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MINIREVIEWS

Pulmonary Disposition of Antimicrobial Agents: Methodological Considerations

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INTRODUCTION

Early studies of the penetration of antimicrobial agents into the sites of infection within the respiratory tract have been criticized for methodological considerations, and the clinical relevance of these measurements has been questioned. Sampling of the respiratory tract by fiberoptic bronchoscopy with bronchial biopsy and bronchoalveolar lavage (BAL) has provided the opportunity to investigate the distribution of antimicrobial agents in additional potential new sites of infection, but again, significant methodological problems exist and the clinical relevance of such measurements must be evaluated. In addition, the nature of these sites of infection is poorly understood, and this can lead to difficulty in interpreting the results that are obtained.

The aims of this minireview are to clarify what is known about the nature of the potential sites of pulmonary infection and to delineate the methodological problems which must be addressed if antimicrobial agent concentrations are to be measured within the sites.

The potential sites of pulmonary infection. Before a potential site of infection can be defined, it is necessary to establish the location of pathogens within a tissue at the various stages of respiratory infection. The pathogens involved in infective exacerbations of chronic bronchitis, acute bronchitis, and bronchiectasis are principally found in two sites, the sputum and bronchial mucosa (7, 24, 47). Figure 1 shows a schematic representation of ciliated respiratory epithelium and depicts the three stages of bronchial infection. In stage 1 (colonization), the pathogens are present only in the sputum or mucous layer, and instead of invading the mucosa, they release toxic products which may be responsible for damage to the mucosa. These toxic products include phenazine derivatives (*Pseudomonas* spp.), pneumolysin (*Streptococcus pneumoniae*), and hyaluronidase (16, 54, 64, 65). In addition, the recruitment of neutrophils by the host may lead to damage by neutrophil-derived factors such as proteinases and superoxides (50, 57). Pathogens which are located solely in bronchial secretions may persist relatively unharmed because they distance themselves from the host defense reactions (45). Stage 2 (adherence) may occur as an initial event or after there has been damage to intact mucosa, which not only prevents clearance of bacteria by cilia (54, 64, 65) but also serves to expose receptor molecules on the mucosal surface (27, 44). Finally, the bronchial mucosal epithelium may be invaded (stage 3); macroscopically, this appears as denudation of the epithelium (Fig. 1). In bronchial mucosal infections, it is likely that all of these stages are represented simultaneously with adjacent areas of normal tissue.

From this model of the stages of bronchial infection, it is reasonable to propose both sputum and bronchial mucosa as sites of infection. Since the organisms involved in a chronic bronchial infection are more often located within the lumen, levels of antimicrobial agents in sputum may be the best predictor of efficacy.

The nature of the infective process in pneumonia has been less well studied, but there is evidence that pathogens are located within the bronchiolar lumen (52) and may lead to tissue damage in a fashion similar to that postulated for bronchitis. This may then be followed by airway obstruction with distal collapse and inflammatory cell infiltration, leading to the established histological findings in a lobar pneumonia. Therefore, epithelial lining fluid (ELF) (see below) is considered to be an important site of infection in pneumonia, and the alveolar macrophage (AM) represents an important site for intracellular infection. In established pneumonia, the area of consolidation bears little resemblance to the ELF, but ELF is a useful model for adjacent areas which may be important for containment of infecting agents.

A plethora of data on antimicrobial agent concentrations in bronchial secretions, sputum, and whole lung tissue (9, 11, 32, 35) exists, and more recently, bronchial mucosal biopsy specimens have been studied. The recent application of BAL has provided the opportunity to obtain samples from two further sites of infection, ELF and AMs. A useful definition of ELF sampled by BAL is that fluid which lines the small airways distal to the point of the wedge of the tip of the bronchoscope and which is recovered by BAL. In order to interpret published data on the concentrations of antimicrobial agents at these sites, it is necessary to understand both the methodological difficulties with the measurement of antimicrobial agent concentrations at the sites of infection and the anatomical and physiological natures of the sites.

The nature of potential sites of infection. An important aspect of the nature of sites of infection is the permeability of the barriers to antimicrobial agent movement which demarcate the sites. For sputum and bronchial secretions, the barrier consists of the relatively permeable bronchial capillary endothelium and the relatively impermeable ciliated respiratory epithelium, whose constituent cells are tightly bound to one another by numerous zonulae occludens. Consequently, sputum and bronchial secretions are discrete from serum, and they not only have compositions different from that of serum but are also likely to have concentrations of antimicrobial agents very different from those found in serum.

A bronchial mucosal biopsy specimen consists of at least

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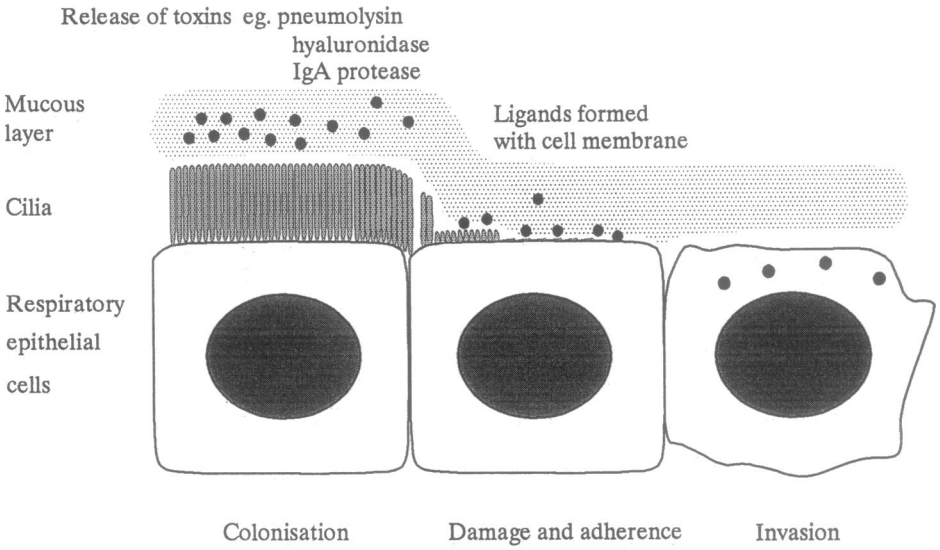

FIG. 1. Proposed stages of infection of the bronchial mucosa. IgA, immunoglobulin A.

two separate compartments: the submucosa, which is composed of mostly acellular material and which is separated from the blood only by the capillary endothelium, and the epithelium, in which the cell membrane of the epithelial cells is an additional barrier. In theory, the interstitial fluid has antimicrobial agent concentrations similar to those in serum for most agents, because the capillary endothelium is relatively permeable, but the concentration in the sample as a whole depends on the ability of the drug to penetrate and concentrate within cells. For whole lung tissue, such as that obtained at thoracotomy, there is an even more confusing situation because more types of tissues are sampled.

In previous reviews (7, 47, 60), the nature of the barriers separating the sputum from the blood has been recognized but the discreet nature of the alveolar lining has not been considered in recent reviews (61). The nature of this important site of infection is therefore described here. Figure 2 shows a schematic representation of the alveolus and the alveolar-capillary membrane. As with bronchial interstitial fluid, the main barrier separating the pulmonary interstitium from the blood is the capillary endothelium, which is nonfenestrated (62). This provides a more significant barrier to antimicrobial agent movement than do the fenestrated capillaries of other organs. These sites are separated from the blood by the alveolar membrane, which is relatively impermeable. The evidence for this is convincing. First, ultrastructural studies of the alveolus by electron microscopy have shown that the epithelial cells are tightly apposed by numerous zonulae occludens (30, 53, 63), whereas in the endothelial cells these are poorly developed. Second, physiological study of the alveolar epithelial membrane has shown permeability characteristics similar to those of an intact cell membrane (14, 15, 59).

Therefore, the ELF is separated from the blood by a very significant barrier, ensuring the production of a microenvironment worthy of study. AMs reside in this microenviron-

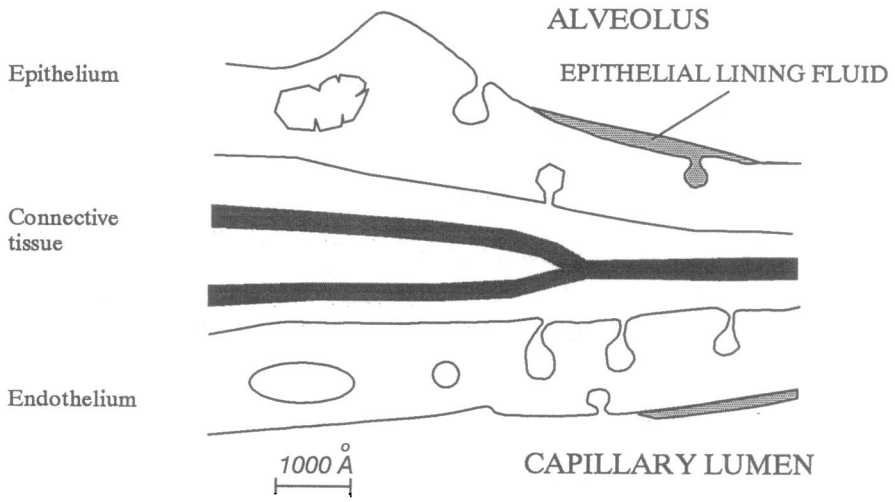

FIG. 2. Detail of the alveolar capillary barrier. The barrier consists of three layers, of which the epithelium constitutes the least permeable because of the presence of numerous zonulae occludens. Epithelial lining fluid lies in pools on the inside surface of the alveolus. 1,000 Å = 100 nm.

ment, and in addition, a further cell membrane must be penetrated in order for antimicrobial drugs to reach the intracellular environment which may be representative of the site of infection for intracellular pulmonary pathogens.

The influence of surface area compared with the volume of the site. For some potential sites of infection, the penetration of antimicrobial agents may be influenced by the ratio of the surface area of the site exposed to concentrations of the drug found in serum relative to the volume of the site of infection (SA/V) as well as the permeability of the barrier demarcating the site. A low SA/V reduces the velocity of penetration, for example, in bone (6). In the sites within the lung, the sites have high SA/Vs, with the exception of pleural effusions and possibly sputum in cases of bronchiectasis, because of the larger air spaces and larger volume of sputum. Thus, SA/V in the lung is unlikely to significantly affect penetration in the majority of cases.

METHODOLOGICAL PROBLEMS

Sputum. Clinical correlates of concentrations of antimicrobial agents in sputum with bacterial eradication were first suggested as early as 1955 by May (42). This small but important study showed that a preparation of penicillin which gave higher concentrations of drug in sputum than those given by other preparations had the greatest efficacy. Since then, other studies have verified this finding and other studies have also shown poor clinical results when subtherapeutic levels of drug are found in bronchial secretions (26, 37, 43, 56). Levels in bronchial secretions differ markedly from concentrations in serum and are higher for those drugs with favorable permeability characteristics. The absolute concentrations of antimicrobial agents in sputum show considerable variability even when the same classes of antimicrobial agents are compared. This is especially true for β-lactams (7, 13, 47). This variability is due at least in part to the well-documented problems with the collection and analysis of sputum. Sputum collected into a sputum pot may be contaminated with saliva and blood. The concentration of antibiotics in saliva may differ markedly from those in sputum. For example, penicillin, ampicillin, and cephalexin all achieve lower levels in saliva than in sputum (19, 20, 26, 37, 43, 55, 56). An alternative to sputum sampling is to collect bronchial secretions by direct aspiration via an endotracheal tube, tracheostomy, or fiberoptic bronchoscope. However, in practice there may still be contamination derived from the suction channel of the bronchoscope or by trauma to the airways, and therefore, careful attention to technique is required.

A significant difficulty arises because bronchial secretions aspirated at bronchoscopy may have been accumulating for some hours (7, 47). This sputum pooling invalidates measurements of antimicrobial agent concentrations with respect to the time of dosing and allows time for the degradation of antimicrobial agents, e.g., by β-lactamases (58). An illustration of the instability of a drug in sputum is given by clavulanic acid. This β-lactamase inhibitor is known to be unstable at physiological temperature and pH. It has been shown that marked differences in the levels of this drug are found when sputum samples are frozen at −20°C immediately after collection as opposed to being stored for 2 h at room temperature. Thus, it is likely that clavulanate activity decreases markedly from the time of sputum production to the time of sampling (18). Concentrations of drugs in sputum may therefore be unreliable as an indicator of clinical efficacy on methodological grounds.

Bronchial biopsy specimens. The methodology for the measurement of total concentrations of drug in bronchial biopsy specimens is now well established and is reliable (25, 39, 40). However, the major criticism is that the most relevant information is the relative amount of drug in the interstitial fluid and cellular fluid (49). This information is not provided by current measurements. The relative proportion of drug in the intracellular and extracellular components is important, because a major determinant of the total drug concentration in a tissue is the degree of intracellular penetration and accumulation. Thus, the fluoroquinolones and macrolides, which accumulate in the intracellular environment, have higher concentrations in bronchial biopsy specimens than in serum, whereas β-lactams which do not penetrate cell membranes to any significant degree have lower concentrations in bronchial biopsy specimens than in serum (25, 39–41). Despite these facts, there are few published data on the constituents of bronchial biopsy specimens in terms of tissue types, and there are no data on the relative amounts of intra- and extracellular water.

ELF. There are two main sources of error attributable to the methodologies that are used: the determination of ELF volume recovered by BAL, which is required to quantify the antimicrobial agent concentrations in ELF, and the BAL procedure that is used. The latter is a relatively well researched problem, and two large reviews of BAL procedures have recommended a standard BAL technique in order to ensure that the results obtained by different centers can be compared (1, 33). In this procedure, 200 to 240 ml of warm isotonic saline is infused in four aliquots into the right middle lobe or lingula. The aspirate from the first 50-ml aliquot contains mainly medium-sized airway fluid (12, 31, 36, 51) and is therefore discarded or processed separately. The remaining three aliquots contain ELF and are pooled for analysis. The use of too little fluid (8) means that, at best, medium-sized airway fluid which is different from the ELF recovered by conventional lavage is sampled (51).

Problems with the accurate quantification of ELF volume mean that previous workers' measurement of antimicrobial agent concentrations in ELF have been unreliable (48). The method used employs urea as an endogenous marker of ELF recovered by BAL and assumes that since urea is a small, relatively nonpolar molecule which travels across membranes freely, it therefore exists at the same concentration in ELF as it does in the blood. The ELF marker concentration is therefore known, hence: volume of ELF = (volume of BAL × [urea] BAL)/[urea] ELF, where [urea] is the concentration of urea.

Several workers have since advocated the use of this method (10, 29, 46), but it has been show that during the prolonged lavage fluid dwell times which are required to sample distal ELF, urea diffuses from the interstitium and blood and therefore falsely elevates the urea concentration in the BAL fluid. The result is an overestimate of ELF volume of approximately 100 to 300% (38, 48) after a 1-min dwell time. To circumvent this problem, a rapid peripheral lavage technique (microlavage) has been described (4). By this method, a 1.7-mm-diameter bronchial brush catheter is used to lavage a distal subsegment rapidly; however, blood contamination causes problems with this technique in some cases. The urea method can therefore be applied and used to calculate the total protein content of the ELF. Protein diffuses sufficiently slowly from the interstitium during conventional BAL and is therefore a reliable marker of ELF. Thus, by using a modified lavage technique to calculate the total protein concentration in ELF, it is possible to use this

as an endogenous marker of ELF in the conventional BAL aspirate as follows: for microlavage, [protein] in ELF/[protein] in lavage = [urea] in ELF/[urea] in lavage; therefore, [protein] in ELF = ([protein] in lavage × volume of lavage)/[urea] of lavage; for conventional lavage (BAL), volume in ELF = ([protein] in lavage × volume of lavage)/[protein] in ELF, where brackets denote concentrations. Unfortunately, there are few published data that were derived by using a method such as this one (2, 3), but it is important that all future studies address this problem and also that the recommended standardized BAL procedures are used to ensure that distal ELF is sampled (1, 33).

AM. A theoretical problem with the measurement of antimicrobial agent concentrations in AMs by using BAL is the loss of AM-associated antimicrobial agents during the lavage procedure because of efflux of the drugs from the AMs, even though cells are always separated immediately by centrifugation. This problem is likely to occur because the saline used to perform BAL effectively washes the AMs. From in vitro studies of antimicrobial agent efflux from neutrophils and AMs, it is apparent that much of the intracellular drug may be lost within 5 min of resuspension of a cell culture in antibiotic-free medium (21, 22, 34). Prior to measuring antimicrobial agent concentrations in AMs, the in vitro efflux characteristics must be examined, and it must be ensured that significant efflux does not occur during the BAL procedure. This important validation step has not been taken for most studies (10, 23, 46). Only two of the published studies of antimicrobial agent concentrations in human AMs in vivo have addressed this problem, and these studies examined azithromycin, which has been shown to efflux from phagocytes very slowly (5, 17), so that significant efflux during BAL is unlikely. Efflux of temafloxacin was shown to be very slow in vivo, a finding which is as yet unexplained (5).

The accurate determination of cell volume is another significant source of error. Perhaps the best method is the use of velocity gradient centrifugation (28), but not all groups have used this method. A further criticism of the use of AMs as a model for intracellular infection is that in bacterial infections, the neutrophil is the predominant cell type. However, from results of in vitro studies, it is likely that AMs concentrate antimicrobial agents in a fashion broadly similar to that of neutrophils, and therefore, the use of AMs is a valid model for the intracellular site of infection.

CONCLUSION

The potential sites of pulmonary infection which have been studied are pertinent to the mechanism of the infection process in the lung, and this is particularly true for the newly examined sites of infection, which may be studied by BAL. In spite of this, methodological problems raise major doubts about the accuracy of these measurements, and in the case of sputum, many investigators have recognized that the values obtained are unreliable even when secretions are sampled directly via the fiberoptic bronchoscope. Measurement of tissue drug concentrations may be misleading because of the uneven distribution of antimicrobial agents within the tissue. Bronchial mucosal biopsy specimens contain less variable tissue types than those found in whole lung specimens and are therefore preferable. The ELF and the AM, which represent pure extracellular and pure intracellular sites, respectively, are particulary relevant not only because they are "pure" but also because of the special nature of the barriers to antimicrobial agent movement which delineate them. It is most important that the sources of error should be circumvented as outlined above, in particular, the use of a standardized lavage procedure, the attention to accurate quantification of ELF volume, and allowance for the efflux of antimicrobial agents from AMs during BAL.

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