Diagnostic Microbiology and Infectious Disease 78 (2014) 168–171

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio

Therapeutic effects of garenoxacin in murine experimental secondary pneumonia by *Streptococcus pneumoniae* after influenza virus infection☆,☆☆,★

Yoshiko Fukuda *, Yuri Furuya, Yusuke Nozaki, Masahiro Takahata, Nobuhiko Nomura, Junichi Mitsuyama

Research Laboratories, Toyama Chemical Co., Ltd., 2-4-1 Shimookui, Toyama 930-8508, Japan

ARTICLE INFO

Article history:
Received 27 August 2012
Received in revised form 10 October 2013
Accepted 5 November 2013
Available online 14 November 2013

Keywords:
Garenoxacin
Influenza
Streptococcus pneumoniae
Secondary bacterial pneumonia
In vivo efficacy

ABSTRACT

In a pneumococcal pneumonia murine model following influenza virus infection, garenoxacin was more effective than other fluoroquinolones and demonstrated high levels of bacterial eradication in the lung, low mortality, and potent histopathological improvements. Garenoxacin could potentially be used for the treatment of secondary pneumococcal pneumonia following influenza.

Influenza virus is one of major causes of severe acute respiratory infections with high morbidity and mortality. Its high mortality is not caused by influenza virus infection itself but rather by secondary bacterial pneumonia, most commonly caused by *Streptococcus pneumoniae* (McCullers, 2006; Morens, et al., 2008). There is a particularly lethal synergism between influenza virus and *S. pneumoniae*, and post-influenza pneumococcal pneumonia increases its severity (McCullers, 2006).

In *S. pneumoniae*, a growing concern is the increasing level of resistance to penicillins, β-lactams, and macrolides. The incidence of multidrug-resistant bacteria has resulted in an increased number of at-risk individuals. Thus, for secondary pneumococcal pneumonia, especially in elder patients or people at a high risk of developing severe complications, it might be necessary to consider treatment not only with antiviral agents but also with antimicrobial agents possessing a high antipneumococcal potency (Gupta et al., 2008). Quinolones with increased antipneumococcal activity are recommended for the treatment of community-acquired pneumonia caused by multidrug-resistant *S. pneumoniae* (File, 2006). Although gatifloxacin reportedly showed a good improvement in survival in a secondary pneumococcal pneumonia murine model (Hayashi et al., 2006), only a few reports have assessed the efficacy of quinolones.

Garenoxacin, a des-fluoroquinolone, exhibits potent antibacterial activity and excellent clinical efficacy against *S. pneumoniae* (Takagi et al., 2008; Weller et al., 2002; Yokota et al., 2009).

In this study, we investigated the efficacy of garenoxacin in the secondary pneumococcal pneumonia murine model and compared it with that of levofloxacin and moxifloxacin. Six-week-old immune normal female BALB/c mice ($n = 10$) were infected intranasally with 20 plaque-forming units of influenza virus A/PR/8/34 [influenza virus A (IAV), H1N1] under anesthesia. On day 7 after infection, the mice were intranasally inoculated with 10^3 CFU of a clinical isolate of *S. pneumoniae* D-979 (categorized as genetic penicillin-resistant *S. pneumoniae* benzylpenicillin MIC: 2 μg/mL). The MICs for garenoxacin, levofloxacin, and moxifloxacin were 0.0313, 1, and 0.125 μg/mL, respectively, which were determined by the microdilution method recommended by the Clinical Laboratory Standards Institute (CLSI). At 2 h after inoculation, a single dose of each quinolone (10 or 30 mg/kg) was administered orally once a day. At 24 h after inoculation (i.e., on day 8 after virus infection), *S. pneumoniae* viable cells in the lungs were counted, and tracheal morphology was observed using a scanning electron microscope (SEM, S-4500; Hitachi High–Technologies Corp., Tokyo, Japan). Histopathological analysis of lungs was performed at 24 h and on day 3 after inoculation. Mortality was observed daily for 14 days after inoculation. The pharmacokinetic parameters at each dose in the serum and lung were calculated from the serum and lung concentrations for a single oral dose of 10 or 30 mg/kg once a day in mice with the secondary pneumococcal pneumonia following IAV infection (WinNonlin, version 5; Pharsight, Mountain View, CA, USA). All procedures involving animals were conducted in accordance with the Laboratory Animal Use Management Regulations at Toyama Chemical Co., Ltd.

☆ We declare no conflict of interest.

☆☆ A part of this study was presented at the 50th Interscience Conference on Antimicrobial Agents and Chemotherapy in Boston (September 12–15, 2010).

★ No funding support for this study was provided.

* Corresponding author. Tel.: +81-76-431-8306; fax: +81-76-431-8208.
E-mail address: yoshiko_fukuda@toyama-chemical.co.jp (Y. Fukuda).

0732-8893/$ – see front matter
http://dx.doi.org/10.1016/j.diagmicrobio.2013.11.003

All of the mice infected with either IAV or *S. pneumoniae* alone survived for 21 or 14 days after inoculation, respectively, while all of the mice inoculated with *S. pneumoniae* on day 7 after influenza virus infection died within 5 days of inoculation (Fig. 1). In the secondarily infected mice, the viable cells counts were 8.71 ± 0.47 Log_{10} CFU/g at 24 h after pneumococcal inoculation, which was higher than that in the mice inoculated with *S. pneumoniae* alone (Table 1). SEM showed that there was a small number of ciliated cells or no desquamation in the tracheal epithelium in mice infected with IAV (Fig. 2B) or *S. pneumoniae* alone (Fig. 2C), whereas there was almost complete desquamation in secondarily infected mice (Fig. 2D). Histopathology also showed that lung injury, which was characterized by marked neutrophil infiltration and pulmonary edema, in the secondarily infected mice (Fig. 3D and H) was more extensive than in mice infected with IAV (Fig. 3B and F) or *S. pneumoniae* alone (Fig. 3C and G).

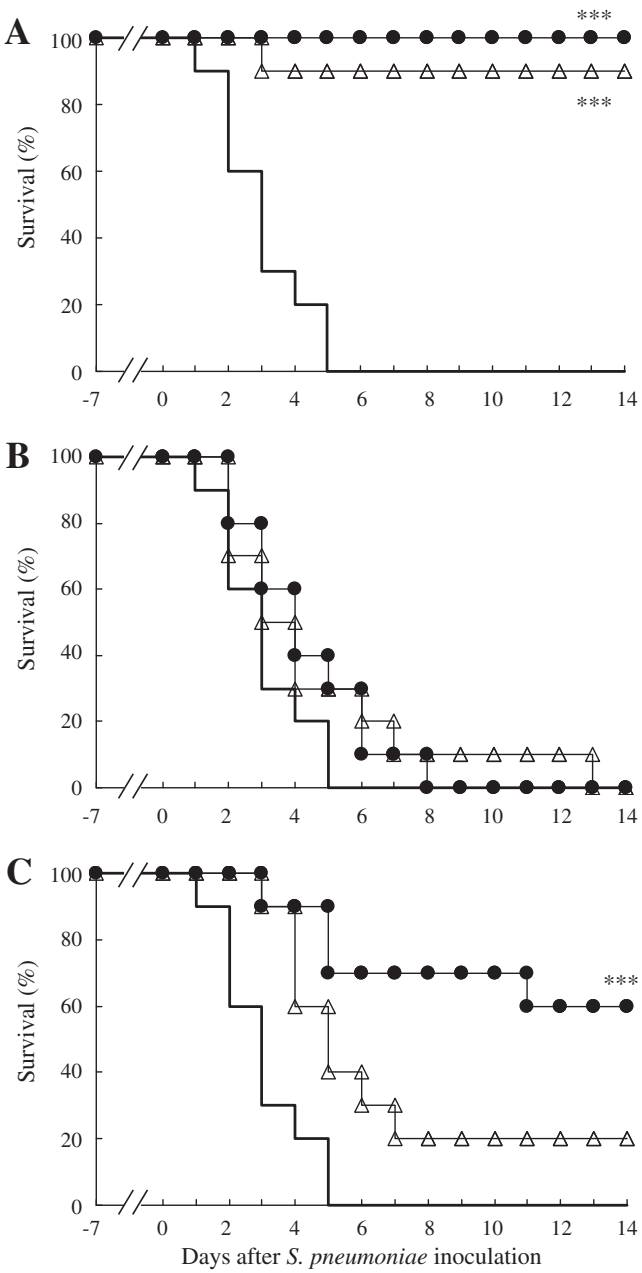

Fig. 1. Survival curves for the infected mice treated with garenoxacin (A), levofloxacin (B), and moxifloxacin (C). Bold line, infected control; open triangle, 10 mg/kg; closed circle, 30 mg/kg. The survival rates of each group of mice were compared with those of the infected control group using the log-rank test (***$P < 0.001$).

Table 1
Therapeutic effect of garenoxacin on experimental secondary pneumococcal pneumonia caused by *S. pneumoniae* D-979 in mice.

Treatment group	MIC[a] (μg/mL)	Dose (mg/kg)	Viable cell counts[b] (Log_{10} CFU/g)	Mortality[c] (%)	$fAUC_{0-24}$[d]/MIC	
					In serum	In lung
S. pneumoniae alone	–	–	2.37 ± 0.10	0	–	–
Infected control[e]	–	–	8.71 ± 0.47	100	–	–
Garenoxacin	0.0313	10	4.57 ± 0.70[f]	10	71.7	106
		30	3.37 ± 0.43[f]	0	288	381
Levofloxacin	1	10	8.12 ± 0.97[g]	100	2.05	4.17
		30	8.48 ± 0.91[g]	100	6.63	11.7
Moxifloxacin	0.125	10	7.00 ± 0.81[f,g,h]	80	9.47	60.4
		30	3.11 ± 1.04[f,i]	40	28.1	127

Six-week-old BALB/c female mice ($n = 10$) were infected with influenza virus A/PR/8/34 and then with *S. pneumoniae* D-979 on day 7 after virus infection.

[a] MIC against *S. pneumoniae* D-979 determined by the microdilution method recommended by CLSI.

[b] Data represented viable cells counts ± SD at 24 h after pneumococcal inoculation.

[c] On day 14 after pneumococcal inoculation.

[d] Calculated from the serum and lung concentrations for a single oral dose of 10 or 30 mg/kg once a day. Serum protein binding ratio in mice: garenoxacin, 69.9%; levofloxacin, 23.9%; moxifloxacin, 38.4%.

[e] Infected with influenza virus and then with *S. pneumoniae*.

[f] $P < 0.001$ compared with infected control, parametric Tukey's test.

[g] $P < 0.001$ compared with the garenoxacin-treated group at the same dose, parametric Tukey's test.

[h] $P < 0.01$ compared with the levofloxacin-treated group at the same dose, parametric Tukey's test.

[i] $P < 0.001$ compared with the levofloxacin-treated group at the same dose, parametric Tukey's test.

Garenoxacin (10 and 30 mg/kg) reduced the viable cell counts in the lungs to 4.57 ± 0.70 and 3.37 ± 0.43 Log_{10} CFU/g at 24 h after inoculation, which were significantly lower than in the infected control and levofloxacin-treated groups (Table 1, $P < 0.001$). Moxifloxacin-treatment reduced the viable cells counts in the lungs, which were significantly less than in infected control mice ($P < 0.01$ and $P < 0.001$). However, at 10 mg/kg, the viable cell count was significantly larger than in the garenoxacin group at the same dose ($P < 0.001$).

As shown in Fig. 1, garenoxacin also significantly prolonged survival compared to the infected control group ($P < 0.001$). Conversely, most of the mice treated with levofloxacin at both doses or moxfloxacin at a dose of 10 mg/kg died within 8 days after pneumococcal inoculation, resulting in no improvement in survival.

Histopathologically, mononuclear cell infiltration was observed for garenoxacin treatment, but only a few neutrophils were observed (Fig. 3I, J, M, and N). On day 3, the alveolar bronchiolization, indicative of the epithelium regeneration, was observed in the garenoxacin-treated group (Fig. 3R). Conversely, the massive infiltration of neutrophils and pulmonary edema that filled the alveolar air spaces were more pronounced in the infected control and levofloxacin groups at 24 h (Fig. 3H and O) and on day 3, and in the moxifloxacin group (10 mg/kg) on day 3 (data not shown).

The area under the unbound serum concentration-time curve over 24 h divided by the MIC ($fAUC_{0-24}$/MIC) is one of the most important predictors for the clinical efficacy of fluoroquinolones (Craig, 1998). In this secondary pneumococcal pneumonia model following IAV infection, oral garenoxacin (10 and 30 mg/kg) had $fAUC_{0-24}$/MIC ratios of 71.7 and 288 in the serum and $fAUC_{0-24}$/MIC ratios of 106 and 381 in the lungs, resulting in effective bacterial eradication and excellent efficacy (Table 1). Although it is not clear yet whether the 3 quinolones show similar efficacy in the same $fAUC_{0-24}$/MIC in the secondary pneumococcal pneumonia model or not, the $fAUC/MIC_{90}$ ratio of garenoxacin at a clinical dose in human for *S. pneumoniae* is ≥352, which is also greater than those of levofloxacin (15.5) and moxifloxacin (107) (Chein et al., 1997; Takagi et al., 2008; Watanabe et al., 2012; Zeitlinger et al., 2003). It was considered that the potent