Animal models of pneumonia for evaluation of antimicrobial therapy

Morbidity and mortality associated with pneumonia result not only from sepsis itself but from life-threatening reductions in pulmonary gas exchange. Thus, potentially reversible infections, when located in lung tissues, may not be amenable to therapy by virtue of the unique pathophysiology associated with pneumonia. Standing in opposition to this particular organ vulnerability during infection is a complex array of pulmonary host defences, perhaps the most elaborate of any human organ (Reynolds, 1983). In many instances, the local pulmonary defence system, as well as local lung tissue response to virulence factors, work independently of systemic responses to infection. It is thus clear that useful information regarding pathogenesis, treatment, or prevention of pneumonia must be collected by studies of lung infection. Extrapolation from data relating to other organs to reach conclusions regarding the lung is simply not reliable. Furthermore, to base conclusions regarding treatment of pneumonia upon in-vitro data is particularly risky, due to the well-known variability of antibiotic penetration into the lungs and respiratory tract (Pennington, 1981).

With these facts in mind, it is not surprising that animal models of experimental pneumonia have been used for many years. The reader is referred to the discussion by Nungester & Jourdonais (1936) for a review of models used before 1936. Early studies with pneumonia models were designed to investigate the pathogenesis of pneumococcal pneumonia, and also to evaluate efficacy of pneumococcal antisera. A consistent problem was the inability to create a reproducible model of fatal pneumococcal pneumonia. Various methods (chemical irritants to the bronchus, mechanical obstructions in the airways) were used to increase the virulence of pneumococcal challenge, but the most practical manoeuvre was that described in 1936 by Nungester & Jourdonais. When pneumococci were suspended in 5% hog gastric mucin, bacterial virulence was increased and fatal pneumonia was produced routinely in rats infected by direct intrabronchial instillations. In 1941, Wood (1941) published the first of his classic series of articles dealing with pathogenesis of pneumococcal pneumonia in rats. Wood used the Nungester-Jourdonais model, and found that by using pneumococci in an earlier growth phase (6h cultures) their virulence in lungs was further enhanced. Wood and co-workers then went on to report what were the earliest experimental studies describing efficacy of antimicrobial agents for pneumonia, beginning with sulphonamides in 1946 (Wood & Irons, 1946) and later penicillin (Smith & Wood, 1956). Thus began the modern era, in which animal models of pneumonia have played an increasingly important role in our assessment of antimicrobial agents.

In recent years, emphasis has been on evaluation of antimicrobial agents with activity against Gram-negative bacilli (Dale et al., 1976; Nishi & Tsuchiya, 1980; Bakker-Woudenberg, van den Berg & Michel, 1982). Also, data regarding treatment of newly recognized pulmonary pathogens, such as Legionella sp., have recently been provided (Fraser et al., 1978; Edelstein, Calarco & Yasui, 1984). Among contemporary pulmonary bacterial pathogens, none are associated with higher mortality than Pseudomonas aeruginosa. Accordingly, we have used a guinea pig model of acute, haemorrhagic bacteraemic pseudomonas pneumonia to evaluate a number of antimicrobial regimens for treating this infection. To illustrate how a specific animal model of pneumonia may be used, the following is a brief description of the guinea pig model, and of several questions addressed in its use.

In contrast to pneumococci, Ps. aeruginosa used in high enough inocula (generally 10^6 cfu) will induce a fatal pneumonia in immunologically competent small animals. The artefact associated with hog gastric mucin suspensions may thus be avoided. Lighly anaesthetized guinea pigs may be intubated, or the trachea entered via a small mid-line incision, and a bolus of pseudomonas in saline instilled directly into the lower respiratory tract with a needle.
and syringe. By three hours after lung inoculation, bacteraemia is present, and by 12 h, intrapulmonary quantities of bacteria will have increased by one log. Deaths occur in untreated animals between 12 and 72 h after infection. Lungs are uniformly haemorrhagic and tissue examination reveals typical lesions associated with pseudomonas pneumonia (Pennington & Stone, 1979). While the tempo of this pneumonia is more rapid than that of most natural human infections, this model does provide a reproducible fatal pseudomonas pneumonia in a non-neutropenic host. Furthermore, the impact of antimicrobial therapy on survival, intrapulmonary killing of bacteria, and incidence of bacteraemia may be evaluated in this experimental system. It must be emphasized that pseudomonas pneumonia does not occur in the normal human host; thus, pathogenic information obtained in this model does not necessarily mimic the sequence of events during infection in compromised humans. However, this model is quite useful for examination of the effects of alterations in host (e.g., neutropenia versus normal), organism (e.g., exotoxin A producer or non-producer), or treatment (e.g., various antimicrobial agents alone or in combination) on the pathogenesis and outcome in pseudomonas pneumonia.

To date, several therapeutic questions have been addressed in this model (Pennington & Stone, 1979; Pennington & Johnson, 1982; Pennington, Johnson & Platt, 1982; Schiff & Pennington, 1984; Schiff, Small & Pennington, 1984; Pennington, in press). These questions include:

(1) Do newer β-lactams with improved in-vitro activity against Ps. aeruginosa perform more efficiently than less active β-lactams in treating pneumonia? In comparing ticarcillin (MICs 16 and 32 mg/l for challenge strains), to piperacillin (MICs 4 and 4 mg/l), azlocillin (MICs 8 and 8 mg/l), latamoxef (moxalactam) (MICs 8 and 16 mg/l), cefotaxime (MICs 4 and 8 mg/l), ceftriaxone (MICs 1 and 2 mg/l), and ceftazidime (MICs 1-5 and 1-6 mg/l), no significant advantage could be ascribed to the newer agents, despite their wider therapeutic ratio. In one instance, however, the newer β-lactam imipenem was found to perform with efficacy superior to that of ticarcillin.

(2) As a class, are aminoglycosides or β-lactams superior agents in treating pseudomonas pneumonia? With high challenge inocula (10^8 cfu), aminoglycosides performed significantly better than β-lactams. The only exception occurred with imipenem. When challenge inocula were reduced to 10^6 cfu, no significant differences in efficacy were noted for either drug class.

(3) Does combining a β-lactam with an aminoglycoside improve outcome (survival) or intrapulmonary killing of bacteria (quantitative lung cultures), as compared to single drug regimens? With high inocula (10^8 cfu), combined regimens were not significantly more efficacious than aminoglycosides alone. At lower inocula (10^6 cfu), additive benefits were observed.

(4) Can in-vitro observations with antimicrobial agents predict for therapeutic efficacy in this model? Clearly, demonstrating lower MICs did not ensure increased efficacy for a number of β-lactams (see 1, above). Nor has in-vitro synergism predicted for in-vivo benefits with combined therapy. On the other hand, a very useful in-vitro test to predict for drug efficacy in this model has been that relating to the inoculum effect, looking for a greater than three-fold increase in MIC when the assay is carried out with 10^7 cfu per ml, as compared to 10^5 cfu per ml. To date, drugs not displaying an inoculum effect have performed more effectively in the treatment of pneumonia (10^8 cfu challenges) than drugs that exhibit the inoculum effect. It is noteworthy that after a 10^6 cfu challenge in this model, the initial concentration of organisms is approximately 4 × 10^8 cfu per ml of homogenized lung. Thus, it appears likely that this in-vitro phenomenon has significance in the treatment of this high-inoculum lung infection. To date, the drugs that have not displayed an inoculum effect against our challenge strains have included tobramycin, gentamicin, imipenem, and ciprofloxacin. As might be predicted from the preceding comments, one of our most recent studies demonstrated efficacy of the quinolone, ciprofloxacin, close to that of tobramycin and exceeding that of ticarcillin.

(5) Are observations in this model applicable to dissimilar pneumonias? The answer is clearly no. It appears that both the host status (e.g., neutropenic versus normal) and the challenge organism (e.g., Ps. aeruginosa versus E. coli), may affect the relative efficacies of β-lactam and aminoglycoside antibiotics. For example, two separate studies of pseudomonas pneumonia in neutropenic hosts (dog, Dale et al., 1976; guinea pig, Rusnak et al., 1984) have demonstrated equal or superior efficacies of β-lactam agents as compared to aminoglycosides. Also, studies in our laboratory with E. coli in high inocula (10^8 cfu) failed to
demonstrate the superior performance of aminoglycosides, previously noted with pseudomonas. It is thus clear that great care in defining the experimental pneumonia system is essential if potentially useful information is to be obtained. It would appear that the lessons derived from the bulk of our studies may be most relevant to the non-neutropenic patient with high concentrations of pseudomonas in the lungs (e.g., an intubated patient receiving intensive care), but may be less relevant to neutropenic patients (e.g., neoplastic chemotherapy).

Although most recent studies have employed animal models of pneumonia for evaluation of treatment for acute Gram-negative bacillary infection, newer models are becoming available which will allow for evaluations of treatment for more chronic respiratory infection (Schiff et al., 1984). Also, a recently described murine model of Haemophilus influenzae pneumonia offers a method for evaluation of newer regimens for treating this relatively common respiratory pathogen (Esposito & Pennington, 1984). Finally, it should be noted that animal models may be useful also in investigation of intrapulmonary pharmacokinetics of antibiotics (Pennington & Reynolds, 1975; Pennington & Stone, 1979). Thus, with the usual but important caveat that caution be used in transposing therapeutic lessons from animal models to clinical settings, it is evident that a number of animal models of pneumonia are available from which significant experimental data may be obtained.

References


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Leading articles


Double \(\beta\)-lactam therapy in the immunocompromised host

For much of the last decade, the emphasis in the initial empiric treatment of the febrile, neutropenic patient has been on selection of effective combinations of antimicrobial agents. The rationale for this therapeutic approach has been most recently restated in a leading article appearing in this journal (Lode, 1983) as well as several other reviews (Klastersky, 1983; Young, 1982). The major concepts expressed in these reviews will not be repeated here, suffice it to state that no single therapeutic agent is reliably effective against the great majority of bloodstream pathogens that commonly appear in patients who have impaired host defenses. Nonetheless, despite the persuasive evidence from human clinical trials and from studies of experimental models of infection that combinations of drugs such as aminoglycosides plus \(\beta\)-lactam agents give the most satisfactory results in empiric therapy, there has been fluctuating interest in the approach which combines two \(\beta\)-lactam agents. The principal justification for such regimens has been avoidance of aminoglycoside toxicity.

'Double \(\beta\)-lactam' therapy is not a new concept. Even in the mid-1970s some attempts were made to combine a first generation cephalosporin and an anti-pseudomonal penicillin in the treatment of febrile, neutropenic patients. Bodey et al. (1977) reported a study in which carbenicillin was paired with either cefalothin or cefazolin to treat 36 bacteremias caused by either Escherichia coli, Klebsiella pneurnoniae, or Pseudomonas aeruginosa. The clinical results in less than half of these bloodstream infections were felt to be 'responses', and therapy of 11 of 12 Klebsiella bacteremias was unsuccessful. In the first multicentre study undertaken by the European Organization for Research and Treatment of Cancer (EORTC), cefalothin plus either carbenicillin or ticarcillin was least effective of three regimens evaluated (EORTC, 1978).

Gurwith et al. (1978) reported that mortality was significantly higher in bacteremic patients given cefalothin plus carbenicillin rather than gentamicin/carbenicillin/methicillin. In the EORTC study, the double \(\beta\)-lactam approach seemed particularly unsatisfactory for Ps. aeruginosa bacteremia.

Viewing the chemotherapeutic alternatives of the mid-1980s, however, one can understand why the revival of interest in double \(\beta\)-lactam therapy has occurred. Newer penicillins and cephalosporins are considerably augmented in their \(\text{in-vitro}\) potency, as compared with their predecessors. Some combinations may even interact synergistically, because of binding to different target proteins. Furthermore, some penicillins or cephalosporins may act as inhibitors of \(\beta\)-lactamase, thereby permitting a partner to exert an even greater therapeutic effect. The first large scale clinical trials of 'modern' double \(\beta\)-lactam therapy in immunocompromised hosts are now reaching publication and some comment is justified. Several investigations have been reported in abstract form but two studies carried out in the United States have recently been published, one from the M. D. Anderson Hospital and Tumor Institute in Houston, Texas and the second from the U.C.L.A. Center for the Health Sciences in Los Angeles, California. The former study compared latamoxef (moxalactam)/ticarcillin with latamoxef/tobramycin (Fainstein et al., 1984) while the U.C.L.A. group evaluated latamoxef plus either penicillin or amikacin (Winston et al., 1984). Both studies have enrolled hundreds of patients and provide interesting if not provocative results.

First, it must be stated that, overall, the results in both therapeutic trials showed no significant difference in response rates between treatment groups receiving aminoglycoside and latamoxef (this might be considered 'standard' or conventional combination therapy) versus the double \(\beta\)-lactam regimen. The large number of patient entries into each study, however, tends to obscure the fact that there were relatively fewer documented bacterial infections. Some groups of microbiologically documented infections were really too small for meaningful assessment. The M. D. Anderson investigators noted that almost one third of blood stream pathogens were organisms such as Staph. epidermidis and Corynebacterium species, that were quite resistant to the agents used. Counterbalancing this observation was the finding that the double \(\beta\)-lactam treatment was significantly less nephrotoxic than the combination containing tobramycin. In the U.C.L.A. study no difference in ototoxicity or renal damage could be detected between the two regimens. The U.C.L.A. study is also inter-