Permeability Values of the Selected Compounds in Cellular Models

Compound	Model system	Apparent permeability (10^{-6} cm/s)
Hydrocortisone	Reconstructed human cornea	5.41 ± 0.4 (ab)
Testosterone	Pig nasal mucosa – liquid mucosal interface	32.24 ± 31.12 (ab)
	Pig nasal mucosa – air mucosal interface	9.82 ± 11.41 (ab)
	HT29-18-C1 intestinal cells	4.8 (ab)
	mucus secreting HT29-MTX	3 (ab)
	Caco-2	4.8 (ab)
	TR146 (human buccal epithelium)	24.1 ± 0.4 (ab)
Dexamethasone	Primary tracheal epithelial cells – Air-interfaced culture	9.8 ± 0.4 (ab)
	Primary tracheal epithelial cells – Liquid-covered culture	8.7 ±0.3 (ab)
	Rabbit cornea	24.2 ± 1.03 (ab)
	Rabbit conjunctival epithelial cells	4.1 ± 0.1 (ab)
		6.6 ± 0.3 (ba)
	Caco-2	11.4 ± 0.9 (ab)
Budesonide	pAEpCs	2.65 ± 0.49 (ab)*
		2.80 ± 0.2 (ba)*
	Caco-2	10.7 ± 2.6 (ab)
		8.7 ± 3.9 (ba)
	Calu-3	5.33 ± 0.95 (ab)
		4.11 ± 0.19 (ba)
	Human nasal cells	17.1 ± 1.9 (ab)
Betamethasone	N.A.	N.A.
Cromolyn	Caco-2	0.1 ± 0.03 (ab)
		0.1 ± 0.02 (ba)
Formoterol	Caco-2	1.9 ± 1.4 (ab)
		2.1 ± 1.5 (ba)
Imipramine	Caco-2	10.8 (ab)
		12.1 (ba)
Losartan	Caco-2	1.0 ± 0.4 (ab)
		3.7 ± 0.3 (ba)
Terbutaline	Caco-2	0.78 ± 0.41 (ab)
		0.8 ± 0.5 (ab)
		0.4 ± 0.25 (ba)
		1.1 ± 0.5 (ba)
Salbutamol	N.A.	N.A.
Zopiclone	N.A.	N.A.
Zaleplon	N.A.	N.A.
Zolpidem	N.A.	N.A.
Leflunomide	N.A.	N.A.
Oxymetazoline	N.A.	N.A.

(Table 2). Contd…..

Compound	Model system	Apparent permeability (10^{-6} cm/s)
Nicotine	TR146 (human buccal epithelium)	65.4 ± 5.2 (ab)
	Porcine buccal mucosa	0.0154 ± 0.012 (ab)
Menthol	N.A.	N.A.
Mannitol	Pig nasal mucosa – liquid mucosal interface Pig nasal mucosa – air mucosal interface	3.12 ± 1.72 (ab) 2.26 ± 1.42 (ab)
	Calu-3	0.27 ± 0.02 (ab)* 0.27 ±0.08 (ba)*
	Rabbit cornea	8.99 ± 1.43 (ab)
	Rabbit sclera	6.18 ± 1.08 (ab)
	Caco-2	0.82 ± 0.09 (ab) 0.54 ± 0.21 (ab) 0.64 ± 0.04 (ab) 0.2 ± 0.3 (ab) 0.5
	Rabbit conjunctival epithelial cells	0.1 (ab) 0.09 (ba)
	Primary tracheal epithelial cells – Air-interfaced culture Primary tracheal epithelial cells – Liquid-covered culture	0.12 ± 0.03 (ab) 0.28 ± 0.06 (ab)
	Rabbit alveolar epithelial primary cells	0.101 ± 0.003 (ab)
	TR146 (human buccal epithelium)	3 ± 1.2 (ab)
	MDCK	2.41 ± 0.06 (ab)
Caffeine	Porcine buccal mucosa	18.9 ± 3.1
Methotrexate	N.A.	N.A.
Vinblastine	Rat alveolar epithelial cells	1.77 (ab) 5.71 (ba)
	Caco-2	5.4 (ab) 16.28 (ba)
	MDCK	1.5 (ab)
Topotecan	N.A.	N.A.
Sulfasalazine	N.A.	N.A.
Digoxin	pAEpCs	1.91 ± 0.27 (ab)* 2.12 ± 0.25 (ba)*
	Calu-3	0.42 ± 0.07 (ab)* 2.07 ± 0.11 (ba)*
Rhodamine123	pAEpCs	0.77 ± 0.27 (ab)* 0.89 ± 0.19 (ba)*
	Calu-3	0.49 ± 0.24 (ab)* 3.01 ± 0.71 (ba)*
	hAEpCs	1.41 ± 0.78 (ab) 4.35 ±2.15 (ba)
	Normal human bronchial epithelial cells	2.82 ± 0.81 (ab) 8.31 ± 2.15 (ba)
	Caco-2	0.116 ± 0.019 (ab) 1.42 ± 0.16 (ba) 10.4 ± 1.5 (ba)

(Table 2). Contd…..

Compound	Model system	Apparent permeability (10^{-6} cm/s)
Fluorescein	Calu-3	0.18 ± 0.03 (ab)* 2.19 ± 0.20 (ba)*
	pAEpC	0.37 ± 0.17 (ab)*
	HT29-18-C1 intestinal cells	0.8 (ab)
	Calu-3 (LCC conditions) Calu-3 (AIC conditions)	1.48 ± 0.19 (ab) 3.36 ± 0.47 (ab)
	hAEpC	0.121 ± 0.019 (ab) 0.104 ± 0.016 (ba)
Propranolol	Calu-3	20.27 ± 4.75 (ab)* 15.26 ± 2.40 (ba)*
	pAEpC	26.61 ± 7.93 (ab)*
	Caco-2	7.4 ± 2.5 (ab) 14.5 (ab) 13.5 ± 0.34 (ab) 41.91 ± 4.31 (ab) 43.03 ± 3.64 (ab) 29.4 ± 2 (ab) 23 (ab) 41.91 ± 4.31 (ab)
	Primary tracheal epithelial cells – Air-interfaced culture Primary tracheal epithelial cells – Liquid-covered culture	31.7 ± 8 (ab) 23.8 ±3 (ab)
Atenolol	Caco-2	0.422 (ab) 0.203 ± 0.004 (ab) 0.2 ± 0.01 (ab) 0.8 ± 0.3 (ab) 0.203 ± 0.004 (ab) 0.47 (ab)
	HT29-18C	4.2 (ab)
Talinolol	Caco-2	0.3 ± 0.01 (ab) 1.2 ± 0.1 (ba)
Metoprolol	pAEpC	18.04 ± 0.3 (ab)*
	Caco-2	10.9 ± 3.7 (ab) 7.3 ± 3.2 (ba) 26.95 ± 0.71 (ab)
	16HBE14o-	25.2 (ab)

ab: apical to basolateral transport direction.
ba: basolateral to apical transport direction
N.A.: Not available
*: own data

Table 3. Metabolism of the Selected Compounds

Compound	Localization	Enzyme	Metabolite	Comments
Hydrocortisone (cortisol)	Target cell	11β-hydroxysteroid dehydrogenase (11HSD)	Cortisol Cortisone	Interconversion cortisol-cortisone
	Liver Adipose tissue	11HSD1	Cortisol	Corticoid regeneration Glucocorticoid receptor activation amplification 11HSD1 inhibited by the growth hormone-insuline-like growth factor 1 axis
	Kidney	11HSD2	Cortisone (inert)	Cortisol inactivation
	Liver	5-α reductase 5-β reductase	Tetrahydrocortisol Tetrahydrocortisone	-
	Liver	CYP3A4	6β-hydroxycortisol	-
Testosterone	Liver Testis Prostate	5-α reductase	5-α dihydrotestosterone (active)	Androgen receptor Hormonal signal amplification
	Muscle Fat tissue	Aromatase	Estradiol	Activity on estrogen receptor
Dexamethasone	Liver	11HSD System	11-dehydro-dexamethasone (active)	-
Budesonide	Lung	Esterase	Budesonide-oleate (active)	Prolonged anti-inflammatory activity Improved airway selectivity and efficacy
	Liver	CYP3A4	6-hydroxybudesonide (inactive)	-
Betamethasone	Liver	CYP3A4	6-β-hydroxybetamethasone 11-ketobetamethasone	-
Cromolyn	-	-	Not identified	-
Formoterol	Liver	CYP2D6 CYP2C19 CYP2C9 CYP2A6	O-demethylated formoterol (inactive glucuronide conjugate) Deformlyated formoterol (inactive sulphate conjugate)	-
Imipramine	Liver	CYP2C19 CYP1A2 CYP3A4 CYP2D6 CYPD2D18	Nortriptyline (active) N-desmethylclomipramine (active) Desipramine (active)	Contribution to general pharmacological effects Serotonin transporter inhibition Norepinephrine reuptake inhibition
	Liver	Uridine-5'-diphosphoglucuronosyltransferase 1A4	Imipramine N(+)-glucuronide	-
	Brain	Flavin-containing monooxygenase	Imipramine N-oxide	Locally modulate pharmacological/toxic effects
Losartan	Liver	CYP3A4 CYP2C10 CYP2C9	EXP3174 (active)	Increased potency as antihypertensive Increased duration of action
			EXP3179	No antihypertensive properties Anti-inflammatory agent

(Table 3). Contd…..

Compound	Localization	Enzyme	Metabolite	Comments
Terbutaline	Intestine	Terbutaline sulphotransferase	Sulphate conjugate	-
Salbutamol	Intestine	Sulphotransferase 1A3	Sulphate conjugate (inactive)	Extensive metabolism leads to low bioavailability
Zopiclone	Liver	CYP3A4 CYP2C8 (*in vitro*)	N-oxidezopiclone (low activity) N-desmethylzopiclone (inactive)	-
Zaleplon	Liver	Aldehyde oxidase CYP3A	5-oxo-zaleplon (inactive) N-desethyl-5-oxo-zaleplon (inactive)	-
Zolpidem	Liver	CYP3A4	Inactive metabolites	-
Leflunomide	Intestine Liver	CYP1A2 CYP2C19	A771726 (active)	Rapid metabolism Metabolite responsible for all the drug's activity *in vivo*
Oxymetazoline	Liver Nose	-	Hydroxilated metabolites Glucuronidated metabolites Glutathione conjugates Imidazole conjugates	-
Nicotine	Liver	CYP2A6 CYP2B6 UDP-glucuronosyltransferase 2B10	Nicotine-N-glucuronide Cotinine Cotinine-N-glucuronide Trans-3'-hydroxycotinine Trans-3'-hydroxycotinine-O-glucuronide	-
Menthol	Liver	UDP-glucuronosyltransferase P450 monooxygenase system	Menthol-glucuronide Mono-, di-hydroxylated menthol	Rapid metabolism
Mannitol	Liver	P450 monooxiygenase system	-	-
Caffeine	Liver	CYP1A2 CYP2E1	More than 25 derivatives, some of which with pharmacological properties	Interindividual variability due to environmental and genetic factors
Methotrexate	Liver	Aldehyde oxidase	7-hydroxy-methotrexate	-
Vinblastine	Liver	CYP3A4	O4-deacetylvinblastine	-
	Serum	Copper oxidase (ceruloplasmin)	Inactive metabolites e.g. Catharinine	Catharinine also obtained in the presence of hydrogen peroxide through Peroxidase
Topotecan	Liver	N-demethylase UDP-glucuronosyltransferase	N-desmethyl topotecan Topotecan-O-glucuronide N-desmethyl topotecan-O-glucuronide	Low extent of metabolism
Sulfasalazine	Intestine	Bacterial azo-reductases	Sulfapyridine Aminosalicylate	Lower activity than parent compound
Digoxin	Liver	CYP3A4	Digoxigenin bis-digitoxoside Digoxigenin mono-digitoxoside Digoxigenin	Minor pathway of metabolism

(Table 3). Contd…..

Compound	Localization	Enzyme	Metabolite	Comments
	Kidney	-	Dihydrodigoxin (inactive)	Drug is mainly renally eliminated
Rhodamine123	-	-	-	Low metabolism
Fluorescein	Liver	UDP-glucuronosyltransferase	Fluorescein glucuronide	Rapid metabolism Fluorescent like parent compound
Propranolol	Liver	CYP2D6 (mainly) CYP1A2 CYP2D	4-hydroxypropranolol (Main metabolite, active) 5-hydroxypropranolol N-desisopropylpropranolol 7-hydroxypropranolol (traces)	Extensive metabolism
Atenolol	-	-	-	Low metabolism
Talinolol	Liver	CYP3A4	4-trans hydrosytalinolol	High metabolism
Metoprolol	Liver	CYP2D6	Alpha-hydroxymetoprolol O-demethylmetoprolol 4-(2-hydroxy-3-isopropylaminopropoxy)-phenylacetic acid Deaminated metoprolol	-

pulmonary models as the basis for permeability determination of the above described compounds.

There are plenty of *in vitro* models of the epithelial barriers of the upper and lower respiratory tract available. Table **4** shows a few highlights. It is not the aim of this article to do an extensive review of each of them, further information can be found in the literature [11, 20]. Below, we briefly describe the two models we have chosen to perform permeability tests for pBCS establishment. Both of them reliably mimic the *in vivo* situation in terms of cell phenotype and

Table 4. Cellular Models for the Respiratory System

Name	Species	Origin	Region	Characteristics
NHBE	Human	Primary cells	Bronchi	Mucus production Functional tight junctions Confluent layers
RPMI 2650	Human	Anaplastic squamous cell carcinoma	Nasal	Expression of tight junctions Features of nasal epithelium
Calu-3	Human	Bronchial adenocarcinoma	Bronchi	Expression of tight junctions Metabolic capacity Mucus production under AIC
16HBE14o-	Human	Transformed bronchial epithelial cells	Bronchi	Basal cell morphology Tight junctions. microvilli. cilia
Alveolar epithelial cells	Rat	Primary cells	Alveoli	Type II to type I transdifferentiation Tight epithelial barrier
Alveolar epithelial cells	Human	Primary cells	Alveoli	Type II to type I transdifferentiation Tight epithelial barrier
Alveolar epithelial cells	Pig	Primary cells	Alveoli	Type II to type I transdifferentiation Tight epithelial barrier

display the typical characteristics of an epithelial barrier, as found in the different parts of the respiratory system.

Calu-3

This human sub-bronchial cell line derived from a 25-year-old Caucasian male adenocarcinoma is used as an upper airway (bronchial) model. After screening for airway secretory proteins and mRNA, it was found to be the only out of 12 cell lines derived from human lung cancers with mRNA and protein contents characteristic of native epithelium. Cells feature epithelial morphology, adherent polarized monolayer growth, and tight junctions under liquid-covered conditions. Additionally, they secrete mucus if cultured under air-interfaced conditions. They express a plethora of efflux proteins which will probably have a high impact on drug absorption such as P-gp, MRP1, 2, 3 and 5, Novel Organic Cation Transporter and also LRP, Peptide Transporter 1, and Cystic Fibrosis Transmembrane Conductance Regulator. The presence of the latter has promoted the use of Calu-3 cells as a model for cystic fibrosis. Tightness of the monolayers is mediated by the intercellular tight junctions, as shown by staining of the ZO-1 protein, a characteristic component of these structures. Cells build desmosomes and zonulae adherents (cell-cell adhesion structures), as detected by immunostaining of their constituent proteins desmoplakin and E-cadherin. The ability of this cell line to form tight junctions and polarized monolayers is critical to their use as an *in vitro* drug absorption model. Indeed, these cells are able to discriminate compounds of different apparent permeabilities (P_{app}), as shown in Fig. (**3**) (P_{app}values of marker molecules are shown). P-gp activity is demonstrated with the help of a P-gp substrate [21-27].

Porcine Alveolar Epithelial Primary Cells

While several cell lines exist that reproduce the phenotype found in the upper respiratory tract, this is not the case for the deep lung. Cell lines such as A549 cells that are frequently used as an alveloar human model in toxicity tests fail to create a tight monolayer and are thus not suitable for permeability studies. Therefore, primary cells are often used in transport models for drug permeability studies. Published data have been obtained mainly with cells of human or rat origin. To overcome ethical problems in the first case and low yield in the second case, we have developed a new model based on porcine cells. The pig closely resembles man in its anatomy and physiology as well as in histological and biochemical aspects. Porcine lung primary cells have been previously used for the study of inflammatory airway disease, of the properties of lung surfactant preparations, or *Actinobacillus pleuropneumoniae* pathogenesis. In our model, cells are able to form a tight epithelial barrier and exhibit a mixture of type I and type II alveolar cell phenotype. As shown in Fig. (**4**), they are able in discriminating compounds of different permeabilities in transport studies, but do not show P-gp activity. This observation is supported by other studies that demonstrated the presence and function of P-gp in bronchial epithelium and in alveolar type I cells, but not in alveolar type II cells [12, 13, 28-33]. It should be noted that the role of P-gp in the lung is still an important matter of discussion in the scientific community. Another controversial point is how to standardize primary cellular models. These models can be highly variable, for instance due to starting material coming from different individuals. Although this is an inherent problem of such models, it should be noted that the models suggested in this review have shown a high degree of reproducibility considering the isolation process as well as in permeability studies as shown in Fig. (**4**).

CONCLUSIONS

Permeability studies performed in the above described *in vitro* cellular systems and with the list of compounds listed

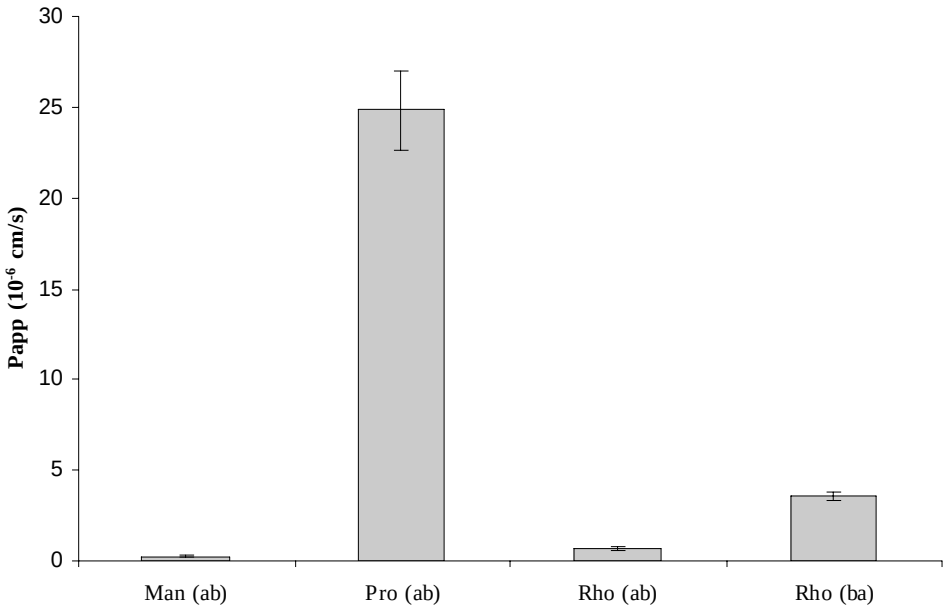

Fig. (3). Apparent permeability values of marker compounds across Calu-3 cell monolayers. Mannitol (Man), low permeability marker; Propranolol (Pro), high permeability marker; Rhodamin 123 (Rho), P-gp activity marker; ab=apical to basolateral transport direction; ba=basolateral to apical transport direction. Data shown are mean values ± SD (n=3).

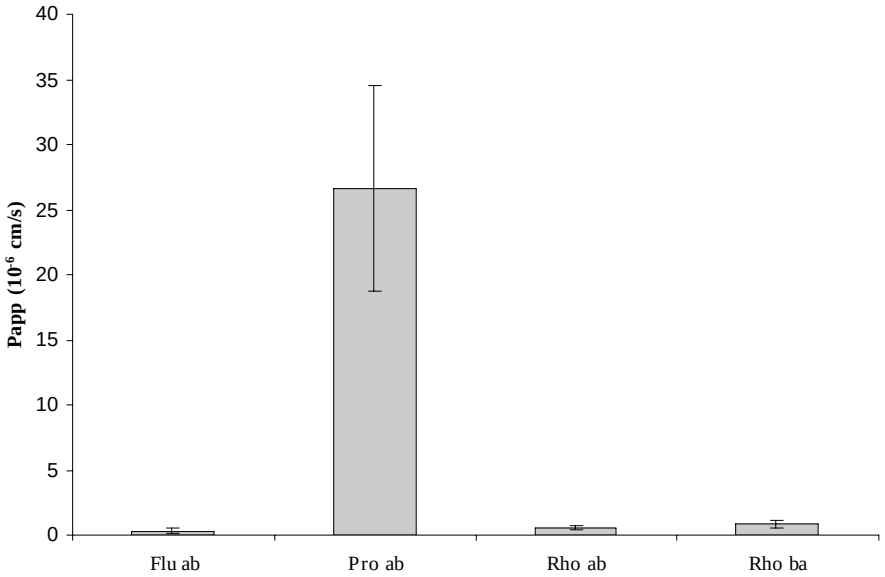

Fig. (4). Apparent permeability values of marker compounds across pAEpC monolayers. Fluorescein (Flu), low permeability marker; Propranolol (Pro), high permeability marker; Rhodamin 123 (Rho), P-gp activity marker; ab=apical to basolateral transport direction; ba=basolateral to apical transport direction. Data shown are mean values ± SD (n=3).

in this review will certainly provide valuable data for further predictions of candidate drugs. Considering solubility, metabolic and efflux transport data, these results should be the basis for the proposed pulmonary Biopharmaceutical Classification System. Certainly, further complementation with dose-dependence and linearity data, and comparison with *in vivo* results will improve our knowledge about the drug absorption process in the lung, which is, without doubt, a drug delivery route to be considered.

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ABBREVIATIONS

API	=	Active Pharmaceutical Ingredient
BCRP	=	Breast Cancer Resistance Protein
BCS	=	Biopharmaceutical Classification System
BDDCS	=	Biopharmaceutics Drug Disposition Classification System
LRP	=	Lung Resistance Protein
MDR	=	Multidrug Resistance
MRP	=	Multidrug Resistance-associated Protein
P_{app}	=	apparent permeability
pBCS	=	pulmonary Biopharmaceutical Classification System
PCS	=	Permeability-Based Classification System
P-gp	=	P-glycoprotein

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