

Mice Model of Penicillin-Resistant Pneumococcal Pneumonia: Facts and Controversies

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ABSTRACT

10 Recently we have established non-compromised CBA/J mice model of penicillin-resistant *Streptococcus pneumoniae* (PRSP) pneumonia. In this symposium comparative efficacy of several antibiotics against PRSP pneumonia was examined in this newly developed animal model. We compared therapeutic efficacies of penicillin-G, cefotaxime, imipenem, and vancomycin on pulmonary clearance, pharmacokinetics and survival in this pneumonia model. In mice 48 hours after intranasal inoculation of strain No.11 (penicillin-G MIC, 0.015 µg/ml), 0.6 mg/kg of penicillin-G (6 times, 1 hour interval) significantly reduced the bacterial number in lungs whereas 40 mg/kg was required to induce similar response in pneumonia with strain No.741 (penicillin-G MIC, 1 µg/ml). Among antibiotics tested, imipenem (MIC, 0.25 µg/ml) was the most active
20 in pulmonary clearance in strain No.741 pneumonia, which were followed by vancomycin (MIC, 0.5 µg/ml), cefotaxime (MIC, 0.5 µg/ml) and penicillin-G. On survival study in strain No.741 pneumonia, survival rates in mice treated with saline (control), penicillin-G (160 mg/kg), cefotaxime (40 mg/kg), imipenem (40 mg/kg) and vancomycin (40 mg/kg) were 0, 40, 30, 90, and 90% respectively. These results indicated that CBA/J mice model may be useful to evaluate efficacies of antibiotics against penicillin-resistant pneumococcal pneumonia in non-compromised individuals. The present data support that imipenem and vancomycin may be the most active agents against penicillin-resistant *S. pneumoniae* pneumonia.

30 INTRODUCTION

Streptococcus pneumoniae continues to be the leading pathogen of bacterial pneumonia with significant morbidity and mortality^{2, 15}). Although this organism was originally exquisitely susceptible to penicillin, the past two decades, particularly the fast few years, have witnessed an alarming increase in the number of strains resistant to penicillin as well as other antimicrobial agents all over the world^{1, 7, 20}). Clinical treatment failure in cases of infection caused by penicillin-resistant *S. pneumoniae* prompted investigators to re-examine therapeutic efficacies of antibiotics in *in vitro* and

in vivo^{4, 11, 16)}

Animal models which closely resemble the features of disease in humans is necessary to find the optimal antimicrobial treatment. In the recent reports, immunocompromised mice were used to evaluate therapeutic efficacies of antibiotics against penicillin-resistant *S. pneumoniae* pneumonia because they failed to find any virulent strains in mice pneumonia model^{3, 17)}. In those reports, mice were kept in leukopenic by cyclophosphamide, and challenged by relatively high number of organisms. Since penicillin-resistant *S. pneumoniae* pneumonia were frequently seen in immunocompetent persons as community-acquired pneumonia^{10, 23)}, animal models without any immunosuppressive drugs may be suitable for precise understanding of pathogenesis and estimation of therapeutic efficacies of antimicrobials in those patients. Recently we have found that CBA/J mice, but not CBA/N, C3H/HeN, C3H/HeJ, C57BL/6, nor ICR, were susceptible to intranasal challenge with penicillin-resistant *S. pneumoniae* strain TUM19. In these experiments, we have observed fatal pneumonia in non-compromised CBA/J mice, with gradual increase of bacterial number in lungs through 10⁴ CFU/lungs at day 1, 10⁵ CFU/lungs at day 3 and 10⁷ CFU/lungs at day 5^{21, 22)}.

In this symposium, we have examined the virulence of several clinical isolates of penicillin-susceptible and -resistant strains in ICR and CBA/J mice. Therapeutic efficacies of penicillin-G, cefotaxime, imipenem, and vancomycin were evaluated on pulmonary clearance, pharmacokinetics and survival study in pneumonia model of CBA/J mice in non-compromised condition.

MATERIALS AND METHODS

Challenge organisms. Ten strains of *S. pneumoniae* isolated from different patients at different date through 1993 to 1995 in Toho university school of medicine were used in the present study. Strain No.39 and No.40 were mucoid-type, whereas the other pneumococci showed rough colonies with varying degrees of alpha-hemolytic activity. They were frozen at -80 C in skim milk until used.

Animals. Five-weeks-old male CBA/J mice (body weight range, 16-20 g) and ICR mice (body weight range, 18-22 g) were obtained from Charles River Japan, Inc. (Kanagawa, Japan).

Antimicrobial agents. Penicillin-G, cefotaxime, imipenem, and vancomycin were obtained from Meiji Seika, Ltd. (Tokyo, Japan), Hoechst Japan, Ltd. (Tokyo, Japan), Banyu Pharmaceutical Co., Ltd. (Tokyo, Japan), and Shionogi & Co., Ltd. (Osaka, Japan), respectively. Imipenem was mixed with cilastatin (Banyu) in the ratio of 1:1, and was used in *in vivo* pneumonia model.

Pneumonia model. Bacteria were inoculated into Todd Hewitt broth (Difco) supplemented with 30% horse serum and incubated for 5-6 h at 37 C until the culture

was turbid to the naked eye. This exponential-growth culture was suspended in 0.9% saline to the desired concentration, which was confirmed by plating serial 10-fold dilutions onto 5% blood agar following incubation for 18 h at 37 C. Mice were anesthetized lightly by intramuscular injection of a mixture of 6 mg/kg of ketamine (Sankyo Pharmaceutical, Ltd., Tokyo, Japan) and 1 mg/kg of xylazine (Bayer Japan, Ltd., Tokyo, Japan), and then each mouse was intranasally challenged with approximately 10^5 logarithmic-phase organisms per mouse.

10 ***Pulmonary clearance and survival studies.*** To evaluate effects of antibiotics on bacterial numbers in lungs, the indicated doses of antibiotics were subcutaneously administered from 48 or 96 hours after infection for 6 times 1 hours interval. Animals were sacrificed 2 hours after final administration of antibiotics. The lungs were removed and homogenized in saline, and 0.1 ml of serial 10-fold dilutions of the homogenates were plated on blood agar for counts of viable bacteria. Results were expressed as the mean log CFU per lung \pm standard deviation for groups (n=5). The lower limit of detection was 2 log CFU per lung, which corresponded to the weakest dilution of tissue homogenates (10^{-1}) that avoided significant drug carryover with control inocula. To compare efficacies of various antibiotics on survival (n=10), the indicated doses of antibiotics were subcutaneously administered from 5 days after
20 infection twice a day for 6 days. Survival rates were recorded daily for 16-18 days after infection.

Pharmacokinetic studies. The pharmacokinetic studies of penicillin-G, cefotaxime, imipenem/cilastatin, and vancomycin were determined in serum and lungs of CBA/J mice (n=3) 48 hours after infection of *S. pneumoniae* No.741. Serum and lungs were collected at 5, 15, 30, 60, and 120 min after 40 mg/kg of subcutaneous administration of antibiotics. Antibiotic concentrations in serum and lung homogenates were determined by the agar well diffusion method using *Bacillus subtilis* ATCC12432 as the bioassay reference strain.

30 **RESULTS**

Penicillin-susceptible and -resistant S. pneumoniae pneumonia in ICR and CBA/J mice.

Three (No.39, 40, and TUH3) of five strains of penicillin-susceptible *S. pneumoniae* caused lethal pneumonia in ICR mice, whereas no death was observed in ICR mice infected with any 5 strains of penicillin-resistant pneumococci tested. In CBA/J mice, 4 of 5 strains of penicillin-susceptible *S. pneumoniae* induced lethal pneumonia. Mice infected with strain No.11, No.39, No.40 and TUH3 died within 8, 5, 5, and 4 days after infection, respectively. Times to death in pneumonia of penicillin-susceptible pneumococci in CBA/J mice were generally shorter than those in ICR mice. In contrast
40 to the results in ICR mice, all 5 strains of penicillin-resistant *S. pneumoniae* were lethal

for CBA/J mice, although these strains showed varying degrees of virulence. These results indicated that CBA/J mice were generally susceptible to pneumococci, not only to penicillin-susceptible strains but also to penicillin-resistant *S. pneumoniae* in pneumonia model.

Efficacy of penicillin-G on bacterial number in lungs of penicillin-susceptible and -resistant pneumococcal pneumonia.

Strain No.11 and strain No.741 were selected for further experiments to compare therapeutic efficacies of penicillin-G because virulence of these strains to CBA/J mice were similar, judged from the results in Fig.2. In strain No.11 pneumonia, 0.6 mg/kg or more of penicillin-G reduced the bacterial number from 6.4 log CFU/lungs to below 3 log CFU/lungs (Fig.4, $P<0.05$). In contrast, strain No.741 pneumonia was apparently more resistant to penicillin-G than that of strain No.11, and at least 40 mg/kg of penicillin-G was required to induce significant decrease of bacterial number in lungs. From these results, penicillin-G doses required for 2 log decrease in bacterial number were calculated to be 30.2 mg/kg in strain No.741 pneumonia and 0.41 mg/kg in strain No.11 pneumonia, respectively. The ratio of penicillin-G doses required (strain No.741/No.11) was 73.7, which was similar to the MICs ratio (1/0.015, 66.7). These results indicated that to obtain the same therapeutic responses as those in strain No.11 pneumonia, proportional increase of penicillin-G dosages in relation to the change of MIC is necessary for treatment of strain No.741 pneumonia.

Comparative efficacies of penicillin-G, cefotaxime, imipenem, and vancomycin on bacterial number in lungs of strain No.741 pneumonia.

Among these antibiotics, imipenem/cilastatin was the most active against penicillin-resistant strain No.741 pneumonia. Minimum dose of imipenem (0.6 mg/kg) reduced bacterial number in lungs from 7.6 log CFU/lungs to 5.0 log CFU/lungs. Doses of imipenem, cefotaxime, vancomycin, and penicillin-G required for 2 log decrease in lungs were calculated to be 0.42, 5.5, 3.3, and 31.0 mg/kg, respectively. Effects of vancomycin were slightly different from others. This agent induced proportional decrease of bacterial number in the tested range of antibiotic doses.

Pharmacokinetic studies.

Pharmacokinetic profiles for penicillin-G, cefotaxime, imipenem, and vancomycin in serum and lungs of mice infected with strain No.741 were investigated after subcutaneous administration of 40 mg/kg of antibiotics. Peak concentrations of penicillin-G, cefotaxime, imipenem, and vancomycin in lungs were 10.5 $\mu\text{g/g}$ at 5 min, 7.3 $\mu\text{g/g}$ at 5 min, 24.3 $\mu\text{g/g}$ at 15 min, and 35.2 $\mu\text{g/g}$ at 15 min, respectively. Half-elimination time of these antibiotics in lungs were 15.0, 14.5, 14.5, and 215.4 min, respectively. Time above MIC of these antibiotics in lungs were 42.6, >60, >60, and >120 min, respectively. Pharmacokinetics of vancomycin in infected lungs were superior to those of penicillin-G, cefotaxime and imipenem in these parameters.

Comparative efficacies of penicillin-G, cefotaxime, imipenem, and vancomycin on survival.

Control mice died between 6-8 days after infection. Survival rates of mice treated with penicillin-G (160 mg/kg) and cefotaxime (40 mg/kg) were 40% and 30% at 18 days after infection, respectively. In contrast, effects of imipenem and vancomycin (40 mg/kg) were more active, and survival rates treated with these antibiotics were both 90%.

10 **DISCUSSION**

Monie P. et al.¹⁷⁾ have used leukopenic mice pneumonia model to evaluate efficacy of ceftriaxone because they failed to induce penicillin-resistant pneumococcal pneumonia in immunocompetent mice. Leukopenic mice developed acute pneumonia and quickly died within 2 or 3 days after intratracheal instillation of 10^7 CFU organisms per mouse, therefore therapy was initiated 3 hours after bacterial challenge. Azoulay-Dupuis E. et al.³⁾ have also used leukopenic mice to investigate therapeutic efficacy of sparfloxacin in pneumonia. On the other hand, our pneumonia model using CBA/J mice was quite different from others. Without any immunosuppressive drugs, CBA/J mice intranasally challenged with relatively low number of organisms developed pneumonia at 2-4 days, and majority of mice died from 5 to 10 days after infection. Moreover, the present study confirmed that CBA/J mice were generally susceptible to several clinical isolates of penicillin-resistant *S. pneumoniae*. Since CBA/J mice pneumonia model is likely to resemble to community-acquired *S. pneumoniae* pneumonia in humans, this model provided an opportunity to investigate antibiotic efficacies and pathogenesis of pneumococcal pneumonia in immunocompetent individuals.

Several investigators have reported the usefulness of penicillin in the treatment of penicillin-resistant *S. pneumoniae* infection. Barry B. et al.⁵⁾ have reported that in a gerbil model of acute middle ear otitis, two injections of 2.5 mg/kg of amoxicillin cured infection due to the penicillin-susceptible strain, whereas 10 and 25 mg/kg were required to eradicate penicillin-resistant and highly penicillin-resistant pneumococci, respectively. In pneumonia model, it has been reported that increasing the dose of amoxicillin from 5 mg/kg to 50 mg/kg improved survival rates in leukopenic mice infected with penicillin-resistant pneumococci³⁾. Clinically, Pallares R. et al.¹⁸⁾ have reported that pneumonia due to penicillin-resistant pneumococci may respond to intravenous high-dose penicillin therapy if MICs are ≤ 2 $\mu\text{g/ml}$, from their experience of 24 adults cases. Although improvement of therapeutic efficacies by increasing penicillin-G dosages was consistent with those of previous reports, it was surprised that 70-fold or more of penicillin-G was required for penicillin-resistant pneumococcal pneumonia to obtain the same therapeutic responses as those of penicillin-susceptible strain. In addition, the ratio of these penicillin-G doses required was similar to the MICs

ratio of two strains. Knudsen J. D. et al.¹³⁾ have reported a highly significant correlation between log MIC and log ED50 in mouse peritonitis model, and that the MIC is highly predictive of the dose of penicillin needed for the drug to have an effect. Our results were consistent with theirs, in spite of the difference of type of infection, pneumonia and peritonitis.

As alternatives to penicillin, efficacies of several antimicrobial agents such as cephalosporins^{6, 17, 19)}, newer quinolones^{3, 14)}, imipenem^{4, 16)}, and vancomycin^{11, 12, 16)} have been investigated in *in vitro* and *in vivo* models. Doit C.P. et al.⁹⁾ have reported that against 15 strains of penicillin-resistant pneumococci, MICs of imipenem (0.12-
10 0.25 µg/ml) were lower than those of penicillin-G (2 µg/ml), and imipenem was the most active drug in *in vitro* killing systems among antibiotics tested. In rabbit model of meningitis with penicillin-resistant pneumococci, a single dose of penicillin (50 or 150 mg/kg) or of ceftriaxone failed to lower the number of organisms in CSF, whereas imipenem (24 mg/kg) or vancomycin (15 mg/kg) reduced the counts in CSF more than 2 log CFU/ml¹⁶⁾. Catalan M.J. et al.⁸⁾ have reported failure of cefotaxime in the treatment of meningitis due to relatively resistant pneumococci, in which cure was achieved with vancomycin, and they recommended that in cases of meningitis caused by strains of *S. pneumoniae* for which MICs are ≥ 1 µg/ml, cefotaxime should be used with caution. The present study showed that in pulmonary clearance study, imipenem
20 was the most active among antibiotics tested. On the other hand, vancomycin was superior to imipenem, cefotaxime and penicillin-G in several pharmacokinetic parameters. Probably reflecting these results, efficacies of imipenem and vancomycin (40 mg/kg) were excellent in survival study, comparing with those of penicillin-G (160 mg/kg) and cefotaxime (40 mg/kg). Our results and the previous reports strongly suggest that imipenem or vancomycin may be a first-line antimicrobial, especially for patients in whom pneumococcal pneumonia with high-level penicillin resistance are suspected, or in systemic infections in critically ill patients.

It must be very careful when we consider the relevance of results obtained from mice model to the treatment of human infections because there are substantial differences in
30 several pharmacokinetic parameters between mice and humans. In particular, half-lives of penicillin-G, cefotaxime, imipenem, and vancomycin in serum of human were reported to be respectively, whereas the present data in mice showed that half-lives of these antibiotics were respectively 8.5, 14.3, 12.1, and 32.2 min in serum, and respectively 15.0, 14.5, 14.5, and 215.4 min in lungs. Nevertheless, as an usual regimen in humans, these antibiotics were administered only twice per day for survival experiments in the present manuscript. Further experiments designed to satisfy human pharmacokinetics, such as frequent dosing and constant infusion, may be necessary to exactly define efficacies of these antibiotics in human infections.

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