CURRENT MICROBIOLOGY Vol. 24 (1992), pp. 349–353

Current Microbiology

Antibacterial Activity of Rufloxacin in the *Staphylococcus aureus* Rat Granuloma Pouch Model

Giuseppe Ravizzola,[1] Gabriele Pinsi,[1] Marina Cesana,[2] Carmela De Rango,[1] Laura Peroni,[1] and Adolfo Turano[1]

[1]Institute of Microbiology, University of Brescia, Spedali Civili, Brescia, Italy; and [2]Medical Department, Mediolanum Pharmaceuticals, Milano, Italy

Abstract. The protective effects of rufloxacin against *Staphylococcus aureus*-induced infections were compared with those of ciprofloxacin in the granuloma pouch model in the rat. Two strains with different in vitro sensitivity to the drugs were studied. Rufloxacin concentrations persisted longer than ciprofloxacin in the exudate in the pouch cavity and were about eight times higher. Equal doses of rufloxacin and ciprofloxacin had similar antibacterial activities. However, rufloxacin inhibited *Staphylococcus aureus* bacterial growth significantly longer than did ciprofloxacin.

Animal models of infectious diseases have proven useful for the evaluation of new agents in the early phases of antimicrobial investigations.

Experimental animal models, which allow standardization of infection for treatment regimens, have been employed by many investigators to analyze the relationship between in vitro susceptibility tests and parameters of in vivo antimicrobial efficacy [7, 9]. However, the reliability of minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) to predict in vivo activity is still problematic; lack of correlation between in vitro and in vivo data is common [14].

Difference in potency of antimicrobial agents can be explained largely by difference in pharmacokinetics. A simple, reproducible animal model that reflects human infectious disease is the granuloma pouch, used by many experimenters to evaluate pharmacokinetic and chemotherapeutic properties of antibiotics [4, 6].

Studies of antimicrobial agents in the experimental granuloma pouch model are particularly useful for establishing two points: the penetration of the drug into the exudate and the bactericidal activity.

We have compared the pharmacokinetic properties and the in vivo efficacy of a new, long-acting quinolone, rufloxacin, with those of ciprofloxacin in the laboratory rat granuloma pouch model. Rufloxacin is a broad-spectrum fluoroquinolone [3, 11] with good tissue penetration [5, 12] and a particularly long half-life [8], which may account for its in vivo efficacy in the face of moderate in vitro activity.

Materials and Methods

Pouch formation. Granuloma pouches were formed by subcutaneously injecting 25 ml of air, according to the method of Dalhoff [4], into the backs of Wistar (Wl) BR male rats (Charles River, Calco, Italy) weighing 120–130 g.

Contamination was avoided by drawing air into a syringe through a sterile filter. Immediately afterwards, 0.5 ml of 0.5% croton oil in olive oil was injected into the air space. After 48 h, the air was removed with a syringe; the pouches were deflated, and the accumulation of fluid in the pouch cavity occurred in 3–6 days. At this point, with the pouches formed, pharmacokinetic studies and quantification of in vivo antimicrobial activity were started.

Drug concentrations. Groups of four rats with exudate in the pouch cavity were given orally, by gavage, a single dose of rufloxacin or ciprofloxacin at 50 mg/kg. At 30 min, 1, 2, 4, 8, 12, and 24 h, 0.5 ml samples of exudate were removed with sterile syringes. Concentrations of each quinolone was assayed in the exudate samples by an agar-well microbiologic assay. Standard samples were prepared in exudate. The indicator microorganism was *Escherichia coli* EES (extra-susceptible) with which one can detect concentrations of rufloxacin as low as 1 mg/L and ciprofloxacin as low as 0.03 mg/L.

Pharmacokinetic analysis. Pharmacokinetic parameters were calculated individually for rufloxacin and ciprofloxacin in purulent

Address reprint requests to: Prof. Adolfo Turano, Director, Institute of Microbiology, University of Brescia, Spedali Civili, Brescia 25100, Italy.

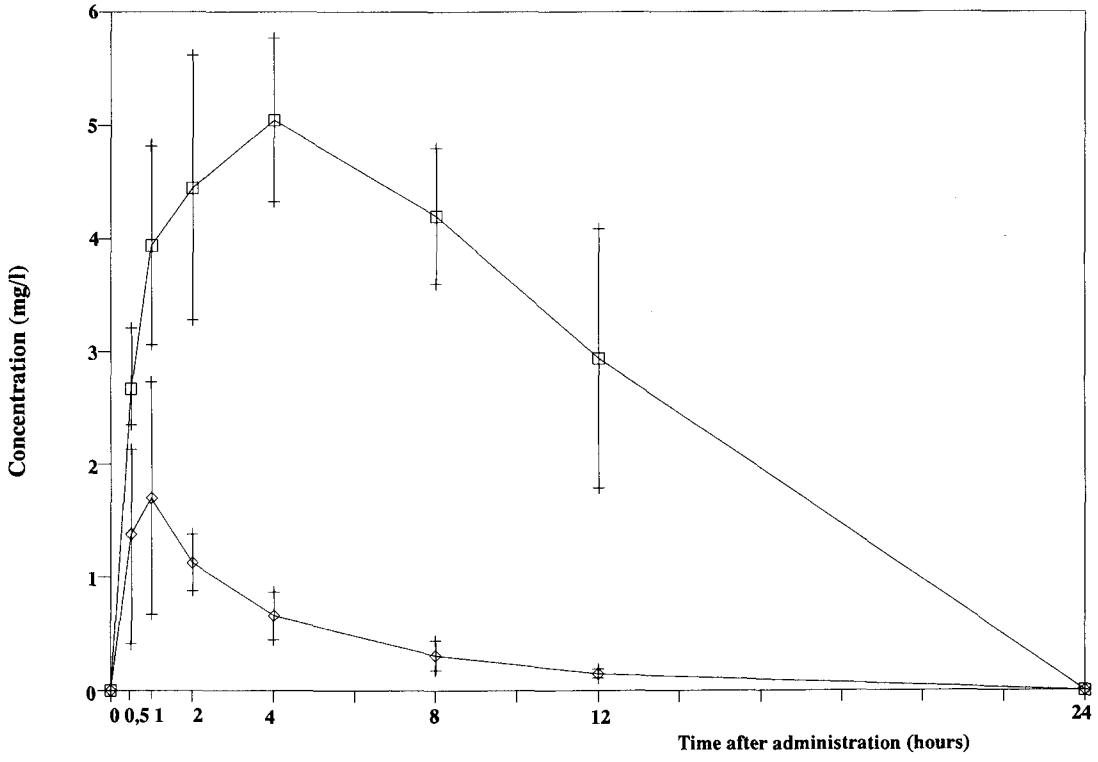

Fig. 1. Concentrations (mg/L) of □ rufloxacin and ◇ ciprofloxacin in granuloma pouch exudates of rats treated with a single 50 mg/kg oral dose of drug.

fluid of the granuloma pouch. The terminal half-life was estimated by log-linear regression of terminal data points. The maximum drug concentration (C_{max}) and the time required to reach the maximum (T_{max}) were determined for each antibiotic. The areas under the concentration–time curve from 0–24 h (AUC_{0-24}) were calculated by the linear trapezoidal rule. Data are presented as means ± standard error of mean.

In vivo antimicrobial activity. After formation of pouches, the exudate was removed by a syringe, pooled, and heated at 56°C for 30 min to inactivate complement. The test strains of staphylococci from overnight cultures in brain–heart infusion were diluted in the inactivated fluid to a concentration of 10^6 cfu/ml, after which 5–6 ml of inoculated diluted exudate was reinjected into the pouches of five rats for each test and control group. The infective microorganims were *S. aureus* BS/87 (MIC for rufloxacin 4 mg/L, MIC for ciprofloxacin 0.5 mg/L) and *S. auareus* BS/90 (MIC for rufloxacin 16 mg/L, MIC for ciprofloxacin 2 mg/L). Samples of exudate for determining counts of the viable infecting microorganisms were removed by syringe at 4, 8, and 24 h after infection. Quinolones were given orally at concentrations of 50 mg/kg (BS/87) and 50 or 70 mg/kg (BS/90) 30 min and 8 h after infection. Antibiotic activity was determined by comparing the numbers of microorganisms (cfu/ml) present at different times (4, 8, 24, and 48 h) in the pouch fluid of treated animals with those in the control group.

Statistical analysis. The number of colony forming units of *S. aureus* were log-transformed to obtain normalization and homogeneity of variance of data [2]. The numbers of colony forming units between treatment groups and times were compared by analysis of variance for a split-plot design. Values of $p < 0.05$ (two-tailed) were considered statistically significant. Calculations were made with the NWA STATPAK statistical package [10].

Results

The antibiotic concentrations of rufloxacin and ciprofloxacin in the granuloma pouch exudate are given in Fig. 1. After a single dose of 50 mg/kg of rufloxacin, the concentrations inside the pouch were 2.7 ± 0.5; 3.9 ± 0.9; 4.5 ± 1.2; 5.1 ± 0.7; 4.2 ± 0.6; and 2.9 ± 1.2 mg/L at 30 min, 1, 2, 4, 8, and 12 h, respectively. At same times the concentrations of ciprofloxacin were 1.4 ± 1.0; 1.7 ± 1.0; 1.1 ± 0.3; 0.7 ± 0.2; 0.3 ± 0.1; and 0.2 ± 0.1 mg/L. The C_{max} for rufloxacin was 5.1 ± 0.4 mg/L and was reached at 3.5 ± 0.5 h. The peak levels for ciprofloxacin were observed earlier than rufloxacin (T_{max} = 1.3 ± 0.3 h) but were only about one third as high (C_{max} = 1.7 ± 0.5 mg/L) as those for the new quinolone. Rufloxacin concentrations were more stable than ciprofloxacin levels ($T_{\frac{1}{2}}$ = 12.8 ± 4.5 vs 3.5 ± 0.3 h). This is reflected in the corresponding AUC_{0-24}

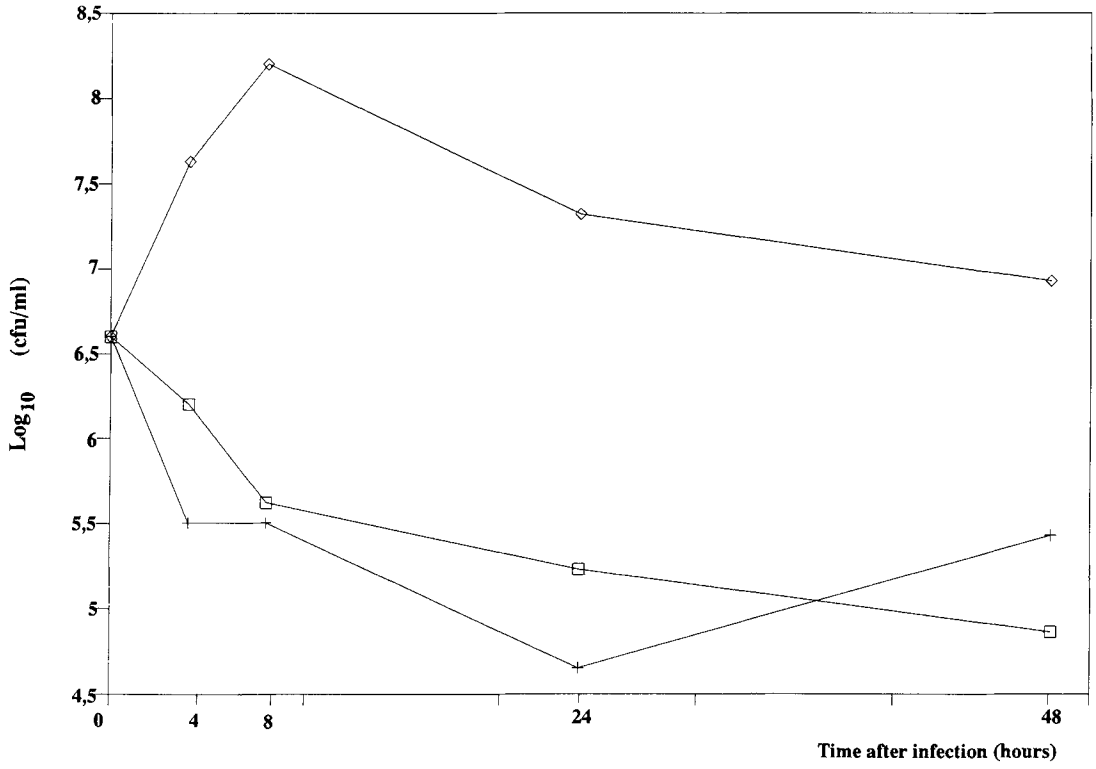

Fig. 2. Antibacterial activity of doses of 50 mg/kg rufloxacin and ciprofloxacin in granuloma pouch infected with *Staphylococcus aureus* BS/87 (MIC for rufloxacin, 4 mg/L; MIC for ciprofloxacin, 0.5 mg/L). □ rufloxacin; + ciprofloxacin; ◇ controls.

values, which were about eight times higher for rufloxacin than for ciprofloxacin (66.5 ± 5.8 vs 8.2 ± 1.4 mg × h/L).

Figure 2 summarizes the data related to experimental infection with *S. aureus* BS/87, for which the MIC for rufloxacin is eight times higher than for ciprofloxacin. Both quinolones significantly ($p \leq 0.001$) inhibited bacterial growth for the entire 48 h of observation. At 4 h, the antibacterial activity of ciprofloxacin was significantly higher ($p \leq 0.05$) than that of rufloxacin. In the pouches of rats treated with rufloxacin, $1.6 \pm 0.9 \times 10^6$ cfu/ml were found, in those treated with ciprofloxacin, $3.2 \pm 2.6 \times 10^5$ cfu/ml. Similar activity was observed at 8 h. At 24 h, there were three times as many viable cells in the pouches of animals treated with rufloxacin as with ciprofloxacin ($p \leq 0.05$). At 48 h, the animals treated with rufloxacin had fewer viable cells, while those treated with ciprofloxacin had an increased number of microorganisms, indicating a significantly ($p \leq 0.001$) longer duration of antibacterial activity for rufloxacin. In granuloma pouches infected with *S. aureus* BS/90 (Fig. 3), 50 mg/kg doses of both quinolones had similar antibacterial activity at 4, 8, and 24 h, while at 48 h the rufloxacin-treated animals had only one-third of the number of microorganisms as the ciprofloxacin-treated ($p \leq 0.05$). With the 70 mg/kg dose, the antibacterial activities at 4 and 8 h were similar, but at 24 and 48 h the number of test organisms/ml in the pouches of rats treated with rufloxacin were one-eight and one-third those in the ciprofloxacin-treated rats ($p \leq 0.05$).

Discussion

Although there are limitations, animal models of infection are the best means presently available for determining the efficacy and safety of an antibiotic before its use in clinical trials and for finding new approaches to the treatment of infections [1, 13]. The technical advantages of using animal models are no less important: studies can be performed in large groups of animals for statistical analysis; they provide reproducible results, and individual effects can be examined separately through variation of the test conditions. We evaluated the activity of rufloxacin, a new long-acting quinolone [8, 12] with in vitro

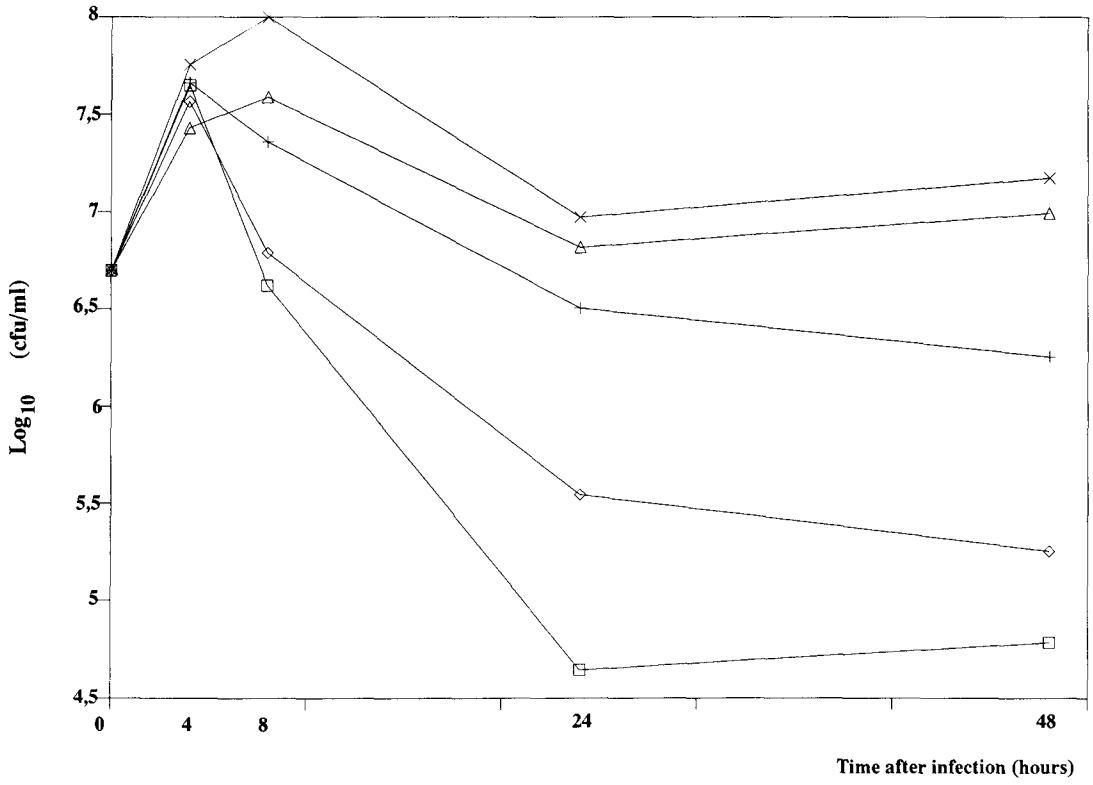

Fig. 3. Antibacterial activity of doses of 50 and 70 mg/kg rufloxacin and ciprofloxacin in granuloma pouch infected with *Staphylococcus aureus* BS/90 (MIC for rufloxacin, 16 mg/L, MIC for ciprofloxacin, 2 mg/L). □ rufloxacin 70 mg/kg; + rufloxacin 50 mg/kg; × controls; ◇ ciprofloxacin 70 mg/kg; △ ciprofloxacin 50 mg/kg.

lower activity than ciprofloxacin and other quinolones [11].

The in vivo antibacterial activity of rufloxacin and ciprofloxacin is related to the dose and depends on the MIC value of the organism test.

S. aureus BS/87 was more susceptible to quinolones than *S. aureus* BS/90 and was subject to higher bactericidal activity.

The in vivo results were surprising because the infecting strains we used have MIC values eight times higher for rufloxacin than ciprofloxacin. Ciprofloxacin had similar antibacterial effect as rufloxacin, but its duration of action was significantly shorter than that of the new quinolone. Although in a comparison of different antibiotics several studies have demonstrated a correlation between MICs and in vivo data, in experimental models and in humans many uncontrolled parameters could explain discrepancies between in vitro susceptibility tests and in vivo outcome. The eight times higher concentrations reached by rufloxacin at the site of infection and its longer half-life provide it with greater antibacterial activity in vivo than would be predicted from its in vitro activity. The animal model studies suggest many potential uses of rufloxacin. The efficacy of rufloxacin has been observed not only in granuloma pouch infection but also against respiratory and subcutaneous infections. Studies with respiratory *Klebsiella pneumoniae* infections in rats and subcutaneous *Pseudomonas aeruginosa* or *S. aureus* infections in guinea pigs showed that the protective effects of rufloxacin are equal to those of ciprofloxacin and ofloxacin [11].

In conclusion, results from animal models have shown that a particularly favorable pharmacokinetic property of rufloxacin is to produce antibacterial activity in vivo in different infections similar to those of other quinolones. Furthermore, its long half-life in the site of infection means that once-a-day therapy is feasible.

Literature Cited

1. Arko JR, Balows A (1986) Animal models of experimental gonococcal infection. In: Zak O, Sande MA (eds) Experimen-

tal models in antimicrobial chemotherapy, vol. 1. London: Academic Press, pp 355–369
2. Bartlett MS (1947) The use of transformations. Biometrics 3:39–52
3. Cecchetti V, Fravolini A, Fringuelli R, Mascellani G, Pagella PG, Palmioli M, Segre G, Terni P (1987) Quinolone-carboxylic acids. 2. Synthesis and anti bacterial evaluation of 7-oxo-2,3-dihydro-7H-pyrido [1, 2, 3-de] [1, 4] benzothiazine-6-carboxylic acids. J Med Chem 30:465–473
4. Dalhoff A (1986) The granuloma pouch. In: Zak O, Sande MA (eds) Experimental models in antimicrobial chemotherapy, vol 1. London: Academic Press, pp 123–137
5. Dirksen MSC, Jol C, Pahlmann W, Bach D, Boerema HBJ (1991) Rufloxacin: penetration in human tissue. J Chemother Suppl. 4:258–259
6. Fantin B, Leggett J, Ebert S, Craigwa (1991) Correlation between in vitro and in vivo activity of antimicrobial agents against Gram-negative bacilli in a murine infection model. Antimicrob Agents Chemother 35:1413–1422
7. Frimodt-Moller N, Thomsen VF (1987) The pneumococcus and mouse-protection test: correlation of in vitro and in vivo activity for beta-lactam antibiotics, vancomycin, erytromycin, and gentamicin. Acta Pathol Microbiol Immunol Scand Sect B 95:159–165
8. Imbimbo BP, Broccali GP, Cesana M, Crema F, Parrinello Attardo G (1991) Inter- and intrasubject variabilities in the pharmacokinetics of rufloxacin after single oral administration to healthy volunteers. Antimicrob Agents Chemother 35:390–393
9. McCabe WR, Treadwell TL (1986) In vitro susceptibility tests: correlations between sensitivity testing and clinical outcome in infected patients. In: Lorian V (ed) Antibiotics in laboratory medicine. Baltimore: The Williams and Wilkins Co., pp 925–936
10. NWA STATPAK Multi-function Statistics Library. Version 3.1. Northwest Analytical, Inc., Portland, Oregon
11. Ravizzola G, Pinsi G, Pirali F, Colombrita D, Foresti I, Peroni L, Turano A (1989) Rufloxacin (MF 934): in vitro and in vivo antibacterial activity. Drugs Exptl Clin Res XV:11–15
12. Segre G, Cerretani D, Cerri D, Moltoni L (1988) A new tricyclic fluoroquinolone, rufloxacin (MF 934), with interesting antibacterial and pharmacokinetic characteristics. Drugs Exptl Clin Res XIV:747–754
13. Zak O, O'Reilly T (1991) Animal models in the evaluation of antimicrobial agents. Antimicrob Agents Chemother 35:1527–1531
14. Zak O, Sande MA (1982) Correlation of in vitro antimicrobial activity of antibiotics with results of treatment in experimental animal models and human infection. In: Sabath LD (ed) Action of antibiotics in patients. Bern, Switzerland: Hans Huber Publishers, pp 55–56