2005

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Laurel C. Preheim

Keith M. Olsen

Mei Yue

Mary U. Snitily

Martha J. Gentry-Nielsen

Pharmacology

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Laurel C. Preheim[a,b,c,*], Keith M. Olsen[d], Mei Yue[a], Mary U. Snitily[a],
Martha J. Gentry-Nielsen[a,b,c]

[a]Infectious Diseases Section, Veterans Affairs Medical Center, Omaha, NE 68105, USA
[b]Creighton University School of Medicine, Omaha, NE 68178, USA
[c]University of Nebraska College of Medicine, Omaha, NE 68198, USA
[d]University of Nebraska College of Pharmacy, Omaha, NE 68198, USA

Abstract

A rat model was used to study the effects of cirrhosis on antibiotic therapy of pneumococcal pneumonia. Cirrhotic and control male Sprague-Dawley rats were infected transtracheally with type 3 *Streptococcus pneumoniae*. Treatment began 18 h later with phosphate-buffered saline (PBS), azithromycin (50 mg/kg), trovafloxacin (50 mg/kg), or ceftriaxone (100 mg/kg) injected subcutaneously twice daily for 5 days. Antibiotic concentrations were measured by high-performance liquid chromatography. Azithromycin, trovafloxacin, and ceftriaxone were all equally effective at preventing mortality in both cirrhotic and normal rats. Free fraction area under the curve to minimum inhibitory concentration ratio (AUC/MIC) and maximum calculated serum concentration to MIC ratio (C_{max}/MIC) and percent time that the serum concentration exceeded the MIC (%T > MIC) were greater for ceftriaxone compared with azithromycin or trovafloxacin. Azithromycin achieved higher concentrations in bronchoalveolar lavage fluid (BALF), epithelial lining fluid (ELF), and BAL white blood cells than ceftriaxone or trovafloxacin in cirrhotic rats. Macrolide, β-lactam, or fluoroquinolone antibiotic efficacy in a pneumococcal pneumonia model does not appear to be affected by hepatic cirrhosis.
Published by Elsevier Inc.

Keywords: Cirrhosis; Antibiotic efficacy; Pneumococcal pneumonia

1. Introduction

Streptococcus pneumoniae (pneumococcus) is the most common bacterial cause of adult pneumonia (File, 2003). Individuals with alcohol-induced cirrhosis are at increased risk for severe pneumococcal infections, including bacteremia (Austrian and Gold, 1964; Mufson et al., 1974). The associated mortality can exceed 50% in patients with severe liver disease even with the use of appropriate antimicrobial therapy (Gransden et al., 1985). Despite the association between cirrhosis and severity of infection, the effect of liver impairment on antibiotic penetration into pulmonary tissues and the outcome of specific forms of antibiotic

treatment of pneumococcal disease are unknown (Bergman et al., 1999).

Ceftriaxone, an expanded-spectrum cephalosporin, is approved for therapy of pneumococcal infections, including pneumonia, bacteremia, and meningitis. It is effective and widely used for infections known or suspected to be caused by penicillin-resistant pneumococci (Kaplan and Mason, 1998).

The macrolide derivative, azithromycin, has excellent in vitro antibiotic activity against a variety of lower respiratory tract pathogens, including *S. pneumoniae* (Zhanel et al., 2001). Pharmacokinetic studies in animals, including rats, have shown that azithromycin has an extended half-life and excellent penetration into lung tissue after oral (Girard et al., 1987) or parenteral (Shepard and Falkner, 1990) administration. Similarly, healthy human volunteers who received oral azithromycin had sustained lung tissue penetration and extensive accumulation in alveolar macrophages after 5 oral doses (Olsen et al., 1996).

Alatrofloxacin is an L-alanyl-L-alanine prodrug that is rapidly cleaved in vivo to the parent fluoroquinolone, trovafloxacin (Brighty and Gootz, 1997). Trovafloxacin, an advanced generation fluoroquinolone, has excellent in vitro and in vivo activity against a variety of bacterial respiratory pathogens, including penicillin-resistant pneumococci (Eliopoulos et al., 1993; Kim et al., 1997; Thomson et al., 1997). Pharmacokinetic studies in animals, including rats, have shown that trovafloxacin has an extended half-life and excellent penetration into pulmonary tissue after oral or parenteral administration (Teng et al., 1996a, 1996b). In a murine model of pneumococcal pneumonia, trovafloxacin was more effective than temafloxacin or ciprofloxacin and achieved excellent concentrations in lung tissue (Girard et al., 1995). Concentrations of trovafloxacin in bronchial mucosa, epithelial lining fluid (ELF), alveolar macrophages, and serum exceeded the MIC_{90} (minimum inhibitory concentration at which the growth of 90% of isolates is inhibited) for common respiratory pathogens after administration of oral doses to patients undergoing fiber-optic bronchoscopy (Andrews et al., 1997). Trovafloxacin is hepatically metabolized by conjugation, with 13% of the administered dose excreted unchanged (Brighty and Gootz, 1997). The effects of severe cirrhosis on the pharmacokinetics or efficacy of fluoroquinolones that undergo hepatic metabolism are unknown.

We previously have shown that cirrhotic rats are more susceptible than control rats to experimental pneumococcal pneumonia and secondary bacteremia (Mellencamp and Preheim, 1991; Preheim et al., 1991, 1992) and that administration of granulocyte colony-stimulating factor does not overcome this defect (Preheim et al., 1996). In addition, we have previously demonstrated that antibiotic pharmacodynamic parameters are an important predictor of response rate in a rat pneumococcal pneumonia model (Gentry-Nielsen et al., 2002). The purpose of this study was to determine 1) the comparative effect of cirrhosis on the efficacy of representatives from 3 antibiotic classes for the therapy of experimental pneumococcal pneumonia in our rat model and 2) the impact of cirrhosis on antibiotic serum pharmacokinetic and pharmacodynamic parameters and intrapulmonary antibiotic concentrations.

2. Materials and methods

2.1. Cirrhosis model

Male Sprague-Dawley rats were fed rat chow and water containing 1.5 mM phenobarbital. When weight reached 200 g, rats were treated weekly by gastric gavage with increasing doses of carbon tetrachloride (cirrhotic) or phosphate-buffered saline (PBS; controls) as previously described (Mellencamp and Preheim, 1991). Rats developing stable, visible ascites fluid (indicative of uncompensated cirrhosis) were rested for 1 week before use in experiments with age-matched controls (Mellencamp and Preheim, 1991).

2.2. Experimental pneumonia

Rats were anesthetized with ether and their tracheas were surgically exposed. Type 3 pneumococci (ATCC 6303) were injected transtracheally in 0.3 mL of saline at $10\times$ the expected lethal dose 50 (2×10^6 for cirrhotics and 2×10^7 for controls) as described (Mellencamp and Preheim, 1991). For survival studies, after antibiotic therapy was initiated mortality was observed and recorded for 10 days as described (Preheim et al., 1996).

2.3. Antibiotic treatment

Cirrhotic and control rats received azithromycin (Zithromax®, Pfizer, New York, NY; 50 mg/kg), alatrofloxacin (Trovan®, Pfizer; 50 mg/kg), ceftriaxone (Rocephin®, Roche Pharmaceuticals, Nutley, NJ; 100 mg/kg), or saline administered subcutaneously twice daily for 5 days beginning 18 h after experimental infection. The MICs of the study antibiotics for the infecting strain were the following: azithromycin, 0.25 mg/L; trovafloxacin, 0.19 mg/L, and ceftriaxone, 0.016 mg/L, as determined by E-test (AB Biodisk, Solna, Sweden). Serum samples for determination of pharmacokinetic and pharmacodynamic parameters were obtained from additional rats not used in mortality studies. On the third day of therapy and after the fifth antibiotic dose, serum samples were obtained at 1, 3, 5, and 12 h. All sera were frozen at -70 °C for antibiotic concentration determination.

2.4. Peripheral white blood cell counts and blood cultures

Blood was obtained using aseptic puncture of foot veins under ether anesthesia (Snitily et al., 1991). Cell counts before and on days 3 and 5 after infection were performed using a hemacytometer. Differential cell counts were performed on stained slides (Diff-Quik; Baxter Scientific Products, McGraw Park, IL). Quantitative bacterial cultures also were performed on days 3 and 5 after infection.

2.5. Bronchoalveolar lavage

Rats not used in mortality studies were killed by an intraperitoneal injection of pentobarbital and exsanguinated by cardiac puncture 1 h after the last antibiotic dose on day 3 of therapy. The lungs and trachea were removed and a 20-gauge catheter was positioned above the carina. Cold saline was injected into the lungs in 8-mL aliquots (approximately 60 mL) and recovered by gravity drainage until a final volume of 50 mL was collected as described (Preheim et al., 1991). Aliquots of bronchoalveolar lavage fluid (BALF) were cultured. Cells were collected by centrifugation, resuspended in PBS, and counted with a hemacytometer. Differential cell counts were performed on Diff-Quik–stained cytospin cell preparations.

2.6. Antibiotic assays

All serum, BALF, and BAL cells collected at steady state were assayed by high-performance liquid chromatography techniques.

Azithromycin concentrations were measured by a previously published method (Olsen et al., 1996; Shepard et al., 1994). This reverse-phase assay used an electrochemical detector set in a series mode with dual glassy carbon electrodes at 600 and 800 mV versus Ag–AgCl with aliquot extracted from the sample matrix injected onto the column. Quality control samples were assayed with each standard curve and daily during the analysis. The intra- and interday percent coefficients of variation were 5.3% and 7.9% for BALF and cell pellets and 4.4% and 5.9% in serum, respectively.

The concentrations of ceftriaxone in BALF, AM, and serum were assayed by a high-performance liquid chromatography assay with ultraviolet detection as previously described (Jehl et al., 1990). The method was validated for linearity, precision, accuracy, and specificity in our laboratory. All samples were prepared before injection on the column. Briefly, after freeze–thaw cycles of the BAL cells, acetonitrile, which contains the internal standard (cephalexin), was added to BAL cells and serum and deproteinated, vortexed, and centrifuged. An aliquot of 50 µl of the supernate was injected onto the column. The BALF samples were prepared by centrifugation and then filtered through a 0.22-µm filter before extraction and injection onto the column. Separation was performed with a C_{18} reverse-phase column (70 × 4.6 mm; particle size 3 µm; Phenomix, Torrance, CA). The mobile phase consisted of 24 mM hexadecyl trimethylammonium bromide–phosphate buffer (pH 7.0)–acetonitrile (43:5:52, v/v/v) at a flow rate of 1.5 mL/min at a temperature of 35 °C. The assay was linear over the range of 0.25–100 µg/mL with the limit of quantification set at 0.10 µg/mL. The inter- and intraday coefficients of variation were determined for each matrix: 7.7% and 3.8% for serum, 8.0% and 5.2% for BAL cells, and 9.8% and 7.8% for BALF, respectively.

Trovafloxacin concentrations in all matrixes were determined by a previously published assay (Teng et al., 1996a, 1996b). This assay used solid-phase extraction and a reverse-phase chromatography method with ultraviolet detection. Before extraction, the BAL cells were exposed to freeze–thaw cycles to release intracellular drug. After the extraction procedure, supernates of all matrixes containing the internal standard (trovafloxacin derivative) were separated by a C_{18} analytical column and equivalent guard column with the wavelength set at 275 nm. The mobile phase consisted of 0.04 M H_3PO_4–acetonitrile–terbutylammonium hydroxide–0.005 M dibutyl amine phosphate reagent (82:16.9:0.05:0.05, v/v) at pH 3. The lower range of quantification was 0.1 µg/mL and was linear over the range of 0.1–20 µg/mL for all matrixes. The intra- and interday coefficients of variation at 1.0 µg/mL were 4.5% and 5.9%

for serum, 6.7% and 8.6% for BAL cells, and 5.6% and 7.8% for BALF, respectively.

Albumin and urea concentrations in serum and BALF were determined by previously described methods (Olsen et al., 1996). The volume of ELF contained in each BALF sample was determined by the urea dilution method and results confirmed by application of the same method with albumin dilution (Renard et al., 1986). The ELF volume (V_{ELF}) is estimated by the following relationship: $V_{ELF} = V_{BAL} \times (Urea_{BAL}/Urea_{serum})$, where V_{BAL} is the volume of BAL fluid, $Urea_{BAL}$ is the urea concentration in the BALF, and $Urea_{serum}$ is the concentration of urea in the serum. The concentration of antibiotic in the ELF (ABX_{ELF}) was determined by the following formula: $ABX_{ELF} = ABX_{BAL}/V_{ELF}$, where the ABX_{BAL} is the total amount of drug in each BALF sample.

The volume of BAL cells in the cell pellet was determined by cytocentrifugation and multiplying the mean cell volume times the total number of BAL cells. The mass concentration of antibiotic divided by the cell volume produced a concentration in micrograms of cell volume (Baldwin et al., 1992; Wilcox et al., 1988).

Separation of free from protein-bound fractions of azithromycin, ceftriaxone, and trovafloxacin were determined by an ultrafiltration technique. Briefly, 0.375 mL of serum from each rat was divided into 2 aliquots, one of which was inserted into an ultrafiltration device (Amicon, Beverly, MA) and centrifuged at a fixed angle for 20 min at $1000 \times g$. The resultant ultrafiltrate and the nonfiltered serum aliquot were then analyzed for the respective antibiotics as described. The ratio of the concentration in the ultrafiltrate to the total concentration in the nonfiltered sample was used to calculate the antibiotic free fraction. With each sample run, a blank containing sterile water, a blank containing mobile phase, and saline with antibiotic spiked at a midrange concentration were analyzed to confirm no binding to the filter membrane.

2.7. Pharmacokinetics and pharmacodynamics

Antibiotic serum concentrations versus time were analyzed with WinNonlin Software, Standard Edition, Version 1.5 (Scientific Consulting, Cary, NC). The azithromycin, ceftriaxone, and trovafloxacin pharmacokinetic parameters were estimated using a noncompartmental extravascular dose input model. The area under the serum concentration time curve for all antibiotics (AUC_{0-24}) was calculated by the trapezoidal rule. Pharmacodynamic parameters were determined by dividing the MIC of the antibiotics for *S. pneumoniae* 6303 into the AUC (AUC/MIC) and the maximum calculated serum concentration (C_{max}/MIC). The percent time that the serum concentration exceeded the MIC (%T > MIC) was calculated using the equation $C_{pmin} = C_{pmax} \cdot e^{-ket}$, where C_{pmin} equals the MIC, C_{pmax} equals the calculated maximum peak serum concentration, and e^{-ket} is the decay parameter.

2.8. Statistical analysis

Mortality statistics were performed by Fisher's exact test. White blood cell (WBC) parameters and mean pharmacokinetic parameters were compared by 1-way ANOVA, with post hoc comparisons determined by Tukey's and Newman-Keuls tests, respectively. The level of significance was set at $P < 0.05$ for all analyses.

3. Results

3.1. Peripheral white blood cell counts and percentage of polymorphonuclear leukocytes

Baseline mean peripheral WBC counts before infection were significantly higher in cirrhotic rats than in controls when rats from all drug treatment groups were combined ($P = 0.002$; Fig. 1). Peripheral WBC counts rose after infection, peaked on day 5, and returned to baseline at day 10 in cirrhotic and control rats treated with azithromycin. Similar trends were seen in cirrhotic rats treated with trovafloxacin. Control rats that received trovafloxacin, however, maintained elevated peripheral WBC counts through day 10. In contrast, peripheral WBC counts did not rise in either cirrhotic or control rats treated with ceftriaxone (Fig. 1).

The mean percent polymorphonuclear leukocytes (PMNLs) in peripheral blood also was significantly higher at baseline in cirrhotic rats from all drug treatment groups when compared with controls ($P < 0.001$; Fig. 2A). In control rats, these percentages rose during infection and treatment, peaking on day 5 in groups treated with azithromycin or trovafloxacin and on day 3 in those receiving ceftriaxone. In cirrhotic rats, mean percent PMNL

remained at baseline serum concentrations through day 5 and fell by day 10 in those receiving azithromycin or trovafloxacin (Fig. 2B). PMNL counts fell more quickly in cirrhotic rats treated with ceftriaxone and were significantly lower at days 3 and 5 in this group compared with other cirrhotic rats receiving azithromycin or trovafloxacin ($P < 0.05$; Fig. 2B).

3.2. White blood cell and polymorphonuclear leukocytes in bronchoalveolar lavage fluid

On day 3 of therapy, there were no significant differences in the numbers of WBC and PMNL recovered in BALF from cirrhotic versus control rats (data not shown). There was a tendency toward higher numbers of PMNL in the BALF of PBS-treated rats in comparison with those receiving any of the antibiotics, but these differences also failed to reach statistical significance.

3.3. Bacteria in bronchoalveolar lavage fluid and peripheral blood

Three days after infection, control rats treated with any antibiotic had similar significant reductions of pneumococci in their BALF cultures when compared with control rats that were sham-treated with PBS ($P < 0.001$; data not shown). The results were similar in antibiotic- and sham-treated cirrhotic rats. All 3 antibiotics were equally effective in clearing pneumococci from the lungs when comparisons were made both within and between cirrhotic and control groups.

All PBS-treated control and 5 of 8 PBS-treated cirrhotic rats had $>10^2$ CFU/mL of *S. pneumoniae* in their bloodstream on day 3. In contrast, blood cultures on day 3 were sterile in all antibiotic treatment groups with the

Mean Peripheral White Blood Cells

Value sig. lower than for all antibiotic-treated groups (p < .05)
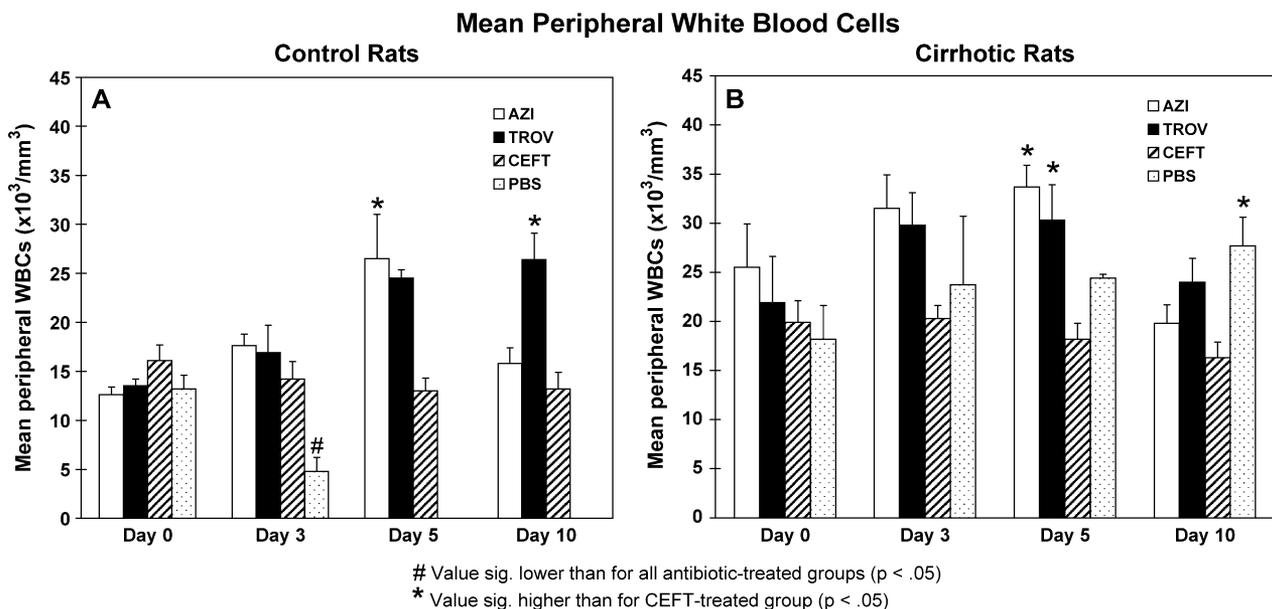* Value sig. higher than for CEFT-treated group (p < .05)

Fig. 1. Numbers of total peripheral WBCs as determined by hemacytometer count before and after infection. AZI, azithromycin; TROV, trovafloxacin; CEFT, ceftriaxone; PBS, phosphate-buffered saline.

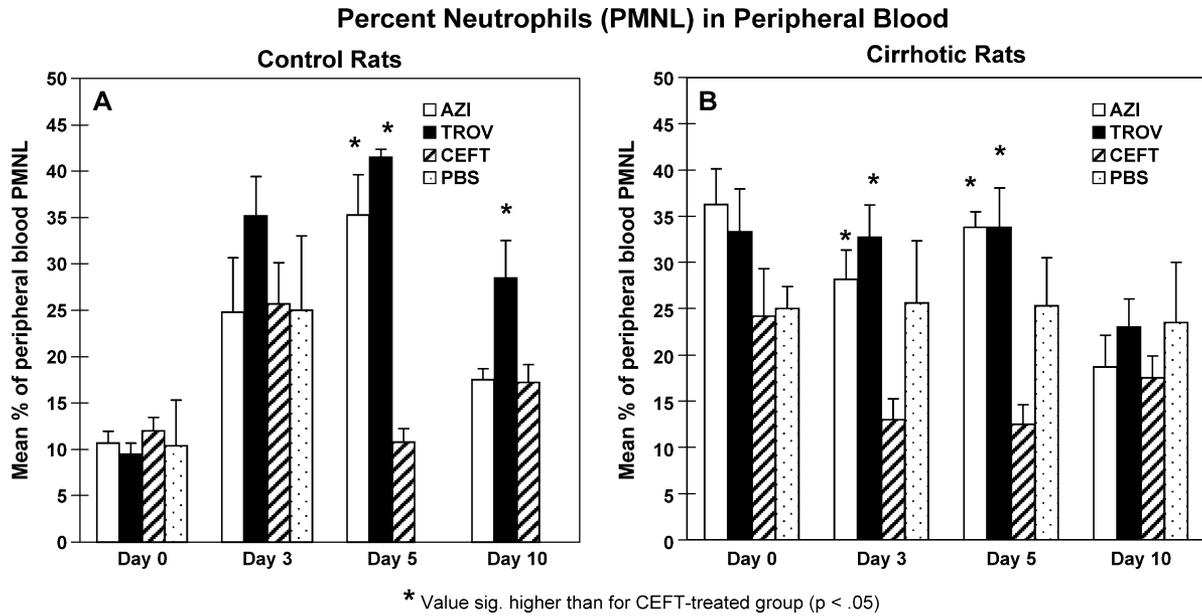## Percent Neutrophils (PMNL) in Peripheral Blood

Fig. 2. Percentage of WBCs that were neutrophils as determined by manual differentials performed on Diff-Quik–stained slides of peripheral blood smears performed before and after infection. AZI, azithromycin; TROV, trovafloxacin; CEFT, ceftriaxone; PBS, phosphate-buffered saline.

exception of 1 control rat receiving trovafloxacin (1.6×10^3 CFU/mL) and 1 control rat receiving azithromycin (4×10 CFU/mL).

3.4. Survival

In survival studies, all control rats and 70% of cirrhotic rats died after sham treatment with PBS (Fig. 3). In comparison, all of the cirrhotic and control rats treated with azithromycin, trovafloxacin, or ceftriaxone survived.

3.5. Pharmacokinetics and pharmacodynamics

Pharmacokinetics parameters varied between antibiotics (Table 1). Cirrhosis increased the free fraction of antibiotic compared with previous studies and controls (Ellbogen et al.,
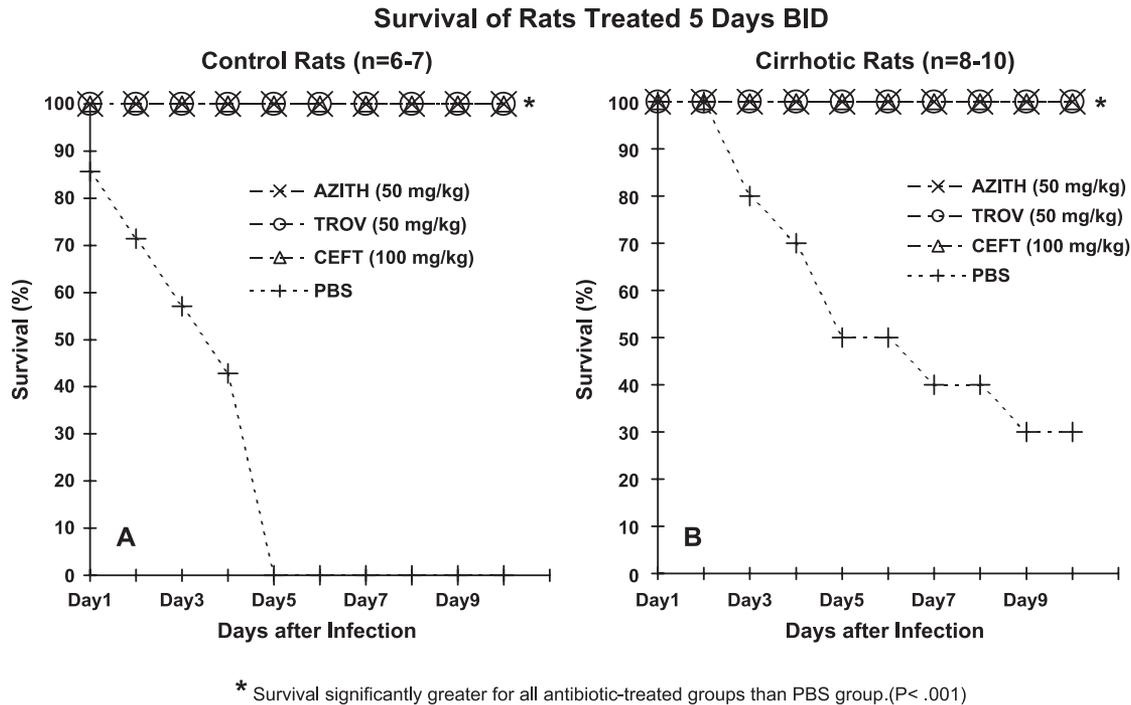

Fig. 3. Cumulative percentage survival of rats recorded for 10 days after experimental challenge. Rats were infected on day 0 with type 3 *S. pneumoniae* (2×10^7 CFU for control rats and 2×10^6 CFU for cirrhotic rats). AZI, azithromycin; TROV, trovafloxacin; CEFT, ceftriaxone; PBS, phosphate-buffered saline.

Table 1
Pharmacokinetic and pharmacodynamic parameters for ceftriaxone, azithromycin, and trovafloxacin

	Mean concentration (mg/L)		
	Ceftriaxone	Azithromycin	Trovafloxacin
Cirrhotic rats			
Serum C_{max}	92.4 ± 16.1	3.4 ± 0.3*	13.2 ± 4.5*
AUC $\pm$	59.7 ± 10.3	13 ± 1.2	41.9 ± 8.7
PB (%)	72 ± 14.5	15 ± 2.4	86 ± 3.4
C_{max}/MIC[a]	1617 ± 278	11.6 ± 1.1	9.7 ± 3.3
%T > MIC[a]	27.6 ± 4.8	12.8 ± 1.3	1.89 ± 0.6
AUC/MIC[a]	1032 ± 178	44.2 ± 5.2	30.8 ± 6.9
BALF	0[b]	1.7 ± 0.8	1.2 ± 0.9
ELF	0[b]	44.2 ± 18.9*	3.2 ± 1.4
BALF WBC	0[b]	121.2 ± 36.7*	38.4 ± 17.1
Control rats			
Serum C_{max}	106.2 ± 18.3	1.2 ± 0.2*	16.4 ± 5.9*
BALF	0[b]	1.6 ± 0.7	1.1 ± 0.9
ELF	0[b]	36.5 ± 14.6*	3.0 ± 1.0
BALF WBC	0[b]	103.2 ± 29.8*	33.5 ± 13.7

All data are expressed as mean $\pm$ SD ($n = 4$).
BALF, bronchoalveolar lavage fluid; ELF, epithelial lining fluid; BALF WBC, white blood cells from bronchoalveolar lavage fluid.
  [a] Value was calculated using antibiotic free fraction.
  [b] Below level of detection.
  * $P < 0.05$ for comparisons between drugs.

2003; Gentry-Nielsen et al., 2002; Shepard and Falkner, 1990; Teng et al., 1996a, 1996b). Using the combined data for cirrhotic and control groups, at 1 h postdose, the mean serum C_{max} was highest for ceftriaxone (92.4 ± 16.1 μg/mL), intermediate for trovafloxacin (13.2 ± 4.5 μg/mL), and lowest for azithromycin (3.4 ± 0.3 μg/mL). The free fraction serum C_{max}/MIC ratios for the ceftriaxone, azithromycin, and trovafloxacin antibiotics were 1617, 11.6, and 9.7, respectively ($P < 0.05$, ceftriaxone vs. azithromycin or trovafloxacin). The free fraction AUC/MIC ratios followed a similar pattern with free fraction values of 1032, 44.2, and 30.8 ($P < 0.05$ ceftriaxone vs. azithromycin and trovafloxacin).

In contrast to results with serum, ceftriaxone concentrations were undetectable in BALF, ELF, or BAL cells (Table 1). The mean concentrations of azithromycin and trovafloxacin in BALF were similar (1.7 ± 0.8 and 1.2 ± 0.9, respectively). This likely represents the short half-life and lack of ceftriaxone uptake into alveolar macrophages. In ELF, however, azithromycin achieved significantly higher concentrations than were observed with trovafloxacin (44.2 ± 18.9 vs. 3.2 ± 1.4 mg/L; $P < 0.05$). Similarly, the ELF concentration/MIC was higher for azithromycin than for trovafloxacin (176.8 vs. 16.3; $P < 0.01$). Intracellular concentrations of azithromycin exceeded those of trovafloxacin by approximately 3-fold in WBC obtained by BAL.

4. Discussion

Cirrhosis increases the susceptibility to and mortality from severe pneumococcal infections in both humans and rats. The precise mechanisms are unknown, but cirrhotic rats demonstrate decreased uptake and killing of pneumococci by phagocytes within their lungs (Gentry et al., 1996) and diminished clearance of complement-activating pneumococcal strains from their bloodstream (Alcantara et al., 1999). However, no published studies to date have determined whether cirrhosis affects the outcome of antibiotic therapy for these infections.

Recent guidelines for the treatment of pneumococcal pneumonia (Mandell et al., 2003) include azithromycin and advance-generation quinolones such as trovafloxacin, and ceftriaxone, which are representatives of 3 different classes of antimicrobials. These agents are highly effective in the normal host but vary with respect to their pharmacokinetics and hepatobiliary clearance.

In normal human volunteers, ceftriaxone demonstrates extensive and saturable protein binding, substantial biliary clearance, and renal elimination almost exclusively by glomerular filtration (Patel et al., 1981). In a single-dose pharmacokinetic study, Stoeckel et al., (1984) found that when compared with normal controls, patients with chronic liver disease had significantly decreased mean nonrenal clearance of unbound ceftriaxone. The elimination half-life ($t_{1/2}\beta$), however, was not influenced by chronic liver insufficiency (Stoeckel et al., 1984). Others (Hary et al., 1989) conducted a similar study in cirrhotic patients with ascites and also found no change in $t_{1/2}\beta$. Plasma concentrations versus time plots (AUC) of ceftriaxone in healthy subjects, however, are significantly higher than those in the cirrhotic patients. The apparent volumes of distribution (V_d) were higher in patients with liver disease. This finding may be because of expansion of the extracellular space by the presence of ascites. Another proposed explanation is reduction in protein binding of the drug, which would allow its movement into the extracellular compartment. Protein binding could be reduced because of decreased synthesis of albumin and/or the accumulation of endogenous binding inhibitors such as bilirubin (Hary et al., 1989). The rats in this study demonstrated similar pharmacokinetics to human subjects with cirrhosis with decreased protein binding among all antibiotics.

Our rat model of CCl_4-induced liver cirrhosis is associated with decreased serum albumin concentration, elevated serum bilirubin levels, and development of ascites (Mellencamp and Preheim, 1991). Cirrhotic rats achieved lower mean peak serum ceftriaxone concentrations compared those observed in control rats, but were not statistically different. However, the free fraction of antibiotic did increase because of the reduced protein binding. These results differ slightly from those observed in the studies with patient volunteers cited above (Jehl et al., 1990; Mazzei et al., 1993; Stoeckel et al., 1984). Unlike the single-dose ceftriaxone pharmacokinetic studies in uninfected cirrhotic patients, we measured peak serum concentrations at steady state on day 3 of therapy in rats with pneumococcal pneumonia. By this time, the infected cirrhotic rats also

had reabsorbed some of their ascitic fluid, thus, reducing the V_d of ceftriaxone. Ceftriaxone demonstrated considerably higher AUC/MIC and C_{max}/MIC ratios as well as the %T > MIC compared with other study antibiotics. Azithromycin values were higher than those of trovafloxacin, but only %T > MIC was statistically different. Although the efficacy was similar among all agents, in rats given ceftriaxone and azithromycin, peripheral WBC normalized to baseline whereas those rats receiving trovafloxacin remain elevated through study day 10. In previous pharmacodynamic studies in animal models, fluoroquinolone AUC/MIC ratios greater than 30 have been associated with efficacy in pneumococcal pneumonia (Lister and Sanders, 1999; Ambrose et al., 2001). Trovafloxacin AUC/MIC ratios barely reached the minimum efficacy value (30.8 ± 6.9) and may partially explain failure of the peripheral WBC to normalize.

Trovafloxacin undergoes primarily phase 2 hepatic metabolism (glucuronidation, N-acetylation, and N-sulfo-conjugation) with minimal oxidative metabolism (Vincent et al., 1998). The drug's pharmacokinetics do not appear to be significantly altered in patients who have acute hepatic dysfunction with elevated liver transaminases (Garey and Amsden, 1999), but patients with severe cirrhosis have not been studied. In our rat model, the presence of cirrhosis did have a small decrease of most pharmacokinetic values compared with normal rats (Teng et al., 1996a, 1996b).

The relative pharmacokinetics of azithromycin, trovafloxacin, and ceftriaxone varied widely as expected. Ceftriaxone reached peak serum concentrations that exceeded by 1–2 logs, respectively (ie, those achieved by trovafloxacin and azithromycin in both cirrhotic and control rats). In contrast, ceftriaxone was undetectable in BALF or ELF obtained minutes after the serum samples were drawn. The MICs for trovafloxacin and azithromycin against the test strain of pneumococcus were 10-fold higher than the MIC for ceftriaxone. The success of azithromycin and trovafloxacin in this pneumonia model is likely related to multiple factors, including antibiotic penetration into bronchial secretions and pulmonary phagocytes, where they achieved concentrations that were well above their MICs for *S. pneumoniae*.

The peripheral WBC is commonly used to help determine the severity of infection and response to therapy in humans. No rise in peripheral WBC counts was observed in control or cirrhotic rats treated with ceftriaxone. In contrast, peripheral WBC counts initially rose before returning to baseline in those receiving azithromycin or trovafloxacin. This result may have been because of a more rapid response to treatment with ceftriaxone, thus averting the initial leukocytosis after infection. The %T > MIC pharmacodynamic parameter at approximately 50% of the dosing interval best describes activity and clinical outcomes of ceftriaxone and other cephalosporins. However, in this study, %T > MIC for ceftriaxone exceeded the MIC for only 27% of the dosing interval, predicting a higher treatment failure, which we did not observe. At very high

AUC/MIC ratios against pneumococci, ceftriaxone acted in a concentration-dependent manner in this model. If a pneumococcal strain with an MIC > 1 had been used, AUC/MIC ratios would have fallen to <100, making them more comparable to azithromycin and trovafloxacin. The persistent elevation in peripheral WBC observed in control rats treated with trovafloxacin was likely because of an inflammatory reaction and the minimally acceptable AUC/MIC ratios required for efficacy. Tender subcutaneous nodules developed at the injection site where rats received alatrofloxacin. No nodules were found in rats treated with ceftriaxone, and only a minimal local reaction to azithromycin was noted. Despite these differences in WBC responses to therapy, all antibiotics were equally effective in removing pneumococci from the lung and the bloodstream. In addition, there was no difference in mortality among rats in any of the antibiotic treatment groups.

The impact of azithromycin on BALF WBC counts was not significantly different from what was observed in the other antibiotic groups. Macrolides, including azithromycin, possess anti-inflammatory properties that could reduce the overall number of neutrophils in the respiratory tract (Yamaryo et al., 2003; Tamaoki, 2004). The macrolide anti-inflammatory response is generally thought to effect cytokine production and neutrophil accumulation in noninfected patients and animal models (Tamaoki, 2004). In a study of azithromycin's effect on neutrophil function in healthy human subjects, inflammatory mediators were found to be reduced, although the circulating WBC did not change acutely or after 28 days of therapy (Culic et al., 2002). In a preliminary study in 30 noninfected subjects with stable chronic obstructive lung disease, clarithromycin therapy resulted in a reduction of airway neutrophils (Basyigit et al., 2004). Despite these studies in noninfected patients, the effect of azithromycin on peripheral and pulmonary neutrophils during infection remains largely unknown. The anti-inflammatory properties of azithromycin did not appear to blunt the WBC response in our model of acute pneumonia.

In summary, uncompensated cirrhosis has no impact on the efficacy of azithromycin, trovafloxacin, or ceftriaxone when administered parenterally in a rat model of pneumococcal pneumonia. These results indicate that, despite their predisposition to severe pneumococcal infections in cirrhotic hosts, efficacy does appear to be affected by antibiotic choice. In this model, azithromycin, ceftriaxone, and trovafloxacin were equally effective at preventing mortality. Additional studies are needed to determine whether cirrhosis affects the pharmacokinetics or efficacy of orally administered azithromycin and fluoroquinolones for the treatment of pneumococcal pneumonia.

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