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EDITORIAL

Airway liquid: a barrier to drug diffusion?

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The paper by HOHLFELD *et al.* [1] in this issue shows that, in rats, bronchoconstriction due to inhaled acetylcholine is inhibited by inhaled aerosols of surfactant. Bronchoconstriction was assessed from changes in total lung resistance; other parameters of airway calibre were not affected. The authors conclude that this inhibition is due to the surfactant setting up a hydrophobic barrier on the surface of the airway liquid that is relatively impermeable to hydrophilic substances such as acetylcholine. The results are important. Not only do they point to possible therapeutic approaches, for example to prevent allergen entry in asthma and even allergic rhinitis, but they also add to our understanding of the way in which inhaled drugs enter the airway mucosa.

Before any inhaled substance can enter the tissues, it must first dissolve in the airway surface liquid (ASL) [2, 3]. This applies to drugs used to treat diseases such as asthma, to agents used to test bronchial responsiveness, to toxic agents such as pollutants, and even to "soluble" particles such as liposomes. The volume and chemical nature of the ASL is crucial in three respects:

1) If the inhaled agent binds to the mucins in the ASL then it may be removed by mucociliary clearance and thus be rendered relatively ineffective. The degree of binding would depend on the quantities of agents and mucins, and on their mutual binding affinities (tightness of binding) and capacities (quantity that can be bound) [4]. Once bound, the agent would be rendered unavailable until it is slowly released, perhaps higher up the respiratory tract. We know almost nothing about the binding properties of airway mucins to the agents used to test and treat disease, and this process is usually ignored or assumed to be insignificant. Airway mucins bind some antibiotics and also diethylenetriamine pentaacetic acid (DTPA) [4], the last with high affinity but small capacity [5]. Since mucins are negatively charged, binding would be greater for positively charged chemicals. If binding occurs it will reduce and delay the effectiveness of a drug.

2) The ASL will constitute a diffusion barrier for inhaled agents. The strength of the barrier will depend on its thickness and physical and chemical nature, and on the properties of the diffusing agent. For example, if the barrier has a hydrophobic layer (see below), penetration by a hydrophilic chemical will be hampered. Most drugs used in treatment and testing of airways' diseases are hydrophilic. However, steroids, for example, are lipophilic. There have been few measurements of diffusion through layers of respiratory mucus [5], but several with gastro-intestinal mucus [4, 6–8]. These indicate that, if the ASL layer is thin, the diffusion barrier should cause only an insignificant delay to drug entry into the airway mucosa. Note that the diffusion barrier will slow down entry of an agent into the tissues but, unlike binding, will not reduce it.

To take an example, if the ASL thickness is 50 μm (see below), and its properties as a diffusion barrier are similar to those of intestinal mucus, the permeability coefficient for the lipophilic agent antipyrine would be about -9×10^{-4} cm·s^{-1} and for the hydrophilic agent phenylalanine about -8×10^{-4} cm·s^{-1} [6]. Permeability coefficients through saline buffer are approximately 2–2.5 times larger (table 1). Thus the diffusion barrier through ASL compared with saline could be appreciable. Similar figures for gastro-intestinal mucus have been reported [7, 8]. If the ASL was thinner than 50 μm, the permeability coefficient would be proportionally greater. These values should be compared with those for the tracheal epithelium, which are given in table 1 [6, 9–11]. Although these figures may not be strictly comparable, due to differences in methodology and agents, they do suggest that for a lipophilic agent a 50 μm layer of mucus is 3–40 times less of a barrier than the epithelium, and for a hydrophilic agent the difference is 3,600–5,000 fold. Unlike a mucus sheet, the epithelium is far more permeable to lipophilic than to hydrophilic compounds. Damage to the epithelium, as may occur in asthma [12], greatly increases the permeability to hydrophilic, but not to lipophilic agents.

3) The volume, and therefore the thickness, of the ASL will determine the rate of entry of a drug into the tissues [2, 3]. For any given quantity (Q) of an aerosolized drug, its concentration (C) will vary with ASL volume (*V*) and, since the airway surface area (S) is fairly

Table 1. – Permeability coefficients for different agents and preparations

Preparation	Permeability coefficient $\times10^{-7}$ cm·s^{-1}	
	Lipophilic	Hydrophilic
Saline (50 μm)	AP: 22000	PA: 18000
Rat intestinal mucus (50 μm)	AP: 8800	PA: 8200
Human sputum (50 μm)*		DTPA: 19300
Sheep trachea *in vivo*	AP: 3300	DTPA: 3
Epithelium damaged	AP: 2600	DTPA: 90
Ferret trachea *in vitro*	AP: 220	DTPA: 5
Epithelium damaged	AP: 220	DTPA: 35

AP: antipyrine; PA: phenylalanine; DTPA: diethylenetriamine penta-acetic acid. *: processed from chronic bronchitis (from [6, 9, 10]; representative values selected from a much larger literature reviewed in [4, 11]).

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constant, with ASL thickness (T). This is represented by the equation:

$$C = Q/(S \times T) \quad (1)$$

The concentration of the drug determines its flux or rate of entry (dQ/dt) into the tissues from the equation:

$$dQ/dt = -P \times \Delta C \times S \quad (2)$$

where P is the permeability coefficient for the drug (an index of the velocity of penetration), ΔC is the concentration gradient, and the negative sign is directional (lumen to submucosa). Combining the two equations we get:

$$dQ/dt = -(P \times Q)/T \quad (3)$$

This assumes that $\Delta C=C$, which can be justified [2, 3]. This leads to the perhaps surprising conclusion that the rate of entry of a drug into the airway mucosa depends only on the load of drug, the permeability coefficient for drug and tissues (which may contain a component for mucus permeability) and, inversely, with the thickness of the ASL. Surface area is irrelevant because, if it is large, the concentration and flux of the drug per unit area will be proportionately smaller.

It is, therefore, most regrettable that we know so little about the binding properties of drugs to mucus in ASL, their diffusion characteristics through it, and even its volume and thickness. All these variables will influence the time course and effectiveness of inhaled drugs and other agents.

With regard to thickness, ASL is usually considered to be a combination of a periciliary layer or sol, and a surface layer or gel. It is often assumed that these have different mucin contents (and therefore presumably different binding and diffusion characteristics). These differences have never been quantified, and the assumption depends on differences in opacity under the electron microscope (EM) [13, 14] and, more recently, on differences in structural appearances on freezing [15]. All authors agree that there is a periciliary liquid layer, as thick as the length of the cilia (5–10 μm). Should this tend to dry out it would be maintained by capillary forces acting between the cilia [16].

For some decades there have been arguments about the thickness, or even the existence, of a gel layer, and whether it is continuous, in plaques or islands, or even absent [17]. Recent studies aggravate rather than resolve the dispute. Of sixteen studies on large airways in various species [13–15, 18–29], values from 0–250 μm were obtained (table 2). There are no values for humans. Thus, the total ASL thickness, sol plus gel, could be as small as 5 μm or as great as 260 μm. This is a 50 fold difference, and it follows that the amount of mucin available for binding, the thickness of the diffusion barrier, and the ASL volume and, therefore, the concentration gradient and flux of an inhaled drug, could lie somewhere within this 50 fold range. In one study of healthy dogs, ASL thickness, albeit assessed by an indirect and invasive method, varied from about 5–180 μm in different dogs [24]. Possible causes of this enormous variation, and the factors that may change and determine ASL thickness, have been discussed [30]. For example, in some of the EM preparations it would take only 1–2 min for the gel to be cleared from the mucosa by mucociliary transport. On the other hand, maximal mucus secretion from glands can increase ASL thickness by about 10 $\mu m \cdot min^{-1}$ [30]. It is also worth noting that almost all the EM studies showed a very thin layer of gel, whereas those using physiological methods usually showed a much thicker layer.

Table 2. – Thickness of airway surface liquid (ASL)

Species	Method	Gel Thickness μm	Reference
Hamster	EM	0	[18]
Monkey	EM	0.1–1	[19]
Cow	EM	0.5–2	[20]
Guinea-pig	EM	~1	[21]
Guinea-pig	EM	~1	[22]
Rat	EM	~1	[22]
Cow	EM	1–2	[23]
Rat	EM	5	[13]
Rat	EM	5–10	[14]
Dog	Volume	5–180	[24]
Cow	EM	20	[15]
Sheep	Probe	30	[25]
Ferret	Tracer	50	[26]
Rabbit	Tracer	40	[26]
Guinea-pig	Tracer	60	*
Guinea-pig	Probe	100–200	[27, 28]
Guinea-pig	Probe	250	[29]

The thickness of the sol would add 5–10 μm to the total ASL thickness. EM: electron microscope. *: S. Duneclift, U.M. Wells and J.G. Widdicombe, unpublished results.

The concept that ASL thickness may determine rate of drug entry and effectiveness is not purely theoretical, but is supported by a number of observations. In sheep, instillation of mucus simulant decreases airways responsiveness to carbachol [31]. Chronic exposure of dogs to sulphur dioxide causes mucus hypersecretion and lessens their bronchial responsiveness to histamine, prostaglandin $F_{2\alpha}$ and methacholine [32–34]. Dogs exposed to cigarette smoke have hypersecretion and reduced bronchial responsiveness to methacholine [24]. In healthy dogs, the effectiveness of methacholine given as an aerosol (compared with the *i.v.* route) in increasing airways resistance varied directly with mucus thickness over a wide range [24]. Mucus hypersecretion, as in chronic bronchitis, may also change the distribution of an aerosol inhaled into the lungs, and this could affect the rate of drug uptake [35–39].

The paper by HOHLFELD *et al.* [1] adds a new factor to be considered. An aerosol of surfactant lessened the bronchoconstriction due to aerosolized acetylcholine. The fact that a similar effect was not seen with *i.v.* acetylcholine strengthens the conclusion that the surfactant is acting as a barrier. If the surfactant were to increase the ASL thickness, for example by stimulation of mucus secretion, or if it could bind or inactivate acetylcholine, this might explain the results, but there is no reason to believe that this occurred. It has been known for a long time that surfactant is present in the airway mucus [38]. It can be secreted by submucosal glands and by the epithelium [39–41]. Early studies indicated that the surfactant lay between the sol and the gel [38], and it was suggested that it might lubricate the movement of the

gel over the cilia. Recent research has indicated a continuous sheet of surfactant on the air surface of the gel [18, 42–44], which can be displaced towards the lumen by inflammatory exudate [43, 44]. It is not known if secretions from submucosal glands that make or thicken the gel also displace a surfactant film to the luminal surface. These studies have been with rodents, which usually have a poor supply of submucosal glands, and their extension to other species is highly desirable [42]. If this surfactant sheet normally exists, then an added aerosol of surfactant might thicken the sheet or plug any holes in it, and make it less permeable to hydrophilic (but not lipophilic) substances such as acetylcholine. It might also make it less permeable to allergens, which raises the interesting possibility that a surfactant aerosol might protect against allergen-induced asthma. Given into the nose it might also have a prophylactic action in allergic rhinitis. Surfactant aerosols are now beginning to have a far wider role in the treatment of respiratory diseases than just as a replacement therapy in infant and adult respiratory distress syndromes.

As HOHLFELD *et al.* [1] point out, we need quantitative measurements of the permeability coefficients of drugs before and after administration of surfactant aerosols. We need to know whether surfactant changes airway surface lining composition and thickness, and whether it has a chemical action on acetylcholine. We need to know whether the results with acetylcholine also apply to other drugs, including lipophilic ones, and to macromolecules such as allergens. A merit of this paper is that it stimulates thought and should also stimulate research.

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