Efficacy of Quinolones against Secondary Pneumococcal Pneumonia after Influenza Virus Infection in Mice

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We established a mouse model of secondary pneumococcal pneumonia after influenza virus infection and investigated the efficacy of several quinolones against pneumonia in this model. Gatifloxacin exhibited the highest efficacy among the quinolones examined and is probably useful for the treatment of secondary bacterial pneumonia.

Influenza virus is a frequent cause of severe acute respiratory infection. According to studies conducted during influenza epidemics, influenza virus infection is one of the causes of excess mortality worldwide (1, 2, 4). The most severe condition associated with influenza virus infection is pneumonia, which is often life threatening (12, 13). Although influenza virus infection by itself can lead to pneumonia, secondary bacterial infection during or shortly after recovery from the influenza virus infection is a much more common cause of pneumonia (4, 7, 15). While anti-influenza virus drugs, such as amantadine, zanamivir, and oseltamivir, have been used for the treatment of influenza virus infection, these agents show no efficacy against secondary bacterial infections. Recently, the respiratory quinolones have been used successfully for the treatment of pneumonia caused by bacteria (3). Gatifloxacin is a respiratory quinolone with broad-spectrum antibacterial activity against pathogens implicated in respiratory infections and is recommended for use in the treatment of community-acquired pneumonia (5). On the other hand, the efficacy of gatifloxacin against secondary bacterial pneumonia has not yet been well studied. In this study, to evaluate the efficacies of quinolones, including gatifloxacin, we established and characterized a mouse model of secondary pneumococcal pneumonia after influenza virus infection.

The secondary pneumococcal pneumonia model was established using 6-week-old female BALB/c mice infected with influenza virus A/PR/8/34 (H1N1) and *Streptococcus pneumoniae* KY9, both of which exhibit high virulence in mice. The mice were lightly anesthetized by intraperitoneal injection of pentobarbital at 1.25 mg/mouse and inoculated intranasally with 20 PFU of influenza virus. Seven days after the influenza virus inoculation, the mice were anesthetized by intraperitoneal injection of pentobarbital at 0.94 mg/mouse and then intratracheally inoculated with *S. pneumoniae* (approximately 10^5 CFU/mouse). All of the mice infected with either influenza virus or *S. pneumoniae* alone survived for at least 10 days after inoculation. In contrast, all of the mice inoculated with *S.*

pneumoniae 7 days after the influenza virus inoculation died within 4 days of the bacterial inoculation.

The bacterial titers in the lungs of mice infected with 8.2×10^4 CFU/lung of *S. pneumoniae* alone were reduced to below the limit of detection ($<5.0 \times 10^2$ CFU/lung) 4 days after the bacterial inoculation. On the other hand, the bacterial titers of mice infected secondarily with *S. pneumoniae* after the influenza virus inoculation increased to 6.6×10^7 CFU/lung 1 day after the bacterial inoculation and remained at this level.

Figure 1 shows representative histological examples of lung tissue samples from infected mice. The lung injury caused by the secondary bacterial pneumonia (Fig. 1E and F) was significantly more extensive than that caused by influenza virus (Fig. 1A and B) or *S. pneumoniae* (Fig. 1C and D) infection alone. The secondary bacterial pneumonia was characterized by extensive inflammatory cell infiltration, focal necrosis, and hemorrhage.

These results indicate that the susceptibility of the mice to *S. pneumoniae* increased markedly by influenza virus infection. Recently, an animal model of secondary bacterial pneumonia after influenza virus infection was established (8, 14). It has been reported that bacterial adhesion to the epithelial cells of the bronchial mucosa is enhanced by influenza virus infection (6, 11) and that virus-mediated alterations in the receptor milieu can create circumstances favorable to pneumococcal infection; for example, viral neuraminidase helps to prime the lungs for pneumococcal infection (9). These findings lend support to our experimental results. In this model, pneumococcal pneumonia was induced in nonimmunocompromised mice, in contrast to the conventional model in which leukopenia is induced in the mice by pretreatment with chemical compounds, such as cyclophosphamide. Therefore, it is suggested that this secondary bacterial pneumonia model may represent human pneumococcal pneumonia better.

In the present work, we evaluated the therapeutic effects of three quinolones—gatifloxacin, levofloxacin, and ciprofloxacin using the secondary infection model of mice infected with the KY9 strain of *S. pneumoniae* (penicillin-susceptible *S. pneumoniae*; penicillin G MIC, 0.063 μ g/ml). The MICs of gatifloxacin, levofloxacin, and ciprofloxacin against KY9 were 0.25, 1, and 1 μ g/ml, respectively. The quinolones were administered orally at a dose of 25, 50, or 100 mg/kg body weight twice daily for 3 days, beginning 18 h after inoculation of *S. pneumoniae*.

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FIG. 1. Pathological examination of lung tissue specimens of mice infected with influenza virus alone (A, B), *S. pneumoniae* alone (C, D), and *S. pneumoniae* after influenza virus infection (E, F). The organs were stained with hematoxylin-eosin. Magnifications, $\times 40$ (A, C, E) and $\times 100$ (B, D, F).

The mice were monitored daily for mortality until 10 days after the bacterial inoculation. Administration of gatifloxacin at doses of 25, 50, and 100 mg/kg induced a significant prolongation of survival in a dose-dependent manner ($P < 0.01$; Fig. 2, left). In addition, the bacterial titers in the lungs of the mice treated with 25, 50, and 100 mg/kg of gatifloxacin also declined

to 7.8×10^6 , 9.5×10^3 , and 9.5×10^2 CFU/lung, respectively, 6 days after the bacterial inoculation. The mice treated with levofloxacin at 100 mg/kg also showed significant prolongation of survival ($P < 0.01$), although those treated with 25 and 50 mg/kg of levofloxacin died within 7 days of the bacterial inoculation. All of the ciprofloxacin-treated mice died within 4 days

FIG. 2. Cumulative survival rates of the infected mice treated with gatifloxacin (A), levofloxacin (B), and ciprofloxacin (C). Mice were infected with influenza virus and then with the KY9 (left) or MBC188 (right) strain of *S. pneumoniae*. Arrows indicate the times of administration of quinolones. Symbols: ●, control; ◆, 25 mg/kg; ▲, 50 mg/kg; ■, 100 mg/kg. The survival rates of each group of mice were compared with those of the control groups using the log rank test. Values that were significantly different from those of the control group are indicated by asterisks (**, $P < 0.01$; ***, $P < 0.001$).

of the bacterial inoculation. Similar results were obtained following secondary infection using another strain of *S. pneumoniae*, MBC188, categorized into penicillin-intermediate *S. pneumoniae* (penicillin G MIC, 0.5 $\mu\text{g/ml}$; Fig. 2, right). The MICs of gatifloxacin, levofloxacin, and ciprofloxacin against MBC188 were 0.5, 2, and 2 $\mu\text{g/ml}$, respectively. Thus, our results revealed that gatifloxacin was more effective than either levofloxacin or ciprofloxacin against infection induced by either strain of *S. pneumoniae* in our secondary pneumococcal infection model.

There have been few reports on the efficacies of antibacterial agents in the secondary bacterial pneumonia model (10). In the present work, several of the quinolones examined showed efficacy in vivo in our secondary infection model. Therefore, it appears that this model may be useful for evaluation of the efficacy of antibacterial agents against secondary bacterial pneumonia. Gatifloxacin exhibited the greatest efficacy among

the quinolones examined in this model and is, therefore, probably useful for the treatment of pneumonia, including secondary bacterial infection. Treatment with appropriate antibacterial agents is necessary for cure in cases of secondary bacterial pneumonia.

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